LIMITATIONS IN ESSENTIAL AMINO ACID

NUTRITION IN COTTON RATS

(*SIGMODON HISPIDUS*) IN

OKLAHOMA

By

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Bachelor of Science

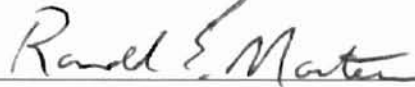Oklahoma State University

Stillwater, Oklahoma

1995

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
MASTER OF SCIENCE
December, 2000

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PREFACE

This study evaluated the hypothesis that the addition of methionine for growth and reproduction is important in determining the densities of wild cotton rats and that dietary methionine for proper function of hematological and immunological parameters is important in wild populations of cotton rats. Chapters included in this thesis were written according to the format specified by The Journal of Wildlife Management.

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Chapter I.

LIMITATIONS IN ESSENTIAL AMINO ACID NUTRITION IN COTTON RATS (*SIGMODON HISPIDUS*) IN OKLAHOMA

Abstract: Numerous studies evaluating quality of dietary protein have proposed that essential amino acids (especially methionine and cysteine) limit small-herbivore populations. Therefore, the hypotheses that methionine is important for growth and reproduction and thus cotton rat (*Sigmodon hispidus*) densities was evaluated. I examined effects of supplemental methionine on density, survival, and reproductive parameters of wild, but enclosed, populations of cotton rats in north-central Oklahoma. Sheet metal enclosures (n=12) were stocked with wild-caught cotton rats and randomly assigned a treatment of no supplementation, low-methionine supplementation, or high-methionine supplementation. In 1997, no treatment differences occurred in density; however, in 1999, addition of methionine resulted in greater densities throughout the year. Treatment effects on survival interacted with date in 1999, with higher survival on methionine-supplemented plots in June, October, and November 1999. In 1997, there were no differences between treatments in per capita recruitment rates, but recruitment in 1999 was highest with supplemental methionine. Treatment effects on proportions of animals in reproductive condition interacted with date in both years, with differences in April and June 1997 and from March to July 1999. High-methionine supplementation also resulted in earlier and longer breeding seasons in 1999. These results suggest that low amounts of dietary methionine limit populations of cotton rats, especially in late summer and autumn.

Many theories have been advanced to explain regulation of herbivore populations. Among those, forage quality and quantity are of central importance in regulating populations of small herbivores (Cole and Batzli 1979, Hansson 1979, Sinclair et al. 1982, Keith 1983, Sullivan et al. 1983, Jones 1990, Batzli and Lesieutre 1991, Doonan and Slade 1995, Eshelman and Cameron 1996). In particular, protein may be the most limiting factor in many herbivore populations (White 1978, Mattson 1980, Randolph et al. 1995, Cameron and Eshelman 1996, Schetter 1996).

Protein in the diet supplies essential amino acids that cannot be synthesized in adequate amounts by an organism. The ability of a dietary protein to supply a proper balance of these essential amino acids determines the quality of that protein (Oser 1959). The ideal protein is perfectly balanced in terms of content of essential amino acids and its supply of nitrogen as nonessential amino acids for an animal's particular requirements (Moughan et al. 1988, Robbins 1993). Studies on the qualities of proteins in diets of northern pintail (*Anas acuta*; Krapu and Swanson 1975), cackling geese (*Branta canadensis minima*; Sedinger 1984), willow ptarmigan (*Lagopus lagopus*; Steen et al. 1988), bobwhite quail (*Colinus virginianus*; Boren 1992, Peoples et al. 1994), cottontail rabbits (*Sylvilagus floridanus*; Lochmiller et al. 1995), and cotton rats (*Sigmodon hispidus*; Schetter 1996) have suggested that essential amino acids (especially methionine and cystine) may be deficient in wild populations.

Cystine is not an essential amino acid, but methionine is an indispensable component of the diet that supplies sulfur for cystine synthesis in the absence or deficiency of the latter (Rose 1937, Williams et al. 1954). In the absence of dietary methionine, animals (i.e., laboratory rats, *Rattus* sp.) rapidly lose weight and eventually die, even if an abundant amount of cystine is supplied (Rose 1937). If methionine is supplied in levels that permit maintenance or slow growth, addition of cystine greatly improves quality of the diet. Thus, cystine stimulates growth only when dietary methionine is present but is in suboptimal quantities (Rose 1937). Cystine can furnish at least half of the total sulfur amino acid requirement of the laboratory rat. However, if dietary methionine levels are adequate, cystine does not need to be present in the diet (Nelson and Evans 1958).

To further understand the role of nutrition in population regulation, I evaluated the hypothesis that the addition of methionine for growth and reproduction is important in determining the densities of cotton rats. My primary objectives were to examine effects of supplemental methionine on density, survival, and reproductive parameters of wild populations of cotton rats. I predicted that methionine supplementation would increase density, survival, and reproduction of wild cotton rats.

STUDY AREA AND METHODS

This study was located about 20 km west of Stillwater, Oklahoma (97°N, 60°E) on the Zoology Research Area. Primary vegetation consisted of little bluestem (*Schizachyrium scoparium*), switchgrass (*Panicum virgatum*),

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indiangrass (*Sorghastrum nutans*), big bluestem (*Andropogon gerardii*), panicum (*Panicum* spp.), dropseed (*Sporobolus* spp.), lespedeza (*Lespedeza* spp.), western ragweed (*Ambrosia psilostachya*), sagewort (*Artemisia ludoviciana*), brome (*Bromus* spp.), and yarrow (*Achillea lanulosa*) interspersed with small (< 1.5 m in height) eastern redcedar (*Juniperus virginiana*), American elm (*Ulmus americana*), winged elm (*Ulmus alata*), winged sumac (*Rhus copallina*), smooth sumac (*R. glabra*), and buckbrush (*Symphoricarpos orbiculatus*).

Twelve 0.1-ha enclosures, constructed with galvanized sheet metal (buried approximately 25 cm in the ground and 2 m in height), were cleared of all mammals. Cotton rats were trapped from local populations (0-18 km from enclosures) using Sherman live traps baited with oats. Sixteen non-reproductive cotton rats, in equal sex ratios, were toe-clipped to indicate unique identification numbers, weighed, and placed in each enclosure in November 1996 and 1998. At the end of 1997, enclosures were cleared of all mammals, and an entirely new study group of study animals was introduced for the second year of the study.

Each enclosure was randomly assigned a treatment of no supplementation (control), supplementation of a low-methionine diet, or supplementation of a high-methionine diet, with 4 enclosures/treatment. The low-methionine diet consisted of 47.0% corn, 50.75% beet pulp, 3.0% dried molasses, and 0.25% vitamin and mineral mix by weight, for a total of 0.20% methionine (slightly below the maintenance requirement of 0.22% for laboratory rats; Williams et al. 1954). The total sulfur-containing amino acid requirement for optimal lactation in the rat is 1.11-1.24% of the diet (Nelson and Evans 1958).

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Therefore, the high-methionine diet was similar to the low-methionine diet except for an added amount of D-L-methionine to obtain a total of 1.20% of the diet. Covered feeders were placed at the center and each corner of each enclosure (i.e., 5 feeders/enclosure). Supplemental food was checked and replenished every 7-10 days.

In 1997 and 1999, vegetation measurements were taken seasonally to determine if differences in habitat existed between treatments. Vegetation cover was estimated using the Daubenmire method (Bonham 1989). Ten 0.1-m^2 quadrats were located randomly in each enclosure to obtain cover estimates of grasses, forbs, legumes, woody plants. standing litter, and fallen litter. Percent cover was classified as 0%, 1-5%, 6-25%, 26-50%, 51-75%, 76-95%, or 96-100% for each vegetation class. Midpoints of cover classes were used in statistical analyses. Height (cm) of the tallest vegetation also was recorded in the quadrat. Biomass estimates (kg/ha) of grasses, forbs, and legumes were determined in quadrats by clipping plants to ground level, but biomass of woody plants was determined by clipping only the current year's growth up to 1 m in height. Clippings were oven-dried at 70°C, and biomass for each vegetation class was recorded (Bonham 1989). Analyses of variance (PROC GLM; SAS Institute Inc. 1996) and multiple comparison tests (least squared means; SAS Institute Inc. 1996) were used to examine treatment differences in vegetation height, cover, and biomass data. Statistical significance was set at $P = 0.05$.

To assess populations, a 5 X 5 grid in each enclosure was trapped monthly, except during the normal breeding season (Apr-Nov) when attempts

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were made to trap grids twice per month. Sherman live traps were set every trapping day in late afternoon, baited with oats, and checked the next morning. For each animal captured, body mass (g), sex, reproductive condition, and toe-clip number were recorded. If the animal was not toe-clipped, it was assigned a new identification number. Age was determined by body mass: <60 g = juvenile, 60-89 g = subadult, or ≥ 90 g = adult (Stafford and Stout 1983). Males were considered reproductively active when scrotal. The female's vaginal condition (perforate or imperforate), pregnancy status (determined by palpation), and lactation status were used to assess reproductive condition.

For each enclosure, the minimum number known alive was calculated by summing the number of unique individuals caught during a census period and all animals that were caught both before and after that period (Krebs 1966, Pollock et al. 1990). Estimates were converted to densities (number of cotton rats per ha). Densities of females and males were analyzed separately and combined. Sex ratios were calculated as proportions of females to males in the populations. Age ratios were calculated as proportions of each age class in the populations. Survival rates were indexed as proportions of animals captured during a census period that were recaptured during any subsequent census period (Schetter 1996). Therefore, survival rates were not indexed for the last census period of each year. Survival rates of females and males were indexed separately and combined. Per capita recruitment was determined as the number of new juveniles divided by the number of mature females (subadults and adults combined; Schetter 1996). Breeding intensity was indexed as proportions of

mature animals in the population that were reproductively active. Reproductive activity of females and males also were indexed separately and combined.

Treatment effects and date by treatment interactions for densities, sex ratios, age ratios. survival rates, per capita recruitment, and proportions of reproductively active cotton rats were determined with repeated-measures analyses of variance (PROC GLM; SAS Institute Inc. 1996). When significant differences occurred. least-squared-means tests were used to determine which treatments differed by date or overall treatment differences (SAS Institute Inc. 1996). Statistical significance was set at P = 0.05. To assess the influence of climatic variables on density, recruitment, reproductive intensity, and survival, I used stepwise multiple regression analyses (PROC REG; SAS Institute Inc. 1996). Density, recruitment. proportions of reproductively active animals, and survival rates as dependent variables (in 4 separate analyses) and climatic conditions as independent variables. Average monthly temperature (C), total monthly precipitation (cm), and total monthly snowfall (cm) were used as independent variables in the stepwise models (National Oceanic and Atmospheric Administration 1997, 1998, 1999). Statistical significance was set at P = 0.15 for inclusion into the models (Ott 1993, Cody and Smith 1997). When independent variables were significant, slopes were compared among treatments using F-tests (Neter and Wasserman 1974, Ott 1993). I also examined relationships with climatic variables using Pearson correlation analyses (PROC CORR; SAS Institute Inc. 1996) and variance inflation factors (Montgomery and

Peck 1992, Allison 1999). Statistically significance was set at $P = 0.05$ for correlation analyses.

Data collected between 23 November 1996 and 22 March 1997 were not included in analyses because of inadequate enclosure maintenance. In 1997 and 1999, 1 enclosure was not used in analyses because excess predation by hawks and owls that perched on the enclosure's boundary and eliminated the population of cotton rats within weeks after stocking. Therefore, in 1997, the high-methionine treatment only included 3 enclosures, and in 1999, the control included 3 enclosures.

RESULTS

In 1997, only percent cover of fallen litter differed ($P = 0.038$) among treatments. In 1999, only woody biomass and percent grass cover differed ($P = 0.008$ and $P = 0.002$, respectively). Of 22 vegetation characteristics measured, only 1 in 1997 and 2 in 1999 were statistically different, suggesting that habitats were the same among treatments. No trends were found upon examination of scatter plots of treatment versus habitat parameters, and habitats were again concluded to be the same among treatments. Because of the minimal number of differences found and lack of trends in plots, I concluded that the small differences in vegetation among treatments were not biologically relevant, and therefore, any differences in biotic and demographic characteristics of cotton rats were assumed to be treatment effects.

In 1997, overall densities of cotton rats (sexes combined) did not differ between treatments ($F_{2,8} = 1.62$, $P = 0.258$; Fig. 1A). In 1999, treatment effects

on density interacted with sampling date ($F_{28,112}$ = 1.86, P = 0.012; Fig. 1B). No climatic variables met the significance level for entry into multiple regression models for the unsupplemented or low-methionine treatments when regressed against density; however, precipitation had a positive effect on density in the high-methionine treatment (R^2 = 0.206, $F_{1,24}$ = 5.98, P = 0.023). Precipitation also was the only significant climatic variable in correlation analyses (Table 1).

Densities of females did not differ between treatments in 1997 ($F_{2,8}$ = 1.50, P = 0.814; Fig. 1C), but in 1999, there was a greater female density in the high- (P = 0.003; Fig. 1D) and low-methionine (P = 0.015) treatments than the control. However, female density in low- and high-methionine populations did not differ in 1999 (P = 0.301). In 1997, male density in the low-methionine treatment was greater than control (P = 0.014; Fig. 1E), but there was no difference between the high-methionine treatment and control (P = 0.420) or low-methionine treatment (P = 0.073). In 1999, male density in the low- (P = 0.009; Fig. 1F) and high-methionine (P < 0.001) treatments were greater than control, but the low- and high-methionine treatments did not differ (P = 0.067).

Sex ratios did not differ between treatments in 1997 ($F_{2,8}$ = 1.08, P = 0.386; Table 2) or 1999 ($F_{2,8}$ = 0.48, P = 0.635). Supplemental methionine did not affect proportions of juveniles in 1997 ($F_{2,8}$ = 2.12, P = 0.183; Table 3) or 1999 ($F_{2,8}$ = 1.84, P = 0.220). In 1997, treatment effects on proportions of subadults interacted with sampling date ($F_{18,67}$ = 3.94, P < 0.001), with treatment effects occurring during the months of April and December; however, treatments did not differ in 1999 ($F_{2,8}$ = 2.11, P = 0.184). Treatment effects on proportions of

adults also interacted with sampling date ($F_{18,67}$ = 6.53, P < 0.001), with treatment effects occurring during the months of April, May, and December 1997. Addition of methionine did not affect proportions of adults in 1999 ($F_{2,8}$ = 0.24, P = 0.790).

No differences occurred in overall survival rates between treatments in 1997 ($F_{2,8}$ = 1.16, P = 0.361; Fig. 2A), but, in 1999, treatment effects interacted with sampling date ($F_{28,112}$ = 1.76, P = 0.021), with treatments differing in June, October, and November (Fig. 2B). No climatic variables met the significance level for inclusion into multiple regression models with survival as the dependent variable. Correlation analyses also failed to show significant effects of climatic variables on survival rates (Table 1).

Female survival did not differ between treatments in 1997 ($F_{2,8}$ = 2.11, P = 0.184; Fig. 2C). In 1999, treatment effects on female survival interacted with sampling date ($F_{28,112}$ = 2.49, P < 0.001), with treatment effects occurring during the months of June and August (Fig. 2D). Male survival rates did not differ between treatments in 1997 ($F_{2,8}$ = 1.00, P = 0.409; Fig. 2E) or 1999 ($F_{2,8}$ = 0.87, P = 0.454; Fig. 2F).

