Adult Cat Flea (Siphonaptera: Pulicidae) Excretion of Host Blood Proteins in Relation to Larval Nutrition

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ABSTRACT Protein content and ion composition of host blood and feces from blood-fed adult cat fleas, *Ctenocephalides felis* (Bouché), were examined. Total fecal protein differed slightly from host blood indicating that there was little digestion of host blood by adult fleas. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis gels of host blood and flea feces also revealed little digestion, as evidenced by the quantitatively and qualitatively similar protein banding patterns. Blood and fecal ion compositions also were similar, except for fleas fed in vivo on cats, where both potassium and chloride compositions of feces were ≈50% lower. Host blood or flea feces alone were not of sufficient food value to allow >12.5% of larvae to develop to adults. The addition of yeast or dog chow increased larval development to the pupal stage to 100% and 87.5% of first instars tested, respectively. Although some larval survival occurred with diets of dog chow + yeast (37.5%), feces + hair (20%), and feces + stratum corneum (16.7%), the period to cocoon formation was twice as long as that of larvae fed diets containing blood or flea feces + yeast. In contrast to fleas, feces of adults of two other hematophagous insects—bed bugs, *Cimex lectularius* L., and mosquitoes, *Aedes aegypti* (L.)—contained significantly less protein and more digested protein than the blood that they had fed upon. We propose that host blood excretion evolved in response to larval nutritional requirements.

KEY WORDS *Ctenocephalides felis*, larval diets, protein composition

ADULT CAT FLEAS, *Ctenocephalides felis* (Bouché), are obligate ectoparasites of a variety of mammalian species including dogs, cats, opossums, and goats (Hubbard 1947, Holland 1949, Benton 1980). Large quantities of blood are both consumed and excreted, particularly by the females (Joseph 1976, Dryden & Gaafar 1991, Hinkle et al. 1991). Like the larvae of many ectoparasites, larval cat fleas live off the host. Cat flea eggs and larvae and adult feces are found in areas frequented by the host, such as the carpeting in a doghouse or in the folds of a sofa cushion (Dryden 1989, Byron & Robinson 1991).

Flea feces or whole blood are necessary for larval development (e.g., Bruce 1948, Moser et al. 1991). Moser et al. (1991) indicated that flea feces alone were sufficient for >79% of larvae to develop to the adult stage. Other organic materials (e.g., yeast and dog chow) improved larval survival when compared with blood alone (e.g., Bruce 1948), but did not significantly affect development in other studies (e.g., Moser et al. 1991). Larval cat fleas also develop when feeding on younger or injured flea larvae (Moser et al. 1991, Strenger 1973; J. S., unpublished data) and flea eggs (Strenger 1973), but will not develop on decaying vegetable matter, feathers, or cat feces (Strenger 1973; J. S., unpublished data).

The objectives of this study were to quantify the dietary requirements of developing larval cat fleas and to investigate the relationship between host blood and adult flea feces composition. The relationship between host blood and feces composition in cat fleas was compared with that in two hematophagous insect species that are ecologically and phylogenetically distinct.

Materials and Methods

Insects. Adult fleas used in in vitro feeding studies and feces produced by fleas fed on cats were obtained from USDA–ARS, Gainesville, FL. Flea larvae used in diet studies were obtained as eggs from C. L. Hendricks, Auburn University College of Veterinary Medicine, Auburn, AL. Nymphal and adult *Cimex lectularius* L. were obtained from Insect Control and Research, Baltimore, MD. Adult *Aedes aegypti* (L.) were provided by G. R. Mullen, Department of Entomology, Auburn University, Auburn, AL. Adult fleas were fed either citrated bovine or feline blood using a device similar to that described by Wade & Georgi (1988). A diffusion cell system (Vanguard Instruments, Melville, NY) was modified so that each water-jacketed cell could be fashioned with stretched parafilm (American Can, Greenwich, CT). Each cell rested above a plastic container (5 cm diameter

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by 3 cm high) housing the insects. The top and bottom of each container were fitted with nylon screen to allow access of the blood to the insects and to collect insect feces. The temperature of the blood was maintained at 38°C. The blood was changed every 24 h, and the feces were collected 3 d after the insects were placed in the feeding chambers. We waited 3 d to accumulate sufficient feces for the assays.

