ESSENTIAL AMINO ACID NUTRITIONAL ECOLOGY OF COTTONTAIL RABBITS (*Sylvilagus floridanus*)

By

DAVID G. PEITZ

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Iowa State University

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Thesis Approved:

Thesis Adviser

D. M. Leslie Jr.

David M. Engle

Thomas C. Collins

Dean of the Graduate College

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CHAPTER I

INTRODUCTION

This thesis is composed of 4 manuscripts formatted for submission to selected scientific journals. Each manuscript is complete as written and requires no additional material for support. Manuscripts are arranged in the order of text, literature cited, tables, and figures. Chapter II, "Assessing quality of dietary protein through analysis of stomach digesta", is formatted for submission to the Journal of Mammology. Chapter III, "Temporal changes and limitations in essential amino acid nutrition of cottontail rabbit (*Sylvilagus floridanus*) populations", is formatted for submission to the journal Ecology. Chapter IV, "Deficiencies in essential amino acids of forages in the diet of cottontail rabbits (*Sylvilagus floridanus*)", is formatted for submission to the Journal of Mammalogy. Chapter V, "Influence of spatial and temporal changes in habitat quality on condition of cottontail rabbits (*Sylvilagus floridanus*)", is formatted for submission to the Canadian Journal of Zoology.

CHAPTER II

ASSESSING QUALITY OF DIETARY PROTEIN THROUGH ANALYSIS OF STOMACH DIGESTA

ABSTRACT--We investigated the feasibility of using essential amino acid (EAA) analyses of stomach digesta from cotton rats (Sigmodon hispidus) to assess the nutritional quality of proteins in their diet. Post-prandial changes (0 - 3 h) in the EAA composition of stomach digesta from animals fed either a high (rodent chow) or low (rabbit chow) quality diet were recorded. Concentrations of EAA in stomach digesta reflected corresponding concentrations in the diet; tyrosine and methionine (plus cystine) were the only EAA that differed significantly with post-prandial time interval. Accuracy of this technique diminished when dry weight of stomach digesta was <0.1 g.

INTRODUCTION

The nutritional quality of diets consumed by free-ranging small mammals is frequently determined by analysis of forages known to occur in the diet (Choo et al., 1981; Randolph et al., 1991). However, technical difficulties often arise because of selective foraging by animals, misidentification of forages consumed, temporal changes in the quality of forages analyzed, and the substantial botanical diversity of many diets. One proposed solution to these difficulties is to indirectly assess the nutritional quality of diets by analyzing the chemical composition of digesta (Goering and Van Soest, 1970; Millar et al., 1990; Servello et al., 1983). In particular, considerable interest has been shown in using stomach digesta of small mammals for indexing digestibility, energy content, or concentrations of fiber, lignin, and protein in diets (MacPherson et al., 1985, 1988; Millar et al., 1990; Servello et al., 1983, 1984; Schreiber 1979).

Quality of protein in the diet is largely dependent on the concentrations of essential amino acids (EAA) relative to an animal's requirement. Crude protein is often a poor measure of the quality of dietary proteins due to deficiencies in one or more EAA and a prominent non-protein nitrogen component in many forages (Holt and Sosulski, 1981; Sedinger, 1984). As a result, we examined the feasibility of using stomach digesta to assess the protein quality or EAA composition of diets consumed by cotton rats (Sigmodon hispidus).

MATERIALS AND METHODS

Wild-caught cotton rats were returned to the laboratory and acclimated to either a commercial rabbit chow (17% protein, A and M Feeds, Stillwater Milling Company, Stillwater, OK) or laboratory rodent chow (23% protein, Purina Formulab Chow 5008, Purina, St. Louis, MO) diet for

1-week prior to initiating experimental feeding trials. All animals were fasted 8 h prior to offering a meal of their respective experimental diet for a 1-h feeding episode. The 8-h fast was utilized in an attempt to normalize the amount of stomach-fill and endogenous nitrogen present (Armstrong et al., 1978). After the 1-hr meal, food was removed from the cage and four animals per diet were terminated (cervical dislocation under ketamine hydrochloride anesthesia) at each of four post-prandial time intervals (0, 0.5, 1.5, and 3.0 h). Stomachs were excised and digesta removed. Hair fibers and parasites were removed from digesta before obtaining a wet weight to the nearest 0.1 mg. Stomach digesta and representative samples of each diet were lyophilized to dryness, weighed and ground to a fine powder with mortar and pestle.

Lipids were extracted from ground samples of digesta and diets using a soxhlet apparatus and diethyl ether solvent (Williams, 1984). Fat-extracted stomach digesta and diets amounting to approximately 40 mg of protein were weighed into 25 X 150-mm glass tubes with teflon caps and hydrolyzed in 15 ml 6N HCL at 110 C for 24 hrs. One ml of the hydrolyzed sample was filtered through a 0.45-um syringe filter (Acrodisc CRPTF, Fisher Scientific, Plano, TX). An internal standard (25 ul 4.0 nm methionine sulfone in 0.1 N HCL) was added to 75 ul of the filtered hydrolosate before derivatization. Pre-column derivatization of amino acids was accomplished using phenylisothiocynate to produce phenylthiocarbamyl amino acids (Pico-Tag Workstation, Millipore Corporation, Milford, MA) and re-filtered through a 0.45-um syringe filter. Concentrations of 10 essential (histidine, arginine, threonine, tyrosine, valine, methionine+cystine, isoleucine, leucine, phenylalanine, and lysine) and six nonessential (aspartate, glutamate, serine, glycine, alanine, and proline) amino acids were determined in derivatized samples using high pressure liquid chromatography (HPLC, Waters Model 820 System Controller and Model 501 Pumps, Millipore Corporation, Milford, MA). Tryptophan was destroyed by acid hydrolysis and therefore not measured. Chromatographic conditions were the following: Waters Pico-Tag Silica/C18 (15 cm X 3.9 mm) column; column temperature of 38 C; flow rate of 1.0 ml/min with pump back pressures of 5500 PSI; system sensitivity of 489 mv/sec (recorder) and 0.5 absorbance units full scale (Waters Model 484 UV Detector set at 254 nm); injection volume of 4 ul; and run time of 27.5 min. Solvents used were Waters Eluent A and Eluent B (catalog no. 88108 and 88112, respectively) under conditions and gradients described for separation of amino acids by Cohen et al. (1988). A casein reference protein (from bovine milk, no. C-0376, Sigma Chemical Co., St. Louis, MO) of known amino acid composition was hydrolyzed and analyzed with samples for comparison of amino acid recovery. Amino acid concentrations were recorded as a relative percentage of the total amino acid pool, on an air-dry weight basis (mg/g dry weight), and as a percent recovered from stomach digesta when compared to their respective concentrations in the diet.

For each experimental ration, post-prandial changes (0, 0.5, 1.5, 3.0 h) in the 10 essential and six nonessential amino acids in stomach digesta were examined separately by one-way analysis of variance (ANOVA). The use of EAA concentrations in stomach digesta to determine differences in concentrations in the diet was examined by two-way ANOVA and multivariate analysis of variance (MANOVA) with diet (rabbit chow, rodent chow) and post-prandial time interval as main factor effects. The Statistical Analysis System (SAS Institute, Inc., 1982) was used for all data anlayses.

RESULTS AND DISCUSSION

Concentrations (mg/g dry weight) of amino acids were approximately 35% greater in rodent chow than rabbit chow; relative concentrations (percent of the total amino acid pool) were similar for the two diets (Table 1). Overall recoveries of digesta for both diets decreased from a mean of 4.6722 g to 0.9448 g over the 3-h time interval due to passage of digesta through the gut. All but one (at the 3-h time interval) animal fed the rabbit chow diet yielded >0.1 g dry weight of stomach digesta. Passage rates were faster for the more soluble rodent chow diet, resulting in the recovery of only small amounts of digesta by the 3-h sample. Samples yielding <0.1 g dry weight of digesta from the stomach appeared to have elevated concentrations of several amino acids, so samples of stomach digesta <0.1 g dry weight were statistically compared (one-way ANOVA) to samples weighing >0.1 g to evaluate the influence of stomach-fill on concentrations of amino acids.

Cotton rats with <0.1 g dry weight of stomach digesta were eliminated from further statistical analysis due to significantly elevated concentrations of the amino acids threonine (P = 0.048), lysine (P = 0.008), glycine (P = 0.046), and serine (P = 0.024) compared to those containing >0.1 g dry weight. Percent recoveries (concentration in stomach relative to diet) of these four amino acids averaged 164% in the <0.1 g group compared with 110% for samples with >0.1 g dry weight of digesta. Dilution of the diet by additions of endogenous sources of amino acids (salivary and gastric secretions) and passage of soluble components of the diet from the stomach undoubtedly accounted for the elevated concentrations of these four amino acids (Zebrowska et al., 1983). Gastric and duodenal secretions in other species have been shown to be relatively high in concentrations of threonine, serine, and glycine (Low, 1979; Snary and Allen, 1971).

In spite of these endogenous additions of some amino acids, the composition of stomach digesta for each diet after removal of animals yielding <0.1 g dry weight (most of these animals were in the 3-h interval) was similar to that of the diets (Table 2). Similar observations have been documented by Low (1979) and Zebrowska et al. (1983) for domestic monogastric animals. Tyrosine, proline, and methionine+cystine in rabbit chow-fed animals were the only amino acids whose concentrations differed significantly ($\underline{P} < 0.05$) among post-prandial time intervals; temporal changes were not significant ($\underline{P} > 0.05$) for those fed rodent chow. Percent recoveries of proline and methionine+cystine were lower at the 3-h interval and tyrosine recoveries were higher at the 1.5-h interval compared to other time intervals. With the exception of the three amino acids above, overall percent recovery of individual amino acids varied from a low of 82% for histidine to a high of 119% for threonine among those fed rodent chow and a low of 84% for arginine and a high of 114% for serine and alanine among those fed rabbit chow.

A partial explanation for the high recoveries of tyrosine and methionine+cystine is that they occurred at low concentrations in the diet but at relatively high concentrations in proteolytic enzymes such as pepsinogen (Arai et al., 1984). Overall mean recoveries of dietary amino acids from stomach digesta for our feeding trials were 102.5% and 101.3% for rodent and rabbit chow diets, respectively (Table 2), suggesting that stomach digesta recovered between 0 and 3.0 h reflect dietary EAA intake.

Usefulness of stomach digesta for evaluating the EAA composition of diets consumed by cotton rats was evident with comparisons of overall concentrations (mg/g dry weight) between a high quality (rodent chow) and low quality (rabbit chow) diet (Table 3). Multivariate analysis of variance indicated that overall EAA composition of stomach digesta was strongly influenced ($\underline{P} = 0.006$) by quality of dietary protein. Mean concentrations of all EAA were highest in digesta samples from those fed the high quality protein source. Histidine was the only EAA that was not found at significantly greater ($\underline{P} > 0.05$) concentrations in the high than low quality diet. Utility of this technique in the field will be limited by residence time of digesta in the stomach, which is dependent upon rate of passage through the gut. However, from our results it seems reasonable to conclude that concentrations of EAA and protein quality in the diet of cotton rats can be measured by using only those animals containing stomach digesta amounting to >0.1 g dry weight (or full stomachs). Animals with considerably less stomach-fill will not provide accurate measures of EAA concentrations in the diet, as endogenous secretions form a greater proportion of the amino acid pool. Phenolics and other protein-binding constituents in plants should not interfere with this technique.

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Table 1.--<u>Concentrations of nonessential and essential amino acids in commercially available rabbit and rodent chow diets used in experimental feeding trials.</u>

Amino acid	Rabbit chow mg/g dry weight	 Relative % of amino acid pool	Rodent chow mg/g dry weight	 Relative % of amino acid pool
Nonessential				
Aspartate (Asp)	20.1	9.7	28.2	10.2
Glutamate (Glu)	35.5	15.6	45.8	15.0
Serine (Ser)	10.4	6.4	13.7	6.3
Glycine (Gly)	9.9	8.5	13.9	8.9
Alanine (Ala)	10.4	7.5	14.7	8.0
Proline (Pro)	14.0	7.9	16.1	6.7
Essential				
Histidine (His)	5.7	2.4	7.5	2.3
Arginine (Arg)	14.9	5.5	19.7	5.4
Threonine (Thr)	8.4	4.6	11.1	4.5
Tyrosine (Tyr)	6.6	2.3	8.2	2.2
Valine (Val)	11.6	6.4	14.5	5.9
Methionine+cystine (Met+Cys)	2.3	1.0	4.6	1.5
Isoleucine (Ile)	9.7	4.8	12.6	4.6
Leucine (Leu)	15.9	7.8	22.4	8.2
Phenylalanine (Phe)	10.4	4.1	13.5	3.9
Lysine (Lys)	12.0	5.3	18.2	6.0

Table 2.--Mean percent recovery (compared to diet composition) of amino acids in stomach digesta of cotton rats (Sigmodon hispidus) at four post-prandial time intervals following consumption of either rabbit (A) or rodent (B) chow diets.

	Post-prandial time intervals (h)									
	0		0.5		1.5		3.0		Overall	
Amino acids[c]	A (n=4)	B (n=3)	A (n=4)	B (n=2)	A (n=4)	B (n=4)	A (n=3)	B (n=1)	A X ± SE	B X ± SE
Nonessential										
Asp	86.8	96.5	91.4	104.3	97.9	90.7	85.7	70.3	90.8 ± 2.8	93.1 ± 5.4
Glu	92.0	92.4	92.7	96.1	92.7	86.2	69.5	56.0	87.9 ± 4.1	87.0 ± 6.3
Ser	108.3	115.6	116.6	135.1	126.9	112.6	100.7	91.8	114.0 ± 3.9	116.0 ± 6.7
Gly	98.2	108.7	107.3	119.7	116.9	102.6	107.0	84.8	107.4 ± 3.2	106.0 ± 5.9
Ala	112.1	124.6	121.0	117.8	120.0	104.6	101.2	99.2	114.4 ± 3.8	112.7 ± 4.9
Pro	94.9[a]	101.1	96.3[a]	96.2	94.8[a]	90.8	66.5[b]	60.4	89.6 ± 4.3	91.9 ± 6.2
Essential										
His	93.1	95.3	93.0	89.5	81.7	76.9	73.2	50.3	86.1 ± 3.7	82.3 ± 6.8
Arg	85.5	97.8	87.0	94.0	89.4	86.0	73.1	48.8	84.4 ± 3.4	87.4 ± 7.0
Thr	103.9	119.9	111.1	136.7	121.9	112.4	106.7	109.7	111.2 ± 3.4	119.2 ± 6.3
Tyr	107.0[a]	136.4	114.4[a]	162.4	147.4[b]	154.7	102.9[a]	94.5	118.9 ± 5.5	144.7 ± 12.9
Val	88.4	103.7	88.4	105.0	86.3	88.2	93.1	89.9	88.8 ± 2.7	96.4 ± 5.0
Ile	86.7	100.9	85.8	103.5	84.1	85.9	94.5	84.9	87.3 ± 3.0	93.8 ± 5.4

Table 2.--(cont., pg 2).

	Post-prandial time intervals (h)									
	0		0.5		1.5		3.0		Overall	
Amino	A	B	A	B	A	B	A	B	A	B
acids[c]	(*n*=4)	(*n*=3)	(*n*=4)	(*n*=2)	(*n*=4)	(*n*=4)	(*n*=3)	(*n*=1)	X ± *SE*	X ± *SE*
Met +cys	139.9[a]	126.8	171.8[a]	138.1	159.3[a]	114.5	92.5[b]	77.0	144.1 ± 9.7	119.1 ± 7.7
Leu	101.7	105.2	105.7	102.9	99.0	84.0	90.7	70.8	99.9 ± 3.1	92.8 ± 5.7
Phe	95.5	106.2	101.9	106.1	102.3	94.4	88.6	72.2	97.7 ± 3.5	98.0 ± 6.0
Lys	84.4	99.5	92.8	118.2	114.9	96.4	105.8	76.7	99.0 ± 4.6	99.7 ± 5.6
Overall means	98.7	108.2	104.8	114.1	108.5	98.8	90.7	77.3	101.3 ± 4.0	102.5 ± 6.5

[a,b] Values in a row with different superscripts are significantly ($P < 0.05$) different between time intervals.

[c] Abbreviations defined in Table 1.

Table 3.--Comparison of overall mean concentrations of essential amino acids in stomach digesta of cotton rats (Sigmodon hispidus) fed either rodent or rabbit chow diets.

	Concentration (mg/g dry weight)			
Amino acids[a]	Rabbit chow (n=15)	Rodent chow (n=10)	Correct rank[b]	P > F[c]
His	4.9	6.1	Yes	0.0529
Arg	12.6	17.2	Yes	0.0124
Thr	9.4	13.2	Yes	0.0001
Tyr	7.8	11.9	Yes	0.0024
Val	10.3	13.9	Yes	0.0003
Met+cys	3.3	5.5	Yes	0.0001
Ile	8.5	11.8	Yes	0.0003
Leu	15.9	20.8	Yes	0.0013
Phe	10.2	13.2	Yes	0.0071
Lys	11.9	18.1	Yes	0.0001

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[a] Abbreviations defined in Table 1.

[b] Correct rank denotes whether analysis of stomach digesta correctly identified the higher quality diet for that particular essential amino acid.

[c] Differences between diets tested by ANOVA.