In 1997, there were no treatment differences in per capita recruitment ($F_{2,8}$ = 0.39, P = 0.691; Table 4). However, in 1999, recruitment in the high-methionine treatment was greater than the control (P = 0.005) and low-methionine treatment (P = 0.008), but no differences were found between control and low-methionine (P = 0.488) populations. Recruitment was related positively to temperature in unsupplemented (R^2 = 0.171, $F_{1,23}$ = 4.53, P = 0.045), low-

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methionine ($R^2 = 0.099$, $F_{1,24} = 2.53$, $P = 0.125$), and high-methionine ($R^2 = 0.175$, $F_{1,24} = 4.86$, $P = 0.038$) treatments. However, slopes did not differ between unsupplemented and low-methionine treatments ($F_{2,16} = 0.45$, $P > 0.050$), unsupplemented and high-methionine treatments ($F_{2,16} = 0.21$, $P > 0.050$), or low- and high-methionine treatments ($F_{2,16} = 0.01$, $P > 0.050$). Therefore, treatments were affected in the same manner, and differences in recruitment were due to supplementation. Correlation analyses showed significant positive relationships between temperature and recruitment in unsupplemented and high-methionine treatments (Table 1).

In 1997, treatment effects on proportions of cotton rats in reproductive condition interacted with sampling date ($F_{18,72} = 1.89$, $P = 0.031$), with differences occurring in April and June (Fig. 3A). In 1999, treatment effects on proportions in reproductive condition also interacted with sampling date ($F_{32,128} = 2.17$, $P = 0.001$), with treatment differences occurring in the months of March, April, May, June, and July (Fig. 3B). Precipitation was positively associated with reproductive intensity in unsupplemented ($R^2 = 0.301$, $F_{1,24} = 9.89$, $P = 0.005$), low-methionine ($R^2 = 0.224$, $F_{1,24} = 6.63$, $P = 0.017$), and high-methionine ($R^2 = 0.248$, $F_{1,24} = 7.57$, $P = 0.011$) treatments. However, slopes did not differ between unsupplemented and low-methionine ($F_{2,16} = 0.01$, $P > 0.050$), unsupplemented and high-methionine ($F_{2,16} = 0.14$, $P > 0.050$), or low- and high-methionine ($F_{2,16} = 0.21$, $P > 0.050$) treatments. Precipitation affected treatments in the same manner, and differences in reproductive intensity were due to supplementation of methionine. Correlation analyses also showed positive

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relationships between precipitation and reproductive activity in all 3 treatments (Table 1).

Proportions of adult females in reproductive condition did not differ between treatments in 1997 ($F_{2,8}$ = 1.63, P = 0.254; Fig. 3C). In 1999, however, treatment effects interacted with sampling date ($F_{32,128}$ = 5.19, P < 0.001), with treatment effects occurring in the months of March, May, June, and July (Fig. 3D). There were no treatment effects on proportions of scrotal males in 1997 ($F_{2,8}$ = 0.05; P = 0.949; Fig. 3E). In 1999, proportions of scrotal males in the high-methionine treatment were greater than control (P = 0.021; Fig. 3F) and low-methionine (P = 0.044) populations; however, the control and low-methionine treatment did not differ (P = 0.522).

DISCUSSION

Results from 1997 were not consistent with the hypothesis that supplemention of high-methionine food results in greater densities of cotton rats The control density generally was lower than low- and high-methionine populations from mid-summer through autumn, but differences were not statistically significant. Results from 1999 supported the hypothesis because the unsupplemented population had the lowest density and supplemental treatments had the highest densities. With the exception of the positive relationship between precipitation and density in the high-methionine treatment (which may have been spurious), there were no significant effects of weather on density. Therefore, differences between treatments were primarily due to supplementation of methionine.

Densities of cotton rats in the enclosures were considerably higher than reported by other authors. In Kansas, supplementation of high-quality food to free-ranging cotton rats resulted in mean densities of 27.7 and 33.6 rats/ha in 2 consecutive years, and control populations averaged 12.4 and 16.8 rats/ha (Doonan and Slade 1995). Mean densities in my study were 71.3 and 57.1 rats/ha for unsupplemented and 81.7 and 115.0 rats/ha for high-methionine populations in 1997 and 1999, respectively.

In habitats that provided high-quality protein, populations of cotton rats in Oklahoma averaged 23, 31, 78, and 37 cotton rats/ha in February, May, August, and November, respectively (Schetter 1996). Mean densities for the high-methionine treatment were 130, 97, and 68 cotton rats/ha in May, August, and November 1997 and 115, 138, 110, and 70 rats/ha in February, May, August, and November 1997, respectively. Schetter (1996) reported average densities in low-quality habitats of about 7, 6, 2, and 4 cotton rats/ha in February, May, August, and November, respectively. Mean densities for my unsupplemented population were 98, 63, and 30 cotton rats/ha in May, August, and November 1997 and 70, 60, 47, and 10 cotton rats/ha in February, May, August, and November 1999, respectively.

There are several possibilities that may explain differences in density between previously obtained results and my study. First, enclosures in the current study were stocked with extremely high densities of cotton rats (160 rats/ha). Habitats in my study also may have been higher quality than in previous studies. Unfortunately, no food habit analyses were performed in my

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study for comparisons between studies. Another possibility is that cotton rats were not exposed to terrestrial predators in my study (due to the enclosures), but populations in the other studies experienced natural predation. Because of low per capita recruitment in my study, I do not believe that increased recruitment was the cause of greater densities. Dispersal might be a major regulatory mechanism of density of cotton rats and is density-dependent (Joule and Cameron 1975, Spencer and Cameron 1983); however, cotton rats in the present study were not allowed to disperse. If cotton rats in my study had not been enclosed, densities might have been high enough for the populations to implement dispersal strategies, thus lowering local densities.

Differences in treatment effects between 1997 and 1999 are difficult to explain. No vegetation parameters measured differed between years. Therefore, habitat was not likely to be the cause of annual differences. One possible explanation for inconsistent results in 1997 is that individuals were supplemented 4 months less (during winter) than 1999. If supplementation could have been provided during those harsh months, differences between treatments might have occurred, especially because densities of supplemented populations were generally higher than the control from late summer through autumn. The fact that only data collected after 22 March 1997 were used in analyses also might explain some differences observed between years.

Regardless of methionine supplementation, densities of cotton rats in my study peaked in mid-spring to early summer and reached a nadir in autumn. However, densities of cotton rats in my study were only monitored from April to

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December 1997 and January to December 1999 and probably would have continued to decrease throughout winter. Previous studies in Oklahoma documented continued declines during winter (Hays 1958a; 1958b, Goertz 1964, Schetter 1996). In Texas, populations of cotton rats also steadily decline in winter and spring before reproduction begins (Joule and Jameson 1972). Unlike the current study, populations of cotton rats in many states experience bimodal density patterns with spring and/or autumn peaks and summer and/or winter lows (Komarek 1937, Odum 1955, Hays 1958b, Raun and Wilks 1964, Petersen 1973, Layne 1974, Cameron 1977, Doonan and Slade 1995). There is a positive correlation between reproduction and density in populations of cotton rats (Layne 1974). In the current study, the decrease in reproduction in late summer led to autumnal lows because of the lack of new recruits entering the population at that time. The autumnal decrease in reproduction could be attributed to the high late-summer densities. In Oklahoma, cotton rats extend the breeding season into late autumn if late-summer densities are low; however, when late-summer densities are high, cotton rats stop breeding in August (Green 1964).

Differences in productivity of cotton rats are largely a consequence of environmental factors (Derting 1997). Patterns of cotton rat density show within-year seasonal changes, probably cued by temperature trends (Odum 1955, Haines 1971, Goertz 1964, Fleharty et al. 1972, Joule and Cameron 1974, Layne 1974). Although temperature did not directly influence density in the current study, indirect effects were seen through recruitment. Extreme high and low temperatures inhibit reproduction (Odum 1955, Haines 1961, Porter and McClure

1984, Langley and Shure 1988) and cotton rat survival (Goertz 1964, Dunaway and Kaye 1961). Temperature did not have any significant effects on reproductive intensity or survival in my study. However, mean monthly temperatures were within 5°C of average temperatures for the area, and no extremely high or low temperatures persisted for more than a couple days (National Oceanic and Atmospheric Administration 1997, 1998, 1999).

Sex ratios in my study were similar to previous studies. In Kansas (Fleharty et al. 1972), Texas (Cameron 1977), and Florida (Layne 1974, Stafford and Stout 1983), the proportion of females reported in populations of cotton rats were 0.43, 0.50, 0.46, and 0.56, respectively. McMurry et al. (1994) reported sex ratios of cotton rats in Oklahoma to be 0.45 female. Mean proportions of females occupying habitats supplying high-quality dietary protein (high-density populations) and habitats limiting availability of high-quality protein (low-density populations) were reported as 0.47 and 0.45, respectively (Schetter 1996). Goertz (1965a) observed that when densities of cotton rats in Oklahoma were high, sex ratios were skewed slightly toward females (0.53), but at lower densities, significantly fewer females were present (0.40).

At high densities, a shift in age distribution toward more young individuals has been reported in populations of cotton rats in Georgia (76% juveniles, 18% subadults, and 6% adults; Odum 1955). However, populations of cotton rats in Oklahoma that occupied habitats providing high-quality dietary protein (high-density populations) had 27% juveniles, 36% subadults, and 37% adults (Schetter 1996). In the current study, high-methionine populations consisted of

26% juveniles, 24% subadults, and 50% adults. At lower densities, populations consisted of 55% juveniles, 30% subadults, and 15% adults (Odum 1955). However, other cotton-rat populations occupying low-quality habitats (low-density populations) consisted of 25% juveniles, 32% subadults, and 43% adults (Schetter 1996). Unsupplemented populations in the current study consisted of 21% juveniles, 33% subadults, and 46% adults. In my study, no decrease in mean age distribution was observed at high densities. However, densities in my study were higher than reported by Odum (1955) and may not have been low enough to experience a change in age structure.

In several other small-herbivore species, populations with access to either high-quality protein in the forage or supplementation had increased survival rates. When high-quality forage was available, life expectancy of prairie voles (*Microtus ochrogaster*) was increased (Cole and Batzli 1979). Townsend voles (*Microtus townsendii*; Taitt et al. 1981) and snowshoe hares (*Lepus americanus*; Boutin 1984) also had increased survival when supplementation was provided. In 1999. low- and high-methionine populations experienced significantly higher survival rates than the control in autumn, presumably because of food quality and/or availability. However, supplementation does not always affect overall survival (Taitt and Krebs 1981), including most months in the present study.

Because enclosures limit dispersal, Schetter (1996) predicted that nutrient-supplemented populations of cotton rats would achieve greater densities because of improved rates of recruitment compared with unsupplemented populations. This prediction was not supported in 1997, but results in 1999 were

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more consistent with the hypothesis because cotton rats on high-methionine had greater recruitment than unsupplemented and low-methionine cotton rats. Although temperature had small effects on recruitment (9-17%), treatment differences in recruitment were likely due to supplementation of methionine.

My estimates of recruitment (range = 0.23-1.42 new juveniles/adult female) were lower than other cotton rat populations in Oklahoma (range = 0.50-3.50 new juveniles/adult female; Schetter 1996). Schetter (1996) reported that total number of juvenile recruits that entered the trappable population were about 5 times more abundant in populations with access to high-quality protein than populations occupying low-quality habitat (62 and 12 total recruits, respectively). Total number of juvenile recruits in my study was 77, 105, and 136 for control, low-methionine, and high-methionine populations, respectively.

My results from 1999 generally support Randolph et al. (1995), who stated that dietary protein might limit reproduction in wild populations of cotton rats. Except for June 1999, the high-methionine treatment resulted in greater reproductive intensity than the control. Precipitation had positive effects on reproductive intensity (22-30%), but differences were primarily due to supplementation of methionine. Eshelman (1991) also demonstrated increased reproductive potential of cotton rats on a diet containing high-protein levels. Results from studies on supplemented white-footed (*Peromyscus leucopus*; Briggs 1986) and house mice (*Mus musculus*; Bomford and Redhead 1987) also were consistent with this hypothesis.

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Cotton rats reproduce primarily between late spring and autumn and rarely in winter (Green 1964, Fleharty et al. 1972, Cameron 1977, Cleveland 1978, Glass and Slade 1980, McMurry 1993, McMurry et al. 1994). There is some evidence that reproductive activity is reduced during periods of extreme heat in mid-summer (Goertz 1965b, O'Farrell et al. 1977). Cotton rats (sexes combined) in my study primarily reproduced from early spring to about mid-summer with no reproduction from mid-September through December. However, in 1999, supplementation of high-methionine food resulted in earlier and longer reproductive seasons than the control. This phenomenon also is common in mouse and vole populations supplied with additional high-quality food. White-footed mice also experience an earlier breeding season with supplementation of high-quality food (Hansen and Batzli 1978, 1979). With the addition of high-quality food, populations of wood mice (*Apodemus sylvaticus*; Akbar and Gorman 1993) and house mice (Bomford 1987) begin breeding earlier and for longer. Supplementation of high-quality food also shortened the winter non-breeding season in populations of Townsend's voles (Taitt and Krebs 1981).

Density also affects length of the mating season in Oklahoma (Green 1964). Dense populations of cotton rats experience shorter mating seasons, which limits growth rate of the population. If summer density is high, cotton rats stop breeding in August; however, the breeding season extends into late fall if the summer population is low (Green 1964). Therefore, high densities in the current study may have caused cessation of breeding in early autumn.

Nutritional requirements increase during reproduction, especially for females, and inadequate nutrition inhibits reproduction (Robbins 1993). My data support these tenets because the addition of high-methionine food resulted in earlier and lengthened female breeding seasons in 1999. This phenomenon also has occurred in female prairie voles (*Microtus ochrogaster;* Cole and Batzli 1979) and white-footed mice (Briggs 1986) with available high-quality protein forage or the addition of high-quality food. McMurry et al. (1994) reported that peak female reproduction in populations of cotton rats occurred in autumn in north-central Oklahoma. However, in other populations in Oklahoma, proportions of reproductively active females peaked in spring (0.80), declined throughout summer (0.64-0.46), peaked again in autumn (0.86), then declined through winter (0.10-0.00; Schetter 1996). In Virginia, peaks in female reproductive activity also were seen in spring and autumn (Rose 1986). In both years of my study, peak female reproduction occurred in mid- to late spring with a slight peak in October 1997.

The hypothesis that an increased availability of high-quality protein results in increased reproductive intensity of female cotton rats generally was not supported in either year. Proportions of reproductively active female cotton rats in the high-methionine treatment were 0.46 in 1997 and 0.15 in 1999. Proportions in unsupplemented populations were 0.37 in 1997 and 0.16 in 1999. Schetter (1996) reported that 24% of females were reproductively active in habitats containing high-quality protein forage versus 16% in low-quality habitats. Reproduction of female house mice also increased with increased availability of

high-protein food (Bomford and Redhead 1987); however, supplementation of high-quality food had no effect on the proportion of reproductively active female pinyon mice (*Peromyscus truei*; Hall and Morrison 1998).

Proportions of scrotal males in populations of meadow voles (*Microtus pennsylvanicus*; Desy and Thompson 1983) and pinyon mice (Hall and Morrison 1998) supplemented with high-quality food did not differ from unsupplemented populations. However, high-methionine supplementation resulted in greater reproductive intensity in adult, male cotton rats in 1999, but did not affect their breeding seasons.