Protein Analysis. For protein quantification and sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE), 1 ml of blood was dispensed in a watch glass and dried in a desiccator for 3 h. Feces collected from fleas fed either bovine or feline blood and feces from bed bugs and mosquitoes fed bovine blood also were dried. We used bovine blood because it was a suitable food source for in vitro rearing (Wade & Georgi 1988). We also wanted to compare bovine blood assimilation with that of a natural host. One mg of dried blood or feces was dissolved in 900 μl diH_2O and 100 μl 1% Triton-X 100 and centrifuged (12,000 rpm) for 1 min. The supernatant was diluted 1:10 in diH_2O, and 50 μl were analyzed for total protein by the Bradford (1976) assay using a standard curve for hemoglobin with absorbance measured at 595 nm. Colored solutions can interfere with the measurement of total protein by spectrophotometric methods. To minimize this difficulty, the assay was calibrated using a hemoglobin (colored) standard (Sigma, St. Louis, MO). Solutions of bed bug and mosquito feces were analyzed similarly for proteins except that they were not diluted 1:10. Six replicates were performed for each blood/feces type for fleas, and three replicates for bed bug and mosquito feces. For SDS-PAGE, samples were electrophoresed in a 4% polyacrylamide stacking and 10% polyacrylamide separating gel containing SDS (Bio-Rad Laboratories, Richmond, CA) on a Hofer (San Francisco, CA) vertical slab apparatus, using the discontinuous buffer system of Laemmli (1970). Sample buffer contained 10% vol/vol glycerol, 0.002% (wt/vol) bromophenol blue (Bio-rad), 2% (wt/vol) SDS, and 5% 2-mercaptoethanol (Sigma) in 63 mM tris-HCL, pH 6.8. Molecular weight standards were from Bio-Rad. Gels were fixed and silver-stained (Health Products Inc., Rockford, IL) according to Merril et al. (1981). Protein bands were quantified with an ultrascan densitometer (LKB), and numerical integration qualitative results from one gel are reported.

Ion Analysis. Fresh bovine and feline blood were collected at the Auburn University College of Veterinary Medicine. Ten 50-μl samples of the citrated whole blood of each species were weighed to the nearest 0.01 mg on an analytical balance and dried at 50°C in an 11-liter desiccator filled with 0.5 kg anhydrous $CaSO_4$ (Drierite, W. A. Hammond Drierite, Xenia, OH). The proportion of total blood water content was calculated as the difference between the initial mass of the fresh blood and the dry mass divided by the initial mass. Fresh blood, dried blood reconstituted with diH_2O to its initial mass, and dried insect feces dissolved in water to give the same concentration (wt/wt) as the blood that the insect had fed upon were analyzed for inorganic ion concentration. The potassium and chloride concentrations of whole blood and dissolved fecal samples (three to six replicates for each ion) were determined with a flame photometer and a chloridometer, respectively (Radiometer, Copenhagen, Denmark). Sodium concentrations were not determined because of the large amount of sodium added to the blood as sodium citrate.

Larval Diet and Development. One- to 2-day-old cat flea eggs were obtained from the Auburn University College of Veterinary Medicine, where flea colonies were maintained according to Silverman et al. (1981). Eggs were cleaned of all debris, and groups of 100 were placed in 100-ml covered plastic cups maintained in an 11-liter desiccator at 25–27°C and 75% RH. Eggs were examined daily to determine hatching. One-day-old unfed larvae were used in all experiments.

Larvae were placed individually into no. 00 gelatin capsules (Eli Lilly, Indianapolis, IN) with 0.33 g of smooth sterile sand (500 μm diameter). The sand was sterilized by heating it to 200°C for 48 h. Rows of capsules containing larvae, sand, and the various diets were attached to index cards with double-sided tape and maintained at 25–27°C, 75% RH, and continuous darkness. Capsules were examined daily for mortality and cocoon formation, and then periodically for adult emergence. Diet experiments were conducted three times using eight individual larvae per treatment for a total sample size of 24 larvae per treatment.