CHAPTER III

TEMPORAL CHANGES AND LIMITATIONS IN ESSENTIAL AMINO ACID NUTRITION OF COTTONTAIL RABBIT (*Sylvilagus floridanus*) POPULATIONS

ABSTRACT. Protein quantity and quality of diets may be the most important factors influencing the dynamics of herbivore populations. Previous studies have indicated that cottontail rabbit (*Sylvilagus floridanus*) populations respond favorably to habitat disturbance. We explored the hypothesis that vegetation in upland hardwood forest-tallgrass prairie habitat 4-5 yr post-disturbance provides higher quality protein resources, as measured by the availability of essential amino acids, than vegetation 7-9 yr post-disturbance. Seasonal concentrations of all essential amino acids as well as gross nutrient categories (crude protein, fat, and non-protein nitrogen) were determined for stomach digesta of cottontail rabbits collected over a 6-year period from areas in various stages of herbicide-induced secondary succession. Composition of diets were not influenced by disturbance intensity, but they were influenced by time since disturbance and season. Habitats 4-5 yr post-disturbance provided diets higher in essential amino acids and crude protein and lower in fat than those 7-9 yr post-disturbance. Winter diets were higher in essential amino acids and crude protein than summer diets. The sulfur-containing amino acids methionine and cystine were the most limiting of the essential amino acids in all years and seasons of study. Histidine was the second most limiting of the essential amino acids required in the diet of cottontail rabbits. The calculated biological value of protein in cottontail rabbit diets was found to be below requirements for growth more frequently in summer than winter and habitats 7-9 yr post-disturbance compared to 4-5 yr post-disturbance. Dietary crude protein estimates overestimated the true nutritional value of diets due to a prominent non-protein nitrogen component to the total nitrogen pool, which averaged 32%. The nutritional value of diets for essential amino acids declined as grasses replaced forbs on disturbed habitats, which coincided with declines in other small herbivore populations.

INTRODUCTION

Studies on the nutritional ecology of lagomorphs have resulted in several theories on how localized populations are nutritionally regulated (Sinclair et al. 1982, Keith 1983). In general, these theories have proposed that lagomorph populations are sensitive to food availability and diet quality, which are considered the primary factors regulating population numbers. Supporting evidence is provided by several studies on hares and jackrabbits that have demonstrated strong links between the availability of suitable forage and the onset of breeding (Vaughan and Keith 1981, Keith 1987, Rogowitz 1992), fecundity (Rogowitz 1992), and juvenile survival (Pease et al. 1979, Keith 1987).

Current evidence suggests that cottontail rabbit populations also respond to changing nutritional conditions in the habitat. Examples in support of this suggestion include: decreased breeding success as nutritional quality of the habitat declines (Trethewey and Verts 1971, Pelton and Provost 1972, Bothma and Teer 1977, Chapman et al. 1977, 1982); variation in population densities across habitat types (Lochmiller et al. 1991); habitat influences on food habits (Turkowski 1975); and increases in home range size and foraging effort with plant maturity (Janes 1959, Trent *and Rongstad 1974*, Anderson and Pelton 1976, Chapman et al. 1982, Allen 1984). However, the proximate nutritional factors responsible for altering these life history events are not known.

Protein has been suggested as the most limiting nutrient category for many wild herbivore populations (White 1978). Although protein nutrition of cottontail rabbits has been evaluated in the laboratory, especially with regard to the consequences of intake restrictions on growth rate (Bailey 1969, Snyder et al. 1976), similar evaluations in natural populations have not been completed. Protein in the diet supplies essential amino acid nutritents, required by an organism due to its inability to synthesize adequate amounts; as such, the ability of dietary proteins to supply a proper balance of these essential amino acids will largely determine its quality (Oser 1959). Investigations into the importance of protein to herbivore populations should consider availability of specific essential amino acid nutrients. Studies on dietary protein quality of northern pintail (Anas acuta) (Krapu and Swanson 1975), snow geese (Chen caerulescens) (Thomas and Prevett 1980, Sedinger 1984), willow ptarmigan (Lagopus lagopus lagopus) (Steen et al. 1988), and primates (Oftedal 1991), although limited in scope, suggest that essential amino acid deficiencies may be seasonally important relative to physiological requirements of many animal species.

Our objective was to elucidate seasonal and annual changes in the essential amino acid composition of diets consumed by cottontail rabbit populations in temperate, upland hardwood forest-tallgrass prairie subjected to various intensities of anthropogenic disturbances. Diet quality of many wild mammalian herbivores increases with habitat disturbances that result in higher levels of disturbance-indicator plant species (Coppock and Detling 1986, Bucyanayandi and Bergeron 1990). However, detailed studies on the dynamics of essential amino acid nutrition in wild mammalian herbivores and in their responses to vegetation disturbance have not been published. Dietary protein quality among different disturbance regimes was compared to ascertain if essential amino acids were deficient and when such deficiencies occurred. We hypothesized that diets of cottontail rabbits were higher in protein quality (balance and concentration of essential amino acids) on more intensely and recently disturbed areas compared to undisturbed areas due to increased availability of forbs (Engle et al. 1991*a* 1991*b*).

METHODS

Study area

We examined changes in protein quality of the diet of cottontail rabbits from replicated treatments on an area located approximately 11 km southwest of Stillwater, Oklahoma (36°2′ to 36°4′ N, 97°9′ to 97°11′ W). Our study area encompassed upland hardwood forest with interspersed tallgrass prairie invaded by *Juniperus virginiana*. This area is representative of the cross timbers vegetation type (Ewing et al. 1984), which accounts for nearly 5 million ha of land in Oklahoma, Kansas, and Texas (Soil Conservation Service 1981). These upland hardwood forests are dominated by *Quercus stellata* and *Q. marilandica* in the overstory, and *J. virginiana*, *Cornus drummondii*, *Cercis canadensis*, *Ulmus americana*, and *Symphoricarpos orbiculatus* in the understory. Herbaceous ground cover is dominated by *Schizachyrium scoparius*, *Sorghastrum nutans*, *Panicum oligosanthes*, and *Ambrosia psilostachya*. Vegetation on tallgrass prairies is dominated by *S. scoparius* followed by *S. nutans* and *Andropogon gerardii*.

Our study area consisted of 20 disturbed and 4 undisturbed areas (32.4-ha; 0.42 x 0.83 km) arranged in a randomized complete block design resulting in six disturbance types blocked into four replications. Five levels of disturbance were created as a result of combinations of removal of woody overstory and burning. Four of these disturbance types were created with applications of the herbicides tebuthiuron or triclopyr applied aerially at 2.2 kg ha^{-1} during spring 1983, and in conjunction with or without late spring burns in 1985, 1986, 1987, and 1990 (Stritzke et al. 1991a, 1991b). The fifth was disturbed only by late spring burns in 1990 and 1991. Undisturbed areas, where herbicide removal of overstory canopy and fire were not used, served as experimental controls.

Average grass and forb production on disturbed areas throughout our study were higher than undisturbed controls (Fig. 1). Forb production showed a greater decline with time since disturbance (4-5 yr vs. 7-9 yr post-disturbance) than grass on disturbed areas. Tebuthiuron was an intense disturbance, resulting in near total removal of the woody canopy, an effect that persisted throughout the study (Stritzke et al. 1991a, 1991b). Efficacy of the two herbicides (tebuthiuron and triclopyr) differed with respect to reduction of resprouting. Triclopyr did not kill as many overstory and understory woody plants and resulted in areas containing a mixture of woody species that were released after removal of dominant Quercus spp.. The Quercus overstory of triclopyr-treated areas was replaced by U. americana and J. virginiana; the woody understory was dominated by S. orbiculatus (Stritzke et al. 1991a, 1991b). During the first several years after disturbance (4-5 yr) by herbicide, the understory was dominated by pioneer forbs and grasses, especially Conyza canadensis. Several years after disturbance (7-9 yr) the understory was dominated by a mixture of annual forbs and grasses, especially P. oligosanthes, with increasing amounts of warm-season perennial grasses toward the end of the study (Engle et al. 1991a, 1991b). Burning had little influence on woody plant composition, except to reduce J. virginiana on tebuthiuron-disturbed areas starting in the 1988 burn year (Engle et al. 1991a, 1991b, Stritzke et al. 1991a, 1991b). However, burning was a disturbance to the understory, where it tended to increase forbs (Engle et al. 1991a, 1991b). Thus, disturbance intensity could be ranked as: tebuthiuron plus burn > tebuthiuron > triclopyr plus burn > triclopyr > burn only > undisturbed.

Data collection

Temporal changes in the nutritional quality of rabbit diets from various disturbed areas were assessed by examining stomach digesta (Schreiber 1979, Servello et al. 1983, 1984, MacPherson et al. 1985, 1988, Millar et al. 1990, Peitz and Lochmiller 1993). A total of 505 cottontail rabbits was collected in summer and winter from 1987 to

1992; rabbits were not collected from winter 1989 to winter 1990. An attempt was made to collect five cottontail rabbits per season from each of two replications of each disturbance type and undisturbed control; four replications were sampled in the summer of 1990. Cottontail rabbits were collected at a distance of >75 m from boundary fences to minimize the chance that individuals were inhabiting two or more different disturbance types. Previous mark-recapture studies indicated that cottontail rabbit movement between disturbance types was negligible (Lochmiller et al. 1991).

Carcasses were placed on ice in the field and returned to the laboratory where stomachs were excised and digesta removed. Digesta was cleared of hair fibers, mucosa, and parasites prior to determining a wet mass (± 0.1 mg). Digesta samples were lyophilized (samples collected prior to summer 1990 were dried in a conventional oven at 50^0C) to constant dry mass (± 0.1 mg), ground to pass through a 20-gage mesh screen, and composited by treatment replication within each season. Composites were created by mixing 1.0 g of digesta from each individual within a replication (Jenks et al. 1989). Digesta samples from stomachs containing <20% of the mean dry mass for each season were excluded from compositing (Peitz and Lochmiller 1993).

Lipids were extracted from digesta composites using a Soxhlet apparatus and diethyl ether solvent (Williams 1984). Fat-extracted samples amounting to approximately 40 mg of protein were weighed into 25- x 150-mm glass tubes with teflon-lined screw caps and hydrolyzed in 15 ml 6 N HCl at 110°C for 24 hr. One ml of the hydrolyzed sample was filtered through a 0.45-um syringe filter (Acrodisc CRPTFE, Fisher Scientific, Plano, TX). An internal standard (25 ul 4.0 nm methionine sulfone in 0.1 N HCL) was added to 75 ul of filtered hydrolysate before derivatization. Pre-column derivatization of amino acids was accomplished using phenylisothiocynate to produce phenylthiocarbamyl amino acids (Pico-Tag Work Station, Millipore Corporation, Milford, MA) and refiltered through a 0.45-um syringe filter. Concentrations of 17 individual amino acids (11 are thought to be essential in cottontail rabbit nutrition) were determined in derivatized samples using high pressure liquid chromatography (HPLC, Waters Model 820 System Controller and Model 501 Pumps, Millipore Corporation, Milford, MA). Concentrations obtained for methionine and cystine were combined as were phenylalanine and tyrosine, because it is known that cystine and tyrosine spare their respective amino acid nutritionally (Lebas 1988). Tryptophan was not measured due to destruction by acid hydrolysis (Sarwar et al. 1983, Gehrke et al. 1985). Chromatographic conditions were: Waters Pico-Tag Silica/C18 (15 cm x 3.9 mm) column; column temperature of 38°C; flow rate of 1.0 ml/min with pump back pressures 5500 PSI; system sensitivity of 489 mv/sec (recorder) and 0.5 absorbance units full scale (Waters Model 484 UV Detector, Millipore Corporation, Milford, MA, set at 254 nm); injection volume 4 ul; and run time 27.5 min. Solvents used were Eluent A and Eluent B (catalog numbers 88108 and 88112, respectively; Millipore Corporation, Milford, MA). Solvent conditions and gradients used for separation of amino acids were those described by Cohen et al. (1988). A casein reference protein (from bovine milk, no. C-0376, Sigma Chemical Co., St. Louis, MO) of known amino acid composition was hydrolyzed and analyzed with samples for determination of recovery efficiency. Sulfur-containing amino acid losses during acid hydrolysis averaged 25%; therefore, methionine + cystine concentrations were adjusted upward 25% to correct for recovery efficiency. Amino acid concentrations were recorded on a dry mass basis (mg/g dry mass).

Non-extracted digesta samples were analyzed for nitrogen content with a Labconco Rapid Kjeldahl System (Labconco Corp., Kansas City, MO) (Williams 1984). Percent nitrogen values were multiplied by 6.25 to estimate crude protein content. Non-protein nitrogen concentration of digesta was a measure of the difference between amino acid nitrogen (HPLC analysis) and total nitrogen (Kjeldahl analysis) concentrations. Non-protein nitrogen was assumed to be all nitrogen not incorporated into amino acids recovered (HPLC analysis) and is made up of a diverse group of compounds (Maynard et al. 1979, Holt and Sosulski 1981, Singh and Jambunathan 1981, Oka and Sasaoka 1985). Unlike other measures of non-protein nitrogen (Holt and Sosulski

1981), we did not include free amino acids (those amino acids not bound as protein) that were recovered during HPLC analysis. Amino acid nitrogen was multiplied by 6.25 to estimate corrected crude protein content to correct for non-protein nitrogen concentration.

The overall adequacy of a protein in the diet depends entirely upon how well it meets essential amino acid requirements of individual cottontail rabbits. Many empirical techniques have been developed to evaluate quality or biological value of dietary proteins based upon their essential amino acid composition. We modified the chemical score technique (Mitchell and Block 1946) to compare the most limiting essential amino acid in each diet against nutritional requirement. Chemical scores have an inverse relationship with the biological value of a protein source (Mitchell and Block 1946). Biological value of dietary proteins was estimated by calculating a modified essential amino acid index and calculated biological value (Oser 1959). This method compared ratios of essential amino acids in each dietary protein to respective amounts required for growth.

Data analysis

Influence of disturbance type on diet quality measures (essential amino acids, non-protein nitrogen, crude protein, and fat) was found to be non-significant ($\underline{P} < .05$) by season when examined by two-way analysis of variance (Proc GLM, SAS 1988, SAS Institute Inc., Cary, NC) with disturbance type and year as main factors. Therefore, digesta composites were grouped across disturbance types within each season (summer, winter) and year to assess the extent of season and annual changes in diet quality (concentrations of essential amino acids, non-protein nitrogen, crude protein, and fat) of cottontail rabbits. Digesta composites remained grouped across disturbance types for the remaining statistical analyses unless noted otherwise.

Our preliminary analyses of annual effects indicated that the influence of disturbance on diet quality was different between 4-5 yr and 7-9 yr post-disturbance. Therefore, season (summer, winter) and time post-disturbance (4-5 yr, 7-9 yr) effects on diet quality were examined using a two-way analysis of variance (Proc GLM, SAS 1988); undisturbed and burned-control areas were excluded from this analysis. Mean differences in diet quality measures were investigated further using a least significant difference test when main effects were significant ($\underline{P} < .05$).

A discriminant analysis procedure (Proc DISCRIM, SAS 1988) was used to further examine the influence of time post-disturbance and season on overall quality of dietary protein (essential amino acids). Discriminant analysis separated subgroups on the basis of a combined group of essential amino acid variables that differed between the subgroups. These discriminator variables (lysine, methionine + cystine, arginine, histidine, leucine, isoleucine, phenylalanine + tyrosine, threonine, and valine) were determined using a data reduction procedure, stepwise discriminant analysis (Proc STEPDISC, SAS 1988). A canonical discriminant analysis procedure (Proc CANDISC, SAS 1988) was used to further evaluate relationships in diet quality based on individual essential amino acids most influenced by time post-disturbance and season.

RESULTS

Seasonal influences

Composited digesta samples (n = 99) from cottontail rabbits collected on replicate disturbance types between 1987-1992 varied seasonally ($P < .05$) for all diet quality measures, except non-protein nitrogen (Table 1, Figs. 2-4). Concentrations for all nutrients that differed seasonally were consistently higher in winter than summer. Concentrations of dietary fat averaged 33% higher and crude protein averaged 41% higher in winter than summer. Overall, concentrations of the nine essential amino acids measured in the diet of cottontail rabbits averaged 47% greater in winter compared to summer. The largest seasonal difference in concentration was observed for methionine + cystine, which averaged 65% more in winter than summer. Low levels of essential amino acids in summer compared to winter were reflected in overall measures of biological value of proteins (Table 2). Calculated biological value of dietary protein was below 100% for all but one summer collection (1988), and values for winter collections were all above 100% except in 1991 (95%).

Essential amino acid deficiencies relative to estimated requirements for growth and lactation of cottontail rabbits were prominent in summer (Figs. 2-4), as suggested by the low calculated biological values (Table 2). Concentrations of methionine + cystine were universally deficient for meeting both growth and lactation requirements during all seasonal collections and were the most limiting of the essential amino acids. Histidine was the second most limiting essential amino acid and concentrations failed to meet growth and lactation requirements in all summer and most winter collections. Lysine was the only essential amino acid nutrient to meet growth requirements in all summer diets. Besides methionine + cystine and histidine, the only other winter deficiencies were threonine for growth and lactation and phenylalanine + tyrosine and leucine for lactation during 1991. Growth and lactation requirements for arginine, valine, isoleucine, and lysine were satisfied in all winter collections.

Habitat disturbance influences

All nutrient categories in cottontail diets appeared to be influenced by changes in vegetation from 4-5 yr to 7-9 yr post-disturbance. Fat concentrations were significantly greater ($P < 0.05$) 7-9 yr post-disturbance compared to 4-5 yr post-disturbance (Table 1). Concentrations of non-protein nitrogen tended to be greater in diets of cottontail rabbits harvested from areas 4-5 yr than 7-9 yr post-disturbance, but differences were not significant ($P = .09$).