In general, densities of cotton rats in this study increased with supplementation of high-methionine food. Except for mid-spring to mid-autumn 1997 and autumn 1999, overall survival rates did not increase with supplementation. In the second year of the study. per capita recruitment and reproductive intensity were increased with the addition of high-methionine food. Supplementation also resulted in earlier and lengthened breeding seasons in 1999, especially for females. Results support the hypothesis that methionine is fundamentally involved in regulating populations of wild cotton rats, but future studies probably need to extend past the 1-year periods included in the current study.

ACKNOWLEDGMENTS

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Resources Division, Oklahoma State University. Oklahoma Department of

Wildlife Conservation, and Wildlife Management Institute, cooperating). I

gratefully acknowledge R. L. Lochmiller, D. M. Leslie, Jr., R. E. Masters, S. S.

Ditchkoff, D. P. Rafferty, and E. C. Hellgren for providing valuable comments

during the course of the study and on earlier drafts.

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Table 1. Correlation coefficients for the relationships between density, survival rates, recruitment rates, and reproductive intensity of wild cotton rat populations with no supplemental food (NS), low-methionine supplementation (LM), and high-methionine supplementation (HM) in north-central Oklahoma and climatic conditions (mean monthly temperature and total monthly precipitation and snowfall), 1997 and 1999 combined.

| | | Treatment | | | | | |
| | | NS | | LM | | HM | |
Variable	Climatic Condition	r	P	r	P	r	P
Density	Temp	-0.148	0.479	-0.054	0.799	0.094	0.655
	Precip	-0.007	0.974	0.173	0.409	0.454	0.023
	Snow	-0.133	0.526	0.044	0.835	0.072	0.733
Surv	Temp	-0.057	0.793	0.147	0.494	0.067	0.757
	Precip	-0.026	0.904	-0.098	0.649	-0.271	0.201
	Snow	-0.166	0.438	-0.248	0.242	-0.145	0.498

Table 1. Continued.

Variable	Climatic Condition	NS		LM		HM	
		r	P	r	P	r	P
Recruit	Temp	0.413	0.045	0.315	0.125	0.418	0.038
	Precip	0.137	0.523	0.181	0.385	0.282	0.171
	Snow	-0.207	0.333	-0.107	0.612	-0.137	0.513
Repro	Temp	0.052	0.806	-0.045	0.831	0.067	0.752
	Precip	0.548	0.005	0.473	0.017	0.498	0.011
	Snow	-0.038	0.858	0.055	0.795	0.120	0.339

Table 2. Mean proportion of females in wild cotton rat populations with no supplemental food (NS), low-methionine supplementation (LM), and high-methionine supplementation (HM) in north-central Oklahoma, 1997 and 1999.

	Treatment								
	NS			LM			HM		
Year	n	Mean	SE	n	Mean	SE	n	Mean	SE
1997	40	0.415	0.041	40	0.585	0.020	30	0.496	0.035
1999	45	0.486	0.027	60	0.486	0.009	60	0.507	0.009

Table 3. Mean proportion of juveniles, subadults, and adults in populations of wild cotton rats with no supplementation (NS), low-methionine supplementation (LM), and high-methionine supplementation (HM) in north-central Oklahoma, 1997 and 1999.

		NS			LM			HM		
Year	Age Class	n	Mean	SE	n	Mean	SE	n	Mean	SE
1997	Juvenile	35	0.217	0.040	40	0.239	0.038	30	0.317	0.047
	Subadult [ab]	35	0.301	0.041	40	0.260	0.038	30	0.233	0.040
	Adult [cde]	35	0.482	0.039	40	0.501	0.050	30	0.450	0.054
1999	Juvenile	48	0.200	0.043	68	0.149	0.025	68	0.210	0.029
	Subadult	48	0.358	0.051	68	0.283	0.033	68	0.251	0.031
	Adult	48	0.446	0.049	68	0.568	0.032	68	0.538	0.033

The column headers span the Treatment grouping.

[a] NS was greater than LM and HM in April ($P < 0.05$).

[b] HM was greater than NS and LM in December ($P < 0.05$).

[c] LM and HM were greater than NS in April ($P < 0.05$).

[d] NS and LM were greater than HM in May and December ($P < 0.05$)

[e] NS was greater than LM in October ($P < 0.05$).

Table 4. Mean per capita recruitment rates of wild cotton rat populations with no supplementation (NS), low-methionine supplementation (LM), and high-supplementation (HM) in north-central Oklahoma, 1997 and 1999.

		Treatment							
	NS			LM			HM		
Year	n	Mean	SE	n	Mean	SE	n	Mean	SE
1997	31	1.058	0.293	33	0.864	0.263	28	1.423	0.365
1999 [a]	33	0.253	0.107	66	0.226	0.069	64	0.493	0.117

[a] HM is greater than NS ($P = 0.005$) and LM ($P = 0.008$).

Figure 1. Mean (± SE) densities of wild cotton rats known to be alive for both sexes combined in (A) 1997 and (B) 1999, mean densities of wild female cotton rats known to be alive in (C) 1997 and (D) 1999, and mean densities of wild male cotton rats known to be alive in (E) 1997 and (F) 1999 in north-central Oklahoma populations with no supplemental diet, supplemental low-methionine diet, and supplemental high-methionine diet. Significant differences ($P < 0.05$) occurred at: [a] High-methionine Diet > No Supplementation; [b] Low-methionine Diet and High-methionine Diet > No Supplementation; and [c] High-methionine Diet > Low-methionine Diet > No Supplementation.

Figure 2. Mean (± SE) survival rates with both sexes combined in (A) 1997 and (B) 1999, mean female survival rates in (C) 1997 and (D) 1999, and mean male survival rates in (E) 1997 and (F) 1999 of wild cotton rats from populations in north-central Oklahoma with no supplemental diet, supplemental low-methionine diet, and supplemental high-methionine diet. Significant differences ($P < 0.05$) occurred at: [a] No Supplementation > Low-methionine Diet and High-methionine Diet; [b] Low-methionine Diet and High-methionine Diet > No Supplementation.

Figure 3. Mean (± SE) proportions of reproductively active cotton rats for both sexes combined in (A) 1997 and (B) 1999, mean proportions of reproductively active female cotton rats in (C) 1997 and (D) 1999, and mean proportions of reproductively active male cotton rats in (E) 1997 and (F) 1999 in wild populations from north-central Oklahoma with no supplemental diet, supplemental low-methionine diet. and supplemental high-methionine diet. Significant differences ($P < 0.05$) occurred at: [a] No Supplementation > Low-methionine Diet; [b] No Supplementation > High-methionine Diet; [c] Low-methionine Diet > High-methionine Diet; [d] High-methionine Diet > No Supplementation; and [e] High-methionine Diet > Low-methionine Diet.

A

B

C

D

E

F

6 Apr
18 Apr
13 May
4 Jun
1 Jul
25 Jul
14 Aug
4 Oct
6 Nov
15 Dec

30 Nov
20 Dec
30 Jan
26 Feb
19 Mar
23 Apr
17 May
1 Jun
20 Jun
3 Jul
26 Jul
11 Aug
30 Aug
14 Sep
24 Oct
26 Nov
13 Dec

1997

1999

No Supplementation   Low-methionine Diet   High-methionine Diet

40

42
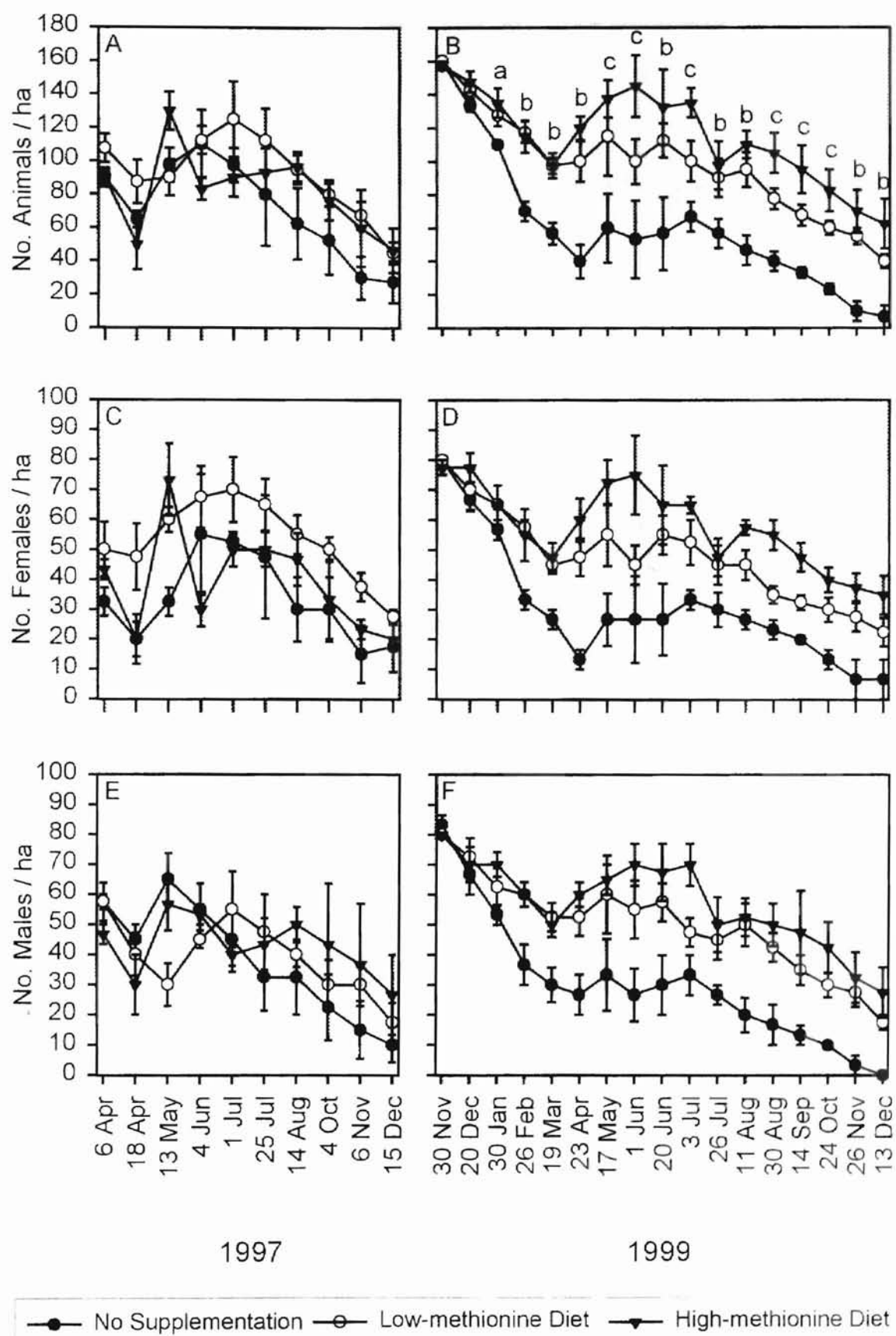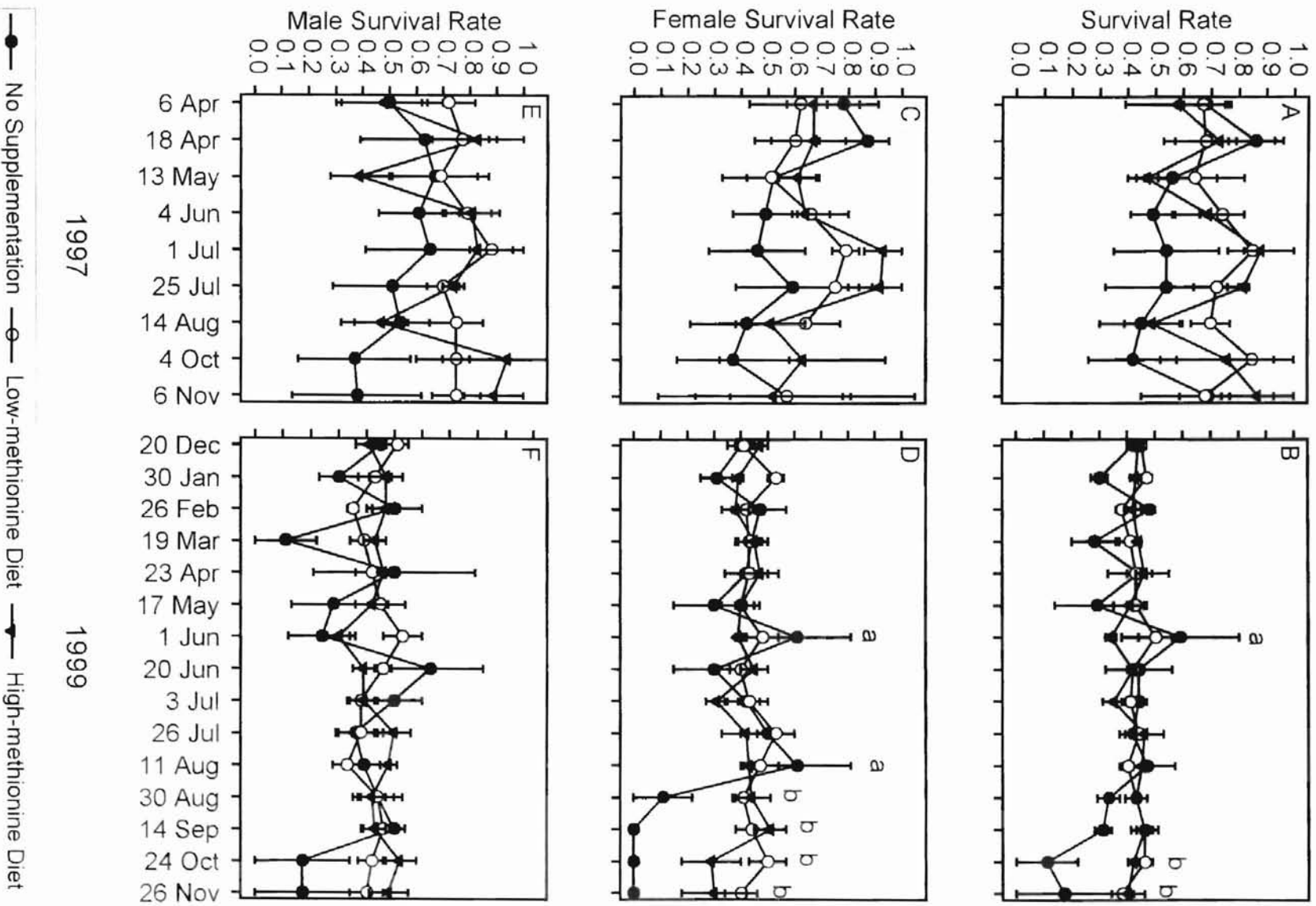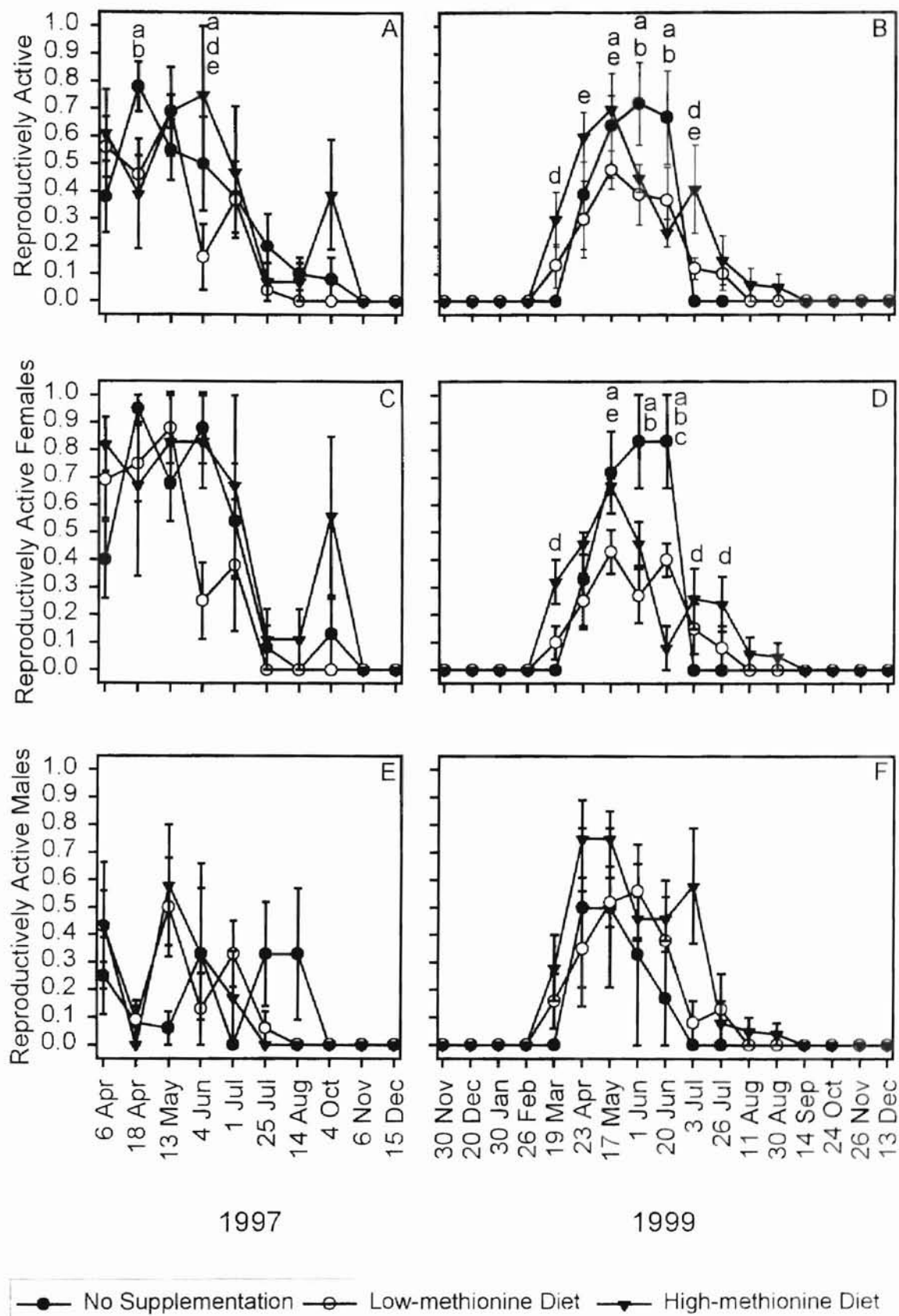
Chapter II.

