*Reprinted from the*
ANNALS OF THE ENTOMOLOGICAL SOCIETY OF AMERICA

# Mallophaga: In Vitro Testing of Artificial Diets[1,2]

D. E. HOPKINS, W. F. CHAMBERLAIN, AND A. R. GINGRICH
U.S. Livestock Insects Laboratory, Agric. Res. Serv., USDA, Kerrville, TX 78028

ABSTRACT

Populations of *Bovicola limbatus* (Gervais) increased 4.5–5.7× over ca. 1.5 generations on diets of goat flesh and wool extract, dehydrated veal and dehydrated lanum, and goat flesh and mohair extract (ratios = 3:1 by wt). Populations of *B. crassipes* (Rudow) increased 4.8× and those of *B. ovis* (Schrank) 2.8× over ca. 1.5 generations on dehydrated veal and wool extract (ratios=4:1 and 3:1, respectively, by wt). The increases were less for lice from field collections than for lice from colonies that had been reared in vitro for 6–7 years.

*Bovicola crassipes* (Rudow), often called the hairy goat biting louse, *B. limbatus* (Gervais), the Angora-goat biting louse, and *B. ovis* (Schrank), the sheep biting louse, can be reared, in vitro, on preparations of skin from the natural host (Hopkins and Chamberlain 1969, 1972a; Hopkins 1970). However, such skins are not always readily obtainable, and diets prepared from them can vary in nutrient value (Hopkins and Chamberlain 1972b). Therefore, we attempted to develop diets composed of commonplace ingredients likely to be consistent in quality. Various tissues and oils were mixed together and their effect upon the fecundity and longevity of lice were evaluated.

## MATERIALS AND METHODS

The tissues used were (1) goat, (2) beef, or (3) sheep flesh chopped to less than 1 $cm^2$, dried up to 8 h at 40°C, shredded in a blender, and sifted through a 14×18-mesh sieve; (4) fishmeal (with 60% protein); (5) pulverized liver (Nutritional Biochemical Corp.); and (6) dehydrated powdered veal (Difco Laboratories). All tissues except the liver and veal were washed 2–4 times with Skelly-Solve B (Sk. B), a mixture of low-boiling hydrocarbon fractions, to remove the organosoluble material.

The oils were Sk. B extracts of raw wool and mohair, lanolin (Mallinckrodt Co.), lanum (hydrated lanolin (25–30% $H_2O$) (Merck and Co.), and dehydrated lanum. We dehydrated the lanum by mixing it with Sk. B (Skelly-Solve B) (10 g/45 ml), letting the mixture stand until it separated into water and solvent layers, and then collecting the solvent layer and evaporating the Sk. B.

To prepare diets, we weighed samples of each oil into glass containers and used a spatula to mix in the desired weights of each tissue. The ratios of tissue to oil by wt were 2:1, 3:1, or 4:1. To facilitate mixing, we added Sk. B (1 μl/mg of tissue) or heated the ingredients to ca. 50°C. The diets containing Sk. B were dried completely and then remixed. However, lice cannot tolerate even small amounts of Sk. B in the diet so care was taken to evaporate this solvent completely (as determined by weighings) when it was contained in diet materials. The materials were heated on a hotplate at ca. 50°C under a stream of $N_2$ or air or placed in a forced air oven at ca. 47°C for 48 or more hours. The diets were stored at room temp for as much as one year.

The lice used in the tests were collected from natural hosts and from colonies that had been maintained in vitro at the U. S. Livestock Insects Laboratory at Kerrville, TX for 6–7 years. The in vitro colonies of *B. limbatus* were maintained on a diet containing dehydrated veal and dehydrated lanum, 3:1; those of *B. crassipes* and *B. ovis* were maintained on a diet containing goat flesh and wool extract, 3:1.

*Survival and Fecundity of Lice from In Vitro Colonies.*—In the tests of survival and fecundity, the ratio of tissue to oil was always 3:1. Duplicate diet samples of 50 mg each were placed in 0.5-dr glass shell vials with several pieces of mohair (for oviposition) plus 2 ♂ and 5 ♀ adult lice of random ages.

The vials containing the diets and lice were held at controlled temperatures in closed polystyrene jars (ca. one liter capacity) that contained saturated aqueous salt solutions to control the RH. *B. limbatus* and *B. crassipes* were held at 35±1.5°C and 72% RH ($NaClO_3$ sol), and *B. ovis* were held at 37±1.5°C and 68% RH ($NH_4Cl$ and $KNO_3$ sol).

At 2 wk the numbers of eggs and of surviving adults were recorded. The adults were removed, and any eggs or nymphs were left. Then at 4 wk and every 2–4 days thereafter, all subsequent adults were removed, and the numbers were recorded. These data could thus be used to determine yields (percentages) of adults from eggs.

*Population Increase of Lice from Field Collections and from In Vitro Colonies.*—Tests were made with

[1] Mention of a proprietary product in this paper does not constitute an endorsement of this product by the USDA.
[2] Received for publication Feb. 24, 1976.

Table 1.—Survival and fecundity of P females after 2 wk and yields of $F_1$ adults of 3 species of biting lice maintained in vitro on diets composed of various tissues and oils (ratio of parts by wt = 3:1). Starting populations in each test were 5 ♀ and 2 ♂. Numbers shown are totals for duplicate tests.

| Diet ingredients | | No. live females | No. eggs | No. $F_1$ adults | % yield of adults from eggs |
|---|---|---|---|---|---|
| | *B. limbatus* | | | | |
| Goat flesh | : wool ext. | 9 | 125 | 100 | 80 |
| | : mohair ext. | 7 | 64 | 32 | 50 |
| | : lanolin | 2 | 61 | 27 | 44 |
| | : lanum | 7 | 91 | 35 | 38 |
| | : dehy-lanum | 4 | 39 | 5 | 13 |
| Cow flesh | : wool ext. | 2 | 83 | 37 | 45 |
| | : mohair ext. | 6 | 51 | 20 | 39 |
| | : lanolin | 6 | 76 | 12 | 16 |
| | : lanum | 1 | 56 | 17 | 30 |
| | : dehy-lanum | 5 | 64 | 15 | 23 |
| Sheep flesh | : wool ext. | 9 | 113 | 84 | 74 |
| | : mohair ext. | 8 | 84 | 18 | 21 |
| | : lanolin | 7 | 83 | 9 | 11 |
| | : lanum | 6 | 56 | 3 | 5 |
| | : dehy-lanum | 6 | 79 | 8 | 10 |
| Dehy-veal | : wool ext. | 7 | 101 | 72 | 72 |
| | : mohair ext. | 6 | 31 | 3 | 10 |
| | : lanolin | 6 | 62 | 15 | 24 |
| | : lanum | 8 | 56 | 39 | 70 |
| | : dehy-lanum | 9 | 95 | 65 | 68 |
| Fishmeal | : wool ext. | 9 | 50 | 0 | 0 |
| | : mohair ext. | 0 | 3 | 0 | 0 |
| | : lanolin | 1 | 10 | 0 | 0 |
| | : lanum | 0 | 5 | 0 | 0 |
| | : dehy-lanum | 0 | 4 | 0 | 0 |
| Liver (pulv) | : wool ext. | 4 | 48 | 0 | 0 |
| | : mohair ext. | 0 | 23 | 0 | 0 |
| | : lanolin | 0 | 9 | 0 | 0 |
| | : lanum | 0 | 13 | 0 | 0 |
| | : dehy-lanum | 0 | 11 | 0 | 0 |
| | *B. crassipes* | | | | |
| Sheep flesh | : wool ext. | 4 | 78 | 37 | 43 |
| | : mohair ext. | 3 | 79 | 60 | 76 |
| | : lanolin | 0 | 21 | 0 | 0 |
| | : lanum | 0 | 23 | 0 | 0 |
| | : dehy-lanum | 0 | 12 | 0 | 0 |
| Dehy-veal | : wool ext. | 5 | 52 | 21 | 42 |
| | : mohair ext. | 8 | 130 | 20 | 15 |
| | : lanolin | 6 | 75 | 30 | 40 |
| | : lanum | 0 | 4 | 0 | 0 |
| | : dehy-lanum | 0 | 18 | 0 | 0 |
| | *B. ovis* | | | | |
| Sheep flesh | : wool ext. | 6 | 34 | 3 | 9 |
| | : mohair ext. | 7 | 28 | 2 | 7 |
| | : lanolin | 6 | 46 | 4 | 9 |
| | : lanum | 0 | 0 | 0 | 0 |
| | : dehy-lanum | 0 | 0 | 0 | 0 |
| Dehy-veal | : wool ext. | 9 | 30 | 8 | 27 |
| | : mohair ext. | 6 | 43 | 1 | 2 |
| | : lanolin | 8 | 18 | 0 | 0 |
| | : lanum | 0 | 0 | 0 | 0 |
| | : dehy-lanum | 0 | 0 | 0 | 0 |

diets containing ratios of tissue and oil of 2:1, 3:1, and 4:1 to determine which best supported increases in the louse populations. Duplicate samples of 200 mg each were placed in 2-dr glass shell vials with some pieces of mohair (for oviposition). Then 3 ♂ and 10 ♀ adult lice of random ages were transferred directly from natural hosts or from in vitro colonies onto the test diets. The diets and lice were held at the conditions previously described for each species. At 6 wk, i.e., after ca. 1.5 generations, the numbers of live lice were recorded, and the rates of population increase were determined by dividing the numbers alive by the starting numbers.

## RESULTS

*Survival and Fecundity of Lice from In Vitro Colonies. (Table 1).*—The best diet for *B. limbatus* was that containing goat flesh and wool extract; also results were generally good when a diet contained sheep flesh and wool extract, dehydrated veal and wool extract, or dehydrated veal and dehydrated lanum. No $F_1$ lice developed to the 4th stadium on diets containing liver or fishmeal.

With *B. crassipes,* results were generally best with diets containing sheep flesh and mohair extract, but relatively good results were obtained with sheep flesh and wool extract. No $F_1$ lice developed to the 4th stadium on diets of sheep flesh and lanolin, lanum, or dehydrated lanum or on diets of dehydrated veal and lanum or dehydrated lanum.

With *B. ovis,* results were best with diets containing dehydrated veal and wool extract. Few or no 4th instars developed on the other diets.

*Population Increases. (Table 2).*—Relatively high rates of increase of in vitro *B. limbatus,* 4.5–5.7×, were obtained on diets containing goat flesh and wool extract, 3:1, dehydrated veal and dehydrated lanum, 3:1, and goat flesh and mohair extract, 3:1. With in vitro *B. crassipes,* a greater rate of increase, 4.8×, was obtained with dehydrated veal and wool extract, 4:1, than with any other diet. With in vitro *B. ovis,* a greater rate of increase, 3.8×, was obtained with dehydrated veal and wool extract, 3:1, than with dehydrated veal and wool extract, 2:1 or 4:1.

With field-collected *B. limbatus,* Table 2 shows the greatest rate of increase, 1.7×, was obtained with goat flesh and wool extract, 3:1, and with sheep flesh and wool extract, 3:1. With field-collected *B. crassipes,* the greatest rate, 2.2×, was obtained with sheep flesh and wool extract, 3:1. With field-collected *B. ovis,* the greatest rate, 0.5×, was obtained with dehydrated veal and wool extract 3:1.

## DISCUSSION

On the basis of performance and availability of ingredients, specific artificial diets could be chosen as standards for in vitro colonies of the 3 species of lice. We chose dehydrated veal and dehydrated lanum, 3:1, for *B. limbatus;* dehydrated veal and wool extract, 4:1, for *B. crassipes;* and dehydrated veal and wool extract, 3:1, for *B. ovis.*

Except with *B. crassipes* on dehydrated veal and

Table 2.—Total numbers of live nymphs and adults of 3 species of lice after 6 wk and rates of population increase (no. lice at 6 wk/no. at start of test) when starting populations of 3 ♂ and 10 ♀ from field collections or from in vitro colonies were maintained on diets composed of various tissues and oils. (Duplicate tests.)

| Diet ingredients and ratio of parts by weight | | Field-collected lice | | | In vitro-colony lice | | |
|---|---|---|---|---|---|---|---|
| | | No. nymphs | No. adults | Rate of increase | No. nymphs | No. adults | Rate of increase |
| | | | *B. limbatus* | | | | |
| Sheep flesh:wool ext. | 3:1 | 21 | 24 | 1.73 | 21 | 39 | 2.31 |
| Goat flesh:wool ext. | 3:1 | 14 | 31 | 1.73 | 40 | 108 | 5.69 |
| Goat flesh:mohair ext. | 3:1 | 0 | 0 | 0 | 26 | 92 | 4.54 |
| Goat flesh:lanolin | 4:1 | 1 | 1 | .08 | 0 | 14 | .54 |
| Dehy-veal:wool ext. | 3:1 | 0 | 1 | .04 | 3 | 1 | .15 |
| Dehy-veal:lanolin | 3:1 | 1 | 0 | .04 | 1 | 1 | .08 |
| Dehy-veal:dehy-lanum | 3:1 | 5 | 7 | .46 | 61 | 62 | 4.73 |
| Dehy-veal:dehy-lanum | 4:1 | 13 | 7 | .77 | 37 | 71 | 4.15 |
| Dehy-veal:lanum | 3:1 | 6 | 14 | .77 | 37 | 11 | 1.85 |
| | | | *B. crassipes* | | | | |
| Sheep flesh:wool ext. | 3:1 | 23 | 35 | 2.23 | 75 | 16 | 3.50 |
| Dehy-veal:wool ext. | 4:1 | 7 | 3 | .38 | 97 | 29 | 4.80 |
| Dehy-veal:lanolin | 4:1 | 27 | 19 | 1.77 | 1 | 6 | 0.30[a] |
| | | | *B. ovis* | | | | |
| Dehy-veal:wool ext. | 2:1 | 5 | 0 | .19 | 3 | 8 | 0.42 |
| Dehy-veal:wool ext. | 3:1 | 8 | 5 | .50 | 33 | 39 | 2.77 |
| Dehy-veal:wool ext. | 4:1 | 0 | 1 | .04 | 14 | 7 | 0.81 |

[a] Single test.

lanolin, 4:1, the rate of increase was greater with in vitro-reared than with field-collected lice. Thus the in vitro colonies were either already adapted or were more adaptable to the test diets than were the lice from field collections. Any of the 3 species taken in field collection could probably be established in vitro on the diets that gave the highest rates of increase, but time for establishment would likely require several generations. The delay would probably be greatest with *B. ovis* since the highest rate of increase of field-collected *B. ovis* was only 0.5×.

The colonies of these 3 species that had been reared in vitro for several years before the present test have now thrived on the new standard diets for well over one year. The rearing procedures are generally the same as those described by Hopkins and Chamberlain (1969) for rearing *B. crassipes* and *B. limbatus* and by Hopkins (1970) for rearing *B. ovis*. The major exceptions were the standard diets as described herein (instead of diets prepared from skins) and the use of 72% RH for rearing *B. limbatus*.

## REFERENCES CITED

**Hopkins, D. E.** 1970. In vitro colonization of the sheep biting louse, *Bovicola ovis*. Ann. Entomol. Soc. Am. 63: 1196–7.

**Hopkins, D. E., and W. F. Chamberlain.** 1969. In vitro colonization of the goat biting lice, *Bovicola crassipes* and *B. limbata*. Ibid. 62: 826–8.

1972a. In vitro colonization of the cattle biting louse, *Bovicola bovis*. Ibid. 65: 771–2.

1972b. Sheep biting louse: Notes on the biology of lice reared off the host. Ibid. 65: 1182–4.