Dietary crude protein concentrations significantly decreased ($\underline{P} < 0.05$) an average of 31% from 4-5 yr to 7-9 yr post-disturbance.

Concentrations of all nine essential amino acids were significantly greater ($\underline{P} < .05$) in diets of cottontail rabbits harvested from areas 4-5 yr post-disturbance than 7-9 yr post-disturbance for both summer and winter (Figs. 2-4). On average, essential amino acid concentrations of diets were 45% greater 4-5 yr than 7-9 yr post-disturbance. Concentrations of methionine + cystine, the most limiting of the essential amino acids, were on average 91% greater in diets of rabbits 4-5 yr than 7-9 yr post-disturbance.

Essential amino acid deficiencies for growth and lactation were common in the diets of cottontail rabbits collected from areas 7-9 yr post-disturbance, especially in summer when concentrations of eight (histidine, arginine, threonine, valine, methionine + cystine, isoleucine, leucine, phenylalanine + tyrosine) of nine essential amino acids in the diet failed to meet one or both requirements (Fig. 2-4). Of the six essential amino acids in diets that failed to meet both growth and lactational requirements (histidine, threonine, phenylalanine + tyrosine, methionine + cystine, isoleucine, and leucine) in summer, deficiencies averaged 25% for growth and 38% for lactation. Greatest deficiencies were observed for methionine + cystine which averaged 78% in summer collections from areas 7-9 yr post-disturbance. Although not as severe as summer, dietary deficiencies in winter 7-9 yr post-disturbance were frequently high; examples include histidine (13% for growth, 40% for lactation) and methionine + cystine (59% for growth and for lactation).

Essential amino acid concentrations in diets of cottontail rabbits from disturbed areas 4-5 yr post-disturbance came closer to meeting nutrient requirements than areas 7-9 yr post-disturbance (Figs. 2-4). When deficiencies were observed, they generally were not as severe as those diets of rabbits from disturbed areas 7-9 yr post-disturbance. Exceptions included histidine (17% for growth, 42% for lactation) and methionine + cystine (54% deficient for growth and lactation) during summer collections. Winter methionine + cystine deficiencies averaged 36% for growth and lactation in areas 4-5 yr post-disturbance. With the exception of histidine requirements for lactation in winter 1988 (5% deficient), concentrations of all other essential amino acids in diets met growth and lactation requirements in winters 4-5 yr post-disturbance (excluding methionine + cystine).

Calculated indices of the biological value of dietary proteins reflected the greater imbalance and lower concentration of essential amino acids in diets as disturbed areas recovered from their initial disturbance (Table 2). Calculated biological values averaged over 100% for both summer (mean = 108%) and winter (mean = 156%) diets in areas 4-5 yr post-disturbance, but were well below 100% in summer (mean = 75%) diets from areas 7-9 yr post-disturbance. Calculated chemical scores, which reflected the percent growth deficiency for the most limiting amino acid (methionine + cystine), showed a similar relationship between post-disturbance times, and the high deficiency estimates compared to calculated biological values reflected an imbalance in amino acid concentrations within diets.

Multivariate analysis

Discriminant analysis classified the nutritional quality of digesta composites into their appropriate post-disturbance and seasonal group with an overall accuracy rate of 97% based on differences in selected essential amino acid concentrations. Canonical discriminant analysis demonstrated highly significant ($P < 0.001$) Mahalanobis distances between all digesta composite centroids (Fig. 5), indicating that essential amino acid nutrition of cottontail rabbits varied as a factor of both time post-disturbance and season. Coefficients for the two canonical variates (Table 3) used in separating digesta composite centroids accounted for 97% of the variance between amino acid profiles from the various post-disturbance periods and seasons. Canonical variate I represented a gradient of increasing concentrations of valine and methionine + cystine and decreasing concentration of phenylalanine + tyrosine. Diets of rabbits from areas 4-5 yr post-disturbance had higher levels of valine and the sulfur-containing amino acids and were significantly separated from 7-9 yr post-disturbance groups along canonical variate I (Fig. 5). The decreasing phenylalanine gradient along canonical variate I was due to the influence of high concentrations of this amino acid in winter diets 7-9 yr post-disturbance. Canonical variate II represented increasing concentrations of isoleucine and phenylalanine + tyrosine and decreasing concentration of valine. Higher levels of the first two amino acids in winter diets significantly separated this group from summer along canonical variate II. The decreasing valine gradient along canonical variate II was due to the influence of high concentrations of this amino acid in summer diets 4-5 yr post-disturbance.

DISCUSSION

Although crude protein concentrations were above the estimated requirement for growth in cottontail rabbits (Snyder et al. 1976), factors such as high levels of non-protein nitrogen indicate that essential amino acid requirements were not always met. Non-protein nitrogen in diets accounted for approximately one third of the total nitrogen pool in diets. While crude protein estimates may be appropriate for ruminant animals that can synthesize protein from non-protein nitrogen sources, they do not accurately reflect protein quality for rabbits who cannot efficiently utilize non-protein nitrogen (NRC 1977, Lebas 1988). A more useful measure of protein quality of rabbit diets would be one that corrects Kjeldahl nitrogen values for the non-protein nitrogen component or directly measures the amino acid content. Crude protein estimates (determined by multiplying Kjeldahl nitrogen by a coefficient 6.25) overestimated available protein in diets an average of 48% (range 38-65). Therefore, a coefficient of 4.22 for converting Kjeldahl nitrogen to protein would provide a more accurate estimate of diet protein available for rabbit nutrition.

Another important factor determining quality of dietary protein for growth and reproduction is diet digestibility. Cottontail rabbits are known to attain normal growth rates (8 g body mass/day) on diets containing 12% digestible protein (Snyder et al. 1976); however, under range conditions where grasses comprise the bulk of dietary protein, crude protein values may overestimate nutritive value due to the high cell wall content in grasses. Cell wall constituents resist enzymatic digestion and subsequent release of cytoplasmic protein for further digestion. Studies with growing domestic rabbits (Crampton and Forshaw 1940, Voris et al. 1940) have demonstrated that grasses are relatively indigestible compared to forbs. Grasses also have been shown to be less digestible than forbs and legumes in feeding trials with young cottontails. Bailey (1969) reported that individuals failed to thrive on domestic grasses (bluegrass [Poa sp.], orchard grass [Dactylis glomerata], timothy [Phleum pratense]) containing apparently adequate crude protein levels, but observed adequate weight gain and survival on wild forbs and legumes. Dry matter digestibility varied among species and season, ranging from 35% to 60% for grasses compared to 60% to 89% for forbs and legumes, and was typically the lowest during summer (Bailey 1969). Analysis of warm-season grasses utilized by rabbits on our study area showed that in vitro dry matter digestibility was 16% lower than for forbs in summer 1985 and 1986 (Bogle et al. 1989). Although there is a lack of digestibility information for winter forages utilized by rabbits, winter diets were probably adequate with respect to most essential amino acids for maintenance and growth; exceptions include histidine and the sulfur-containing amino acids (especially 7-9 yr post-disturbance). Essential amino acid requirements for adult maintenance in rabbits have not been published, but digestible protein requirements are 25% lower than those for growth (NRC 1977).

Essential amino acid concentrations as a measure of the quality of cottontail rabbits diets declined as herbaceous weedy forbs were replaced by warm-season grasses between 4-5 yr and 7-9 yr post-disturbance. Calculated biological values and chemical scores also reflected this decline in diet quality, especially in summer. Previously, forbs were found to contain higher concentrations of crude protein than grasses on the study area (Bogle et al. 1989), and disturbed areas had reached a vegetative community dominated by grasses by 7-9 yr post-disturbance. Similar declines in range nutrient quality after initial disturbances have been noted for prairies grazed by meadow voles (Microtus pennsylvanicus) (Bucyanayandi and Bergeron 1990), black-tailed prairie dog (Cynomys ludovicianus) towns grazed by bison (Bison bison) (Coppock and Detling 1986), and disturbed sagebrush sites fertilized with various levels of nitrogen (McLendon and Redente 1992). Decreases in range nutrients in the first two studies were attributed to an increase in grasses corresponding to declines in pioneer stage forbs, as occurred on our study area. The later study suggested that soil nitrogen determined the rate at which grasses of low tissue nitrogen, compared to forbs, replace forbs during secondary succession.

Declining essential amino acid concentrations from 4-5 yr to 7-9 yr post-disturbance had a probable role in the nutritional ecology of cottontail rabbits and may help explain their preference for disturbed habitat types (Chapman et al. 1982). Concentrations of essential amino acids may regulate population numbers through decreased breeding success or survival because various essential amino acids were limiting even though crude protein estimates were above requirements. Deficiencies in one or more essential amino acid can lead to reduced growth, reproductive performance, or survival in rabbits (Spreadbury 1978, Lebas 1988, Schultze et al. 1988). The higher population densities of cottontail rabbits and other small mammalian herbivores observed on disturbed habitats 4-5 yr post-disturbance compared to undisturbed areas (Lochmiller et al. 1991, McMurry et al. 1993) could have been in response to changing amino acid nutrition. Decreased breeding success of cottontail rabbits due to diet quality has been reported (Trethewey and Verts 1971, Pelton and Provost 1972, Bothma and Teer 1977, Chapman et al. 1977, 1982). Cottontail rabbits may also limit breeding to those seasons when essential amino acid requirements are met by available resources.

Our data supports the proposition of Chapman et al. (1982) that availability of sulfur-containing amino acids may be a critical determinant of life history events in cottontail rabbit populations. Overall classification accuracy (97%) for the function that discriminates among disturbance and seasonal groups was high indicating that the essential amino acid nutrition of cottontails was strongly influenced by these extrinsic environmental factors. Biological value estimates and discriminant analysis provided useful insight into the true complexity of the changing nutritional environment. No single essential amino acid consistently explained a large amount of the seasonal and post-disturbance variability in quality of dietary proteins of rabbits. To the contrary, these temporal changes in quality represented a collective alteration in concentrations of, and relationships among (amino acid balance), many essential amino acids. Our results and the suggestions of others (Krapu and Swanson 1975, Sedinger 1984, Steen et al. 1988, Oftedal 1991) enforces the need to consider the role of essential amino acids in influencing the dynamics of herbivore populations. Simple monitoring of crude protein levels, especially in light of substantial non-protein nitrogen constituents in natural forages, may not provide the level of sensitivity required to detect and interpret the complex interrelationships among nutrients and population responses.

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Table 1. Mean (± SE) concentrations for crude protein, non-protein nitrogen, corrected crude protein, and fat recovered from stomach digesta of cottontail rabbits (*Sylvilagus floridanus*) from areas 4-5 yr or 7-9 yr post-disturbance, by year and season.

	4-5 yr post-disturbance				7-9 yr post-disturbance			
	1987		1988		1990	1991		1992
	Winter (*n*=10)	Summer (*n*=10)	Winter (*n*=10)	Summer (*n*=10)	Summer (*n*=23)	Winter (*n*=12)	Summer (*n*=12)	Winter (*n*=12)
Crude protein (%)[a]	26.8 ± 1.27	17.2 ± 0.34	24.8 ± 1.09	23.3 ± 0.52	12.8 ± 0.68	16.8 ± 1.04	14.4 ± 0.94	22.5 ± 1.60
Non-protein nitrogen (%)[ab]	34.7 ± 3.38	31.6 ± 1.04	29.4 ± 1.02	38.9 ± 2.42	30.1 ± 1.03	29.4 ± 1.06	27.9 ± 1.84	33.2 ± 1.98
Corrected crude protein (%)[a]	17.3 ± 0.01	11.8 ± 0.01	17.5 ± 0.01	14.1 ± 0.01	8.9 ± 0.01	11.7 ± 0.01	10.4 ± 0.01	14.9 ± 0.01
Fat content (%)[a]	4.0 ± 0.27	2.6 ± 0.21	4.6 ± 0.18	4.6 ± 0.14	4.1 ± 0.10	5.8 ± 0.55	4.2 ± 0.18	6.3 ± 0.72

[a] Variables were significantly ($P < .05$) influenced by time post-disturbance.

[b] Variable was not influenced significantly ($P < .05$) by season.

Table 2. Estimated overall nutritional quality of proteins by season and year as calculated by the essential amino acid index (EAAI), calculated biological value (CBV), first limiting amino acid (FLAA), and calculated chemical score (CCS, an inverse estimate of the biological value) in cottontail rabbit (*Sylvilagus floridanus*) diets from 1987-1992.

Diet	% Crude protein	EAAI[a]	CBV[b]	FLAA	CCS[c]
Summer					
1987	17.22	99	96	Methionine + cystine	65
1988	23.29	122	121	Methionine + cystine	42
1990	12.78	73	68	Methionine + cystine	78
1991	14.44	85	81	Methionine + cystine	77
Winter					
1987	26.81	153	155	Methionine + cystine	32
1988	24.84	154	156	Methionine + cystine	40
1991	16.78	97	95	Methionine + cystine	70
1992	22.47	128	128	Methionine + cystine	48

[a] $\mathrm{EAAI} = \sqrt[n]{\frac{Arg_p}{Arg_s} \times \frac{His_p}{His_s} \times \dots \frac{Val_p}{Val_s}}$ (Oser 1959)

Superscript refers to the digesta protein (p) and growth requirement (s); and *n* = 9 is the number of essential amino acids (methionine and cystine, and phenylalanine and tyrosine were counted as one). Histidine, arginine, threonine, valine, leucine, isoleucine, lysine, methionine +

Table 2. (cont. pg2)

cystine, and phenylalanine + tyrosine were included in all computations.

[b] Calculated biological value = 1.09(EAAI) - 11.7 (Oser 1959).

[c] Calculated chemical score is the percent deficiency of the most limiting essential amino acid in meeting growth requirements.

Table 3. Canonical variate coefficients for the amino acid variables used in separating cottontail rabbit (Sylvilagus floridanus) diets relative to protein quality into areas 4-5 yr (1987-1988) or 7-9 yr (1990-1992) post-disturbance and season (summer and winter).

	Canonical variate	
	I	II
Lysine	1.20	-0.89
Methionine + cystine	3.21	-2.05
Arginine	-0.33	1.06
Histidine	-0.67	0.35
Leucine	-1.61	-0.46
Isoleucine	-2.01	2.99
Phenylalanine + tyrosine	-4.11	2.88
Threonine	0.25	0.42
Valine	6.19	-3.57

Figure 1. Average grass and forb production on undisturbed and disturbed upland hardwood forest-tallgrass prairie, by time post-disturbance (years 1987-1988 are 4-5 yr and 1990-1992 are 7-9 yr post-disturbance).

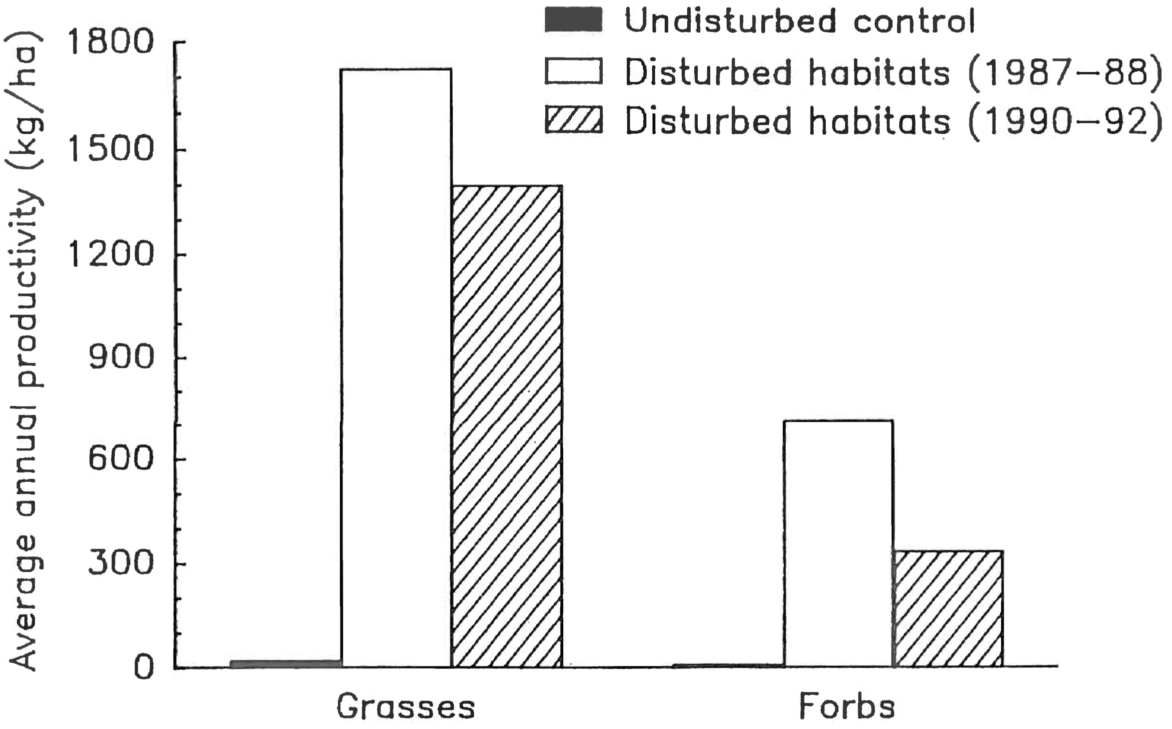

Undisturbed control
Disturbed habitats (1987–88)
Disturbed habitats (1990–92)
Average annual productivity (kg/ha)
1800
1500
1200
900
600
300
0
Grasses
Forbs

Figure 2. Annual changes in the mean (± SE) concentrations of histidine, arginine, and threonine recovered from stomach digesta of cottontail rabbits relative to nutrient requirements for growth (NRC 1977) and lactation (INRA 1984). Years 1987-1988 are 4-5 yr and 1990-1992 are 7-9 yr post-disturbance.