IMMUNE FUNCTION AND HEMATOLOGY OF WILD COTTON RATS (*SIGMODON HISPIDUS*) IN RESPONSE TO INCREASED METHIONINE

Abstract: Numerous studies have proposed that quality of dietary protein influences hematology and immune function of animals. Therefore, I examined effects of supplemental methionine on hematological and immunological parameters of wild, but enclosed, populations of adult, male cotton rats (*Sigmodon hispidus*) in north-central Oklahoma. Sheet metal enclosures ($n=12$) were stocked with wild-caught cotton rats and randomly assigned a treatment of no supplementation, low-methionine supplementation, or high-methionine supplementation. Methionine did not influence numbers, size, or hemoglobin content of red blood cells, but a positive relationship was present between methionine and platelet counts. Supplemental methionine resulted in elevated leukocyte numbers, but the difference was not due to increased percentages of any 1 type of white blood cell. Delayed-type hypersensitivity did not differ between treatments, but the control differed seasonally. Hemolytic-complement activity was not affected by supplemental methionine and all treatments varied seasonally. Although delayed-type hypersensitivity and hemolytic-complement activity varied seasonally, no apparent trend was present. These results suggest that methionine alone does not affect many hematological or immunological parameters of wild cotton rats.

Many theories have been advanced to explain regulation of herbivore populations. Among those, forage quality and quantity can be of central

importance in regulating populations of small herbivores (Cole and Batzli 1979, Hansson 1979, Sinclair et al. 1982, Keith 1983, Sullivan et al. 1983, Jones 1990, Batzli and Lesieutre 1991, Doonan and Slade 1995, Eshelman and Cameron 1996). In particular, protein may be the most limiting factor in many herbivore populations (White 1978, Mattson 1980, Randolph et al. 1995, Cameron and Eshelman 1996, Schetter 1996).

Protein in the diet supplies essential amino acids that cannot be synthesized in adequate amounts by an organism. The ability of a dietary protein to supply a proper balance of these essential amino acids determines quality of that protein (Oser 1959). The ideal protein is perfectly balanced in terms of its content of essential amino acids and supply of nitrogen as nonessential amino acids for an animal's particular requirements (Moughan et al. 1988, Robbins 1993). Studies on qualities of proteins in diets of northern pintail (*Anas acuta*; Krapu and Swanson 1975), cackling geese (*Branta canadensis minima*; Sedinger 1984), willow ptarmigan (*Lagopus lagopus*; Steen et al. 1988), bobwhite quail (*Colinus virginianus*; Boren 1992, Peoples et al. 1994), cottontail rabbits (*Sylvilagus floridanus*; Lochmiller et al. 1995), and cotton rats (*Sigmodon hispidus*; Schetter 1996) have suggested that essential amino acids (especially sulfur-containing methionine and cystine) may be deficient in wild populations.

Deficiencies of sulfur-containing amino acids lead to reduced immune function (Gershoff et al. 1968, Jose and Good 1973, Gross and Newberne 1980), and involvement of host immunocompetence can influence survival of individuals within herbivore populations (Lochmiller 1996). Reduced immunocompetence

might be involved in regulating populations of small mammals, including cotton rats (Mihok et al. 1985, Vestey 1991, Lochmiller et al. 1994). Therefore, I evaluated effects of supplemental methionine on hematological and immunological parameters in wild populations of cotton rats. I predicted that methionine supplementation would enhance nutritional and immunological condition of wild cotton rats.

STUDY AREA AND METHODS

This study was located about 20 km west of Stillwater, Oklahoma (97°N, 60°E) on the Zoology Research Area. Primary vegetation consisted of little bluestem (*Schizachyrium scoparium*), switchgrass (*Panicum virgatum*), indiangrass (*Sorghastrum nutans*), big bluestem (*Andropogon gerardii*), panicum (*Panicum* spp.), dropseed (*Sporobolus* spp.), lespedeza (*Lespedeza* spp.), western ragweed (*Ambrosia psilostachya*), sagewort (*Artemisia ludoviciana*), brome (*Bromus* spp.), and yarrow (*Achillea lanulosa*) interspersed with small (< 1 5 m in height) eastern redcedar (*Juniperus virginiana*), American elm (*Ulmus americana*), winged elm (*Ulmus alata*), winged sumac (*Rhus copallina*), smooth sumac (*R. glabra*), and buckbrush (*Symphoricarpos orbiculatus*).

Twelve 0.1-ha enclosures, constructed with galvanized sheet metal (buried about 25 cm in the ground and 2 m in height), were cleared of all mammals in November 1996. Cotton rats were trapped from local populations (0-18 km from enclosures) using Sherman live traps baited with oats. Sixteen non-reproductive cotton rats, in equal sex ratios, were toe-clipped to indicate unique identification numbers, weighed, and placed in each enclosure in

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November 1996 and 1998. At the end of 1997, enclosures were cleared of all mammals, and an entirely new study group of study animals was introduced for the second year of the study.

Each enclosure was randomly assigned a treatment of no supplementation (control), supplementation of a low-methionine diet, or supplementation of a high-methionine diet, with 4 enclosures/treatment. Because methionine is an indispensable component of the diet that supplies sulfur for cystine synthesis in the absence or deficiency of the latter (Rose 1937, Williams et al. 1954), only methionine was manipulated in the present study. The low-methionine diet consisted of 47.0% corn, 50.75% beet pulp, 3.0% dried molasses, and 0.25% vitamin and mineral mix by weight, with 0.20% methionine (slightly below the maintenance requirement of 0.22% for laboratory rats, *Rattus* sp.; Williams et al. 1954). The total sulfur-containing amino acid requirement for optimal lactation in the rat is 1.11-1.24% of the diet (Nelson and Evans 1958). Therefore, the high-methionine diet was the same as the low-methionine diet except for an added amount of D-L-methionine to obtain 1.20% of the diet. Covered feeders were placed at the center and each corner of each enclosure (i.e., 5 feeders/enclosure). Supplemental food was provided ad libitum and replenished every 7-10 days.

Vegetation measurements were taken seasonally to determine if differences in habitat existed between treatments. Vegetation cover was estimated using the Daubenmire method (Bonham 1989). Ten 0.1-m^2 quadrats were located randomly in each enclosure to obtain cover estimates of grasses,

forbs, legumes, woody plants, standing litter, and fallen litter. Percent cover was classified as 0%, 1-5%, 6-25%, 26-50%, 51-75%, 76-95%, or 96-100% for each vegetation class. Midpoints of cover classes were used in statistical analyses. Height (cm) of the tallest vegetation also was recorded in the quadrat. Biomass estimates (kg/ ha) of grasses, forbs, and legumes were determined in quadrats by clipping plants to ground level, but biomass of woody plants was determined by clipping current year's growth ≤ 1 m in height. Clippings were oven-dried at 70°C, and biomass for each vegetation class was recorded (Bonham 1989). Analyses of variance (PROC GLM) and least squared means were used to examine treatment differences in vegetation height, cover, and biomass data (SAS Institute Inc. 1996). Statistical significance was set at $P = 0.05$.

Density was assessed seasonally using a 5 x 5 trapping grid in each enclosure. Sherman live traps were set every trapping day in late afternoon, baited with oats, and checked the next morning. If the animal was not toe-clipped, it was assigned a new identification number. For each enclosure, the minimum number known alive was calculated by summing the number of unique individuals caught during a census period (3 consecutive nights/period) and all animals that were caught both before and after that period (Krebs 1966, Pollock et al. 1990). Estimates were converted to densities (number of cotton rats per ha).

In June, September, and December 1997 and March, June, September, and December 1999, non-scrotal, male cotton rats (>75 g) were anesthetized by methoxyflurane inhalation (metofane, Pitman-Moore, Mundelein, Illinois, USA).

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Only mature, non-scrotal males were used in the study because hematological values and immunocompetence of cotton rats can vary with age, sex, and reproductive status (Bickerstaff 1967, Vestey 1991, Katahira and Ohwada 1993, Vestey et al. 1993, Lochmiller et al. 1994, Robel et al. 1996). A general measurement of nutritional status should include a complete blood count (Miller et al 1947, Harper 1971. Gershwin et al. 1985); therefore, a blood sample (<0.4 ml) was collected from the retro-orbital sinus (Stone 1954) in 3-ml evacuated EDTA-K tubes (Monojet; Sherwood Medical, St. Louis, Missouri, USA). Whole blood samples were transported to the laboratory where counts of red blood cells (RBC), hematocrit levels, mean corpuscular volume (MCV), hemoglobin concentrations, mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet counts, and counts of total white blood cells (WBC) were measured on a Serono 9000 automated cell counter (Serono-Baker Diagnostics, Allentown, Pennsylvania, USA). Because the differential count is a quick, easy, and inexpensive method for assessing health of an animal (White and Cook 1974), a blood smear was prepared for differential leukocyte counts, which were performed by classifying 100 cells after Diff-Quik staining (American Scientific Products, McGaw Park, Illinois, USA). Whole blood samples were centrifuged (12 min, 2,400 rpm) and plasma collected and frozen at $-80°C$ for future analysis.

Delayed-type hypersensitivity is a consistently and profoundly altered measure of immunocompetence in malnutrition (McMurray 1984, Gershwin et al. 1985, Buckley 1986) and is a simple, cost-effective method to evaluate cellular

immunity (Gershwin et al. 1985, Buckley 1986, Vestey 1991); therefore, following blood collection, *in vivo* cell-mediated immunity was indexed using a hypersensitivity reaction (Williams et al. 1979, Sinclair and Lochmiller 2000). An intradermal injection of 100 μl of phytohemagglutinin (PHA; Sigma, St. Louis, Missouri, USA; 2.5 mg/ml phosphobuffered saline (PBS)) was administered to 1 shaved hip of each non-scrotal male cotton rat. The opposite hip was challenged with an equal amount of sterile PBS to serve as the control. Double-skin-fold thickness was measured to the nearest 0.001 inch (1 inch = 25.4 mm) with a pressure-sensitive micrometer. Animals were housed separately in cages on-site for 24 hr, and double-skin-fold thickness was again measured. The DTH response was expressed as the relative percent increase in double-skin-fold thickness of the stimulated side over the control (Vestey et al. 1993).

Presence of adequate amounts of functional complement is an important component of normal immune function (Chandra 1976, Gershwin et al. 1985), and measurement of hemolytic-complement activity is a convenient and quantitative clinical test for evaluating functional integrity of the complement sequence (Renshaw et al. 1978). Hemolytic-complement activity can help describe effects of protein restriction on overall immunocompetence of cotton rats (Davis et al. 1995). Therefore, innate, nonspecific immunity was assessed by measuring hemolytic-complement activity using the CH_{50} method because it is highly accurate (Gershwin et al. 1995). Hemolytic-complement activity in plasma was determined by a slight modification of the methods of DeWaal et al. (1988), as described by Sams et al. (1996) and Sinclair and Lochmiller (2000). Briefly, 5

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µl of plasma diluted 1:80 in vernal buffer was serially diluted 2-fold in a 96-well round-bottomed microtiter plate; 25 µl of a 0.6% suspension of washed sheep red blood cells (SRBC; Colorado Serum Company, Denver, Colorado, USA) in vernal buffer and 25 µl of a 1:40 dilution of a rabbit-anti-SRBC antibody (Nordic Immunological Laboratories, Capistrano Beach, California, USA) in vernal buffer were added to each well. Plates were vortexed and incubated for 1.5 hr at $37^{\circ}C$ and then centrifuged (5 min. 500 rpm). Absorbance (414 nm) was measured using a Titertek Multiscan II plate reader (Flow Laboratories, Inc., Mclean, Virginia, USA). Hemolytic-complement activity was expressed as CH_{50} units/ml plasma, where 1 CH_{50} unit equaled the amount of plasma required to lyse 50% of the SRBC in culture (Kabat and Mayer 1961, Ruddy 1986).