All combinations of the following foods were used: brewer's yeast (Dayspring Health Food, Auburn, AL), ground dog chow (Purina, St. Louis, MO), bovine blood, and adult flea feces collected from the colony. Bovine blood was obtained by venipuncture from live cows and dried in glass petri dishes at 37°C. In addition, samples of cat skin flakes (stratum corneum) and shed hair were combined with flea feces in some tests. Exactly 0.50 mg of each diet component was used in each capsule. Thus, for example, a three-component diet contained 1.5 mg of material. The relationship between the quantity of flea feces and larval longevity was determined by placing 0.025, 0.05, 0.075, 0.1, 0.2, or 0.5 mg of flea feces into capsules containing one larva and 0.33 g of sand.

Statistical Analysis. Protein composition of host blood and insect feces, ion concentrations, and the effects of the various diet treatments on larval longevity and period to cocoon formations were compared using analysis of variance

Table 1. Total protein composition of host blood and hematophagous insect feces

Host blood	Parasite feces	Type of feeding	μg protein per mg blood or feces
Bovine	—	—	788 ± 47.7a
	Cat flea	in vitro	841 ± 51.4a
	Bed bug	in vitro	68 ± 25.0b
	Mosquito	in vitro	81 ± 29.9b
Feline	—	—	801 ± 30.3x
	Cat flea	in vitro	664 ± 73.6y
	Cat flea	in vivo	718 ± 82.8y

Means ± SD within host-blood type followed by the same letter are not significantly different (K = 100; Waller-Duncan k-ratio t-test [SAS Institute 1988]). For flea feces and blood samples, n = six determinations; n = three determinations for bed bug and mosquito feces.

(ANOVA) and the Waller-Duncan k-ratio t-test (K = 100) (SAS Institute 1988). Probit analysis was used to estimate the LT_{50} (median lethal time) of larvae fed single diet constituents. Regression was used to determine the relationship between amount of flea feces and larval longevity. Correlation analysis was used to relate percentage cocoon formation to duration of the larval period.

Results

Blood and Fecal Proteins and Ions. Adult *C. felis* commenced feeding through the Parafilm membrane within 5 min after blood was provided. Feces were visible within the first 30 min after feeding. The total protein in feces from fleas did not differ significantly from that of the bovine blood on which these adults fed (Table 1). In contrast, the protein content of bed bug and mosquito feces was 8.6 and 10.2%, respectively, of the blood that was ingested. The protein content of feces from fleas fed cat blood through a membrane was 83% of the blood on which they had fed. The feces produced by fleas feeding directly on the host had 90% of the protein found in the original blood. The similarity in protein content between in vitro and in vivo derived feces indicates that citrating host blood had no effect. The protein content of bovine and feline blood was not different; however, less protein was excreted by fleas fed on feline blood than by those fed on bovine blood (ANOVA; F = 5.21; df = 2, 15; P < 0.05).

Analysis of blood and fecal protein by SDS-PAGE revealed very little difference in the protein composition of bovine blood and the feces produced by fleas fed on this food source (Fig. 1). Blood components of >43 kilodaltons (KD) represented nearly 65% of the total proteins present on the gel. Feces from fleas fed bovine blood had 15% less of the total proteins >43 KD than did bovine blood, whereas bed bug feces contained 32% and mosquito feces contained 26% less of

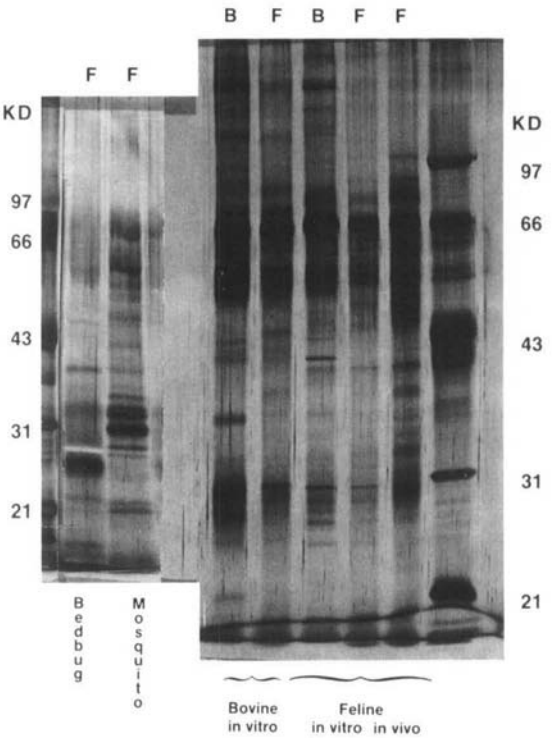

Fig. 1. Silver-stained polyacrylamide gel of whole host blood (B) and flea feces (F) derived from bovine and feline blood. Left and right lanes contain standard molecular weight (KD) markers.