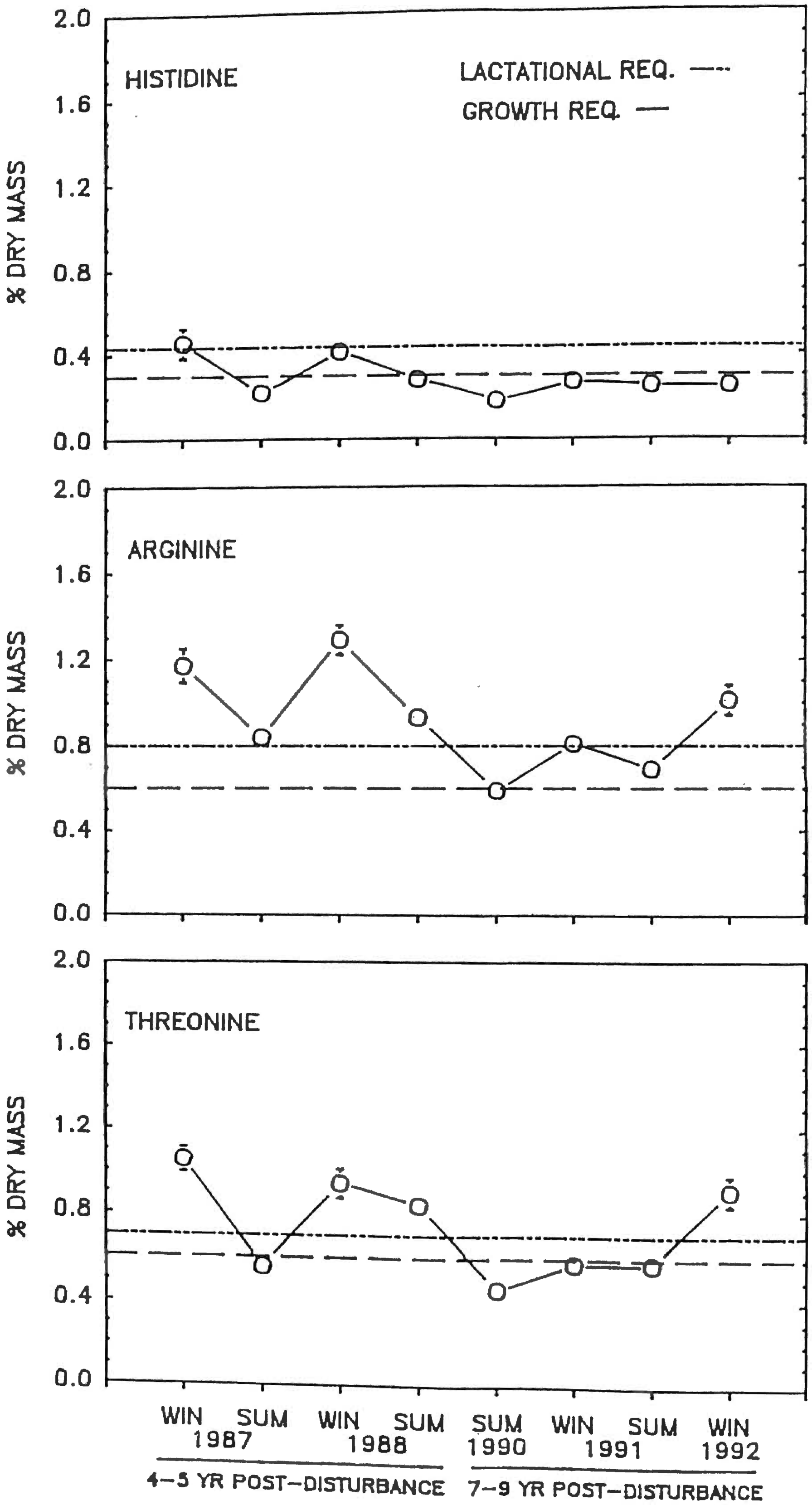
2.0
1.6
1.2
0.8
0.4
0.0
HISTIDINE
LACTATIONAL REQ.
GROWTH REQ.
% DRY MASS
ARGININE
THREONINE
WIN 1987
SUM
WIN 1988
SUM
SUM 1990
WIN 1991
SUM
WIN 1992
4–5 YR POST–DISTURBANCE
7–9 YR POST–DISTURBANCE

Figure 3. Annual changes in the mean (± SE) concentrations of lysine, phenylalanine + tyrosine, and methionine + cystine recovered from stomach digesta of cottontail rabbits relative to nutrient requirements for growth (NRC 1977) and lactation (INRA 1984). Years 1987-1988 are 4-5 yr and 1990-1992 are 7-9 yr post-disturbance. Estimated requirements of methionine + cystine for growth and lactation are equal.

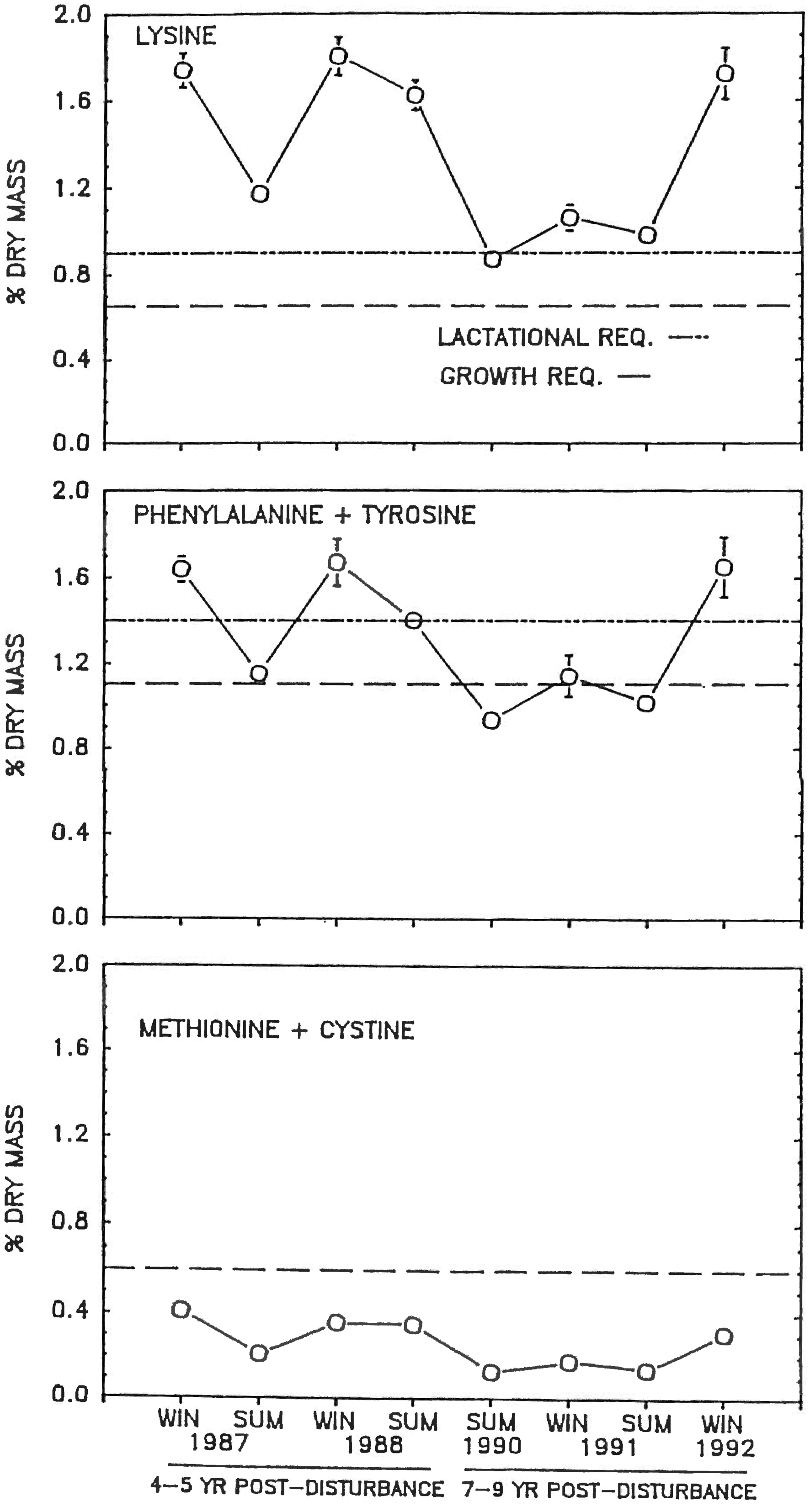

LYSINE
LACTATIONAL REQ. ----
GROWTH REQ. —
PHENYLALANINE + TYROSINE
METHIONINE + CYSTINE
% DRY MASS
2.0
1.6
1.2
0.8
0.4
0.0
WIN
SUM
WIN
SUM
SUM
WIN
SUM
WIN
1987
1988
1990
1991
1992
4–5 YR POST–DISTURBANCE
7–9 YR POST–DISTURBANCE

Figure 4. Annual changes in the mean (± SE) concentrations of valine, isoleucine, and leucine recovered from stomach digesta of cottontail rabbits relative to nutrient requirements for growth (NRC 1977) and lactation (INRA 1984) are given also. Years 1987-1988 are 4-5 yr and 1990-1992 are 7-9 yr post-disturbance.

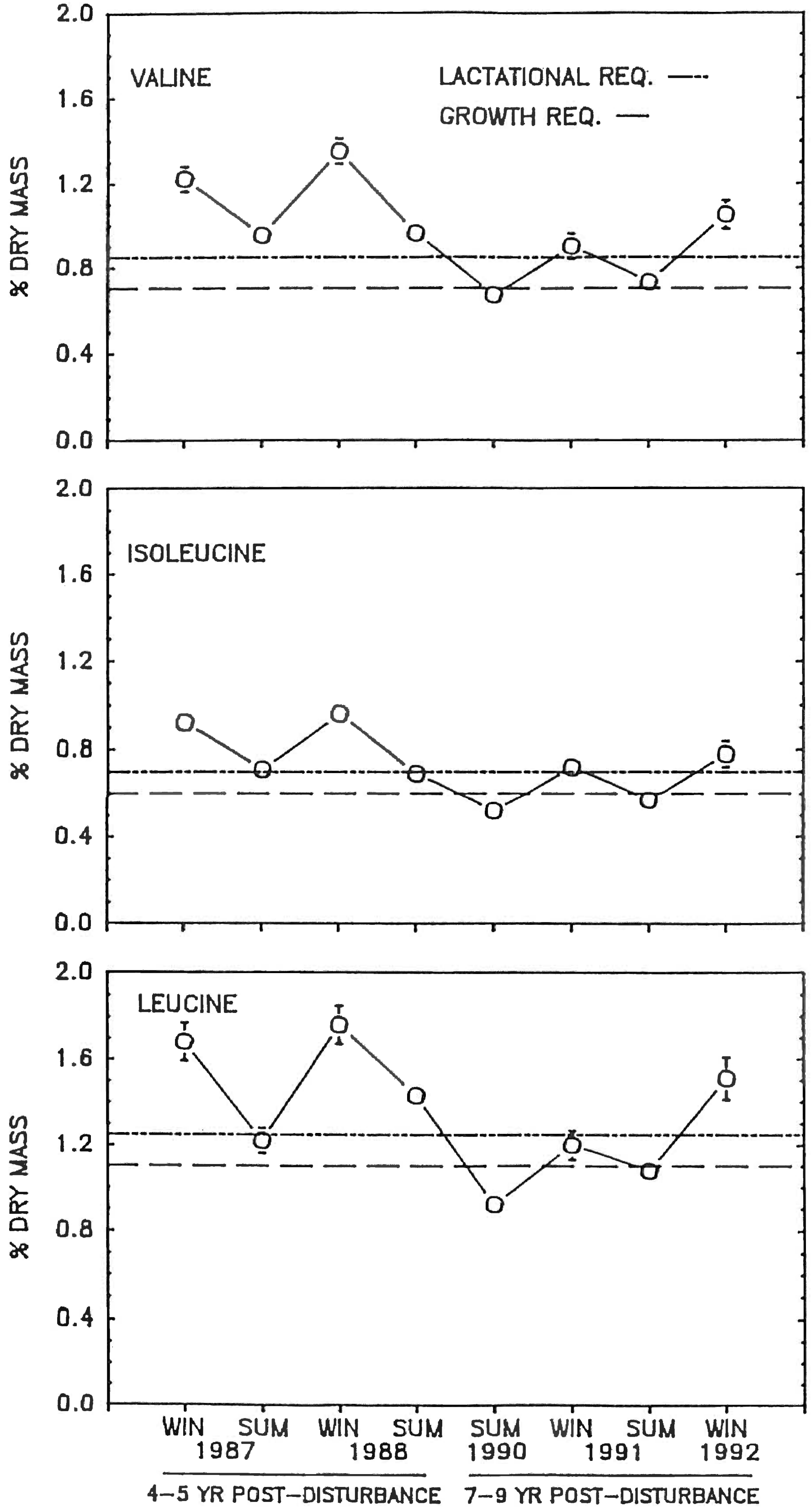
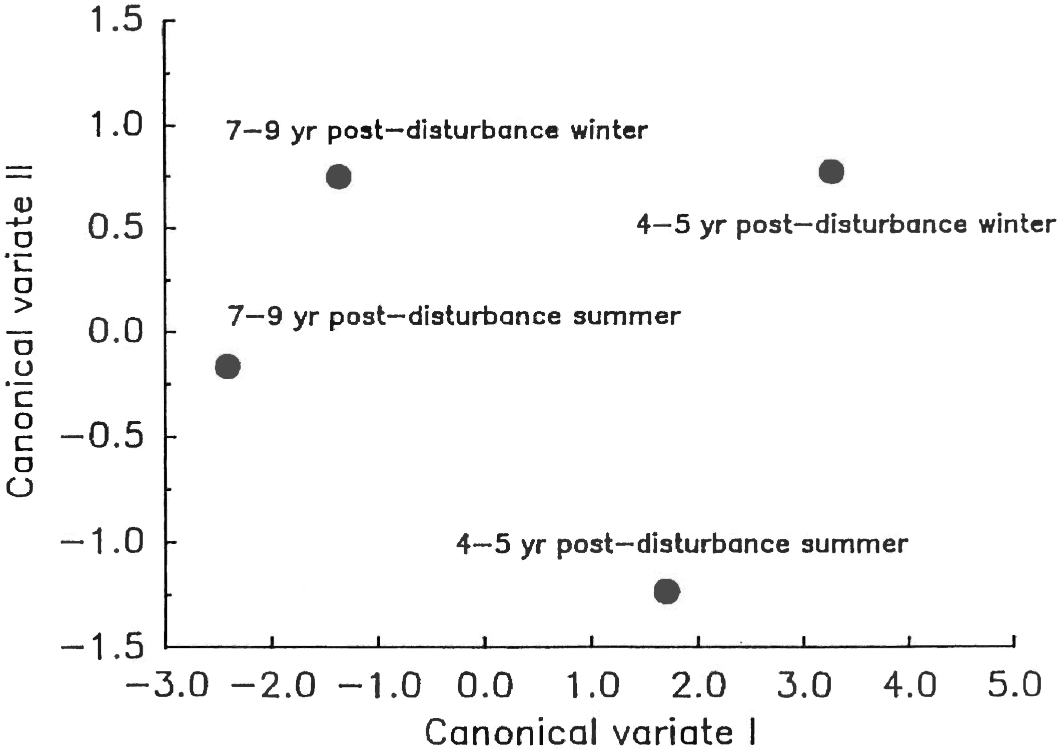

VALINE
LACTATIONAL REQ. ----
GROWTH REQ. —
ISOLEUCINE
LEUCINE
% DRY MASS
2.0
1.6
1.2
0.8
0.4
0.0
WIN 1987
SUM
WIN 1988
SUM
SUM 1990
WIN 1991
SUM
WIN 1992
4–5 YR POST–DISTURBANCE
7–9 YR POST–DISTURBANCE

Figure 5. Graphical representation of canonical variate (see Table 2) means determined by canonical discriminant analysis for nutritional groups separated by time post-disturbance (1987-1988 are 4-5 yr and 1990-1992 are 7-9 yr post-disturbance) and season. Separation was based on selected essential amino acid concentrations recovered from stomach digesta of cottontail rabbits collected in disturbed areas from 1987 to 1992.