Treatment effects, seasonal effects within treatments, and interactions between treatment effects and season on hematological and immunological parameters were determined by analyses of variance (PROC GLM; SAS Institute Inc. 1996). Where significant differences occurred ($P < 0.05$), least squared means tests were used to determine which treatments or seasons differed (SAS Institute Inc. 1996). Regression analyses (PROC REG; SAS Institute Inc. 1996; Allison 1999) were used to assess the influence of density, mean monthly temperature, total monthly precipitation, and total monthly snowfall (National Oceanic and Atmospheric Administration 1997, 1998, 1999) on hematological and immunological parameters. Relationships between temperature, precipitation, snowfall, and density were examined using Pearson correlation

analyses (PROC CORR; SAS Institute Inc. 1996, Allison 1999). Statistical significance was set at $P = 0.05$ for regression and correlation analyses.

Data collected between 23 November 1996 and 22 March 1997 were not included in analyses because of inadequate enclosure maintenance. In 1997 and 1999, 1 enclosure was not used in analyses because excess predation by hawks and owls that perched on the enclosure's boundary apparently eliminated the population of cotton rats within weeks after stocking. Therefore, in 1997, the high-methionine treatment included 3 enclosures, and in 1999, the control included 3 enclosures.

RESULTS

In 1997, only percent cover of fallen litter differed ($P = 0.038$) among treatments. In 1999, only woody biomass and percent grass cover differed ($P = 0.008$ and $P = 0.002$, respectively). Of 22 vegetation characteristics measured, only 1 in 1997 and 2 in 1999 were statistically different, suggesting that habitats were similar among treatments. No trends were found upon examination of scatter plots of treatment versus habitat parameters, and habitats again were deemed similar among treatments. Because of the minimal number of differences found and lack of trends in plots, I concluded that the small differences in vegetation among treatments were not biologically relevant, and therefore, any differences in biotic and demographic characteristics of cotton rats were assumed to be treatment effects of methionine supplementation.

Density, temperature, precipitation, and snowfall were highly correlated ($0.75 \geq r \geq 0.49$); therefore. only density (Table 1) was used as an independent

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variable in regression models (Allison 1999). Only hematocrit values in the high-methionine treatment ($r^2 = 0.12$; $P = 0.006$) and percent lymphocytes in the low-methionine treatment ($r^2 = 0.09$; $P = 0.017$) were affected by density and climate. Of 15 hematological and immunological parameters regressed, only 2 were statistically related and no trends were found upon examination of scatter plots of hematological and immunological parameters versus density, suggesting that density and climate did not influence hematology or immunocompetence of cotton rats. Because of the minimal number of differences found and lack of trends in plots, I concluded that the small influence of density and climate on measured parameters was not biologically relevant, and therefore, any differences in hematology and immunocompetence of cotton rats were assumed to be treatment effects of methionine supplementation.

Counts of red blood cells were greater in the control ($P = 0.010$) and high-methionine treatment ($P = 0.009$) than in the low-methionine treatment, but control and high-methionine treatments did not differ ($P = 0.786$; Fig. 1A, B). No seasonal differences occurred in the control ($F_{5,27} = 1.09$; $P = 0.451$), low-methionine ($F_{6,50} = 0.88$; $P = 0.553$), or high-methionine ($F_{6,47} = 1.65$; $P = 0.263$) treatments for RBC counts (Fig. 1A, B). Hematocrit levels were higher in the control ($P = 0.005$) and high-methionine treatment ($P = 0.003$) than the low-methionine treatment, but control and high-methionine treatments did not differ ($P = 0.801$; Fig. 1C, D). No seasonal differences occurred in control ($F_{5,27} = 1.08$; $P = 0.455$), low-methionine ($F_{6,50} = 0.91$; $P = 0.535$), or high-methionine ($F_{6,47} = 1.92$; $P = 0.206$) treatments for hematocrit levels (Fig. 1C, D) Mean corpuscular

volume did not differ between treatments ($F_{2,93}$ = 0.63; P = 0.535), and there were no seasonal effects in control ($F_{5,27}$ = 1.15; P = 0.427), low-methionine ($F_{6,50}$ = 0.89; P = 0.551), or high-methionine ($F_{6,47}$ = 0.35; P = 0.887) treatments (Fig. 1E, F).

Treatment effects on hemoglobin concentration interacted with season ($F_{12,51}$ = 2.13; P = 0.031), with treatment effects occurring during the months of Sep and Dec 1997 and Dec 1999 (Fig. 2A, B). Concentrations of hemoglobin did not differ between seasons in control ($F_{5,27}$ = 1.67; P = 0.274), low-methionine ($F_{6,50}$ = 1.06; P = 0.465), or high-methionine ($F_{6,47}$ = 1.68; P = 0.255) treatments (Figure 2A, B). Mean corpuscular hemoglobin ($F_{2,93}$ = 1.11; P = 0.337; Figure 2C, D) and MCHC ($F_{2,93}$ = 1.05; P = 0.359; Figure 2E, F) did not differ between treatments. There was no seasonal effects for MCH in control ($F_{5,27}$ = 0.83; P = 0.573), low-methionine ($F_{6,50}$ = 0.85; P = 0.571), or high-methionine ($F_{6,47}$ = 1.02; P = 0.483) treatments (Fig. 2C, D). Mean corpuscular hemoglobin concentrations also did not differ between seasons in control ($F_{5,27}$ = 1.11; P = 0.442), low-methionine ($F_{6,50}$ = 1.19; P = 0.407), or high-methionine ($F_{6,47}$ = 1.78; P = 0.235) treatments (Fig. 2E, F).

Platelet counts were greater in high- (P = 0.002) and low-methionine (P < 0.001) treatments than the control, but high- and low-methionine treatments did not differ (P = 0.338; Fig. 3A, B). Platelet levels did not differ across seasons in control ($F_{5,27}$ = 0.74; P = 0.622), low-methionine ($F_{6,50}$ = 1.16; P = 0.422), or high-methionine ($F_{6,47}$ = 1.21; P = 0.399) treatments (Fig. 3A, B). Total WBC counts were greater in the high-methionine treatment than control (P = 0.005), but

control (P = 0.087) and high-methionine treatments (P = 0.133) did not differ from the low-methionine treatment (Fig. 3C, D). No seasonal differences occurred in control ($F_{5,27}$ = 2.71; P = 0.129), low-methionine ($F_{6,50}$ = 1.35; P = 0.351), or high-methionine ($F_{6,47}$ = 1.39; P = 0.336) treatments for total WBC counts (Fig. 3C, D). Percent neutrophils ($F_{2,93}$ = 1.53; P = 0.226), eosinophils ($F_{2,93}$ = 2.76; P = 0.073), monocytes ($F_{2,93}$ = 0.72; P = 0.492), lymphocytes ($F_{2,93}$ = 2.55; P = 0.088), and basophils ($F_{2,93}$ = 0.57; P = 0.570) did not differ between treatments (Table 2). Percentages of neutrophils, eosinophils, monocytes, lymphocytes, or basophils also did not differ between seasons in any treatment ($F_{12,93} \leq 1.68$; $P \geq 0.100$).

Delayed-type hypersensitivity response did not differ between treatments ($F_{2,93}$ = 2.19; P = 0.122; Fig. 4A, B) but differed seasonally in control ($F_{6,20}$ = 4.52; P = 0.011) but not in low- ($F_{6,37}$ = 1.68; P = 0.177) or high-methionine ($F_{6,36}$ = 0.81; P = 0.573) treatments (Fig. 4A, B). Hemolytic-complement activity also did not differ between treatments ($F_{2,93}$ = 0.82; P = 0.445) but differed seasonally in control ($F_{6,21}$ = 4.83; P = 0.008), low-methionine ($F_{6,37}$ = 14.66; $P < 0.001$), and high-methionine ($F_{6,35}$ = 61.82; $P < 0.001$) treatments (Fig. 4C, D).

DISCUSSION

Studies that evaluated effects of protein quality on hematological and immunological parameters of animals have reported mixed results. Some authors reported no treatment differences, while others reported a decrease or increase in values in response to changes in dietary protein.

There were small differences between treatments in my study, but they were probably not caused by methionine. As RBC counts decrease, erythrocyte size increases (Bickerstaff 1967), but no treatment differences occurred for MCV (the average volume of erythrocytes) in my study. In addition, as RBC counts decrease, MCH increases (Bickerstaff 1967), but MCH did not differ between treatments, and therefore, the small difference ($\leq 0.2 \times 10^6/\mu l$) was probably not caused by methionine. Because small amounts of variation in hematological values were to be expected (White and Cook 1974) and means of all treatments were in general agreement with the literature, this contention is further supported. Dotson et al. (1987) observed a mean RBC count of $5.7 \times 10^6/\mu l$ for cotton rats in the laboratory. Slightly higher RBC counts were found for male cotton rats in the laboratory (mean = $6.48 \times 10^6/\mu l$, Tanaka and Shibuya 1989; mean = $6.2 \times 10^6/\mu l$, Katahira and Ohwada 1993). Hankins (1951) reported RBC counts of $5.7-8.8 \times 10^6/\mu l$ for cotton rats from Kansas and Oklahoma. Hepworth (1966) reported a mean erythrocyte count of $4.17 \times 10^6/\mu l$ for adult, male cotton rats in Oklahoma, but Robel et al. (1996) reported a mean of $6.0 \times 10^6/\mu l$ for wild cotton rats in north-central Oklahoma.

Abnormal health can lower RBC counts (Dudzinski et al. 1962, Bush et al 1981), but differential leukocyte counts, DTH responses, or hemolytic-complement activity did not differ between treatments in my study. Therefore, cotton rats in the low-methionine treatment were probably not more infected with disease and/or parasites than control and high-methionine treatments. Positive relationships exist between erythrocyte counts and condition in mule deer

(*Odocoileus hemionus*; Anderson et al. 1970), black bears (*Ursus americanus*; Hellgren et al. 1993), and wild rabbits (*Oryctolagus cuniculus*; Dudzinski et al. 1962). White-tailed deer (*Odocoileus virginianus*; Seal et al. 1972, 1978b) and pronghorns (*Antilocapra americana*; Seal and Hoskinson 1978) with available high-protein food also exhibit higher erythrocyte counts than animals consuming low-protein food; however, weanling cotton rats exhibit no variation in RBC counts due to protein restriction (Davis et al. 1995), which supports my results. Thus. methionine did not appear to affect erythropoiesis.

Erythrocyte counts are generally lower during winter in caribou (*Rangifer tarandus*; Hawley and Peden 1982) but are higher during winter in gray wolves (*Canis lupus*; Seal and Mech 1983), collared peccaries (*Tayassu tajacu*; Lochmiller et al. 1985), and white-tailed deer (Seal et al. 1978a). In Arkansas, mean RBC counts for cotton rats were highest in December (Bickerstaff 1967). Sealander (1964) also reported higher erythrocyte counts in cotton rats during winter, which could be correlated with decreased ambient temperature (Sealander 1964) because the erythrocyte count is greatly influenced by the animal's metabolic rate (Goldfinger and Goldfinger 1964). In the present study, temperature did not influence RBC counts, and no seasonal differences occurred. Dehydration can elevate RBC counts (Mitruka and Rawnsley 1981, Hawley and Peden 1982, Foreyt et al. 1983, Brown 1993). In the present study, cotton rats were placed in the shade before blood collection, but some animals may have been dehydrated during collections in summer. If animals were

dehydrated, RBC counts might have been elevated and possibly disguised summer lows of circulating red blood cells.

In my study, treatment differences in hematocrit levels were expected because of differences in RBC counts, but probably were not affected by supplemental methionine. Because MCHC did not differ and small amounts of variation were expected (White and Cook 1974), this contention is supported. Mean hematocrit levels were in general agreement with the literature. Dotson et al. (1987) observed a mean hematocrit level of 39.9% for cotton rats in the laboratory, but Tanaka and Shibuya (1989) and Katahira and Ohwada (1993) reported means of 47.5 and 43.5%, respectively, for male cotton rats in the laboratory. Hankins (1951) reported hematocrit levels of 24-48% for wild cotton rats in Kansas and Oklahoma. In Oklahoma, Hepworth (1966) observed a mean hematocrit of 42.5% for male cotton rats, and Robel et al. (1996) reported a mean of 41.9%. Lochmiller et al. (1994) reported a mean hematocrit level of 40.1% for wild male cotton rats in north-central Oklahoma. Mean hematocrit levels in my study (38.8, 37.8, and 38.6% for the control, low-, and high-methionine treatments, respectively) were slightly lower than values reported by Hepworth (1966), Robel et al. (1996), and Lochmiller et al. (1994) but were within the range of Hankins (1951). High hematocrit levels have been reported for bears (Franzmann and Schwartz 1988), white-tailed deer (Seal et al. 1972, Smith and Rongstad 1980), pronghorns (Seal and Hoskinson 1978), mule deer (Rosen and Bischoff 1952, Anderson et al. 1970), bighorn sheep (*Ovis canadensis*; Franzmann 1972), moose (*Alces alces*; Franzmann and LeResche 1978),

caribou (McEwan 1968, Bjarghov et al. 1976), and black-tailed jackrabbits (*Lepus californicus*; Henke and Demarais 1990) in good nutritional condition. Davis et al. (1995) reported lower hematocrit levels in weanling cotton rats with severe protein restriction, and Vestey et al. (1993) observed reduced hematocrit percentages in cotton rats fed a 4% crude protein diet. Therefore, dietary methionine probably did not affect hematocrit levels in this study.

Previous studies have observed higher percentages of hematocrit in winter for humans (Watanabe 1958), white-tailed deer (Bahnak et al. 1979, DeLiberto et al. 1989), caribou (Hawley and Peden 1982), collared peccaries (Lochmiller et al. 1985a), gray wolves (Seal and Mech 1983), and cottontail rabbits (Lepitzki and Woolf 1991) than during other seasons. Sealander (1962) also reported higher hematocrits in winter for deer mice (*Peromyscus* sp.), harvest mice (*Reithrodontomys fulvescens*), and cotton rats, presumably in response to metabolic acclimation to seasonal temperature changes, but temperature was not correlated with hematocrit levels in my study. In Arkansas, percentages of hematocrit in cotton rats were lowest in December (Bickerstaff 1967). Kitts et al. (1956) also observed that hematocrit levels decreased at low ambient temperatures. Sealander (1964) reported hematocrit values of 45.9, 45.5, 46.7, and 47.6% in winter, spring, summer, and autumn, respectively. Hematocrit values also were highest in summer for *Clethrionomys glareolus* (Newson 1962) and lowest in winter for moose (Franzmann and LeResche 1978). Lochmiller et al. (1994) observed peak hematocrit levels in summer and autumn for male cotton rats in north-central Oklahoma. Although not significant,

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the trend of lower values in spring and summer and higher values in autumn and winter in my study were similar to the former studies.

Supplemental methionine did not influence MCV in my study, presumably because dietary methionine was not limiting. Lower nutritional condition (Anderson et al. 1970, Davis et al. 1995) can decrease MCV values. Davis et al. (1995) reported decreased MCV values with protein restriction in weanling cotton rats, which supports this contention. In my study, mean MCV values of 67.0-67.5 fl were slightly below values in the literature, but variation should be expected (White and Cook 1974). Dotson et al. (1987) and Katahira and Ohwada (1993) reported mean MCV values of 69.5 and 70.0 fl, respectively, for cotton rats in the laboratory, and Robel et al. (1996) reported a mean of 71.3 fl for wild cotton rats in Oklahoma.

White-tailed deer (Seal et al. 1978a) and caribou (Hawley and Peden 1982) exhibit increased RBC size during winter, but Lochmiller et al. (1985a) observed lowest MCV values in winter for collared peccaries. Seasonal variation did not occur in my study, which agrees with Seal and Mech (1983; gray wolves) and Sealander (1962; deer mice, harvest mice, and cotton rats).