total proteins >43 KD than did bovine blood, indicating the digestion of proteins with high molecular weight. Nearly 58% of the total proteins in feline blood had molecular weights of >43 KD; the fecal proteins from fleas fed on feline blood were similar in composition to the feline blood except that there was a 12% and 13% reduction in >43 KD proteins in flea feces from fleas fed in vivo and in vitro, respectively. Co-electrophoresis with several of the major components of whole blood revealed no noticeable change in the level of serum albumin (66 KD). The bands appearing at about 55 KD matched those of the gamma globulins. These appeared to be somewhat less abundant in flea feces, especially from cat blood ingested in vitro. Several darker bands appeared only in flea feces collected in vivo from cats. These were most likely breakdown products of larger proteins or host-derived proteins that adhered to flea feces.

Bovine whole blood contained 78.95 ± 0.01% water (n = 10) and feline blood contained 82.63 ± 0.001% water (n = 10). There were no differences between the ion composition of fresh whole blood and reconstituted blood (t-test; P > 0.05); therefore, only the data for fresh blood are presented. Bovine and feline blood contained similar (P > 0.05) concentrations of potassium, but feline blood had a significantly greater con-

Table 2. Inorganic ion composition of host blood and hematophagous insect feces

Host blood	Sample	Potassium	Chloride
Bovine	Blood	15.8 ± 2.0c	101.5 ± 6.1b
	Flea feces, in vitro	16.1 ± 3.3c	103.8 ± 8.1b
	Bed bug feces	40.6 ± 1.9a	69.7 ± 4.0c
	Mosquito feces	30.3 ± 2.7b	181.8 ± 7.8a
Feline	Blood	26.7 ± 5.8x	148.8 ± 1.3x
	Flea feces, in vivo	11.8 ± 0.6y	84.9 ± 13.0z
	Flea feces, in vitro	15.5 ± 0.7y	119.1 ± 10.2y

Means ± SEM within host-blood type followed by the same letter are not significantly different (K = 100; Waller-Duncan k-ratio t-test [SAS Institute 1988]). Concentrations are expressed in milliequivalents per liter.

centration of chloride (t = 7.58, df = 18, P < 0.0001) (Table 2). Feces from adult fleas fed bovine blood contained concentrations of potassium and chloride similar to those of the host blood (P > 0.05) (Table 2). Bed bugs fed on bovine blood excreted significantly different concentrations of measured ions (i.e., ≈69% of the chloride, but 260% of the potassium in the blood they consumed). Mosquitoes fed bovine blood excreted greater concentrations of potassium (200%) and chloride (180%). Feces from adult fleas fed cat blood in vitro contained less of both ions than fleas fed cat blood in vivo. Feces from adult fleas fed cat blood in vivo contained ≈50% less potassium and chloride (Table 2). There were no differences in fecal ion composition between fleas fed bovine or feline blood in vitro (t tests; P > 0.05).

Larval Development. The duration of cat flea larval development ranged from 2.5 ± 0.5 d for the feces + yeast diet to 14.2 ± 1.20 d for the feces + stratum corneum diet (Table 3). There was no difference in developmental time among larvae fed any single diet component (blood, dog chow, and yeast), but 87.5 to 100% of these larvae died before they formed cocoons (Table 3). There was also no difference in developmental time and in time to >50% mortality between larvae fed feces or bovine blood or among larvae fed yeast, dog chow, or yeast + dog chow. Diets containing any single component or combinations without yeast or dog chow and a blood-derived component were inadequate for most larvae to develop. There was 100% mortality among larvae fed on flea feces, yeast, and dog chow diets; however, 12.5% of larvae that fed on blood and 37.5% of those that fed on yeast + dog chow formed cocoons (i.e., did not die as larvae).