7–9 yr post–disturbance winter
4–5 yr post–disturbance winter
7–9 yr post–disturbance summer
4–5 yr post–disturbance summer
Canonical variate II
Canonical variate I
1.5
1.0
0.5
0.0
−0.5
−1.0
−1.5
−3.0
−2.0
−1.0
0.0
1.0
2.0
3.0
4.0
5.0

CHAPTER IV

DEFICIENCIES IN ESSENTIAL AMINO ACIDS OF FORAGES IN THE DIET OF COTTONTAIL RABBITS (Sylvilagus floridanus)

ABSTRACT--Seasonal changes in the botanical composition and quality of diets consumed by cottontail rabbits (Sylvilagus floridanus) were monitored in disturbed and undisturbed upland hardwood forest-tallgrass prairie habitats. Our primary objective was to evaluate the essential amino acid nutrition of animals relative to *habitat quality and season*. Microhistological analysis of stomach digesta indicated that summer diets were dominated by Panicum oligosanthes, Croton spp., and Sporobolus asper; winter diets were dominated by Bromus spp., P. oligosanthes, and Antennaria spp. Phenological changes in the concentration of essential amino acids of sampled forages, previously identified as important in the diet of cottontails, were minimal in both summer and winter. Exceptions included Andropogon gerardii, S. asper, and Sorghastrum nutans which had significant declines (approximately 27%) in crude protein from early to late summer; no differences in crude protein content were observed throughout winter. Differences in the botanical composition and quality of diets between disturbed and undisturbed habitats were of little biological significance. However, levels of crude protein and essential amino acids in diets from both habitats failed to meet requirements for growth and lactation of cottontail rabbits in both summer and winter. Sulfur-containing amino acids (methionine + cystine) were the most deficient, failing to meet rabbit requirements by as much as 92% in summer and 70% in winter. Winter diets met more of the essential amino acid requirements of rabbits than summer diets.

INTRODUCTION

Advances in the study of nutritional ecology of lagomorphs has yielded several theories on population regulation (Keith, 1987; Sinclair et al., 1982). These theories propose that lagomorph populations are sensitive to food availability and diet quality, which are considered primary factors regulating population numbers. The availability of suitable forage can influence the onset of breeding and fecundity in snowshoe hares (Lepus americanus) (Keith, 1983; Vaughan and Keith, 1981) and white-tailed jackrabbits (L. townsendii) (Rogowitz, 1992). Juvenile survival in lagomorphs also has been linked to both forage quantity and quality (Gibb, 1977; Keith, 1983; Pease et al., 1979).

Although much of it is indirect, current evidence suggests that cottontail rabbit (Sylvilagus floridanus) populations also respond to changing nutritional conditions in their habitat. This is supported by observations that food habits (Turkowski, 1975), home range size (Anderson and Pelton, 1976; Janes, 1959; Trent and Rongstad, 1974), foraging effort (Allen, 1984; Anderson and Pelton, 1976; Chapman et al., 1982; Janes, 1959; Trent and Rongstad, 1974), breeding success (Bothma and Teer, 1977; Chapman et al., 1977; Chapman et al., 1982; Pelton and Provost, 1972; Trethewey and Verts, 1971), and population density (King et al., 1991; Lochmiller et al., 1991) vary among habitat types. Despite these relationships, our understanding of the proximate nutritional factors regulating these life history events remains elusive.

White (1978) proposed that protein was the most limiting nutrient category for many wild herbivore populations. Dietary protein supplies amino acids that rabbits cannot synthesize (termed "essential"); therefore, the ability of dietary protein to supply a proper balance of these essential amino acids will largely determine the nutritional quality of that protein (Oser 1959). Studies on quality of proteins in diets of northern pintail (Anas acuta) (Krapu and Swanson, 1975), willow ptarmigan (Lagopus lagopus lagopus) (Steen et al., 1988), snow geese (Chen caerulescens) (Sedinger, 1984; Thomas and Prevett, 1980), and primates (Oftedal, 1991) suggest that essential amino acids may be seasonally deficient relative to physiological requirements of many herbivores. Diets of cottontail rabbits are characteristically high in monocots and low in digestibility and crude protein, indicating that essential amino acid limitations may be important. As a result, Chapman et al. (1982) suggested that sulfur-containing amino acids may be important nutrients limiting cottontail populations.

Our primary objective was to elucidate temporal changes in the essential amino acid composition of major forage proteins consumed by cottontail rabbits from temperate, upland hardwood forests-tallgrass prairie. We seasonally compared protein quality of diets of cottontail rabbits from disturbed and undisturbed habitats. Temporal changes in protein quality due to plant phenology were monitored for preferred forages consumed by cottontail rabbits. Food habit analyses were used to reconstruct overall essential amino acid concentrations in seasonal diets, which were compared to known requirements to identify nutritional deficiencies. We hypothesized that forages in early growth stages and diets from disturbed habitats more closely met essential amino acid requirements of cottontail rabbits for growth and lactation than forages in late growth stages and diets from undisturbed habitats.

MATERIALS AND METHODS

Study area

We examined changes in protein quality of forages in the diet of cottontail rabbits from two disparate habitat types, located approximately 11 km southwest of Stillwater, Oklahoma ($36^{0}2'$ to $36^{0}4'$ N, $97^{0}9'$ to $97^{0}11'$ W). Our two habitat types, disturbed and undisturbed, were arranged in a randomized complete block design with two fenced 32.4-ha (0.42 x 0.83 km) replications of each habitat type. Our study area encompassed land originally composed of upland forest with interspersed tallgrass prairie (Ewing et al., 1984). This "cross timbers" vegetation type accounts for nearly 5 million ha of rangeland in Oklahoma, Kansas, and Texas (Soil Conservation Service, 1981). Undisturbed upland forest habitats were dominated by *Quercus marilandica*, *Q. stellata*, and *Juniperus virginiana* in the overstory, and *Cornus drummondii*, *Celtis* spp., and *Symphoricarpos orbiculatus* in the woody understory. Herbaceous ground cover was dominated by *Schizachyrium scoparius*, *Sorghastrum nutans*, *P. oligosanthes*, and *Ambrosia psilostachya*. Vegetation on tallgrass prairies was dominated by *S. scoparius* followed by *S. nutans* and *Andropogon gerardii*.

Disturbed habitats were maintained in an early seral community by removal of woody overstory vegetation with the herbicide triclopyr (Dow Chemical Co., Midland, MI) applied aerially at 2.2 kg ha^{-1} in June 1983 and by burning in the springs of 1985, 1986, 1987, and 1990. Disturbed habitats were dominated by *Ulmus americana* and *J. virginiana* in the overstory, *S. orbiculatus* in the woody understory, and *P. oligosanthes*, *Conyza canadensis*, and a mix of annual forbs in the herbaceous ground layer (Engle et al., 1991*a*, 1991*b*; Stritzke et al., 1991*a*, 1991*b*). Densities of cottontails were greater on disturbed than undisturbed habitat types

(Lochmiller et al., 1991). Annual production of grass, forb, and browse on undisturbed habitats was 2%, 1%, and 20%, respectively, of that on disturbed habitats. All habitats were seasonally grazed by yearling cattle from early spring to early to mid-fall.

Data collection

Key forages (arbitrarily determined to be all identifiable food items in the diet) in summer (July 1990) and winter (January 1991) diets of cottontail rabbits were identified through microhistological analysis of stomach digesta (Davitt and Nelson, 1980). A total of 10 digesta samples was obtained seasonally from stomachs of animals harvested from each of the two habitat replications (5 animals per replicate). Cottontail rabbits were collected at a distance of >75 m from boundary fences to minimize the chance that individuals were inhabiting both habitat types. Previous mark-recapture studies indicated that cottontail rabbit movement between habitat types was negligible (Lochmiller et al., 1991). Digesta samples were individually dried to constant weight by lyophilization, ground to pass through a 20-mesh screen and mixed, and composited by habitat replicate (0.5 g stomach digesta/rabbit/habitat replication). Composited digesta samples were used to identify key forages in the diet. Composites were bleached, soaked for one week in lactophenol blue, and rinsed with water. Six representative subsamples from each composite were mounted on microscope slides. All slides were permanently fixed in glycerin gel, covered with a cover slip, and sealed with fingernail polish.

Botanical composition of each composited digesta sample was determined by (1) randomly locating 25 microscope fields on each of six microscope slides, (2) identifying the center-most plant fragment in each field at 100x by comparing with plant reference slides, and (3) counting the number of square microns of coverage for each fragment. Relative percent composition of each plant species in the diet was calculated by summing the total number of square microns of coverage per plant species and dividing by the total number of square microns counted per digesta composite.

Key forages that were identified in 1990-1991 digesta samples were hand-collected a year later from each habitat replication during early (early-June or late-December) and late (mid-August or mid-March) growth stages within each season (summer and winter, respectively). Early and late growth stages did not necessarily represent immature and mature phenological stages of plants, but merely represented the phenological stage available at the beginning and end of a season (summer, winter). Herbaceous vegetation was clipped to ground level; bark and leaves of woody material were collected at a height accessible to cottontail rabbits. Juniper berries were collected from the ground because these were generally the only ones available to cottontail rabbits. Forage samples were comprised of a minimum of 15 plants per species collected randomly from each habitat replicate. Herbaceous forages collected during summer and winter were actively growing when collected. Collected forages were sorted to remove dead material and foreign debris, frozen, lyophilized to constant mass, and ground through a 20-mesh screen. Lipids were extracted from ground forage by using a Soxhlet apparatus and diethyl ether as a solvent (Williams, 1984).

Protein quality was assessed by measuring concentrations of individual essential amino acids in forage samples. Fat-extracted samples amounting to approximately 40 mg of protein were weighed into 25- x 150-mm glass tubes with teflon-lined screw caps and hydrolyzed in 15 ml 6 N HCl at 110°C for 24 hrs. One ml of the hydrolyzed sample was filtered through a 0.45-um syringe filter (Acrodisc CRPTF, Fisher Scientific, Plano, TX). An internal standard (25 ul 4.0 nm methionine sulfone in 0.1 N HCL) was added to 75 ul of filtered hydrolysate before derivatization. Pre-column derivatization of amino acids was accomplished using phenylisothiocynate to produce phenylthiocarbamyl amino acids (Pico-Tag Work Station, Millipore Corporation, Milford, MA) and refiltered through a 0.45-um syringe filter. Concentrations of 17 individual amino acids (11 are thought to be essential to cottontail rabbits) were determined in derivatized samples by using high pressure liquid chromatography (HPLC, Waters Model 820 System Controller and Model 501 Pumps, Millipore Corporation, Milford, MA). Concentrations obtained for methionine and cystine were combined as were phenylalanine and tyrosine, because it is known that cystine and tyrosine spare their respective amino acid nutritionally (Lebas, 1988). Tryptophan was not measured due to destruction by acid hydrolysis (Gehrke et al., 1985; Sarwar et al., 1983). Chromatographic conditions were the following: Waters Pico-Tag Silica/C18 (15 cm x 3.9 mm) column; column temperature of 38°C; flow rate of 1.0 ml/min with pump back pressures of 5500 PSI; system sensitivity of 489 mv/sec (recorder) and 0.5 absorbance units full scale set at 254 nm (Waters Model 484 UV Detector); injection volume 4 ul; and run time of 27.5 min. Eluent A and eluent B (catalog numbers 88108 and 88112, respectively, Millipore Corporation) were used as solvents. Solvent conditions and gradients used for separation of amino acids were those described by Cohen et al. (1988). A casein reference protein (from bovine milk, no. C-0376, Sigma Chemical Co., St. Louis, MO) of known amino acid composition was hydrolyzed and analyzed with forage samples for determination of amino acid recovery efficiency. Sulfur-containing amino acid loss during acid hydrolysis averaged 25%, therefore methionine + cystine concentrations recovered were adjusted upward 25%. Amino acid concentrations were recorded on a dry mass basis (mg/g dry mass).

Non-extracted forage samples were analyzed for nitrogen content with a Labconco Rapid Kjeldahl System (Labconco

Corp., Kansas City, MO) (Williams, 1984). Percent nitrogen values were multiplied by 6.25 to estimate crude protein content. Non-protein nitrogen concentrations in forages were a measure of the difference between amino acid nitrogen (HPLC analysis) and the total nitrogen (Kjeldahl analysis) pools. Non-protein nitrogen was assumed to be all nitrogen not incorporated into one of the amino acids recovered (HPLC analysis) and was made up of a diverse group of compounds (Holt and Sosulski, 1981; Maynard et al., 1979; Oka and Sasaoka, 1985; Singh and Jambunathan, 1981). Unlike other measures of non-protein nitrogen (Holt and Sosulski, 1981), we did not include free amino acids (those amino acids not bound in protein) that were recovered during HPLC analysis. Crude protein estimates were corrected (corrected crude protein) for non-protein nitrogen concentrations.

Diet quality

Protein quality of seasonal diets was reconstructed from food habits data. Concentrations of each essential amino acid in the diet from each habitat type was calculated as the sum of the products of the relative contribution of each identified forage item in the diet and its corresponding seasonal average (early, late) amino acid concentration. Seventy-five percent of the diets contained between 2% and 19% unidentified plant material that could not be specifically factored into profiles of diet quality, therefore, diet profiles were calculated with identifiied forages equalling 100% of the diet. Published nutrient requirements of domestic rabbits were compared to estimated dietary levels to evaluate the nutritional adequacy of seasonal diets for cottontail rabbits.

The overall quality of a protein in the diet depends entirely upon how well it meets essential amino acid requirements of the individual animal. Many empirical techniques have been developed to evaluate the quality or biological value of dietary proteins based upon their essential amino acid composition. We modified the chemical score technique (Mitchell and Block, 1946) to compare the most limiting essential amino acid in each diet against growth requirements instead of whole egg protein. Chemical scores have been demonstrated to have an inverse relationship with the biological value of a protein source (Mitchell and Block, 1946). Biological value of dietary protein was determined by using a modification of the techniques described by Oser (1959). Briefly, we first calculating an essential amino acid index by comparing the concentrations of essential amino acids in each dietary protein to rabbit growth requirements, instead of their respective amounts in a reference protein.

Data analysis

Differences in nutrient concentrations (including amino acids) of key forages between early and late growth stages were tested by one-way analysis of variance (Proc ANOVA, SAS, 1988) by season. Differences in nutrient concentrations between habitat types were examined by one-way ANOVA for those forages occurring in diets of rabbits from both disturbed and undisturbed areas. A few species that occurred in small amounts in the diet were difficult to locate on our study areas during sample collections; in these cases, samples from both early and late collections were composited by equal mass into one sample for nutrient analyses. Season and habitat type differences in overall quality of proteins in diets were evaluated qualitatively by comparing calculated biological value estimates and extent of essential amino acid deficiencies. All statistical comparisons were considered significant at $\underline{P} < 0.05$.

RESULTS

Forage quality

Differences in concentrations of either crude protein, non-protein nitrogen, or fat between early and late growth stages were observed in only 7 of 17 forages collected, indicating gross nutritional conditions remained largely unchanged within a season (Table 1). No ubiquitous trends in phenological alterations of gross nutrient composition of plant species were evident from our comparisons between early and late growth stages. In general, alterations occurred more frequently on undisturbed than disturbed habitats. With summer maturity, concentrations of crude protein decreased significantly in *S. nutans* (32% decline) and *S. asper* (23% decline) on undisturbed habitats and *A. gerardii* (27% decline) on disturbed habitats (Fig. 1). Non-protein nitrogen concentrations in *P. oligosanthes* from undisturbed habitats significantly increased 36% with summer maturity, but levels declined 19% on disturbed habitats in winter (Table 1). Concentrations of non-protein nitrogen significantly declined 24% in _Antennaria_ spp. from undisturbed habitats in winter. Fat content of _Carex_ spp. significantly declined 13% but increased 73% in _P_. _oligosanthes_ with maturity during winter on undisturbed habitats (Table 1).

During summer, most of the collected forages used by rabbits showed phenological changes in only ≤ 2 essential amino acids, except _S_. _nutans_ which varied considerably in composition (Table 2). Essential amino acid concentrations of _S_. _nutans_ from undisturbed habitats significantly declined an average of 48% with maturity (Fig. 1). Changes in summer occurred most frequently in histidine concentration (4 of 8 species) (Table 2). _Andropogon_ _gerardii_ from disturbed habitats demonstrated significant declines in methionine + cystine (55%) and histidine (31%), which exceeded declines in its crude protein content (Table 2, Fig. 1). On undisturbed habitats histidine concentrations were observed to significantly decline 42% in _S_. _asper_ and 33% in _P_. _oligosanthes_ during summer. Phenological changes in essential amino acid concentrations were not observed during summer for _Cocculus_ _carolinus_, _Croton_ spp., and _Paspalum_ _setaceum_. Methionine + cystine, and to some degree histidine, were consistently the least concentrated of all essential amino acids in forages examined during summer.

Unlike summer, concentrations of essential amino acids tended to remain stable or increase with forage maturity during winter (Table 3). Arginine (60% increase) and threonine (70% increase) concentrations significantly increased in *Poa* spp. with maturity on undisturbed habitats (Fig. 2). Histidine and lysine significantly increased 124% and 62% in *Antennaria* spp., and 119% and 74%, respectively in *Carex* spp. with maturity on undisturbed habitats during winter. *Antennaria* spp. on disturbed habitats had significant increases of 75% for arginine and 95% for lysine during the winter growing season. No differences in concentrations of essential amino acids were observed during winter for woody plant forages. In winter, histidine was the least concentrated essential amino acid in grasses; methionine + cystine remained the least concentrated essential amino acid for forbs, browse, and berries (Table 3).

For the 10 forages occurring in diets of rabbits from both disturbed and undisturbed habitats, only 3 showed significant differences between habitats. During summer, concentrations of arginine and phenylalanine + tyrosine in *S. asper* averaged 60% and 49% greater on undisturbed than disturbed habitats. Concentration of phenylalanine + tyrosine in *Carex* spp. was 46% greater, and concentration of histidine in *Bromus* spp. was 59% greater on disturbed than undisturbed habitats in winter (Table 3). Habitat disturbance had no significant influence on concentrations of crude protein, non-protein nitrogen, and fat in any forage.