The small differences in hemoglobin concentrations (≤ 0.4 g/dl) between treatments probably were caused by the small increases in RBC counts because treatments did not differ in the average weight of hemoglobin in the erythrocyte (MCH). Average concentration of hemoglobin in erythrocytes (MCHC) also did not differ between treatments, which further supports this contention, and small amounts of variation are to be expected (White and Cook 1974). Hemoglobin

concentrations in my study are similar to reports from the literature. In the laboratory, hemoglobin levels range from 13.0 to 17.1 g/dl in cotton rats (Dolyak and Leone 1953, Dotson et al. 1987, Tanaka and Shibuya 1989, Katahira and Ohwada 1993). In Arkansas, hemoglobin levels of cotton rats range from 11.1 to 15.8 g/dl (Sealander 1962, 1964). Hankins (1951) reported hemoglobin concentrations of 11.0-17.0 g/dl for cotton rats in Kansas and Oklahoma. Hepworth (1966) and Robel et al. (1996) observed mean hemoglobin concentrations of 13.3 and 13.4 g/dl, respectively, for wild cotton rats in Oklahoma. Low nutritional condition in humans (Gershwin et al. 1985), caribou (McEwan 1968), moose (Franzmann and LeResche 1978), white-tailed deer (Seal et al. 1972), pronghorns (Seal and Hoskinson 1978), wolves (Seal et al. 1975). coyotes (*Canis latrans*; Smith and Rongstad 1980), bears (Franzmann and Schwartz 1988, Hellgren et al. 1993), wild rabbits (Dudzinski et al. 1962), and cotton rats (Davis et al. 1995) can cause lower hemoglobin concentrations. Thus, methionine probably did not affect hemoglobin production in my study.

In winter, highest hemoglobin concentrations occur in gray wolves (Seal and Mech 1983), caribou (Hawley and Peden 1982), collared peccaries (Lochmiller et al. 1985a), and voles (*Microtus agrestis*; Newson 1962, Newson and Chitty 1962). Sealander (1962) also observed this phenomenon in deer mice, harvest mice, and cotton rats, presumably in response to metabolic acclimation to seasonal temperature changes; however, in moose (Franzmann and LeResche 1978) and pregnant caribou (Timisjarvi 1981), hemoglobin levels are lowest in early winter. Sealander (1964) reported hemoglobin concentrations

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of 15.2, 14.9, 13.9, and 15.3 g/dl in winter, spring, summer, and autumn, respectively, in populations of cotton rats from various parts of the United States and Canada. Hemoglobin concentrations in cotton rats from northwestern Arkansas were 14.0, 12.8, 12.0, and 12.7 g/dl in March, June, October, and December, respectively (Bickerstaff 1967), but Anderson et al. (1970) observed no seasonal effect on hemoglobin concentrations in mule deer, which agrees with my findings.

Bickerstaff (1967) reported MCH values of 16.5-24.0 pg for cotton rats in Arkansas. Dotson et al. (1987) and Katahira and Ohwada (1993) reported means of 22.7 and 27.6 pg, respectively, for cotton rats in the laboratory. Mean MCH values for wild cotton rats in Oklahoma were 21.1 pg (Robel et al. 1996), which was slightly below means for the present study (22.0-22.2 pg). Davis et al (1995) observed no difference in MCH values in weanling cotton rats under any level of protein restriction, which suggests that dietary methionine did not affect how hemoglobin was packaged in erythrocytes in my study. Lochmiller et al. (1985a) reported lowest MCH values in winter for collared peccaries, but caribou exhibit higher MCH values in winter (Hawley and Peden 1982). However, no seasonal effects occurred in my study.

Dotson et al. (1987) and Katahira and Ohwada (1993) reported mean MCHC values of 32.6 and 39.4%, respectively, for cotton rats in the laboratory. Mean MCHC values in the current study (32.8-33.1%) were slightly above means for cotton rats in Arkansas (31.9%; Sealander 1964) and north-central Oklahoma (31.9%; Robel et al. 1996). Weanling cotton rats exhibit no difference in MCHC

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values under protein restriction (Davis et al. 1995), which supports the contention that dietary methionine did not affect the packaging of hemoglobin into erythrocytes in my study. In gray wolves (Seal and Mech 1983), caribou (Hawley and Peden 1982), and cotton rats (Sealander 1964), MCHC values are highest in winter. Sealander (1962) reported similar trends for deer mice, harvest mice, and cotton rats, but no seasonal differences occurred in my study.

Mean platelet count for the control (383.4 x $10^3/\mu l$) was considerably lower than the low- and high-methionine treatments (528.4 and 505.2 x $10^3/\mu l$, respectively). Thus, methionine appears to affect platelet production; however, the low-methionine diet may not have been restrictive enough to result in different platelet counts than the high-methionine treatment. Davis et al. (1995) also reported increased platelet counts with increased protein quality and quantity in weanling cotton rats. Dotson et al. (1987) and Katahira and Ohwada (1993) reported means of 537 and 551 x $10^3/\mu l$, respectively, for platelet counts of cotton rats in the laboratory. In my study, mean platelet counts in the low- and high-methionine treatments are very similar to levels reported by Robel et al. (mean = 537 x $10^3/\mu l$; 1996) for wild cotton rats in north-central Oklahoma.

Total WBC counts of cotton rats are known to exhibit much variation (Katahira and Ohwada 1993, Lochmiller et al. 1994). Means of 7.8 (Dotson et al. 1987), 4.8 (Tanaka and Shibuya 1989), and 7.2 x $10^3/\mu l$ (Katahira and Ohwada 1993) have been reported for total WBC counts of cotton rats in the laboratory. Total WBC counts for cotton rats range from 4.7 to 7.4 x $10^3/\mu l$ in Arkansas (Bickerstaff 1967) and from 4.0 to 11.7 x $10^3/\mu l$ in Kansas and Oklahoma

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(Hankins 1951). Hepworth (1966) reported a mean of $6.9 \times 10^3/\mu l$ for cotton rats in Oklahoma. Robel et al. (1996) reported a mean of $10.2 \times 10^3/\mu l$ (range = 1.8-15.5 $\times 10^3/\mu l$) for cotton rats in north-central Oklahoma. In my study, the mean leukocyte count for the control ($11.9 \times 10^3/\mu l$) was similar to counts in the literature, but counts were higher in supplemented treatments (14.0 and 16.2 $\times 10^3 \mu l$ for the low- and high-methionine, respectively). Thus, supplemental methionine appears to elevate total leukocyte counts, but no difference in relative percentages of differential leukocytes occurred. Therefore, the increase in total leukocytes was not due to increases in any 1 type of white blood cell. Although not significant, total leukocyte counts tended to be greater in adult cotton rats fed a 16% crude protein diet than among those fed a 4% diet (Vestey et al. 1993). Seal and Hoskinson (1978) reported increased WBC levels in pronghorns with available high-protein forage, and Davis et al. (1995) observed decreased WBC counts in weanling cotton rats as a result of severe protein restriction. Total leukocyte counts quickly increase due to stress in coyotes (Smith and Rongstad 1980), red foxes (*Vulpes vulpes*; White et al. 1991), wild rabbits (Dudzinski et al. 1962), and white-tailed deer (Bubenik and Brownlee 1987). Although high densities might induce social stress, density did not influence WBC counts in this study. Disease and/or infection also increase WBC counts in caribou (McEwan 1968), gazelles (*Gazella dorcas*; Bush et al. 1981), and white-tailed deer (Bubenik and Brownlee 1987), but no treatment differences occurred in differential leukocyte counts, DTH response, and hemolytic-complement activity.

Therefore, disease and/or infection probably did not elevate WBC counts in my study.

Numbers of circulating white blood cells tend to increase in small mammals with increases in ambient temperature (Bickerstaff 1967). The same phenomenon also occurs in mule deer (Anderson et al. 1970) and caribou (Hawley and Peden 1982), but no temperature effects were observed in my study. Lochmiller et al. (1994) reported lower WBC counts during winter and summer and elevated counts in autumn and spring for cotton rats in north-central Oklahoma; however, Lochmiller et al. (1985a) found no seasonal variation in total WBC counts for collared peccaries. Goldfinger and Goldfinger (1964) also observed constant WBC counts across seasons in *S. araneus* and *C. glareolus*, which agrees with my study.

High-protein nutrition increases percentages of neutrophils in white-tailed deer (Bubenik and Brownlee 1987) and cotton rats (McMurry et al. 1994), but Henke and Demarais (1990) reported increased percentages of neutrophils in black-tailed jackrabbits as a result of food restriction. Supplemental methionine did not influence neutrophil percentages in my study. Bickerstaff (1967) reported percentages of neutrophils of 43.9-56.0 for cotton rats in Arkansas. In Oklahoma, Hankins (1951) observed a range of 5-60% (median = 25%) neutrophils in cotton rats. Mean percentages of neutrophils in the current study (35.1-38.4) were higher than values reported by Hepworth (32.5%; 1966) and Robel et al. (27.5%; 1996) for cotton rats in Oklahoma. Stress can increase percentages of peripheral neutrophils (White et al. 1991), but high densities

(social stressor) in the current study did not influence percentages of neutrophils. Bacterial infections also can increase percentages of neutrophils (Bush et al. 1981), which might have caused higher percentages of neutrophils than in the literature. In my study, percentages of neutrophils did not differ between treatments. and therefore, bacterial infections probably did not cause elevated total WBC counts in supplemented populations. Bickerstaff (1967) reported highest percentages of neutrophils in March for cotton rats in Arkansas; however, no seasonal changes were observed in neutrophil counts in mule deer (Anderson et al. 1970), collared peccaries (Lochmiller et al. 1985a), or cotton rats in my study.

In Arkansas, percentages of eosinophils range from zero to 2.4% (Bickerstaff 1967), which agreed with values reported for Kansas and Oklahoma (0-2%; Hankins 1951). However. Hepworth (1966) and Robel et al. (1996) reported means of 5.9 and 4.9%, respectively, for cotton rats in Oklahoma, which were similar to my study (4.6-5.6%). Low percentages of eosinophils indicate the animal is stressed (Mitruka and Rawnsley 1981) and/or is either uninfected or moderately infected with parasites (Bickerstaff 1967, McEwan 1968, Mitruka and Rawnsley 1981). Density (stress) did not influence percentages of eosinophils of cotton rats in the current study. Unfortunately, endoparasites were not evaluated in my study, but lack of variation of percentages of eosinophils between treatments indicated that parasitic infection did not cause elevated total leukocyte counts. Protein deprivation leads to atrophy of lymphoid tissue, and thus eosinophil counts are decreased (Beisel 1996). Mean eosinophil counts are

higher in cotton rats with available high-protein food (McMurry et al. 1994) and lower in food-restricted black-tailed jackrabbits (Henke and Demarais 1990). Supplemental methionine also increased absolute numbers of eosinophils in my study but did not affect relative percentages of eosinophils. Bickerstaff (1967) observed higher eosinophil counts in June for cotton rats in Arkansas; however, no seasonal variation occurred in collared peccaries (Lochmiller et al. 1985) or cotton rats in my study.

Because percentages of monocytes were not affected by food restriction in black-tailed jackrabbits (Henke and Demarais 1990), it was not completely unexpected that nutritional differences in my study probably did not influence percentages of monocytes. Bickerstaff (1967) reported percentages of monocytes of 3.0-6.3% for cotton rats in Arkansas, and cotton rats in Oklahoma had 0-10% (Hankins 1951). In north-central Oklahoma, Robel et al. (1996) reported <0.1% monocytes in wild cotton rats, but my results (4.1-4.3%) more closely agree with Hepworth (Mean = 4.2%; 1966) who also studied cotton rats in Oklahoma. Increased numbers of monocytes generally occur with microbial and fungal infections or when the animal is wounded (Brown 1993). Because percentages of monocytes did not differ between treatments, microbial infection probably did not elevate total WBC counts. Peak monocyte counts occur in summer for mule deer (Anderson et al. 1970) and cotton rats in Arkansas (Bickerstaff 1967), but no seasonal variation occurred in monocytes for collared peccaries (Lochmiller et al. 1985a) or cotton rats in my study.

Lymphocytes generally increase in circulation in the presence of chronic illness (Bubenik and Brownlee 1987) and viral infections (Brown 1993), but they decrease when the animal is under stress (Jacobson et al. 1978, White et al. 1991). Because treatments did not differ, chronic illness or viral infections probably did not elevate total leukocyte counts. Bickerstaff (1967) observed a range of 36.0-51.0% lymphocytes for cotton rats in Arkansas. Lymphocyte levels in cotton rats range from 32 to 92% in Oklahoma (Hankins 1951). Mean lymphocyte levels in my study (52.0-56.0%) were slightly below means reported by Hepworth (57.4%; 1966) and Robel et al. (57.8%; 1996). Therefore, social stress due to high densities probably did not influence cotton rats in the present study more than populations of cotton rats reported in the literature. Percentages of lymphocytes increase during food restriction (Henke and Demarais 1990, McMurry et al. 1994, Beisel 1996). McMurry et al. (1994) reported reduced lymphocyte numbers in cotton rats on a 16% crude protein diet compared with a 4% diet, but no treatment differences occurred in the present study. Bickerstaff (1967) observed highest lymphocyte percentages in October for cotton rats in Arkansas; however, my results agree with Anderson et al. (1970) and Lochmiller et al. (1985a), who reported no seasonal variation in lymphocyte numbers for mule deer (Anderson et al. 1970) or collared peccaries (Lochmiller et al. 1985a).

Hankins (1951) reported 0-2% basophils for cotton rats in Kansas and Oklahoma; means of <0.1% and 0.1% were reported by Hepworth (1966) and Robel et al. (1996), respectively, in Oklahoma. Mean percentages of basophils in my study (<0.13%) were similar to those findings. Low percentages are

expected because basophils usually are not found in significant numbers, if at all, in peripheral blood (Bickerstaff 1967, Brown 1993). Lochmiller et al. (1985a) observed lower basophil counts in winter for collared peccaries, but no seasonal variation was found in basophil levels for mule deer (Anderson et al. 1970) or cotton rats in my study.

Studies have reported mixed results for DTH responses. Detection of decreased DTH function does not identify cause of impairment, but decreased DTH responses can occur during infectious diseases (Hildemann 1984), especially during viral infections and after viral immunization (Buckley 1986). Because DTH responses did not differ between treatments, infectious diseases probably were not more prevalent in any 1 treatment. Protein malnutrition also can reduce DTH responses (Chandra 1976, Buckley 1986). Glick et al. (1983) reported decreased DTH responses in domestic chickens fed low-protein diets; however, Gershwin et al. (1985) stated that cell-mediated immunity in the protein-deprived host either remains intact or enhanced. Vestey et al. (1993) reported enhanced DTH responses in weanling and subadult cotton rats fed a 4% crude protein diet. but no effect was observed in adult animals. Cotton rats provided a 4% crude protein diet exhibited greater DTH reactions than animals on a 16% diet (McMurry et al. 1994). Chronic, moderately-severe protein insufficiency enhances cell-mediated immunity in laboratory mice (Cooper et al. 1974), but none of those studies assessed essential amino acid content of their diets. Bounous and Kongshavn (1978) reported enhanced DTH responses in laboratory mice (*Mus* sp.) receiving diets deficient in multiple amino acids.