Table 4. Effects of larval cat flea diet constituents on mortality times (d)

Treatment	n	LT_{50} (95% CI)	Slope ± SEM
Blood[a]	20	10.14 (8.95–11.26)	5.73 ± 0.84
Dog chow	20	3.64 (3.04– 4.16)	9.68 ± 2.50
Feces	20	14.96 (11.39–24.07)	1.81 ± 0.42
Yeast	20	4.32 (3.22– 5.32)	3.16 ± 0.47

[a] Bovine blood.

Although single diet components were inadequate, prolonged larval survival on suboptimal diets might provide opportunities to locate critical supplemental nutrients in nature. Therefore, LT_{50}s of larvae fed single diet components were compared. There was no difference in LT_{50} between flea larvae fed dog chow (3.65 d) and yeast (4.32 d) (Table 4). Larval mortality when reared on the dog chow diet was more uniform than that on the yeast diet, as indicated by the greater slope of the probit line (Table 4). Unfed larvae died ≈3 d after eclosion, indicating that dog chow alone provided only slightly more nutrition than no diet. There was a significant difference between the LT_{50} values for larvae reared on

Table 3. Effects of diet constituents on larval cat flea mortality and development

Treatment	Mortality		Cocoon formation		Adult emergence,[a] %
	Time, d	%	Time, d	%	
Blood[b]	9.86 ± 0.94ab	87.5	10.00 ± 0.00c	12.5	100
Dog chow	4.38 ± 0.46b	100	—	—	—
Flea feces	13.88 ± 2.74a	100	—	—	—
Yeast	6.00 ± 1.58ab	100	—	—	—
Blood + yeast	—	—	6.88 ± 0.35d	100	75
Blood + dog chow	4.00 ± 0.00b	12.5	9.00 ± 0.53c	87.5	100
Blood + yeast + dog chow	7.00 ± 0.00ab	12.5	7.00 ± 0.00d	87.5	100
Dog chow + yeast	3.60 ± 0.68b	62.5	19.67 ± 0.33a	37.5	67
Feces + dog chow	4.00 ± 0.00b	12.5	9.00 ± 0.22c	87.5	100
Feces + yeast	2.50 ± 0.50b	25	7.33 ± 0.33d	75	83
Feces + yeast + dog chow	—	—	7.00 ± 0.27d	100	100
Feces + blood + yeast + dog chow	5.50 ± 3.50ab	25	7.17 ± 0.31d	75	100
Rearing media	—	—	6.50 ± 0.19d	100	100
Feces + hair	5.00 ± 2.00ab	80	13.00 ± 0.00b	20	100
Feces + stratum corneum	14.20 ± 1.20a	83.3	13.00 ± 0.00b	16.7	100

Means ± SEM within host-blood type followed by the same letter are not significantly different (K = 100; Waller-Duncan k-ratio t-test [SAS Institute 1988]); n = 24 for all treatments.

[a] Percentage based on number of cocoons formed.

[b] Bovine blood.

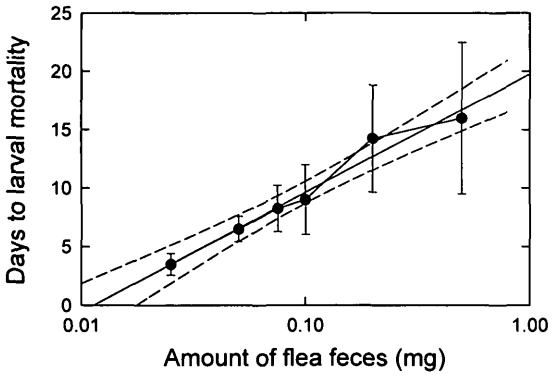

Fig. 2. Relationship between the amount of flea feces fed to individual larvae and period (d) to larval mortality. Each point represents the mean ± SEM. Broken line represents confidence interval.

bovine blood (10.14 d) and flea feces (14.96 d) based on the nonoverlap of the 95% CI. Larvae fed bovine blood responded more uniformly (greater slope of the probit line) than did larvae fed flea feces.

Larval survival increased logarithmically with increasing amounts of adult feces (Fig. 2). For larvae fed >0 mg of feces, larval longevity y = 0.095 (±0.008)$\log_{10}x$ − 1.919 (±0.089) (adjusted r^2 = 0.962, F = 127.74, P = 0.0003), where x is mg of feces. Both the mean longevity and variance increased with increasing amounts of feces (Fig. 2); however, none of the larvae fed feces alone formed cocoons. There were uneaten feces in the gelatin capsules containing >0.1 mg of feces, indicating that those larvae did not starve, but that a diet of flea feces alone was inadequate for development.

Cocoon Formation and Adult Emergence. Cocoon formation periods ranged from 6.5 to 19.7 d for larvae fed complete rearing media and yeast and dog chow, respectively (Table 3). Percentage cocoon formation was negatively correlated with duration of the larval period (r = −0.632, df = 10, P = 0.05). Cocoon formation ranged from 0% for larvae fed dog chow, flea feces, or yeast alone to 100% for larvae fed the rearing media, blood + yeast, or feces + yeast + dog chow. Diets consisting of either blood or feces and yeast or dog chow resulted in ≈75% cocoon formation.

When yeast or dog chow was added to feces or blood, ≈75% of the larvae that formed cocoons successfully pupated and emerged as adults. However, only 67% of the fleas that were fed the dog chow + yeast diet and formed a cocoon emerged as adults (Table 3). Cocoons from which an adult did not emerge were dissected and found to contain either pupae that did not complete the molt from pupa to adult or, rarely (n = 3), a preemerged adult.

Discussion

The similarity in nutrients between host-derived blood and adult flea feces, combined with our knowledge of larval nutritional requirements, may indicate that a unique mechanism of parental investment has evolved in this insect. In two unrelated adult hematophagous insects studied here, host blood was digested more completely than by adult fleas with digested nutrients presumably invested in egg production rather than postembryonic development. This is not surprising, because immature mosquitoes and bed bug nymphs have a diet that does not include adult feces. Bed bugs and mosquitoes take single blood meals before ovarian development. It would be interesting to analyze the excreta of adult *Haematobia irritans* (L.), which blood feed frequently (Harris et al. 1974), but the feces are not part of the larval diet. One might expect that a strategy of excreting nutrients for larval benefit would occur at the expense of maximum egg production. Osbrink & Rust (1984) reported an average of 158 eggs per female *C. felis* over its lifetime, whereas the sticktight flea, *Echidnophaga gallinacea* (Westwood), whose larvae also require blood, produced ≈300 eggs (Suter 1964). Bell et al. (1988) found a maximum of 40 *Glaciopsyllus antarcticus* Smit & Dunnett eggs in the nest of its host, the Southern Fulmar. The larvae of this flea also were observed feeding on blood feces. With the exception of ticks, which produce up to 18,000 eggs and do not provide fecal nourishment for their offspring, other hematophagous arthropods, including those assessed in this study, deposit <500 eggs per female (James & Harwood 1969). Therefore, the fecundity of hematophagous arthropods does not seem to be related to adult investment of undigested nutrients in immatures.

Joseph (1976) reported that during feeding up to 340% of the weight after feeding of female *C. f. orientis* Jordan was excreted. Eggs and feces are deposited at similar times, and therefore have similar spatial distributions within host habitats. Fecal nutrients would be more efficiently utilized and nutrient investment selected for, if a female invested nutrients in her own progeny (i.e., more eggs or more nutrient-filled eggs) rather than invested equally in the progeny of conspecifics. However, if most larvae are related to the adults that are feeding them, there is a strong selective advantage to nutrient excretion. It is likely, at least in fleas that inhabit the nests of their hosts, that all of the individuals are related closely. Hosts (including cats and dogs) that range over large areas could acquire fleas from a large gene pool. However, the habitats that support larval development are limited by microclimate (Silverman & Rust 1983) and nutrient availability (Kern et al. 1992). Therefore,

most adult fleas are probably derived from populations originating at or near host resting sites (locations with adequate nutrition and humid microclimate). In addition, most feline hosts (70%) have relatively few (<7) fleas (Osbrink & Rust 1985), which further implies a more limited gene pool. Thus, flea populations on a host and in a host habitat could be considered a structured deme (Wilson 1980). Adult investment of nutrients probably has few costs, once feeding begins, other than the energy required to pump blood and to avoid host grooming. However, the benefits to larvae are considerable. Even though adult feces cannot be directed to particular larvae, the overlapping spatial distribution of eggs and feces is probably sufficient. Selective benefits may exist even if adult feces are nutrients for future generations. An argument could be made to support the addition of nutrients to unrelated larvae, particularly at low population densities to assure their survival to mate with related individuals. Thus, the risk of no progeny reaching maturity is reduced by producing offspring with some relatedness. Also, feeding any larva may benefit an individual's offspring by reducing cannibalism.

An alternate hypothesis for blood excretion by *C. felis* is the harvesting of key nutrients generally found at low levels in blood, such as B vitamins. Vaughn et al. (1991) indicated that the bloody excreta of anopheline mosquitoes may relate to the concentration of red blood cells in the gut, consequently increasing dietary protein and egg production. Although concentrating a limiting resource may be accomplished for a selfish reason, the outcome may be vital for the offspring. Therefore, our observations with *C. felis* may represent an initial evolutionary step toward true parental investment.

Hinkle et al. (1991) reported that the protein levels for bovine blood, sphere-shaped flea feces, and coiled feces were 5, 7.4, and 11%, respectively. Even assuming that these values were based on whole blood, they are considerably lower than those reported in Table 1. In our study, bovine blood, expressed as percentage (wt/vol), was 16.4% total protein, well within the range of 14–23% (hemoglobin + plasma proteins) reported elsewhere (Merck Veterinary Manual 1986). When adult fleas were fed bovine blood, there was a nonsignificant (110%) increase in total feces protein and no differences in ion composition. The protein concentrations reported here are substantially lower than the increase (150 to 220%) in fecal protein reported by Hinkle et al. (1991). The protein assay methodology they employed, especially their choice of a colorless protein standard, rather than differences in flea physiology or blood source, most likely accounted for the discrepancies between the two studies. Excreta of fleas fed cat blood had 83–90% of the protein and ≈44–80% of the ions found in the original blood. Because cats, not cattle, are the natural host for *C. f. felis*, fleas may better assimilate cat blood proteins and ions, thereby excreting less. This may, in part, account for the gross in vivo and in vitro differences in fecundity reported by Hinkle et al. (1991); however, lack of differences in reproductive output of fleas fed in vitro on cattle or dog blood (Wade & Georgi 1988) indicated that host-related factors, other than blood source, such as tactile cues provided by the host or breakdown of the blood affect fecundity more profoundly than host blood composition.

A curious distinction between the results described here and those reported by others relates to larval development. Although both Bruce (1948) and Moser et al. (1991) obtained adult fleas from larvae fed only dried bovine blood or adult flea feces, we found that these unsupplemented diets were inadequate for larval development. We reared larvae singly, apart from the majority of the hatched chorion, whereas cohort rearing was employed by the other researchers. A chorion was recovered from each larvae; however, there may have been minimal consumption during the eclosion process. Bruce (1948) and Moser et al. (1991) obtained 78–85% adult emergence on these blood diets indicating that nutrients derived from cannibalism and chorion ingestion might have supplemented the base diet. It is interesting that, although both Bruce (1948) and Moser et al. (1991) found that whole blood was suitable, only Bruce (1948) had success with the major protein constituents of blood, hemoglobin and albumin. Again, differences in materials and methods, rather than *C. felis* strains, probably accounted for these disparities.

The addition of yeast or dog chow to dried blood or feces vastly improved larval survivorship. Dog chow contains yeast as well as other vitamin and mineral supplements. Blood alone could not sustain larvae of the northern rat flea, *Nosopsyllus fasciatus* (Bosc); however, by adding yeast almost 100% emergence was obtained (Sharif 1937). Adding yeast to a casein diet increased *Xenopsylla cheopis* (Rothschild) larval weight gain (Pausch & Fraenkel 1966).

Although host-derived materials likely to be found in larval environments such as hair or dried stratum corneum improved survival when added to adult flea feces, the effect was small. In more natural environments, flea larval nutrient supplements would be expected in the form of fungi and other microorganisms, either free or attached to adult feces, host hair and dander, host diet residues deposited in resting areas, and so forth. The incomplete utilization of host blood by adult fleas, the excretion of protein- and ion-rich feces, and the benefit derived from larvae consuming adult feces strongly indicates a unique form of parental investment in *C. felis*.

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