Diet composition and quality

Cottontail rabbit diets were dominated by *P. oligosanthes*, *Croton* spp., and *S. asper* in summer and *P. oligosanthes*, *Bromus* spp., and *Antennaria* spp. in winter (Table 4). Monocots comprised approximately 65% of the identified forages in diets in summer and 72% in winter. In summer, monocots made up 67% of the diet in rabbits from disturbed habitats, and 63% in rabbits from undisturbed habitats. Monocots were found in higher quantities in rabbit diets from disturbed (78%) than undisturbed (66%) habitats during winter. Dicots (forbs) and woody browse (leaf, bark, berries) collectively comprised 32% of the annual diet. Woody plants were not included in summer diets of rabbits but contributed approximately 13% to winter diets. Dicots contributed a larger portion to diets consumed by cottontail rabbits during summer than winter (33% of diets from disturbed and 37% of diets from undisturbed habitats during summer compared to 14% of diets from disturbed and 16% of diets from undisturbed habitats during winter). Forages in diets from disturbed and undisturbed habitats differed to a greater degree in summer than winter (5 of 8 forage species differed between diets in summer compared to 1 of 9 forage species differing between diets in winter).

Diets of cottontail rabbits from both disturbed and undisturbed habitats failed to completely meet their essential amino acid requirements for growth and lactation (Table 5). No essential amino acid met estimated requirements in summer, regardless of habitat type. Compared to growth and lactation requirements, methionine + cystine deficiencies were as high as 92% in summer and 70% in winter, making sulfur-containing amino acids the most limiting group of amino acids in cottontail rabbit diets. Methionine + cystine deficiencies in diets were equally severe on both disturbed and undisturbed habitats during summer. In winter, disturbed habitats satisfied 10% more of the dietary requirement for sulfur-containing amino acids than undisturbed habitats.

Histidine was the second most limiting amino acid, with dietary deficiencies for growth estimated at 33% and 77% on disturbed habitats and 53% and 70% on undisturbed habitats in winter and summer, respectively. All essential amino acid concentrations (except methionine + cystine) in the diet of rabbits from disturbed habitats were lower than those from undisturbed habitats in summer. Crude protein levels were 13% lower while essential amino acid concentrations averaged 16% lower on disturbed than undisturbed habitats in summer. The reverse was true for winter diets; diets of cottontails from disturbed habitats contained 23% more crude protein and averaged 36% higher in concentrations of essential amino acids than those from undisturbed habitats.

Similar to individual forages, estimated non-protein nitrogen content of cottontail rabbit diets was high, averaging 31% of the total nitrogen pool as determined by Kjeldahl analysis (Table 5). Correcting for non-protein nitrogen, diets were estimated to contain an average of 4.9% protein in summer and 10.2% protein in winter (Table 5). Calculated biological value of dietary proteins (based on essential amino acid composition) was 29% higher on undisturbed than disturbed habitats in summer. However, both calculated biological value and chemical score reflected extremely poor quality protein sources in the diets of rabbits from both habitats in summer. Diets of rabbits from disturbed habitats had biological values 43% higher than those from undisturbed habitats in winter. Biological value of winter diets was 163% higher than summer diets.

DISCUSSION

Diets of cottontail rabbits were dominated by grasses in both summer and winter, with variable amounts of herbaceous forbs, browse, and cedar berries. Botanical composition resembled previously reported diets for this species in other locations (Korschgen, 1980). Use of browse and cedar berries by cottontail rabbits in winter suggests an inadequacy of herbaceous vegetation in meeting winter dietary requirements; browse and berries are generally thought to be eaten during periods of food stress and high energy demands (Korschgen, 1980). The seasonal dietary shift from summer to winter was characterized by replacement of warm-season grasses with cool-season grasses in winter.

Within seasons, forage quality did not change appreciably, indicating that plants matured by early summer or remained in a dormant, immature state throughout winter. This suggests that diet quality of rabbits in upland hardwood forest-tallgrass prairie remains largely unchanged over much of the summer and winter. More frequent and dramatic temporal changes in diet quality may be associated with seasons (spring and fall) when many plants are actively growing or producing seed.

Summer diets dominated by warm-season grasses can present nutritional problems because grasses generally contain less high quality protein than forbs and decline in quality more rapidly with maturity than other forage classes (Bailey, 1969; Bogle et al., 1989). Young cottontails show a distinct preference for higher quality forbs compared to grasses (Bailey and Siglin 1966). Bogle et al. (1989) found that digestibility remained high in forbs throughout summer but not in grasses. Nutritionally, rabbits are able to efficiently utilize protein in fibrous forages (Crampton and Forshaw, 1940; Miller et al., 1954; NRC, 1977; Schurg et al., 1977; Voris et al., 1940), but growth and survival are impaired when crude protein and digestibility are both low

(Bailey, 1969). Considering nutritional needs for growth and high preference for forbs, Chapman et al. (1982) postulated that an abundant source of amino acids, particularly sulfur-containing amino acids, is critical to juvenile survival. Although Bailey (1969) reported that cottontail survival on diets of warm-season grasses was poor, cool-season grasses in our study contained much higher quality proteins and were probably adequate for meeting maintenance requirements of adults in winter. The lower protein quality of mature summer forages compared to immature winter forages were the probable result of a dilutional effect from accumulating cell wall material like lignin, cellulose, and hemicellulose (Lindroth et al., 1986).

High levels of non-protein nitrogen constituents in the total nitrogen pool of most forages exemplifies the need for exercising caution when interpreting crude protein values due to their tendency to exaggerate available protein for rabbit nutrition. Non-protein nitrogen in many plants is a result of the plant storing nitrogen in the form of toxic metabolites for protection against herbivory; this nitrogen is redistributed as amino acids when required for growth (Mooney et al., 1983). Phenological changes in non-protein nitrogen concentrations were not apparent in 14 of 17 forages we examined. Exceptions included _P_. _oligosanthes_ from undisturbed sites in summer, which increased non-protein nitrogen concentrations with maturity, and _P_.

oligosanthes from disturbed sites and Antennaria spp. from undisturbed sites in winter, which showed decreased concentrations of non-protein nitrogen with maturity. Decreases in the non-protein nitrogen component in P. oligosanthes and Antennaria spp. corresponded with increases in concentrations of certain amino acid constituents, especially lysine, supporting Mooney et al. (1983) suggestion that some forages may store nitrogen for later plant growth.

Rabbits, unlike ruminants, cannot efficiently utilize non-protein nitrogen in forages (Lebas, 1988); therefore, a more accurate and useful measure of protein quality would be one that corrects Kjeldahl nitrogen values for the non-protein nitrogen component of forage or directly measures the amino acid content. Crude protein estimates (determined by multiplying Kjeldahl nitrogen values by a coefficient of 6.25) overestimated available forage protein an average of 52% (range 30%-81%). As a result, a coefficient of 4.25 for converting Kjeldahl nitrogen to protein would provide a more accurate estimate of forage protein available for rabbit nutrition.

Another important nutritional consideration in the overall quality of proteins for growth and reproduction is diet digestibility. Snyder et al. (1976) estimated that cottontail rabbits require 12% digestible protein for growth. All forages in the summer diet of rabbits had both crude and corrected crude protein levels well below 12%. In contrast, winter cool-season grasses contained crude and corrected crude protein levels at or above the growth requirement before correcting for digestibility. Protein digestibility of commonly fed forages in commercial rabbit operations average 64% (NRC, 1977). Dry matter digestibility of forages used by cottontails has been shown to be extremely variable among seasons and species (grasses 35-60%, legumes 61-89%, wild forbs 60-84%; Bailey, 1969), but is typically lowest in summer. Digestibility considerations indicate that summer forages and corresponding reconstructed diets were extremely deficient in all essential amino acids for all life processes. Although there is a paucity of digestibility information for winter forages used by rabbits, winter forages and diets were probably adequate with respect to most essential amino acids for maintenance and growth on disturbed habitats; exceptions included histidine and the sulfur-containing amino acids. Winter diets of rabbits from undisturbed habitats probably provided most essential amino acids for adult maintenance. Essential amino acid requirements for adult maintenance in the rabbit have not been published, but digestible protein requirements are 25% lower than those for growth (NRC, 1977).

Given the essential amino acid content of diets failed to meet requirements for growth and lactation, a paradoxical dilemma arises concerning how cottontail rabbits can reproduce and develop on natural forages of low biological value. Bailey (1969) clearly demonstrated the inability of young cottontails to survive on grass-dominated diets, especially in summer. Sinclair et al. (1982) proposed three alternative responses an individual may make to low diet quality: (1) maintain a constant rate of food intake by including more low quality forage; (2) increase the quantity eaten to compensate for the low quality of forage; or (3) selectively eat only high quality plants or plant parts, and decrease overall quantity eaten. The second option is widely thought to be a universal optimal foraging strategy of animals. However, a review of the literature on consumption rates in hares and rabbits suggests that intake of cottontail rabbits declines with declining protein quality of diets (Adamson and Fisher, 1971; Bookhout, 1965; Cheeke, 1971; Gaman, 1970; McWard et al., 1967; Sinclair et al., 1982). Snowshoe hares studied by Sinclair et al. (1982) showed no consistent decline in rate of intake as crude protein levels declined, but no animal studied increased intake with declining crude protein content.

Cottontail rabbits may adopt the first alternative above and maintain a constant consumption rate with seasonal declines in diet quality, and limit reproduction to seasons when adequate essential amino acid resources become available, as suggested by Ecke (1955). Reproductive status of adult rabbits harvested on our study area indicated that 41% of females (73 of 177) and 28% of males (40 of 144) were not reproductively active during July. Cottontail rabbits may compensate for periods of dietary protein deficiencies by storing protein in the form of muscle mass during periods of good nutrition and catabolizing protein reserves for critical amino acids during periods of nutritional stress. The evidence in support of such a strategy is limited; however, cottontail rabbit nutritional indices are known to peak one season prior to a period of nutritional stress (Chapman et al., 1977). Low maintenance requirements of adults permit them to endure periods of nutritional stress better than young, as indicated by greater juvenile mortality in lagomorphs when diet availability or quality is low (Gibb, 1977). Under the first and third foraging strategies of Sinclair et al. (1982), the nutritional status of cottontail rabbits could be expected to decline, leading to reductions in physical condition and reproductive activity. The existence of chronic nutritional deficiencies on our study site was suggested by observed reductions in population densities in recent years (Lochmiller et al., 1991).

On technical grounds, it remains likely that cottontail rabbits have the ability to forage more selectively than we could simulate via hand-collected plants, although, not to the extent necessary to compensate all essential amino acid deficiencies observed. Even though we exercised care in collecting and sorting (removal of dead stems and leaves) each forage sample for analysis, rabbits are thought to be efficient in selecting particularly nutritious plant parts

(Allen, 1984; Chapman et al., 1982). A second technical, but unavoidable problem was that forages were hand-collected the year after they were identified as important food items in the diet. Diets consumed by cottontail rabbits probably change from year to year in response to availability and quality of vegetation (Chapman et al., 1982). Even with some technical error in sampling, it remains clear that certain essential amino acid deficiencies are common in winter and summer forages and diets from both disturbed and undisturbed habitats. Our data support the proposition of Chapman et al. (1982) that availability of sulfur-containing amino acids may be a critical determinant of juvenile survival. Our results and similar studies with other herbivores (Krapu and Swanson, 1975; Oftedal, 1991; *Sedinger, 1984;* Steen et al., 1988; Thomas and Prevett, 1980) reinforces the need to reconsider the importance of protein quality in regulating the dynamics of animal populations.

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Table 1.--Concentrations (mean ± SE) of fat, crude protein and non-protein nitrogen in selected forages in summer and winter diets of cottontail rabbits (*Sylvilagus floridanus*) from upland hardwood forest-tallgrass prairie habitat. Correction factors for converting Kjeldahl nitrogen values to protein (corrected crude protein) are also presented for each forage.

Forage	n	Fat (% DW)	Crude protein (% DW)	Nonprotein nitrogen (% of total N)	Corrected crude protein (% DW)	Correction factor
Summer						
Andropogon gerardii^{a}	4	2.4 ± 0.17	4.9 ± 0.45^{b}	37.2 ± 4.71	3.1 ± 0.42	3.96
Cocculus carolinus^{a}	4	2.1 ± 0.23	9.1 ± 0.83	40.7 ± 0.20	5.4 ± 0.47	3.70
Croton spp.	8	3.8 ± 0.01	9.1 ± 0.49	27.5 ± 3.22	6.6 ± 0.57	4.56
Sporobolus asper	8	1.9 ± 0.14	4.5 ± 0.33^{c}	35.5 ± 1.75	2.9 ± 0.26^{d}	4.06
Panicum oligosanthes	8	4.5 ± 0.25	6.8 ± 0.35	31.4 ± 2.82^{c}	4.7 ± 0.37	4.32
Paspalum setaceum^{a}	4	2.2 ± 0.16	6.1 ± 0.50	44.7 ± 1.06	3.4 ± 0.34	4.82
Sorghastrum nutans^{e}	4	2.6 ± 0.28	4.9 ± 0.56^{c}	27.0 ± 5.23	3.7 ± 0.59^{c}	4.63
Plantago spp.^{fe}	2	3.6 ± 0.45	7.7 ± 1.40	31.4 ± 0.47	5.3 ± 0.92	4.29
Winter						
Poa spp.	8	3.4 ± 0.80	18.6 ± 1.58	25.4 ± 3.05	13.9 ± 1.29	4.65
Bromus spp.	8	3.4 ± 0.24	19.3 ± 1.24	23.3 ± 2.17	14.9 ± 1.25	4.82
Panicum oligosanthes	8	2.2 ± 0.22^{c}	17.5 ± 1.01	34.1 ± 1.67^{b}	11.5 ± 0.65	4.10
Antennaria spp	8	2.4 ± 0.34	10.6 ± 0.67	32.3 ± 3.72^{c}	7.3 ± 0.80	4.31
Carex spp	8	2.0 ± 0.09^{c}	14.8 ± 1.01	28.2 ± 2.79	10.6 ± 0.79	4.49
Juniperus virginiana						
berry^{f}	4	14.8 ± 1.25	5.9 ± 0.34	40.1 ± 4.26	3.5 ± 0.24	3.72
leaf^{f}	4	10.0 ± 0.60	7.9 ± 0.11	33.4 ± 2.22	5.3 ± 0.24	4.17

Table 1.--(cont., pg 2).

bark[fe]	2	10.6 ± 0.75	4.7 ± 1.05	48.0 ± 3.88	2.6 ± 0.73	3.29
Rhus copallina						
bark[f]	4	8.8 ± 0.59	4.8 ± 0.13	29.9 ± 2.43	3.1 ± 0.10	4.37

[a] Collected from disturbed habitat only.

[b] Protein or non-protein nitrogen content of this species differed ($P < 0.05$) between collection periods on disturbed habitat.

[c] Protein, fat, or non-protein nitrogen content of this species differed ($P < 0.05$) between collection periods on undisturbed habitat.

[d] Corrected crude protein content of this species differed ($P < 0.05$) between disturbed and undisturbed habitat types.

[e] Collected from undisturbed habitat only.

[f] Early and late growth stages were composited prior to analysis.

Table 2.--Concentrations (% dry mass) of amino acids in selected forages consumed by cottontail rabbits (Sylvilagus floridanus) in summer. Values represent mean ± SE concentrations for plant samples collected during early and late growth stages from two disturbed and two undisturbed upland hardwood forest-tallgrass prairie habitats.

Amino acid	*Andropogon gerardii*[a] (*n*=4)	*Cocculus carolinus*[a] (*n*=4)	*Croton* spp. (*n*=8)	*Sporobolus asper* (*n*=8)	*Panicum oligosanthes* (*n*=8)	*Paspalum setaceum*[a] (*n*=4)	*Sorghastrum nutans*[b] (*n*=4)	*Plantago* spp.[bc] (*n*=2)
Essential amino acid								
Histidine	0.04 ± 0.01[d]	0.12 ± 0.02	0.11 ± 0.01	0.04 ± 0.01[e]	0.07 ± 0.01[e]	0.05 ± 0.01	0.05 ± 0.01[e]	0.11 ± 0.03
Arginine	0.21 ± 0.03	0.38 ± 0.04	0.49 ± 0.04	0.19 ± 0.02[f]	0.31 ± 0.03	0.23 ± 0.02	0.23 ± 0.05[e]	0.32 ± 0.07
Threonine	0.14 ± 0.01	0.22 ± 0.02	0.31 ± 0.03	0.14 ± 0.02	0.23 ± 0.02	0.14 ± 0.03	0.18 ± 0.02[e]	0.29 ± 0.05
Valine	0.28 ± 0.04	0.44 ± 0.03	0.54 ± 0.05	0.26 ± 0.02	0.39 ± 0.03	0.29 ± 0.03	0.32 ± 0.06[e]	0.31 ± 0.03
Methionine + cystine	0.04 ± 0.01[d]	0.04 ± 0.01	0.05 ± 0.01	0.03 ± 0.01	0.05 ± 0.01	0.04 ± 0.01	0.04 ± 0.01	0.06 ± 0.03
Isoleucine	0.21 ± 0.03	0.38 ± 0.03	0.47 ± 0.04	0.20 ± 0.02	0.31 ± 0.03	0.22 ± 0.03	0.25 ± 0.05[e]	0.24 ± 0.02
Leucine	0.38 ± 0.06	0.62 ± 0.04	0.76 ± 0.08	0.34 ± 0.03	0.52 ± 0.04	0.39 ± 0.05	0.43 ± 0.08[e]	0.57 ± 0.08
Phenylalanine + tyrosine	0.34 ± 0.06	0.63 ± 0.05	0.72 ± 0.08	0.28 ± 0.03[f]	0.47 ± 0.04	0.35 ± 0.05	0.35 ± 0.07[e]	0.73 ± 0.14
Lysine	0.28 ± 0.03	0.55 ± 0.05	0.61 ± 0.07	0.25 ± 0.02	0.41 ± 0.03	0.33 ± 0.02	0.33 ± 0.04[e]	0.53 ± 0.08
Non-essential amino acid								
Aspartic acid	0.38 ± 0.05	0.67 ± 0.09	0.75 ± 0.07	0.37 ± 0.04[f]	0.56 ± 0.04	0.40 ± 0.04	0.44 ± 0.07[e]	0.58 ± 0.16
Glutamic acid	0.47 ± 0.06	0.72 ± 0.06	1.01 ± 0.09	0.44 ± 0.04	0.75 ± 0.07	0.51 ± 0.05	0.56 ± 0.10[e]	0.72 ± 0.16
Serine	0.09 ± 0.03	0.14 ± 0.03	0.27 ± 0.03	0.10 ± 0.02	0.21 ± 0.02	0.07 ± 0.04	0.16 ± 0.02	0.29 ± 0.10
Glycine	0.24 ± 0.04	0.38 ± 0.03	0.47 ± 0.04	0.22 ± 0.02	0.35 ± 0.03	0.26 ± 0.03	0.27 ± 0.05[e]	0.32 ± 0.05
Alanine	0.30 ± 0.04	0.42 ± 0.02	0.52 ± 0.05	0.29 ± 0.02[f]	0.43 ± 0.03	0.32 ± 0.03	0.34 ± 0.05[e]	0.41 ± 0.06
Proline	0.23 ± 0.03	0.40 ± 0.02	0.42 ± 0.04	0.21 ± 0.02[f]	0.34 ± 0.03	0.26 ± 0.02	0.24 ± 0.04[e]	0.49 ± 0.03

Table 2.--(cont., pg 2).