Severe methionine restriction compromises cell-mediated immunity in laboratory mice (Jose and Good 1973). Methionine supplementation also improves DTH responses in domestic chicks (Tsiagbe et al. 1987), but Petro and Bhattacharjee (1981) observed no influence of cellular immune function of laboratory mice fed diets deficient in essential amino acids. In my study, DTH response did not differ between treatments, and therefore, dietary methionine was not low enough in any treatment to influence DTH function. This was not completely unexpected because individual cells involved in cellular immunity may be hyperactive during protein deficiency, but there may be a severe reduction in cell numbers in the whole animal (Gershwin et al. 1985). In addition. even normal animals show considerable variation in their ability to mount a DTH response (Gershwin et al. 1985).

Domestic dogs exhibit peak cell-mediated function during summer (Shifrine et al. 1980). Temperature stressors affect Holstein calves by altering cell-mediated immunity (Kelley et al. 1982), but temperature has no effect on cell mediated immunity in deer mice (Demas and Nelson 1998), bobwhite quail (Dabbert et al. 1997), or cotton rats in my study. Vestey (1991) also reported no seasonal variation in immune function in cotton rats from Oklahoma, which agrees with results from the supplemented populations of cotton rats in my study. Delayed-type hypersensitivity varies temporally in prairie voles in Kansas (Sinclair and Lochmiller 2000) and cotton rats in Oklahoma (Vestey et al. 1993), but no seasonal trend was apparent, which agrees with the control in my study In 1999, autumn delayed-type hypersensitivity response was greater than

summer in the control, but no seasons differed in supplemental treatments. Therefore, supplemental methionine might have stabilized delayed-type hypersensitivity responses in cotton rats over seasons.

Presence of adequate amounts of functional complement is important for normal immune function (Chandra 1976, Gershwin et al. 1985), especially in humoral defense against microbial pathogens (Renshaw et al. 1978, Merino et al. 1994). Dietary protein has a potent effect on development of humoral immunity because antibody molecules consist largely of protein; therefore, a deficiency of protein can affect synthesis and secretion of immunoglobulins (Gershwin et al. 1985). In laboratory mice, chronic, moderately severe protein restriction has a variable effect on humoral immunity, either depressing it or not affecting it (Cooper et al. 1974). Davis et al. (1995) also observed increases in hemolytic-complement activity in weanling cotton rats with increases in dietary protein. McMurry et al. (1994) observed lower humoral immunocompetence in cotton rats maintained on a 4% crude protein diet compared with animals fed a 16% diet. Decreased hemolytic-complement activity was demonstrated in weanling cotton rats fed a 4% crude protein diet, but no effect was observed in subadult or adult animals (Vestey et al. 1993). My results agree with Vestey et al. (1993), but they did not assess dietary amino acid contents of their diets. Isolated deficits of a single essential amino acid can be reflected by functional changes in humoral immunity (Beisel 1982). In particular, sulfur-containing amino acids are essential for humoral immune responses (Gershoff et al. 1968), and deficiencies of methionine and cysteine lead to depressed humoral immunity (Beisel 1982).

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Jose and Good (1973) reported decreased humoral immune responses in laboratory mice provided with insufficient concentrations of sulfur-containing amino acids, and Tsiagbe et al. (1987) observed elevated humoral immune responses in broiler chicks supplemented with methionine. However, dietary methionine did not affect hemolytic-complement activity in the current study.

Hemolytic-complement activity varies throughout the year in prairie voles, but no clear seasonal trend is apparent (Sinclair and Lochmiller 2000). Temperature stressors also alter humoral immune function in Holstein calves (Kelley et al. 1982); however, temperature did not influence humoral immunity of bobwhite quail (Dabbert et al. 1997) or cotton rats in my study. In all treatments, June 1997 was considerably higher than all other dates, but no seasonal trend was observed in cotton rats in the present study. which agrees with (Vestey 1991). Although samples collected in June 1997 were collected and stored in the same manner as in other seasons, these extreme values could be a laboratory artifact.

Treatment differences in RBC numbers, hematocrit levels, and hemoglobin concentrations were small, and supplemental methionine did not affect erythrocyte size or MCH of cotton rats. Platelet counts were very low in the control but were increased by supplemental methionine, which suggested that addition of sulfur-containing amino acids positively influenced platelet production in cotton rats. Although total WBC counts for the control were similar to the literature, supplemental methionine resulted in elevated leukocyte numbers, but the difference was not due to increased percentages of any single

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type of white blood cell. Total leukocyte production also might be affected by sulfur-containing amino acids in cotton rats. Treatment differences did not occur for delayed-type hypersensitivity and hemolytic-complement activity. Although delayed-type hypersensitivity varied seasonally in the control and hemolytic-complement activity varied seasonally in all treatments, no apparent seasonal trend occurred. Therefore, supplemention of sulfur-containing amino acids in outdoor enclosures generally did not affect immune function of cotton rats in north-central Oklahoma.

ACKNOWLEDGMENTS

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Table 1. Density of populations of wild cotton rats with no supplemental food (NS), low-methionine food (LM), and high-methionine food (HM) in north-central Oklahoma, 1997 and 1999.

			NS			LM			HM	
						Treatment				
Year	Month	n [a]	Mean [b]	SE	n	Mean	SE	n	Mean	SE
1997	Jun	4	110.0	20.4	4	112.5	8.5	3	83.3	6.7
	Sep	4	52.5	20.6	4	80.0	7.1	3	76.7	12.0
	Dec	4	27.5	12.5	4	45.0	6.5	3	46.7	13.3
1999	Mar	3	70.0	5.8	4	117.5	6.3	4	115.0	9.6
	Jun	3	56.7	21.9	4	112.5	10.3	4	132.5	22.5
	Sep	3	33.3	3.3	4	67.5	6.3	4	95.0	14.4
	Dec	3	10.0	5.8	4	55.0	5.0	4	70.0	12.9

[a] Number of replicates.

[b] Number of cotton rats per ha.

Table 2. Mean total leukocyte and differential leukocyte counts for populations of wild cotton rats with no supplemental food (NS), low-methionine food (LM), and high-methionine food (HM) in north-central Oklahoma, 1997 and 1999 combined.

| | Treatment | | | | | | | | |
| | NS | | | LM | | | HM | | |
	n [a]	Mean	SE	n	Mean	SE	n	Mean	SE
Neutrophils (%)	40	38.38	2.00	64	37.27	1.34	61	35.13	1.79
Eosinophils (%)	40	5.53	0.42	64	5.64	0.34	61	4.59	0.32
Monocytes (%)	40	4.23	0.33	64	4.11	0.28	61	4.30	0.35
Lymphocytes (%)	40	52.00	2.20	64	53.09	1.45	61	56.02	1.86
Basophils (%)	40	0.13	0.06	64	0.09	0.04	61	0.05	0.04
Total (10^3/mm^3) [b]	40	11.90	0.62	64	14.02	0.82	61	16.22	0.80

[a] Number of samples examined

[b] HM is greater than NS ($P=0.005$).

Figure 1. Mean red blood cell counts (RBC; ± SE) in (A) 1997 and (B) 1999,

hematocrit levels (± SE) in (C) 1997 and (D) 1999, and mean corpuscular

volumes (MCV; ± SE) in (E) 1997 and (F) 1999 of wild cotton rats in north-central

Oklahoma populations with no supplemental diet, supplemental low-methionine

diet, and supplemental high-methionine diet.

Figure 2. Mean hemoglobin concentrations (± SE) in (A) 1997 and (B) 1999,

mean corpuscular hemoglobin values (MCH; ± SE) in (C) 1997 and (D) 1999,

and mean corpuscular hemoglobin concentrations (MCHC; ± SE) in (E) 1997 and

(F) 1999 of wild cotton rats in north-central Oklahoma populations with no

supplemental diet, supplemental low-methionine diet, and supplemental high-

methionine diet. Significant differences (P < 0.05) occurred at: [a] No

Supplementation > Low-methionine Diet and [b] High-methionine Diet > Low-

methionine Diet.

Figure 3. Mean counts of platelets (± SE) in (A) 1997 and (B) 1999 and total

leukocyte (WBC; ± SE) counts in (C) 1997 and (D) 1999 of wild cotton rats in

north-central Oklahoma populations with no supplemental diet, supplemental

low-methionine diet, and supplemental high-methionine diet.

Figure 4. Mean delayed-type hypersensitivity response (± SE) in (A) 1997 and

(B) 1999 and hemolytic-complement activity (± SE) in (C) 1997 and (D) 1999 of

wild cotton rats in north-central Oklahoma populations with no supplemental diet,

supplemental low-methionine diet, and supplemental high-methionine diet.

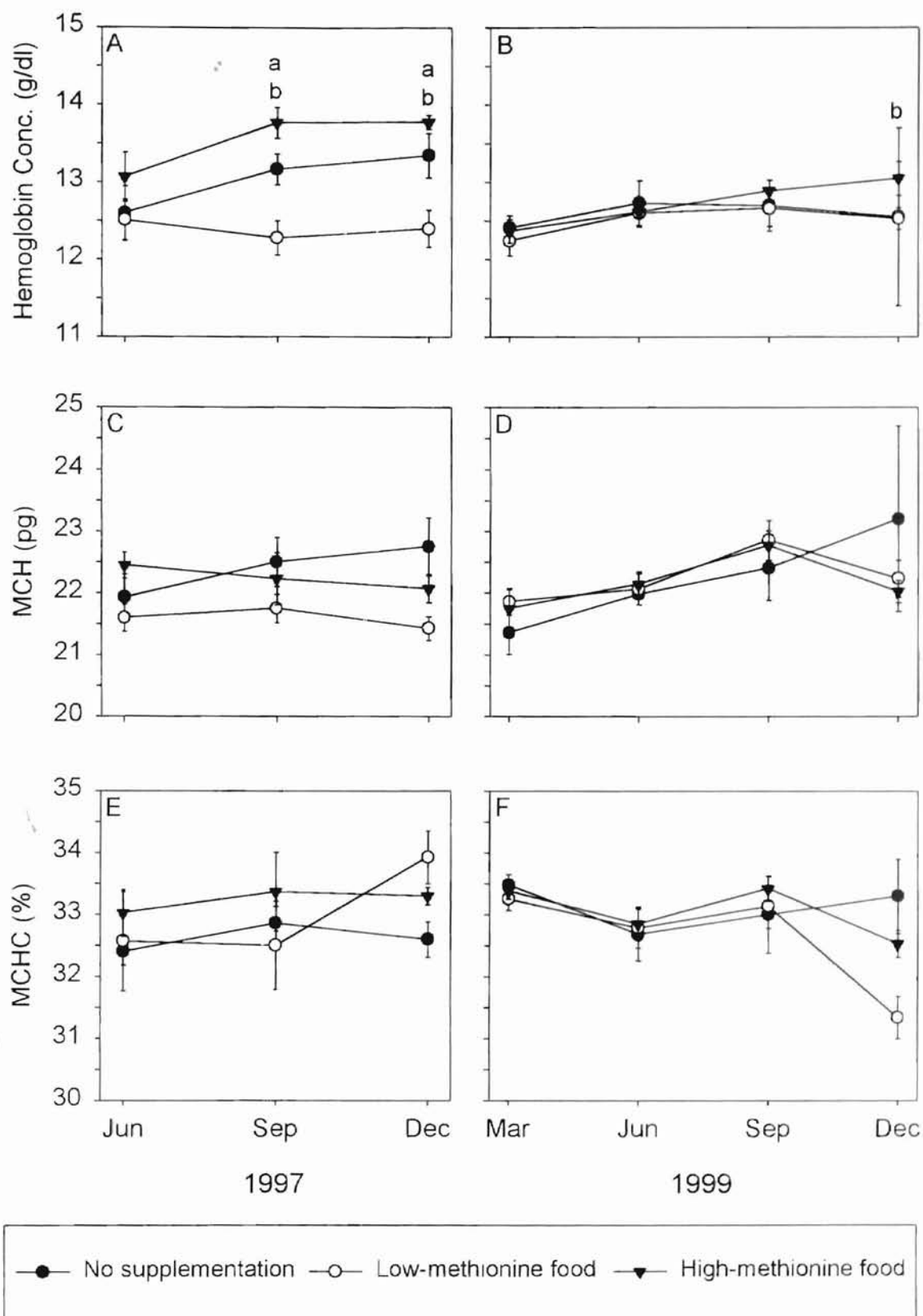Significant differences ($P < 0.05$) occurred at: [a] June 1997 > all other months in

all treatments.

1997

1999
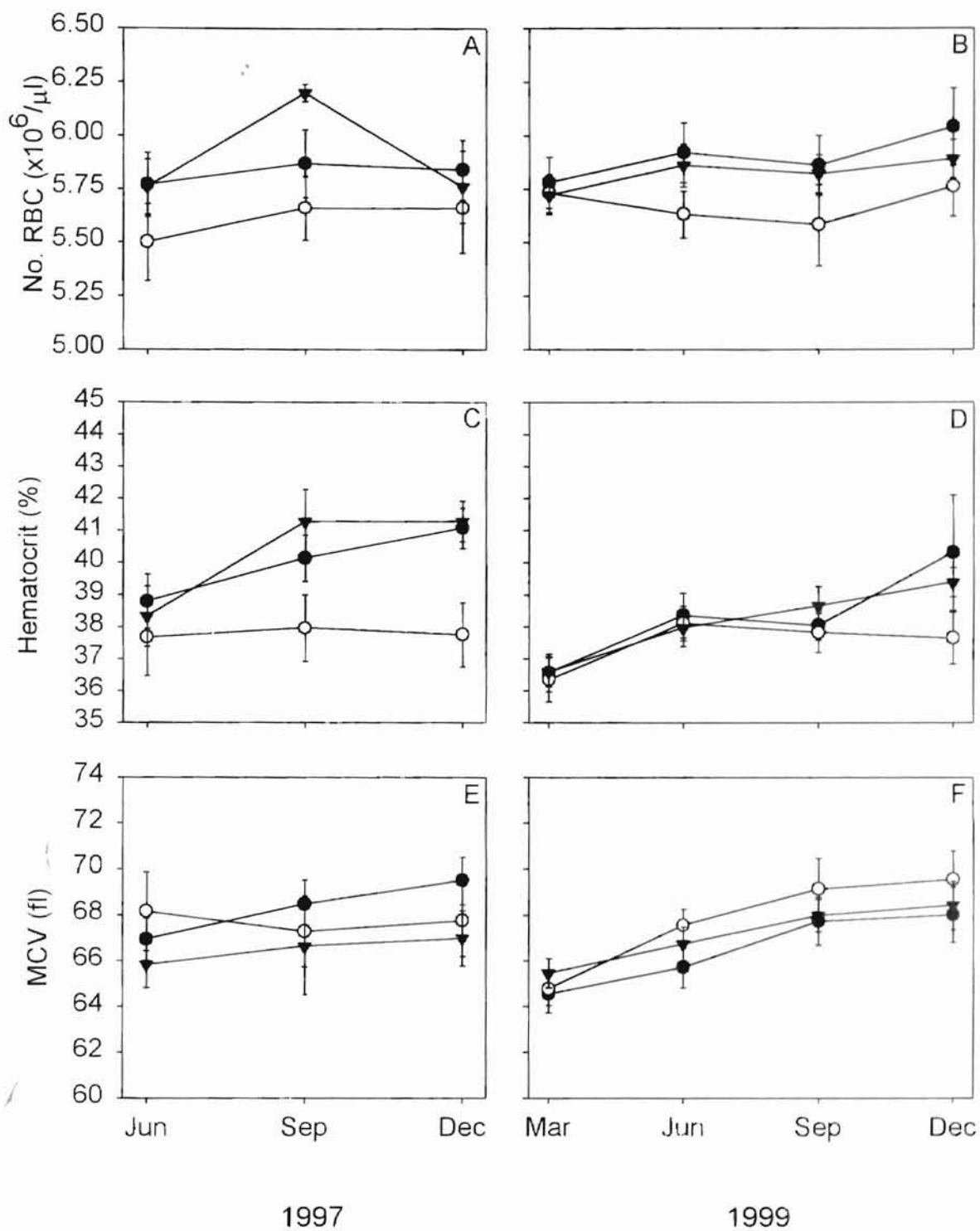
No supplementation —○— Low-methionine food —▼— High-methionine food
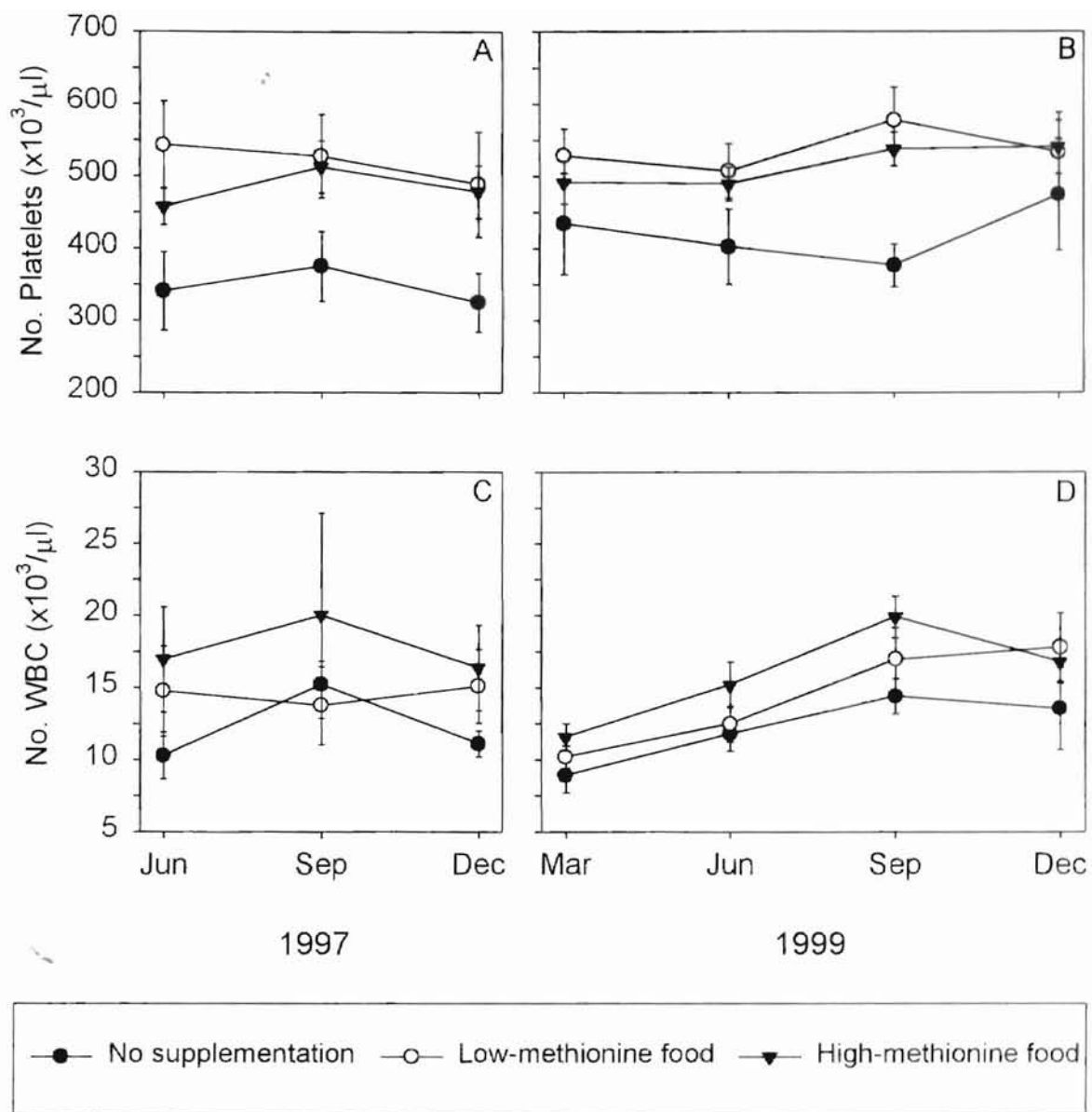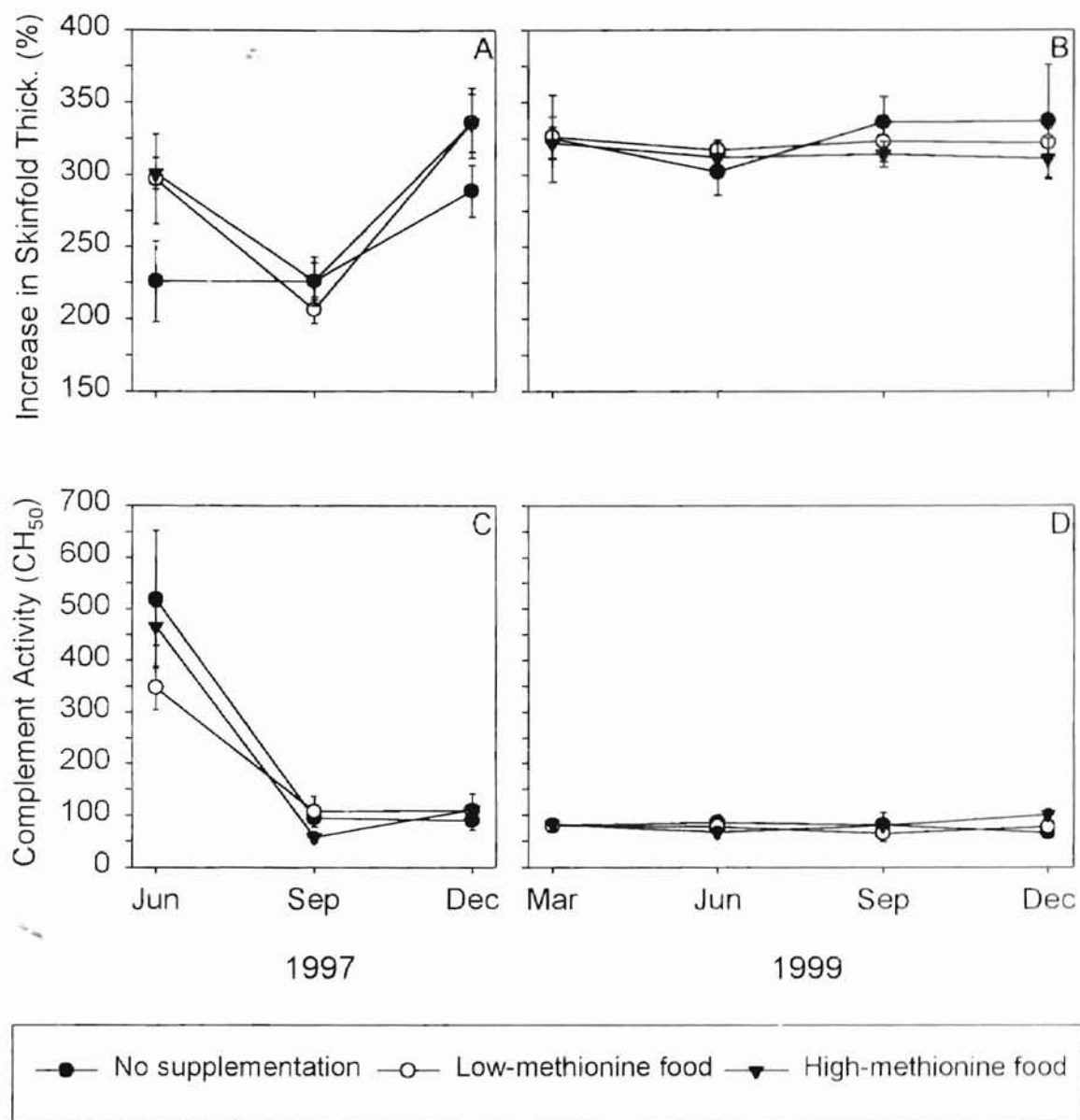
Appendix A. Mean maximum vegetation height (cm), percent cover, and biomass (kg/ha) measured in no

supplementation (NS), low-methionine supplementation (LM), and high-methionine supplementation (HM) treatments in

north-central Oklahoma, 1997 and 1999.

			Treatment					
			NS		LM		HM	
Year	Month	Vegetation parameter	Mean	SE	Mean	SE	Mean	SE
1997 [a]	Jun	Height	44.50	2.26	37.38	1.94	39.33	2.36
		Grass cover	20.69	3.04	18.94	2.65	14.75	2.90
		Forb cover	2.94	0.67	4.63	1.14	4.33	1.01
		Legume cover	0.44	0.15	0.50	0.16	0.50	0.19

Appendix A. Continued.

1997	Jun	Woody plant cover	3.81	1.43	4.31	1.22	1.75	1.25
		Standing litter cover	16.88	2.38	17.50	2.59	14.00	2.42
		Fallen litter cover	72.25	2.99	68.19	3.14	72.42	3.71
		Grass biomass	602.73	58.02	511.93	55.97	371.27	54.01
		Forb biomass	86.63	13.95	103.15	19.82	90.27	13.84
		Legume biomass	7.15	3.45	9.23	3.47	7.13	3.19
		Woody plant biomass	179.40	58.87	178.70	44.06	65.20	35.85
	Sep	Height	43.88	3.21	43.25	1.68	43.83	2.35
		Grass cover	29.38	3.99	23.50	3.27	24.33	4.21
		Forb cover	5.06	1.39	6.56	1.48	8.83	2.62
		Legume cover	0.62	0.38	0.37	0.14	1.66	1.24
		Woody plant cover	9.12	2.79	5.81	1.69	4.16	2.87
		Standing litter cover	8.62	2.57	10.87	2.28	7.75	1.76

Appendix A. Continued.

1997	Sep	Fallen litter cover	38.18	4.17	47.56	3.75	62.33	5.19
		Grass biomass	961.72	94.38	658.55	82.01	953.53	103.79
		Forb biomass	142.75	25.11	128.02	18.10	141.00	26.87
		Legume biomass	8.70	5.09	6.72	2.84	23.50	14.59
		Woody plant biomass	376.22	110.05	247.90	68.28	145.73	76.74
	Dec	Height	35.12	1.89	30.00	1.21	34.00	1.71
		Grass cover	5.12	1.04	3.37	0.87	3.66	0.96
		Forb cover	0.37	0.14	0.43	0.15	0.58	0.19
		Legume cover	0.06	0.06	0.00	0.00	0.00	0.00
		Woody plant cover	2.56	0.84	1.25	0.40	0.91	0.51
		Standing litter cover	31.75	3.23	29.00	3.21	29.16	4.15
		Fallen litter cover	83.31	2.17	77.18	2.73	82.58	2.98
		Grass biomass	391.55	74.34	114.32	25.00	214.66	36.09

Appendix A. Continued.

1997	Dec	Forb biomass	15.52	6.02	14.77	5.17	18.46	6.64
		Legume biomass	0.80	0.80	0.00	0.00	0.00	0.00
		Woody plant biomass	82.92	25.68	50.95	12.31	28.63	12.35
1999 [b]	Mar	Height	33.33	2.51	37.62	2.36	35.12	2.44
		Grass cover	2.33	0.81	2.37	0.69	1.81	0.39
		Forb cover	1.50	0.51	2.06	0.51	2.37	0.60
		Legume cover	0.00	0.00	0.00	0.00	0.00	0.00
		Woody plant cover	2.50	1.39	0.43	0.37	0.43	0.37
		Standing litter cover	29.33	5.10	17.75	3.21	33.62	3.66
		Fallen litter cover	88.66	2.66	89.87	1.94	81.87	3.22
		Grass biomass	6.93	2.29	70.85	21.16	16.90	6.94
		Forb biomass	6.16	3.53	7.95	3.52	10.37	3.52
		Legume biomass	0.00	0.00	0.00	0.00	0.00	0.00

Appendix A. Continued.

1999	Mar	Woody plant biomass	40.96	18.42	11.60	10.43	9.52	7.19
	Jun	Height	37.16	2.07	38.87	1.79	42.25	2.13
		Grass cover	21.66	3.06	12.68	1.91	22.50	3.17
		Forb cover	4.75	1.41	5.00	0.87	3.62	0.78
		Legume cover	1.33	0.51	0.56	0.16	0.62	0.17
		Woody plant cover	4.08	1.03	8.25	2.13	2.56	1.09
		Standing litter cover	16.58	2.15	11.06	1.54	13.31	2.00
		Fallen litter cover	73.75	2.59	75.43	3.12	74.87	2.67
		Grass biomass	443.83	69.51	585.63	80.83	626.78	67.89
		Forb biomass	126.93	19.69	153.22	13.89	104.82	13.46
		Legume biomass	20.00	5.93	9.07	3.18	14.42	4.46
		Woody plant biomass	193.43	35.18	283.95	67.59	106.85	38.01
	Sep	Height	40.16	1.81	41.37	1.66	44.12	2.17

Appendix A. Continued.

1999	Sep	Grass cover	30.00	3.34	14.75	1.91	30.87	3.31
		Forb cover	5.16	1.11	7.37	1.26	4.31	1.16
		Legume cover	0.41	0.17	0.81	0.39	0.81	0.39
		Woody plant cover	6.25	1.62	10.43	2.60	2.62	0.84
		Standing litter cover	6.66	1.09	5.93	0.89	7.43	1.20
		Fallen litter cover	55.75	3.91	56.18	4.32	63.37	3.96
		Grass biomass	1010.00	308.55	912.40	67.86	879.83	68.43
		Forb biomass	126.40	20.78	170.12	17.68	109.67	18.87
		Legume biomass	4.20	1.81	11.80	6.06	7.65	4.07
		Woody plant biomass	190.23	45.17	350.90	82.26	90.95	25.54
	Dec	Height	32.83	1.49	32.87	1.38	34.62	1.81
		Grass cover	5.75	1.13	2.43	0.69	5.75	0.98
		Forb cover	0.83	0.21	0.43	0.15	0.62	0.17

Appendix A. Continued

1999	Dec	Legume cover	0.08	0.08	0.06	0.06	0.12	0.08
		Woody plant cover	2.08	0.67	2.43	0.77	0.43	0.15
		Standing litter cover	27.41	2.92	24.18	2.58	29.50	2.61
		Fallen litter cover	80.58	2.91	82.81	2.35	78.75	2.42
		Grass biomass	131.30	25.72	374.18	63.76	176.25	26.02
		Forb biomass	19.26	5.45	12.57	4.67	17.35	5.07
		Legume biomass	0.36	0.36	0.20	0.20	0.62	0.43
		Woody plant biomass	58.70	15.50	59.05	16.24	23.10	8.84

[a] $n=40$ for NS and LM, $n=30$ for HM.

[b] $n=40$ for LM and HM, $n=30$ for NS.

VITA

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