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A COMPARISON OF FECAL PROTEIN CONTENT IN MALE AND FEMALE CAT FLEAS, *CTENOCEPHALIDES FELIS* (BOUCHE´) (SIPHONAPTERA: PULICIDAE)

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The diet of the larval cat flea, *Ctenocephalides felis* (Bouche´), has been the focus of study by a number of researchers (Bruce 1948; Hsu et al. 2002; Moser et al. 1991; Richman et al. 1999). The consensus is that adult flea feces are the essential nutritional requirement for developing cat flea larvae. Various other organic materials found within the micro-habitat of larvae, previously thought to be of importance, have proven to have no significance in the diet (Strenger 1973).

There is an incomplete utilization of host blood imbibed by adult cat fleas (Hinkle et al. 1991; Silverman & Appel 1994). Hinkle et al. (1991) reported that the protein content of cat flea feces was actually higher than the bovine blood upon which they fed, while Silverman & Appel (1994) found only a slight difference. The fact that adult flea feces are nutritionally necessary for larval development has led to the suggestion that there may be a unique form of parental investment exhibited in cat fleas (Hinkle et al. 1991; Silverman & Appel 1994).

The objectives of this study were to compare protein content in the feces of adult male and female *C. felis* over a 10-day feeding period and to examine the extent to which male fleas may provide protein for developing flea larvae.

Adult male and female cat fleas were reared from eggs at the Missouri Research Center Laboratory in Fulton, Missouri. Fleas were held in an incubator at 28°C and 85% RH and exposed to a 12:12 light/dark regime until used for study purposes.

At 25 days following the egg collection date, fleas were sorted to sex on a vacuum stage under a stereomicroscope for identification. Males and females were aspirated from the stage separately and placed individually into test tubes.

A total of 190 female and 285 male fleas were fed bovine blood containing a 20% solution of sodium citrate in an artificial membrane system (FleaData, Inc., Freeville, NY) similar to the one described by Wade & Georgi (1988). Blood was obtained from a Holstein calf that had no history of exposure to parasiticides. Blood in the feeding sleeves was maintained at approximately 38°C to simulate blood temperature of the live host. Fleas were provided with fresh blood daily from a container refrigerated at 4°C. After six days, fresh blood was obtained from the same calf.

Flea feeding cages were 6 mm in diameter and 1.5 mm deep and consisted of two chambers. The upper chamber containing fleas had a fine nylon mesh top through which fleas imbibed the blood and a bottom of coarse mesh that allowed feces to fall through but which also prevented fleas from escaping. A very thin layer of clean cat hair was placed in the upper chamber with the fleas to facilitate movement onto the feeding screen. The lower chamber was fastened to the upper chamber but was removable. The lower chamber had a very fine mesh bottom, and its sole purpose was to collect feces that fell from fleas in the upper chamber. Every 24 h for a 10-day period, the lower chamber was removed from each feeding cage and the feces were transferred to ½-dram glass vials. Flea feeding cages were shaken vigorously before removal of the lower chamber to make sure all feces were captured for each particular day. All vials were immediately placed in a freezer at –20°C after collection.

One milligram aliquots of adult male and female feces from each of 10 consecutive feeding days were dissolved in 1 ml of de-ionized water, vortexed, and centrifuged for five minutes at 3000 rpm to move any foreign material to the bottom of the test tube. Thirty microliters of supernatant from each sample were combined with 1.5 ml Coomassie Blue reagent (Pierce) in a modified Bradford (1976) total protein assay. Samples were vortexed again and then transferred to square disposable 10-mm cuvettes (Elkay Products) where they were allowed to incubate at room temperature for 10 minutes.

Six replicate samples from each of the 10 days for male and female fleas were measured by absorbance for total protein content with a spectrophotometer (Shimadzu UV-1601) at a wavelength of 595 nm. Protein concentrations were estimated with reference to absorbance values obtained for a series of standard protein dilutions of known concentration, which were assayed along with the flea fecal samples.

A standard curve was prepared by plotting the average blank-corrected 595 nm measurement for each standard versus its concentration in (µg/mg). Total fecal protein concentrations for male and female fleas on each of the 10 days was analyzed with a t-test ($\alpha = 0.05$).

In comparing total protein concentration between adult male and female feces over a 10-day period, a significant difference was found only on day 1 of the study with males having a higher total protein concentration (Table 1). The mean to-

TABLE 1. MEAN ± SE PROTEIN CONCENTRATION (µG/MG) MEASURED DAILY FROM C. FELIS DRY MALE AND FEMALE FECES.

Sex	Day 1	2	3	4	5	6	7	8	9	10
Male	2979.8 ± 123.4*	2639.8 ± 94.1	2633.2 ± 93.7	2635.8 ± 96.1	2561.2 ± 87.6	2621.8 ± 98.6	2647.0 ± 107.7	2479.5 ± 30.7	2646.0 ± 92.8	2598.2 ± 107.6
Female	2597.8 ± 27.4*	2613.3 ± 101.7	2507.5 ± 34.59	2483.0 ± 26.0	2559.2 ± 70.9	2656.2 ± 89.7	2647.0 ± 99.3	2634.2 ± 81.0	2530.5 ± 27.4	2622.2 ± 105.2

Means on the same day followed by an asterisk are significantly different ($\alpha = 0.05$).

tal protein concentration between males and females for the other 9 days of the feeding period resulted in no significant difference between the two sexes.

SUMMARY

This study determined that male feces contain the same amount of protein as female feces when measured as total protein. Protein content in male feces was as high as, or significantly higher than, female feces throughout the entire period of this study. These results demonstrate that male *C. felis* are equally capable of providing protein for developing larvae, as are females.

Whether or not the inefficient use of host blood warrants a form of parental investment is debatable. An alternate hypothesis is that adult fleas are imbibing large volumes of blood to glean nutrients that may be at low levels in the blood. A comparison of a wider range of host blood nutrients to the feces excreted could support such a hypothesis.

The significance of the differences in fecal protein content observed on day 1 can only be conjectured without further investigation. While the data presented here indicate that there is equal qualitative investment between the sexes, the greater volume of feces produced by the female may be indicative of a greater quantitative investment.

In conclusion, these data demonstrate that male *C. felis*, while providing a smaller volume of food, are providing a food source as equally rich in total protein as the female of the species.

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