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[a] Collected from disturbed habitat only.

[b] Collected from undisturbed habitat only.

[c] Early and late growth stages were composited prior to analysis.

[d] Amino acid content of this species differed ($P < 0.05$) between collection periods on disturbed habitat.

[e] Amino acid content of this species differed ($P < 0.05$) between collection periods on undisturbed habitat.

[f] Amino acid content of this species differed ($P < 0.05$) between disturbed and undisturbed habitat types.

Table 3.--Concentrations (% dry mass) of amino acids in selected forages consumed by cottontail rabbits (*Sylvilagus floridanus*) in winter. Values represent mean ± SE concentrations of plant samples collected during early and late growth stages from two disturbed and two undisturbed upland hardwood forest-tallgrass prairie habitats.

Amino acid	*Poa* spp. (*n*=8)	*Bromus* spp (*n*=8)	*Panicum oligosanthes* (*n*=8)	*Antennaria* spp. (*n*=8)	*Carex* spp. (*n*=8)	*Rhus copallina* bark[a] (*n*=4)	*Juniperus virginiana* berry[a] (*n*=4)	*Juniperus virginiana* leaf[a] (*n*=4)	*Juniperus virginiana* bark[ab] (*n*=4)
Essential amino acid									
Histidine	0.18 ± 0.02	0.23 ± 0.03[c]	0.18 ± 0.02	0.12 ± 0.02[d]	0.16 ± 0.02[d]	0.04 ± 0.01	0.04 ± 0.01	0.07 ± 0.01	0.03 ± 0.01
Arginine	1.04 ± 0.10[d]	1.11 ± 0.10	0.73 ± 0.08	0.46 ± 0.05[e]	0.76 ± 0.06	0.22 ± 0.02	0.37 ± 0.03	0.40 ± 0.02	0.13 ± 0.05
Threonine	0.76 ± 0.07[d]	0.83 ± 0.08	0.64 ± 0.03	0.44 ± 0.05	0.62 ± 0.05	0.15 ± 0.01	0.16 ± 0.02	0.29 ± 0.01	0.15 ± 0.06
Valine	1.06 ± 0.09	1.11 ± 0.08	0.73 ± 0.04	0.52 ± 0.06	0.71 ± 0.07	0.24 ± 0.01	0.20 ± 0.03	0.37 ± 0.02	0.17 ± 0.06
Methionine + cystine	0.30 ± 0.02	0.34 ± 0.03	0.24 ± 0.01	0.10 ± 0.01	0.20 ± 0.01	0.01 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.03 ± 0.02
Isoleucine	0.84 ± 0.07	0.85 ± 0.05	0.56 ± 0.03	0.40 ± 0.05	0.59 ± 0.06	0.15 ± 0.01	0.15 ± 0.02	0.31 ± 0.01	0.11 ± 0.04
Leucine	1.48 ± 0.13	1.58 ± 0.12	1.12 ± 0.06	0.79 ± 0.09	1.13 ± 0.09	0.26 ± 0.01	0.30 ± 0.03	0.58 ± 0.02	0.27 ± 0.06
Phenylalanine + tyrosine	1.71 ± 0.16	1.76 ± 0.14	1.14 ± 0.08	0.81 ± 0.09	1.17 ± 0.11[c]	0.34 ± 0.02	0.37 ± 0.03	0.62 ± 0.02	0.29 ± 0.06
Lysine	1.25 ± 0.11	1.31 ± 0.13	1.10 ± 0.08	0.70 ± 0.09[de]	1.03 ± 0.08[d]	0.42 ± 0.03	0.26 ± 0.03	0.59 ± 0.04	0.24 ± 0.03
Non-essential amino acid									
Aspartic acid	1.56 ± 0.14	1.79 ± 0.18	1.71 ± 0.11	0.88 ± 0.10[d]	1.42 ± 0.14	0.28 ± 0.01	0.38 ± 0.02	0.52 ± 0.03	0.16 ± 0.06
Glutamic acid	2.08 ± 0.24	2.16 ± 0.20	2.13 ± 0.13	1.20 ± 0.14[d]	1.64 ± 0.11	0.42 ± 0.03	0.63 ± 0.04	0.71 ± 0.03	0.30 ± 0.12
Serine	0.66 ± 0.09	0.77 ± 0.09	0.69 ± 0.02	0.48 ± 0.04[d]	0.57 ± 0.04	0.19 ± 0.01	0.26 ± 0.02	0.28 ± 0.03	0.28 ± 0.10
Glycine	0.87 ± 0.08	0.99 ± 0.07	0.73 ± 0.02	0.50 ± 0.05	0.68 ± 0.05	0.16 ± 0.01	0.23 ± 0.02	0.33 ± 0.02	0.18 ± 0.06
Alanine	1.19 ± 0.11	1.24 ± 0.11	0.94 ± 0.05	0.56 ± 0.05	0.85 ± 0.06	0.19 ± 0.01	0.23 ± 0.02	0.41 ± 0.02	0.25 ± 0.07
Proline	0.87 ± 0.08	0.94 ± 0.08	0.75 ± 0.04	0.50 ± 0.04[d]	0.66 ± 0.05	0.30 ± 0.01	0.26 ± 0.02	0.36 ± 0.01	0.20 ± 0.05

Table 3.--(cont., pg 2).

---

[a] Early and late growth stages were composited prior to analysis.

[b] Collected from undisturbed habitat only.

[c] Amino acid content of this species differed ($\underline{P} < 0.05$) between disturbed and undisturbed habitat types.

[d] Amino acid content of this species differed ($\underline{P} < 0.05$) between collection periods on undisturbed habitat.

[e] Amino acid content of this species differed ($\underline{P} < 0.05$) between collection periods on disturbed habitat.

Table 4.--Percent contribution of forage items to the diet of cottontail rabbits (Sylvilagus floridanus) from disturbed and undisturbed upland hardwood forest-tallgrass prairie habitats during summer and winter.

	Summer		Winter	
Species	Disturbed habitat	Undisturbed habitat	Disturbed habitat	Undisturbed habitat
Monocots				
Andropogon gerardii	3.2	0	0	0
Sporobolus asper	16.0	3.7	0	0
Panicum oligosanthes	34.1	30.6	25.7	38.5
Paspalum setaceum	5.3	0	0	0
Sorghastrum nutans	0	7.0	0	0
Poa spp.	0	0	2.0	1.7
Bromus spp.	0	0	30.3	7.2
Carex spp.	0	0	5.1	16.8
Dicots				
Cocculus carolinus	6.8	0	0	0
Croton spp.	21.7	19.5	0	0
Plantago spp.	0	4.8	0	0
Antennaria spp.	0	0	11.8	15.2

Table 4.--(cont., pg 2)

Woody				
Juniperus virginiana				
berry	0	0	5.1	14.0
leaf	0	0	1.2	0.9
bark	0	0	0	2.2
Rhus copallina				
bark	0	0	0.3	1.0
Unknowns	12.9	34.4	18.5	2.5

Table 5.--Estimated nutritional quality of reconstructed diets consumed by cottontail rabbits (*Sylvilagus floridanus*) from upland hardwood forest-tallgrass prairie habitats, as influenced by habitat disturbance and season.

	Summer		Winter		Requirements[a]	
	Undisturbed habitat	Disturbed habitat	Undisturbed habitat	Disturbed habitat	Growth (NRC, 1977)	Lactation (INRA, 1984)
Crude protein	7.58	6.60	13.41	16.48	16.00	18.00
Non-protein nitrogen	28.93	34.45	34.68	27.80	0	0
Corrected crude protein	5.19	4.51	9.16	11.26	--	--
Fat	4.05	3.32	4.35	3.81	2	2
Histidine	0.09	0.07	0.14	0.20	0.30	0.43
Arginine	0.38	0.29	0.61	0.83	0.60	0.80
Threonine	0.25	0.22	0.50	0.69	0.60	0.70
Valine	0.41	0.36	0.60	0.83	0.70	0.85
Methionine + cystine	0.05	0.05	0.18	0.24	0.60	0.60
Isoleucine	0.38	0.30	0.46	0.63	0.60	0.70
Leucine	0.62	0.50	0.91	1.24	1.10	1.25
Phenylalanine + tyrosine	0.58	0.46	0.94	1.29	1.10	1.40
Lysine	0.49	0.41	0.83	1.10	0.65	0.90
EAAI[b]	43	36	74	102	--	--
CBV[c]	36	28	69	99	100	100
CCS[d]	92	92	70	60	0	0

Table 5.--(cont., pg 2).

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[a] Requirements are based on the assumption that a high quality diet is fed.

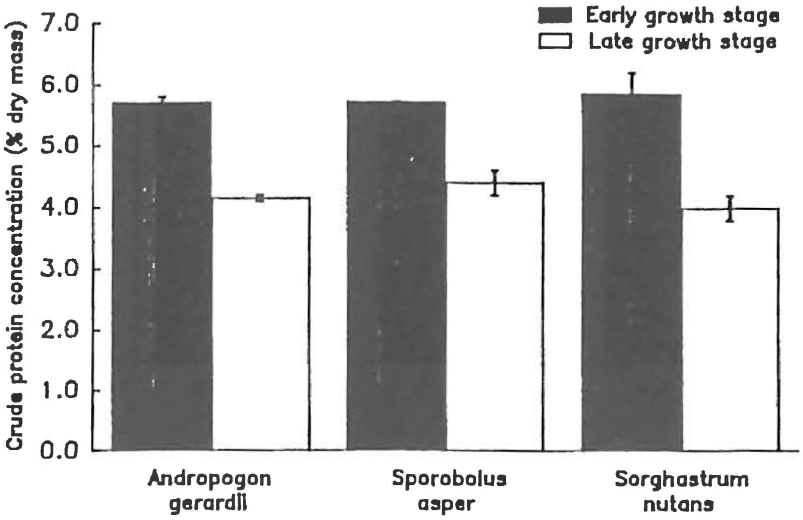
[b] $$\text{EAAI} = \sqrt[n]{\frac{\text{Arg}^p}{\text{Arg}^s} \times \frac{\text{His}^p}{\text{His}^s} \times \cdots \frac{\text{Val}^p}{\text{Val}^s}}$$ (Oser, 1959);

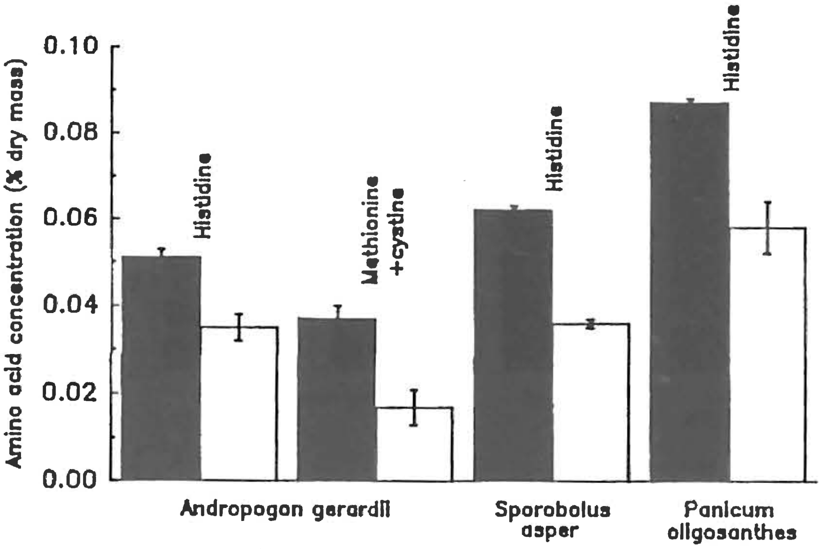
superscripts refer to dietary protein (p) and growth requirement (s); and n = 9 (histidine, arginine, threonine, valine, leucine, isoleucine, lysine, methionine + cystine, and phenylalanine + tyrosine were included in all computations).

[c] Computed biological value = 1.09 (EAAI) - 11.7 (Oser, 1959).

[d] Computed chemical score = the percent deficient of the most limiting essential amino acid for meeting growth requirements.

Figure 1.--Crude protein and essential amino acid concentrations of Andropogon gerardii from disturbed and Sporobolus asper and Sorghastrum nutans from undisturbed upland hardwood forest-tallgrass prairie habitats by growth stage (early, late) in summer. Also shown is histidine concentration of Panicum oligosanthes from undisturbed habitats by growth stage in summer. All concentrations shown varied significantly ($P < 0.05$) between early and late growth stage.


Early growth stage
Late growth stage
Crude protein concentration (% dry mass)
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
Andropogon gerardii
Sporobolus asper
Sorghastrum nutans


Amino acid concentration (% dry mass)
0.10
0.08
0.06
0.04
0.02
0.00
Histidine
Methionine +cystine
Histidine
Histidine
Andropogon gerardii
Sporobolus asper
Panicum oligosanthes

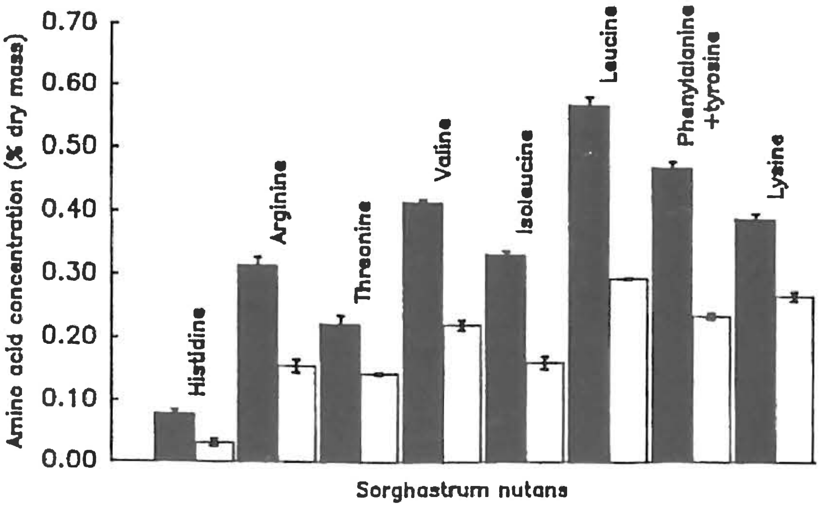
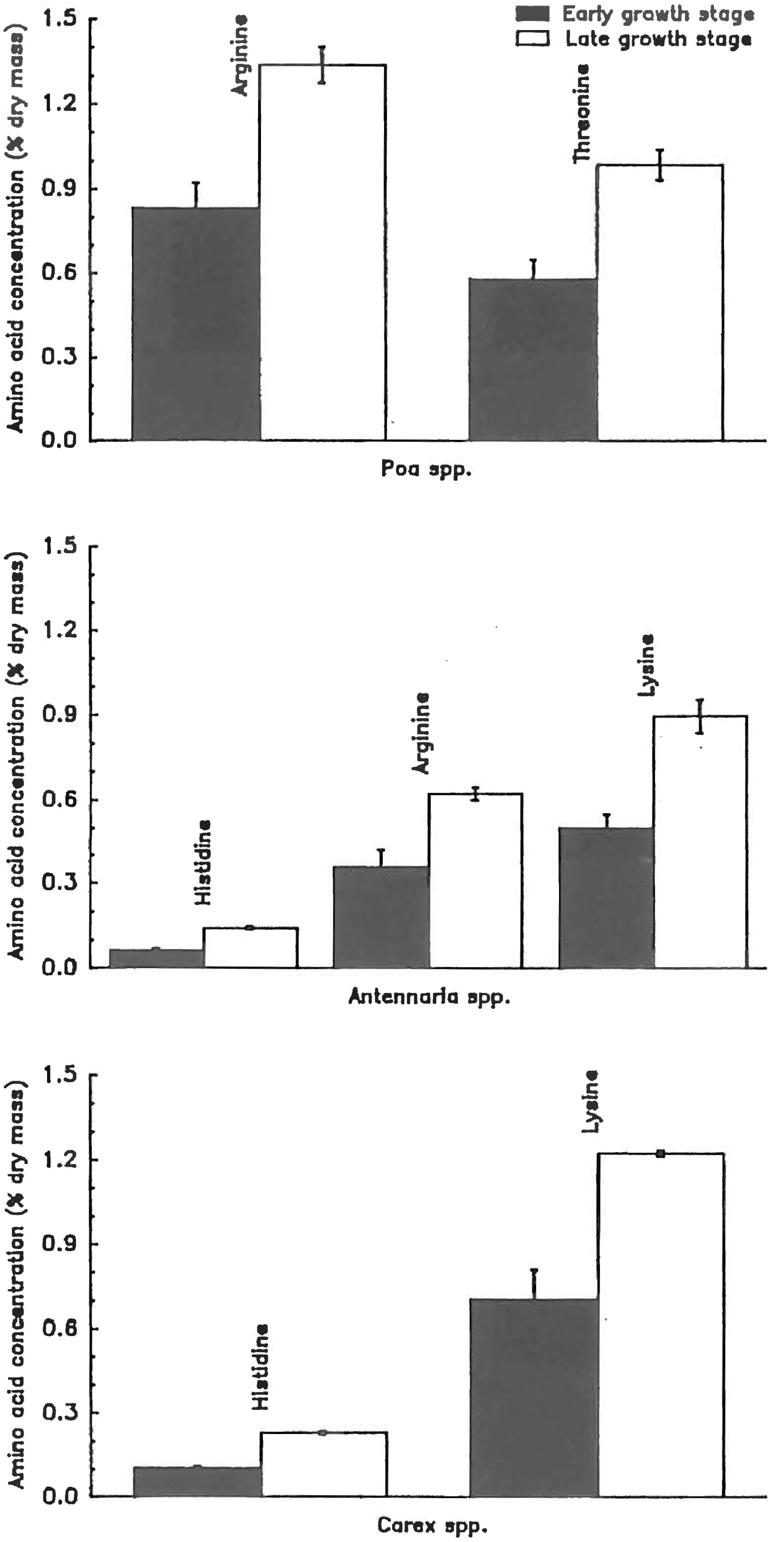

Amino acid concentration (% dry mass)
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
Histidine
Arginine
Threonine
Valine
Isoleucine
Leucine
Phenylalanine +tyrosine
Lysine
Sorghastrum nutans

Figure 2.--Essential amino acid concentrations of *Poa* spp., *Antennaria* spp., and *Carex* spp. from undisturbed upland hardwood forest-tallgrass prairie habitats by growth stage (early, late) in winter. Lysine concentrations for *Antennaria* spp. are average values for samples collected from both disturbed and undisturbed habitats, as concentrations of this essential amino acid varied by growth stage on both habitat types. All concentrations shown varied significantly ($P < 0.05$) between early and late growth stage.



▬ Early growth stage
☐ Late growth stage
Amino acid concentration (% dry mass)
1.5
1.2
0.9
0.6
0.3
0.0
Arginine
Threonine
Poa spp.
Amino acid concentration (% dry mass)
1.5
1.2
0.9
0.6
0.3
0.0
Histidine
Arginine
Lysine
Antennaria spp.
Amino acid concentration (% dry mass)
1.5
1.2
0.9
0.6
0.3
0.0
Histidine
Lysine
Carex spp.

CHAPTER V

INFLUENCE OF SPATIAL AND TEMPORAL CHANGES IN HABITAT QUALITY ON CONDITION OF COTTONTAIL RABBITS (*Sylvilagus floridanus*)

ABSTRACT--Although nutrition is thought to be an important factor in the regulation of cottontail rabbit (*Sylvilagus floridanus*) populations, experimental evidence for such a relationship is limited. We used discriminant analysis to investigate responses of overall physical condition of cottontail rabbits (*n* = 422 adults) to habitat quality and succession on replicated disturbed and undisturbed upland hardwood forest-tallgrass prairie over a 6-yr period. Five different disturbed habitat types were experimentally created using herbicides (tebuthiuron or triclopyr), fire, or a combination of both. Condition parameters that were important indicators of rabbit physical condition included indices of kidney fat and parasitism, and relative masses of spleen, liver, and dried stomach digesta. Rabbits harvested from disturbed habitats were in better physical condition than those from undisturbed habitats, but the type of habitat disturbance was not important. Positive effects of burning disturbed habitats were not apparent until later seral stages when monocots replaced herbaceous

dicots and vegetative composition resembled that of undisturbed areas.

INTRODUCTION

Although nutritional ecology of hares (Lepus spp.) has been well studied (Dunn et al. 1982; Bittner and Rongstad 1982), only limited experimental interest has been given to the nutritional ecology of cottontail rabbits (Sylvilagus floridanus). Experimental nutritional restrictions in the laboratory are known to result in suppressed growth (Bailey 1969; Snyder et al. 1976) and reproduction (Kirkpatrick and Baldwin 1974). However, similar experimental documentation in wild cottontail rabbit populations is limited to non-replicated comparisons between different habitats or correlated responses to climatic events, demonstrating differences in reproductive output (Bothma and Teer 1977; Chapman et al. 1977; Pelton and Provost 1972; Trethewey and Verts 1971), home range size (Anderson and Pelton 1976; Janes 1959; Trent and Rongstad 1974), foraging effort (Allen 1984; Anderson and Pelton 1976; Chapman et al. 1982; Janes 1959; Trent and Rongstad 1974), and physical condition (Bailey 1968).

Our recent work in an upland hardwood forest-tallgrass prairie suggests that habitat disturbance provides important resources for permitting cottontail rabbit populations to increase (Lochmiller et al. 1991). Disturbance effectively alters both cover and nutrient attributes of the habitat. Recent analyses of forage quality (Bogle et al. 1989) and availability (Engle et al. 1991a, 1991b; Stritzke et al. 1991a, 1991b) on our study area suggest that nutrient alterations are proximate resource factors that stimulate increases in population density following habitat disturbance (Lochmiller et al. 1991).

We hypothesized that improvements in the nutritional quality of forage resources on recently disturbed habitats facilitate an increase in overall physical condition of cottontail rabbits. Condition parameters have been widely used to assess responsiveness of lagomorphs to changes in diet quality. Length-weight ratios (Bailey 1968), fat reserves (Henke and Demarais 1990; Lord 1963; Warren and Kirkpatrick 1978), body mass (Bailey 1969; Snyder et al. 1976), and organ-glandular masses (McCreedy and Weeks 1992) have been used to assess changes in habitat quality, dietary intake, or forage quality. Because no single condition index appears universally robust for differentiating among nutritional subpopulations, the use of multivariate approaches that regress several nutritional indices at once has been recommended to improve classification accuracy of subpopulations (Hawley 1987; Hellgren et al. 1989; Jenks 1991; Lochmiller et al. 1985; Lochmiller et al. 1986; Watkins et al. 1991). Discriminant analysis of condition indices has been applied successfully in differentiating among diet groups, metabolic states, and habitat types in a variety of animal species (Hawley 1987; Hellgren et al. 1989; Jenks 1991). Our objective was to use a similar multivariate approach to evaluate the sensitivity of overall physical condition of cottontail rabbits to spatial and temporal alterations in habitat quality on replicated disturbed and undisturbed temperate upland hardwood forest-tallgrass prairie. We were particularly interested in physical condition of rabbits in response to post-disturbance secondary succession.

MATERIALS AND METHODS

Study area

We examined differences in physical condition of cottontail rabbits from replicated habitat types on an area located approximately 11 km southwest of Stillwater, Oklahoma (36°2′ to 36°4′ N, 97°9′ to 97°11′ W). Our study area encompassed upland hardwood forest dominated by blackjack oak (*Quercus marilandica*) and post oak (*Q. stellata*) intermixed with tallgrass prairie invaded by eastern redcedar (*Juniperus virginiana*). This area is representative of the cross timbers vegetation type (Ewing et al. 1984), which accounts for nearly 5 million ha of land in Oklahoma, Kansas, and Texas (Soil Conservation Service 1981). Our study area consisted of 24 32.4-ha (0.42 x 0.83-km) experimental units representing five distinct habitat types in various stages of secondary succession, and undisturbed control habitats. Experimental units were arranged in a randomized complete block design with four replications of each habitat type. The five disturbed habitat types were experimentally created using herbicides, prescribed fire, or a combination to initiate secondary succession. Experimental alterations included: 1) tebuthiuron or 2) triclopyr applied aerially at 2.2 kg ha^{-1} in 1983; 3) tebuthiuron or 4) triclopyr applied as above followed by late-spring burns in 1985, 1986, 1987 and 1990; and 5) late-spring burns only with no herbicide application (burned-controls). Undisturbed habitats received no herbicide or prescribed burning and represented the original upland hardwood forest-tallgrass prairie. All experimental units were moderately grazed by yearling cattle from early spring to fall with the goal of 50% utilization of annual forage production (Stritzke et al. 1991a).

Tebuthiuron caused an intense disturbance and resulted in near total removal of the woody overstory canopy, an effect which persisted throughout the study (Stritzke et al. 1991a, 1991b). Triclopyr was not as efficient as tebuthiuron in controlling resprouting after removal of the oak overstory. Thus, triclopyr habitats contained a mixture of woody species that were released after removal of the dominate oaks in the overstory. The oak overstory of triclopyr habitats was replaced by American elm (Ulmus americana) and eastern redcedar; the woody understory was dominated by buck brush (Symphoricarpus orbiculatus) (Stritzke et al. 1991a, 1991b). During the first several years after disturbance by herbicide, the understory of habitats was dominated by pioneer forbs and grasses, especially horseweed (Conyza canadensis) and pokeweed

(*Phytolacca americana*). Several years after disturbance the understory was dominated by a mixture of annual forbs and grasses, especially rosette panicgrass (*Panicum oligosanthes*), with increasing amounts of warm-season perennial grasses toward the end of the study (Engle et al. 1991a, 1991b). Burning had little affect on the woody plant communities, except to reduce eastern redcedar invasion on tebuthiuron-altered habitats starting in the 1988 burn year (Engle et al. 1991a, 1991b; Stritzke et al. 1991a, 1991b). However, burning tended to increase forbs in the herbaceous understory (Engle et al. 1991a, 1991b). In general, annual productivity of grasses and forbs increased greatly by disturbance compared to undisturbed areas and was greater in early seral (1987-1988) than late seral (1990-1992) communities (Fig. 1). Disturbance intensities of each habitat type could be ranked as: tebuthiuron plus burned > tebuthiuron > triclopyr plus burned > triclopyr > burned-control > undisturbed.

Data collection

A total of 422 adult cottontail rabbits (224 female, 198 male) was harvested from experimental habitat types in January and July from 1987 through 1992. Rabbits were not collected in 1989, January 1990, or July 1992 which resulted in eight sampling periods overall. Adults were individuals of >800 g body mass or lighter individuals in reproductive condition. Rabbits were only collected from burned-control habitats in January and July 1990-1992. An attempt was made during each sampling period to harvest five rabbits from each of two replicate habitat types, except summer 1990 when four replicates of each habitat type were sampled. All rabbits were harvested shortly after sunset with the aid of a spotlight. To minimize collecting rabbits whose home range encompassed two or more different experimental habitat types, all individuals were harvested at a distance >75 m interior to boundary fences. A previous mark-recapture study indicated that cottontail rabbit movement between habitat types was negligible (Lochmiller et al. 1991).

Gender, body mass, total length (from tip of snout to last caudal vertebrae), and hind-foot length were recorded. Carcasses were necropsied, and masses of spleen, liver, thymus, paired kidneys, paired adrenals, and kidney fat were determined. Intensity of parasitism was indexed by enumerating stomach worms (_Obeliscoides cuniculi_). Rabbits also were classified as nonreproductive, pregnant, lactating, nonscrotal, or scrotal. Stomach digesta was removed, and both a wet and dry (lyophilization to a constant mass) mass determined.

Data analysis

To standardize allometric parameters, relative mass of stomach digesta and organ-glandular tissue was calculated as a percent of whole body mass prior to analysis. Kidney fat was expressed as a percent of total paired kidney mass to derive a kidney fat index of nutritional condition (Riney 1951). Bailey’s (1968) index of physical condition was calculated for all individuals. Indices of parasitism and kidney fat, lengths, body mass, and relative masses of kidney, spleen, and thymus were rank transformed to normalize their distribution prior to statistical analysis (Conover and Iman 1981).

Influence of habitat type and vegetative succession on individual condition parameters was assessed using a two-way analysis of variance (ANOVA; PROC GLM; SAS Institute Inc. 1988) with habitat type and succession as main factors. Successional stages were defined as early seral (years 1987-1988) and late seral (years 1990-1992). A least significant difference test was performed when means for habitat type or successional stage were found to be significantly different ($\underline{P} < 0.05$). Single degree of freedom specific contrasts were used to identify by season, differences in condition parameters between two specific treatment categories (unburned herbicide-altered vs. burned herbicide-altered; burned vs. unburned; undisturbed vs disturbed; tebuthiuron-altered vs. triclopyr-altered).

Stepwise discriminant analysis (PROC STEPDISC, SAS Institute Inc. 1988) was used to select reduced sets of discriminator parameters from our overall data set that provided the best overall separation of individual populations among habitat types based on nutritional condition indices. Discriminant analysis (PROC DISCRIM, SAS Institute Inc. 1988) with reduced sets of discriminator parameters (determined by stepwise discriminant analysis) and a jackknife procedure were used to investigate overall condition of rabbits from different habitats and successional stages by season as described by Rice et al. (1983). Two-group comparisons were made for these analyses, with habitats compared as follows: unburned herbicide-altered vs. burned herbicide-altered, burned vs. unburned, undisturbed controls vs. disturbed, tebuthiuron-altered vs. triclopyr-altered, undisturbed controls vs burned-controls. Differences in overall classification accuracies between early and late seral stages for two-group comparisons were analyzed by season using a Z-test of probabilities. Overall comparisons of the effects of spatial and successional alterations in habitat quality on cottontail rabbit condition were investigated by season using a five-group comparison consisting of undisturbed habitats, early and late seral stages of herbicide-altered habitats, and early and late seral stages of burned herbicide-altered habitats. Canonical discriminant analysis on five-group comparisons was used to further determine relationships among the influences of spatial and successional alterations on condition parameters determined to be important indicators of rabbit condition.

RESULTS

No clear trends were evident from univariate comparisons of the 13 condition parameters that we measured in adult cottontail rabbits with respect to influences of habitat type and succession (Tables 1 and 2). During summer, body mass, total body length, and relative masses of spleen and thymus of cottontail rabbits differed ($\underline{P} < 0.05$) among habitat types. During winter, relative masses of liver, kidney, spleen, thymus, and dried stomach digesta differed ($\underline{P} < 0.05$) among habitat types. Relative masses of spleen and thymus were the only two condition parameters of rabbits to vary among habitat types in both summer and winter.

Successionally, the parasitism index and relative wet mass of stomach digesta were greater ($\underline{P} < 0.05$) in rabbits harvested from early seral stages than late seral stages during summer and winter (Tables 1 and 2). During summer, relative thymus mass and hind foot length of rabbits were greater, and total body length was less ($\underline{P} < 0.05$) in early than late seral stages. Total body length of rabbits in winter was greater and relative masses of thymus and liver and indices of physical condition and kidney fat were less ($\underline{P} < 0.05$) in early than late seral stages. Interactions between habitat type and succession ($\underline{P} < 0.05$) were observed for relative stomach digesta wet mass of rabbits harvested in both summer and winter, and relative masses of spleen, dried stomach digesta, liver, and kidney in winter. Least significant difference tests of means when main effects were significant revealed no consistent trends in the influence of habitat types on cottontail rabbit condition (Tables 1 and 2).

Single degree of freedom contrasts helped clarify differences ($P < 0.05$) in condition parameters among habitat types within each season. During summer, altering habitats with tebuthiuron increased body mass (10%) over those altered with triclopyr; total body length was 3% larger on tebuthiuron- than triclopyr-altered habitats (Table 1). These differences were not reflected in the physical condition index. Relative masses of thymus (33%) and kidney (6%) of rabbits were greater on triclopyr-altered than tebuthiuron-altered habitats in summer. On average, relative spleen mass of rabbits was 50% greater on burned than unburned habitats in summer. Rabbits harvested from disturbed habitats had a kidney fat index that averaged 36% and a relative thymus mass that averaged 22% greater than those harvested from undisturbed habitats during summer.

During winter, relative masses of spleen (34%) and kidneys (5%) of cottontail rabbits were greater ($P < 0.05$) on disturbed than undisturbed habitats (Table 2). Relative masses of dried stomach digesta (20%) and liver (7%) were larger in rabbits harvested from unburned than burned habitats during winter. Relative masses of dried stomach digesta (18%) and liver (6%) also were greater in rabbits harvested from unburned herbicide-altered than burned herbicide-altered habitats during winter. Relative liver mass averaged 8% greater for rabbits harvested from tebuthiuron-altered than triclopyr-altered habitats.

Seasonal two-group comparisons of condition indices between habitat categories using discriminant analysis provided overall classification accuracies that were indicative of a positive influence of habitat disturbance on cottontail rabbit condition (Table 3). Highest overall classification accuracies (78% and 77%) were associated with comparisons of condition between rabbits from undisturbed habitats and those from either disturbed or burned-control habitats. