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Estimating nutritional status of German cockroaches, *Blattella germanica* (L.) (Dictyoptera: Blattellidae), in the field

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Abstract

Nutritional status of German cockroaches from the field (HUD apartments) was estimated using uric acid content to measure amount of protein consumed, and respiratory quotient (RQ) to measure fat and carbohydrate metabolized. Initial trials demonstrated the stability of these two indicators as nymphal cockroaches grow and with timing of meals. Nutrient consumption (and presumed availability) was estimated by comparing uric acid content and RQ of nymphal cockroaches collected from kitchens of HUD apartments with those reared in the laboratory and provided a series of meridic diets. Uric acid content was linearly related to percentage of dietary protein ($y=6.2x-32.07$, $r^2=0.96$) and RQ was linearly related to $\log_{10}(\% \text{ fat}:\% \text{ carbohydrate})$ ($y=-0.148\text{Log}(x)+0.790$, $r^2=0.68$). Field-collected German cockroaches contained 10.9 ± 7.7 to 22.9 ± 5.1 $\mu\text{g}/\text{mg}$ uric acid and RQ of 0.770 ± 0.024 to 0.803 ± 0.260 . Comparatively, cockroaches provided rodent chow had greater uric acid content (125.1 ± 9.6 $\mu\text{g}/\text{mg}$) and RQ (0.878 ± 0.022). Employing linear calibration and these regressions, diet consumed by German cockroaches in the field was estimated at $7\pm 3\%$ to $9\pm 3\%$ protein and equivalent amounts of carbohydrates and fat as an energy source. German cockroaches in the field consume less protein and carbohydrates, and more fat compared to those provided a standard laboratory diet such as rodent chow. Diet available in the field is considered suboptimal, resulting in physiological stress; the biological implications of this stress are discussed. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: Nutrition; Nutrient availability; Stress; Uric acid; Respiratory quotient

1. Introduction

Nutritional ecology of the German cockroach, *Blattella germanica* (L.), has been examined extensively under laboratory conditions. Past studies include factors affecting diet consumption, foraging behavior, and diet selection (Rivault and Cloarec, 1991; Silverman, 1986; Valles et al., 1996; Demark and Bennett, 1994, 1995). Nutrient deprivation results in increased mortality, extended development time, altered foraging behavior, and changes in fecundity (Barcay and Bennett, 1991; Durbin and Cochran, 1985; Gordon, 1959; Hamilton et al., 1990; Kunkel, 1966; Willis and Lewis, 1957). However, these laboratory studies may not accurately reflect the behavior of cockroaches in the field (residences and other areas prone to infestation), especially if cockroach

populations are not receiving nutrients required for optimal growth, development and reproduction. There is little information on the actual diet available to field cockroaches, or understanding of the nutritional status of German cockroaches in infested premises.

Although the nutritional status of field German cockroaches is unknown, there is evidence for poor nutrient availability in residences and commercial establishments. Female German cockroaches collected from the field have fewer progeny per ootheca than laboratory reared individuals (Ross and Wright, 1977). In areas of low sanitation, population composition of German cockroaches appears skewed toward more adults, indicating nutritional stress (Sherron et al., 1982). Observations of cockroaches collected in apartments and placed in colony indicate longer times of development than for cockroaches routinely reared in the laboratory (personal observation). This suggests that cockroaches in laboratory colonies were provided a better source of nutrition than those in the field.

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Two physiological indicators used to determine nutritional status in insects are uric acid content and respiratory quotient (RQ) (Lighton, 1989; Mullins and Cochran, 1975a,b). Uric acid was extensively examined in cockroaches (Engebretson and Mullins, 1983; Kramer et al., 1990; Valvolage and Brooks, 1979; Cochran, 1985). Uric acid, a product of nitrogen excretion, is stored in mycetocytes located in the fat body of the cockroach and provides a source of nitrogen for growth and reproduction during times of nitrogen stress (Cochran, 1979; Mullins et al., 1992). Uric acid content in the fat body of American, *Periplaneta americana* (L.), and German cockroaches varies with quantity of protein in the diet (Kramer et al., 1990; Mullins and Cochran, 1975a,b). The RQ indicates the relative proportions of dietary carbohydrate and fat (or protein) used for metabolism and is calculated from the ratio of CO₂ released and O₂ consumed by an organism (Lighton, 1989). An organism with an RQ of 1.0 is metabolizing carbohydrates for energy, and increasing amounts of fat or protein used for energy production causes the RQ to decline (Southwood, 1978).

The objective of this study was to determine amounts of protein, fats, and carbohydrates consumed by field German cockroaches, by comparing urate composition and RQ of cockroaches collected from the field with those reared in the laboratory and provided a series of meridic diets. Defining nutritional status of cockroaches in the field will enable further studies on diet selection, foraging behavior and population growth by using a diet more representative of the field situation.

2. Materials and methods

2.1. Stability of uric acid content and RQ analyses

Trials were performed to evaluate the effects of nymphal age and food deprivation on stability of uric acid content and RQ, using nymphs from a laboratory colony [Johnson Wax strain (Jwax) (Koehler and Patterson, 1986)], provided a diet of rodent chow (HT8604, Harlan Teklad, Madison, WI). Nymphs (>3rd instar) were separated from the colony at the time of molt and held in cohorts for 1, 4, or 7 d in Petri® dishes (polystyrene, 9 cm dia.×2.5 cm). Cockroaches were provided harborage, rodent chow, and water, ad libitum, and held at 25±2°C, a photoperiod of 12L:12D and 40–60% RH. Nymphs were analyzed for uric acid content or RQ after a prescribed time elapsed. Changes to uric acid content and RQ among nymphal instars were evaluated by regressing the response from the analysis on mass (AE 100, Mettler Balance, ×10⁻⁴ g) of the nymph. Cockroaches were randomly sampled from field samples and laboratory colonies (JWax). In food deprivation trials, nymphs (>3rd instar, Jwax) were maintained individually or in groups

of eight per Petri dish (dish and conditions described previously). Treatments (deprived or control) were assigned randomly to each dish. Cockroaches in the control treatment received rodent chow and water, but starved treatments received only water. Eight nymphs from randomly selected dishes were analyzed on 0, 4, 7, 11, and 14 d for uric acid and 1, 2, 4, and 7 d for RQ. Initial studies determined starvation period and frequency of sampling.

2.2. Estimating field nutrient availability

German cockroach nymphs were trapped from public housing apartments in Muncie, IN, and Tallassee, AL. In Muncie, beer and bread-baited jar traps were placed in kitchens for 24 h. Jars (140 ml) were coated on the upper inside 1.5 cm with grease (80:20 mineral oil: petrolatum) and bait was sealed in plastic Petri dishes (35 mm dia.×10 mm high) to prevent consumption. A 25 mm diameter hole was cut in the lid of the Petri dish, and covered with nylon screen to allow release of attractant odors and exclude cockroaches from consuming the bait. The lid and bottom of the Petri dish were glued together at 2 points to completely exclude cockroaches from the bait. Before setting the traps, beer (2.5 ml) was added to the bread through the screen. In Tallassee, cockroaches were collected directly into Petri dishes (9 cm dia.×2.5 cm). Cockroaches were collected from various locations throughout the kitchen including: under the sink, by the stove, by the refrigerator, in cabinets above the sink, in cupboards, and in the pantry.

After trapping, cockroaches were sorted into two groups. One group was placed into plastic tubs (36×24×15 cm), provisioned with food, water, and harborage, and in conditions mentioned previously. Half of the second group was frozen (-70°C) for uric acid analysis and the other half analyzed for respiration directly from the traps. Individuals in the latter group represent nutritional status in the field, while those placed into colony were later used to estimate this nutritional status.

Nymphs (<2nd instar) from field-collected cockroaches and from the JWax strain were each separated into seven sub-colonies provided water, harborage, and one of seven diets, ad libitum (Table 1). The seven diets included rodent chow (HT8604, Harlan Teklad, Madison, WI), three meridic diets varying in protein and carbohydrate (protein series), and three meridic diets varying in fat and carbohydrate (fat series) (Table 1). The balance of nutrients in the meridic diets were formulated at levels similar to rodent chow, contributing 7.3% (vitamins and minerals, 5%, and other growth factors, 2.3%) of the formulation. Vitamins and minerals included 1% vitamin mix (TD40060), 3.5% mineral mix (AIN 93M-MX), and supplemented with 0.3% calcium phosphate (monobasic), 0.1% calcium carbonate, 0.06%

Table 1
Ingredients and levels of nutrients in seven diets fed to German cockroaches

Diet	Diet composition					
	Protein		Carbohydrate		Fat	
	Ingredient ^a	Nutrient ^b	Ingredient	Nutrient	Ingredient	Nutrient
Rodent chow ^c		23		50		4
	EW, SP, WG ^d		CS, M, Su ^e		Sh, CO ^f	
5% Protein	3, 2, 1	5	51, 14, 5	65	2, 2	4
20% Protein	12, 7, 5	20	34, 14, 5	40	2, 2	4
40% Protein	25, 14, 11	40	11, 14, 5	20	2, 1	4
10% Fat	8, 4, 3	12	34, 14, 5	40	8, 2	10
30% Fat	8, 4, 3	12	12, 14, 5	30	28, 2	30
50% Fat	8, 4, 3	12	0, 4, 5	10	48, 2	40

^a Percent of ingredient (rounded to nearest whole number) added to the diet to achieve the desired level of nutrient available to cockroaches.

^b Desired level of nutrient available to cockroaches.

^c Rodent chow #8604, consisting of non-purified ingredients. Nutrient levels available from the guaranteed nutrient analysis, Harlan Teklad, Madison, WI.

^d Protein sources: EW=Egg white solids, SP=Soy assay protein, WG=Wheat gluten.

^e Carbohydrate sources: CS=Corn starch, M=Maltodextrin, Su=Sucrose.

^f Fat sources: Sh=Shortening, CO=Corn oil.

potassium citrate, 0.03% magnesium oxide, 0.03% Vitamin B₁₂ (0.1% trituration), 0.01% folic acid and 0.001% biotin. Additional growth factors contributing included 0.7% inositol, 0.2% choline chloride, 0.2% betaine, and two sources of sterol (0.6% cholesterol, 0.6% β -sitosterol). Addition of cellulose varied 10–11% (protein series) and 13–17% (fat series) to compensate for ingredient change in the protein and carbohydrate, or fat and carbohydrate. Preliminary feeding trials demonstrated consumption of all diets by German cockroaches.

To evaluate nutrient status of cockroaches in the field, standard curves were constructed by regressing uric acid content and RQ of cockroaches feeding on the meridic diets versus the amount of related nutrients in the diet. For uric acid content, the related nutrient (independent variable) was % dietary protein, while a ratio of % fat:% carbohydrate was employed as the independent variable for RQ. Nutrient availability and its corresponding 95% confidence interval (for sample mean) was estimated using linear calibration with the mean responses of uric acid content and RQ from each sampling session and strain (Muncie, Tallassee and JWax).

2.3. Uric acid analysis

Uric acid content in cockroach nymphs was determined using uricase enzyme, which produced a decrease in absorbance as uric acid was converted to allantoin (Dubbs et al., 1955). Cockroaches were dried for 3 d at 60°C then ground in 2 ml of glycine buffer (0.1 M, pH 9.4, ICN Biochemicals Inc., Aurora, OH). After heating (80±2°C) and centrifugation (1400g for 10 m), aliquots (35 μ l) were placed into two reaction tubes with new

glycine buffer. The first tube received 35 μ l uricase enzyme (Sigma, 0.04 units/ml: 1 unit converts 1 μ M of uric acid to allantoin per min at pH 8.5 and 25°C) and the second tube received 35 μ l glycine buffer as a control. Absorbance (292 nm, Perkin Elmer spectrophotometer, Lambda 2 UV/VIS) readings were taken after 1 h incubation (23°C) to assure the enzyme reaction reached end-point. Final absorbance for each sample was calculated by the following equation:

$Abs_{final} = Abs_{no\ enzyme} - (Abs_{enzyme} - Abs_{enz\ blank})$. Concentration of uric acid in each cockroach was then calculated using a standard curve obtained from dilutions of a uric acid standard (Sigma Chemical Co.), assayed concurrently with the cockroach samples.

2.4. Respiratory quotient analysis

Respiratory quotients (RQ) of individual cockroaches were determined using closed system respirometry. Oxygen and CO₂ were measured with a Sable Systems TR3 respirometry system that included an infrared CO₂ analyzer (LiCor Li-6262) and a fuel-cell type oxygen analyzer (Applied Electrochemistry Model E3). Output from the analyzers was collected by a data acquisition system (Datacan 5.1, Sable Systems) running on a computer.

Outside air was drawn through scrubbers containing Drierite[®] and Ascarite[®] to remove water vapor and CO₂, respectively. Dry, CO₂ free air was drawn through an injection port, into the CO₂ detector, through a small Ascarite/Drierite column and into the O₂ analyzer. Air exiting the O₂ analyzer flowed through a mass flow controller and an air pump, which maintained a constant flow rate of 150 ml/min. Carbon dioxide analyzer was

calibrated with 104 ppm span gas (Air Products, Inc.) and the oxygen analyzer was adjusted to read 20.95%.

Respirometer chambers were constructed of 5 ml plastic syringes with a stopcock attached to the Luer tip and a 3 mm vent hole above the 5 ml mark (Lighton, 1991). A 5 ml transfer tube consisting of a syringe with the Luer end removed was used to move cockroaches into the respirometer without undue stress or movement. Once the cockroach was transferred, the plunger was replaced in the chamber, leaving the vent hole open. Respiration chambers were purged for 2 min with dry, CO₂ free air and then the stopcock was closed and chamber was sealed by moving the plunger to the 5 ml mark, sealing off the vent hole. Chambers were incubated in the dark at 26°C and were observed under red light to ensure lack of movement of cockroaches. Cockroaches were incubated a minimum of 30 min between sealing the syringe and injection of the air into the respirometer.

After incubation, chambers were randomly selected for analysis. Sampling air consisted of attaching a 22 ga. needle to the stopcock and purging 1 ml of air from the chamber. The needle was then inserted into the rubber septum of the injection port and 1 ml of air was injected. The incubation period was noted upon time of injection and recorded immediately after injection. The next chamber was analyzed after the CO₂ reading returned to baseline and the O₂ level stabilized. Rates of O₂ consumption and CO₂ production (in ml/h) were quantified by integrating the peaks produced by the analysis. Data-can software corrected for atmospheric pressure, flow rate, flow temperature and integrated the peak areas. Peak area, mass of insect, and volume of chamber were used to calculate V_{CO_2} and V_{O_2} resulting in the respiratory quotient (V_{CO_2}/V_{O_2}) (Lighton, 1991).

3. Results

3.1. Stability of uric acid and RQ analyses

There was no relationship between uric acid content and body mass for nymphs fed rodent chow ($F=1.59$, $df=36$, $P>0.20$) or from field collected nymphs ($F=0.36$, $df=61$, $P>0.50$, Fig. 1). Log-transforming uric acid content also resulted in a lack of significant relationship for cockroaches collected from the field ($F=0.04$, $df=61$, $P>0.80$), and only a weak relationship for those fed rodent chow ($F=4.202$, $df=36$, $P=0.0477$, $r^2=0.10$). Similarly, there was no relationship between RQ and body mass for nymphs fed rodent chow ($F=0.889$, $df=77$, $P>0.30$) or collected from the field ($F=2.337$, $df=72$, $P>0.10$, Fig. 2). Lack of significant relationship also occurred when RQ was log-transformed for cockroaches collected from the field ($F=2.06$, $df=72$, $P>0.10$) or those fed rodent chow ($F=0.50$, $df=77$, $P>0.40$).

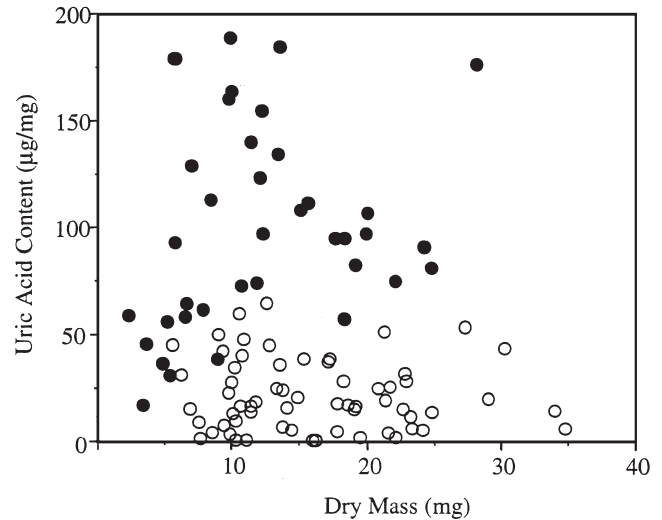


Fig. 1. The relationship between uric acid content and dry mass for German cockroaches collected from residential apartments (○) or sampled from a colony supplied with rodent chow (●). In either case, no significant regression was found in colony ($F=1.59$, $df=36$, $P>0.50$) or field collected nymphs ($F=0.36$, $df=61$, $P>0.80$).

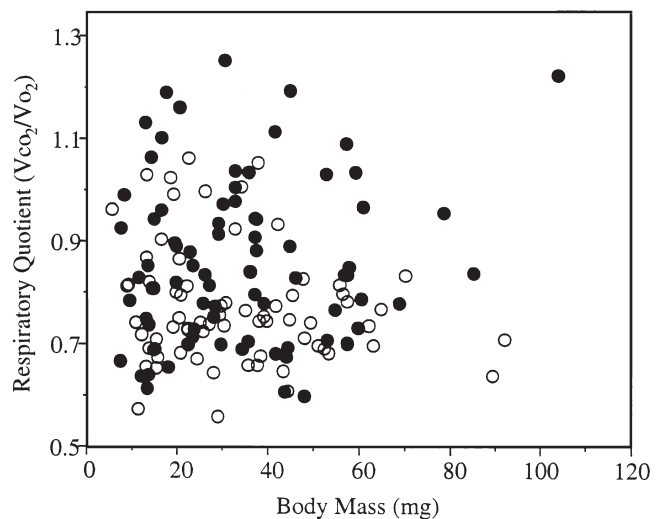


Fig. 2. The relationship between respiratory quotient and body mass for German cockroaches collected from residential apartments (○) or sampled from a colony supplied with rodent chow (●). In either case, no significant regression was found in colony ($F=0.889$, $df=77$, $P>0.30$) or field collected nymphs ($F=2.337$, $df=72$, $P>0.10$).

Because body mass and age are correlated in nymphal German cockroaches (Reid, 1989), the stability of uric acid content and RQ over different instars permits the use of nymphs of unknown age for analysis.

As nymphal stadia progressed, uric acid content of German cockroach nymphs did not change significantly between 24 h (71.1 ± 5.3 µg/mg) and 7 d (76.7 ± 6.9 µg/mg) ($P>0.05$, Table 2). However, stability of RQ did not occur in nymphs through the stadium as the first half of the instar had an RQ of 0.955 ± 0.073 which declined

Table 2

Effect of time within instar on uric acid composition and respiratory quotient of German cockroaches provided rodent chow

Time into instar	Uric acid ($\mu\text{g}/\text{mg}$)			Respiratory quotient		
	<i>n</i>	Mean ^a	$\pm\text{SE}$	<i>n</i>	Mean	$\pm\text{SE}$
24 h	14	71.1a	5.3	6	0.963a	0.073
4 d	10	70.8a	5.1	10	0.958a	0.029
7 d	12	76.7a	6.9	10	0.769b	0.018

^a Means with the same letter within the column were not significantly different using ANOVA procedures with protected LSD ($\alpha=0.05$).

significantly to 0.76 ± 0.018 between 4 and 7 d after the molt ($P<0.05$, Table 2).

For starved cockroaches held in groups of eight, uric acid content was initially not significantly different from cockroaches receiving rodent chow and similarly grouped. However, after one week of starvation, uric acid content of starved cockroaches (in groups) increased significantly, and levels continued to increase after 11 d and 14 d ($P<0.05$, Fig. 3). Uric acid content (y) of starved cockroaches (in groups) increased exponentially after day 4 ($y=54.08e^{0.105x}$, $P<0.005$, $r^2=0.93$). In contrast, the uric acid content did not significantly change over the two weeks for cockroaches provided food or starved in isolation ($P>0.05$). The RQ of starved nymphs significantly ($P<0.05$) declined over 24 and 48 h then stabilized at about 0.70 (0.672 ± 0.028 to 0.727 ± 0.019), for the remainder of the period ($P>0.05$, Fig. 4).

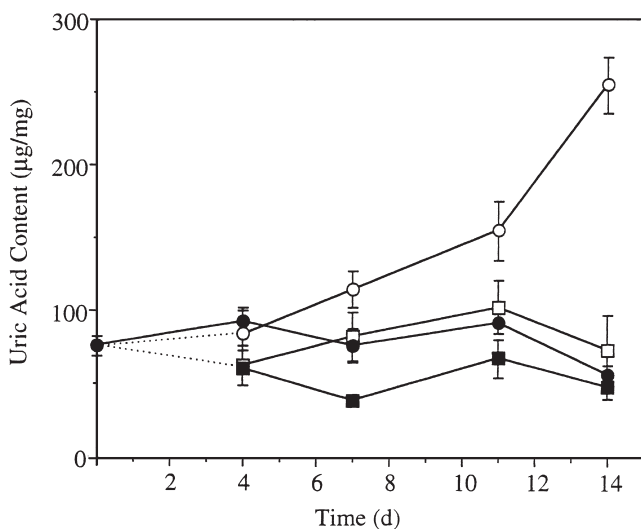


Fig. 3. Comparison of uric acid levels in German cockroaches subjected to starvation in groups (○) and isolation (□), or fed rodent chow in groups (●) and isolation (■). Uric acid content of cockroaches starved in groups began to increase significantly at day seven, while the other treatments remained relatively stable ($\alpha=0.05$). Each point represents a mean of five observations.

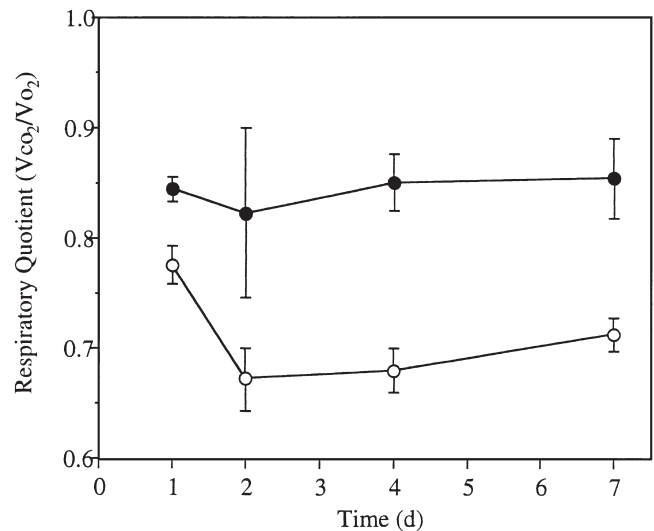


Fig. 4. Comparison of respiratory quotient (RQ) from German cockroaches subjected to starvation (○) or fed rodent chow (●). The RQ of starved cockroaches declines significantly over 24 and 48 h, then was stable for the rest of the period ($\alpha=0.05$). Each point represents a mean of ten observations.

3.2. Estimating field nutrient availability

Linear regressions were calculated for uric acid content versus percentage of dietary protein and RQ versus the ratio of dietary fat:carbohydrate from the cockroaches provided meridic diets. Uric acid content increased linearly with percentage of dietary protein ($y=6.2x-32.1$, $F=386.95$, $df=16$, $P<0.001$, $r^2=0.96$, Fig. 5). Similarly, RQ was linearly related to $\log_{10}(\text{fat:carbohydrate})$ ($y=-0.148\log_{10}(x)+0.790$, $F=15.29$, $df=16$, $P<0.001$, $r^2=0.68$, Fig. 6). From these relationships, nutrient utilization and presumed availability was calculated for field cockroaches.

Field-collected German cockroaches contained 10.9 ± 7.7 to 22.9 ± 5.1 $\mu\text{g}/\text{mg}$ uric acid, whereas cockroaches provided rodent chow had significantly greater uric acid contents of 125.1 ± 9.6 $\mu\text{g}/\text{mg}$ ($P<0.05$). Using the regressions constructed from the analyses of cockroaches provided meridic diets (Figs. 5 and 6), estimated levels of field dietary protein were $7\pm 3\%$ to $9\pm 3\%$ (Table

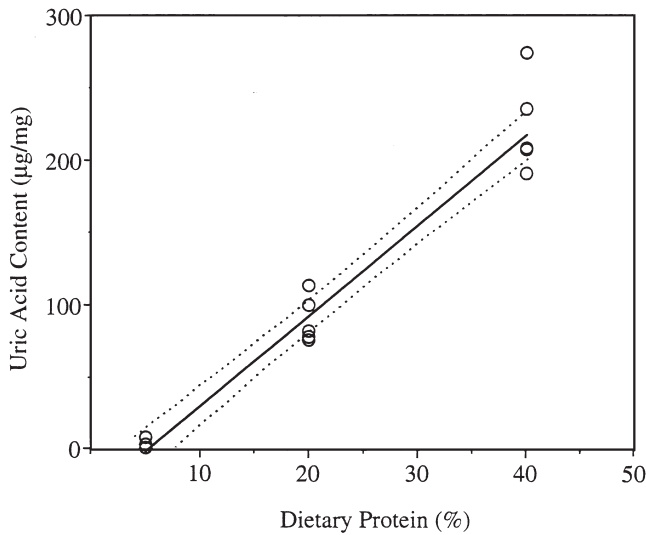


Fig. 5. Regression of mean uric acid content versus percent of protein in the diet for German cockroaches provided three diets that varied protein and carbohydrates. The linear relation ($y=6.2x-32.1$, $r^2=0.96$) represented by the solid line (—) was bounded by the 95% confidence limits (- - -) for the population mean. Each point represents a mean of ≥ 5 observations from each sampling session and strain.

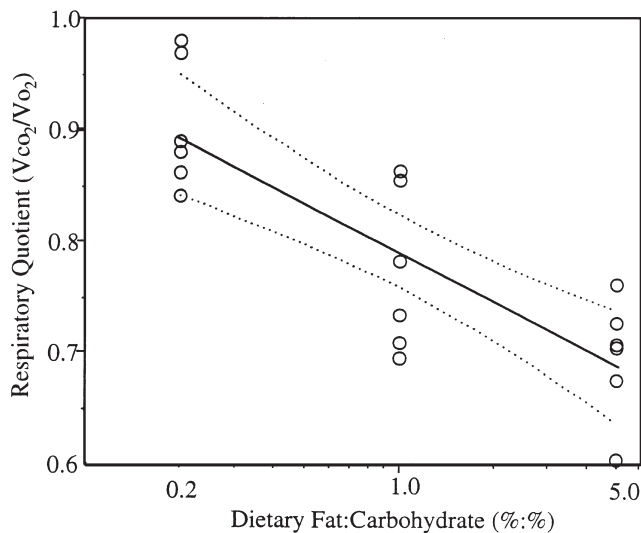


Fig. 6. Regression of mean respiratory quotient versus the ratio of dietary % fat:% carbohydrate for German cockroaches. The three diets varied fat and carbohydrates; protein was constant at 12%. The linear relation ($y=-0.148\log_{10}(x)+0.790$, $r^2=0.68$) represented by the solid line (—) was bounded by the 95% confidence limits (- - -) for the population mean. Each point represents a mean of ≥ 6 observations from each sampling session and strain.

3). Cockroaches provided rodent chow had estimated dietary protein of $24\pm 2\%$ and is consistent with the 23% protein contained in the rodent chow diet.

Respiratory quotients of field cockroaches were 0.803 ± 0.260 (Muncie sample) and 0.770 ± 0.024 (Tallassee sample) compared with individuals provided rodent chow that exhibited a significantly greater RQ of 0.878 ± 0.022 . The resulting estimated ratio (and 95%

confidence limits) of dietary F:C consumed by German cockroaches in the field were 0.79 (0.41, 1.58) and 1.33 (0.74, 2.40) for cockroaches from Muncie and Tallassee, respectively. However, these two estimates were not significantly different and with the confidence intervals for the estimates encompassing 1.0, the cockroaches were utilizing equivalent amounts of carbohydrates and fat as an energy source ($P>0.05$).

4. Discussion

4.1. Stability of uric acid content and RQ analyses

In field collected populations of German cockroaches, age (within instar) and timing of last meal prior to sampling are unknown factors that may increase variability in measurement of physiological parameters. To reduce variability, nymphs were selected for this study because uric acid levels change in adults with reproductive status (Mullins et al., 1992). Estimating nutritional status in the field using analysis of adults was not possible because of the unknown number of reproductive events that sampled males or females had experienced. The nymphal stage would be more reliable because their activities are restricted to functions, such as resting, or foraging for food and water (Demark and Bennett, 1994). However, determining age and instar of field collected cockroaches is impractical because of the variable number of instars that may be encountered. There are five distinct nymph size-classes in the Jwax strain, whereas nymphs from the Muncie strain have at least 8 distinct sizes. Variable number of instars of 5–7 have been reported by several authors and depend on rearing conditions, diet, and injuries (in Ross and Mullins, 1995). Using nymphs of unknown age requires establishing stability of uric acid (and RQ) between instars.

Effect of age on uric acid was demonstrated by regressing uric acid content (and RQ) on mass. Mass increases linearly with age in German cockroaches, with the exception of brief intermissions during molt events (Reid, 1989). As nymphs gained mass there was a proportional increase in total body uric acid, and the amount of uric acid per dry mass remained constant within and between instars. Change in uric acid with body mass was similar to that found in American cockroach nymphs, where uric acid concentration increased only $10\ \mu\text{g}/\text{mg}$ over 60 d, whereas body mass increased by 28.3 mg during the same period (Cochran, 1979). The lack of change in uric acid content over stadia permits us to use cockroach nymphs of an undefined age.

Respiratory quotient of nymphs did not change with mass; they continued to metabolize carbohydrates and fats in an apparently consistent ratio with increasing instar. However within stadia, RQ declined in the second half of the instar, prior to the next molt. This decline in

Table 3
Estimated level of protein and ratio of fat:carbohydrate in the diet of cockroaches from two public housing complexes

Location trapped	Analyses				Estimated diet	
	<i>n</i>	Mean	SE	CV ^a		
		<i>Uric acid content (µg/mg)</i>			<i>% Protein^b</i>	<i>95% CL</i>
Muncie, IN (1994)	32	21.5	3.2	84	9a	7, 11
Muncie, IN (1996)	8	22.9	5.1	62	9a	6, 12
Tallassee, AL (1996)	6	10.9	7.7	174	7a	4, 10
Colony (rodent chow)	23	125.1	9.6	37	24b	22, 26
				<i>Respiratory quotient</i>	<i>Ratio F:C^b</i>	<i>95% CL</i>
Muncie, IN (1996)	24	0.803	0.260	24	0.81a	0.41, 1.58
Tallassee, AL (1996)	50	0.770	0.024	22	1.35a	0.74, 2.40
Colony (rodent chow)	79	0.878	0.022	22	0.24b	0.11, 0.56

^a Coefficient of variation.

^b Means with the same letter were not significantly different because the 95% confidence interval (CL) encompasses other means. Statistical analysis of dietary protein was independent of the analysis for RQ.

RQ corresponds with the feeding activity where consumption begins to decline around the fifth day of the stadia (Valles et al., 1996; Demark and Bennett, 1994). The result caused by decreasing RQ as the stadium progresses increases variability in the regression models and skewed estimates of fat and carbohydrates to favor indications of increased consumption of fat. However, to minimize the effect of skewed data, individuals were selected randomly, with cockroaches from the field and the colonies analyzed during the same period.

As the last feeding period was unknown for any sampled individual, a starvation trial determined the effects of the non-feeding period between foraging and when the cockroach was sampled. Starved cockroaches did not have significantly different levels of uric acid from fed controls after 4 d of starvation. Beyond 4 d of starvation, cockroaches starved in groups experienced an increase in uric acid content attributed to scavenging of exuviae or cannibalism due to extreme nutrient stress (Cochran, 1985). The respiratory quotient changed faster than uric acid content, significantly declining after 24 h of starvation and continuing to decline until 48 h. After 2 d of starvation the cockroaches had depleted their reserves of carbohydrates and were apparently relying on fat (or protein) as the principle metabolic source.

The change of RQ within 2 d of starvation may underestimate dietary F:C ratio for cockroaches sampled from the field. From the starvation trial, RQ decreased by a factor of 0.09 [1-(mean RQ starved/mean RQ fed)] after 24 h of starvation. This factor provides a “worst case” estimate, assuming all cockroaches were starved for a period of 24 h prior to sampling. The corrected F:C ratio for nymphs from Muncie was 0.56 and from Tallassee, 0.71. Based on the meridic diets fed to cockroaches in colony where fat and carbohydrates account for 60% of the diet, the lowest estimated fat content available in the diet was 21%, with carbohydrates accounting for 39%. Still, cockroaches in the field

receive higher amounts of fat than their counterparts in colony that receive 5% fat from rodent chow. With the availability of fats and oils in apartments, evident by visual inspection, we concluded that fat and carbohydrate usage approaches the initial estimate.

As mentioned, timing of the last meal is unknown in the field situation, but within 24 h, cockroaches (except those approaching apolysis) would have fed or attempted to feed on some item regardless of nutritional quality. Support for this assumption was demonstrated by several behavioral studies. First, German cockroaches have two feeding periods during the scotophase (Silverman, 1986), but will also feed during the photophase depending on lighting conditions (Demark and Bennett, 1994). Second, with the onset of nutritional stress (starvation), German cockroaches increase their rate of movement and foraging area (Barcay and Bennett, 1991). Therefore, with increased activity and sampling propensity, cockroaches would have sampled or consumed some food item within 24 h.

4.2. Estimating field nutrient availability

In residential apartments, German cockroaches are exposed to less protein (≈9% versus 24%) and a higher F:C ratio (1.06 versus 0.24) than those reared in laboratory colonies and provided rodent chow. There is also greater variability in uric acid content and RQ values of field-collected cockroaches compared with laboratory reared cockroaches. This variability is likely a result of the unpredictable manner in which food is added to the environment of a kitchen and encountered during foraging. Increased variability of protein was particularly evident with uric acid content where the coefficient of variation for colony reared cockroaches was 37% versus >62% for field cockroaches. Evidence of stress imposed by nutritional status was available from previously reported nutrition studies involving cockroaches, related

insects, and other animals adopting an omnivorous feeding strategy.

For protein, the acceptable dietary range for German cockroaches was between 11 and 40%; diets containing at least 5% protein were sufficient for only limited survival (Haydak, 1953; McCay, 1938; Noland and Baumann, 1951). In female German cockroaches, diets containing 15 to 40% protein provide maximal oocyte growth and the shortest preoviposition periods, both an indication of favorable nutritional status (Cooper and Schal, 1992). Diets containing $\leq 10\%$ protein resulted in decreased oocyte size and increased delay of reproductive cycle (Hamilton and Schal, 1988; Cooper and Schal, 1992). At 5% dietary protein, American cockroaches experienced a continual loss of stored uric acid as mobilization occurred for protein production (Mullins and Cochran, 1975b; Cochran, 1985). German cockroaches in the field situation stored only 14% of the uric acid that they potentially could accumulate if provided a more suitable diet like rodent chow (24% protein). Based on cited evidence and our results of field protein availability, populations of German cockroaches are nutritionally stressed for protein.

Compared with dietary protein, dietary carbohydrate and fat levels have been reported to a more limited extent. German cockroaches require dietary carbohydrate of at least 14% suitable carbohydrate (500 μM glucose/g diet), but greater than 25% for adequate development and reproduction (Gordon, 1959). Low carbohydrate levels were achieved using a diet consisting of 15 to 30% protein and 3% fat, which also provided substrates for metabolism. Optimal levels of carbohydrate for a German cockroach diet are probably greater than 25%. Cohen et al. (1987), in a diet self-selection study involving the brownbanded cockroach, found a carbohydrate level of 84.5% was optimal for growth. For fats, the German cockroach required at least 2% corn oil for growth and reproduction (Gordon, 1959). Any stress imposed on cockroaches from excessive amounts of fat and reduced carbohydrate could be revealed in further behavioral studies.

This study defines the level of nutrition used by field German cockroaches and confirms that these populations were exposed to sub-optimal diets compared with colony-reared individuals (or alternately colony-reared individuals are exposed to an artificially enriched diet). Unfortunately, based on diets available to cockroaches in the field, past laboratory studies may not accurately reflect the condition of cockroaches in the field. The majority of laboratory studies on the German cockroach use cockroaches supplied with a standard diet, such as rodent chow, or subjected to starvation. Rodent chow (or similar commercially available diets) results in acceptable growth of cockroaches in a minimum amount of time. However, population characteristics of cockroaches fed rodent chow (Reid, 1989) do not reflect

characteristics of field populations (Ross and Wright, 1977; Sherron et al., 1982). Starvation as a treatment in studies also does not represent nutritional status of German cockroaches in the field. When German cockroaches were starved for 3 d they greatly increase their food consumption regardless of amount of dilution from non-nutrient material (Van Herrewege, 1974). Behaviors such as foraging, diet consumption, and intraspecific behaviors may change with dietary stress, and may not be accurately represented by cockroaches fed rodent chow or starved.

Field populations of German cockroaches consume (and presumably have available) a diet of lower protein, lower carbohydrate, and higher fat levels than their lab-reared counterparts provided rodent chow. Based on this study and others (Ross and Wright, 1977; Sherron et al., 1982), we consider nutrient availability in the field situation (residences) to be suboptimal resulting in physiological stress. Further studies on diet selection should include diets that reflect field conditions because of the interaction of nutrition with German cockroach population development and behavior. This study was limited to low income housing because populations of cockroaches and similarities among apartments permitted replication. Further studies in other apartments and commercial kitchens will be critical for establishing a broader understanding of how nutrition affects field populations of the German cockroach, and how the use of a control agent affects nutrient availability.

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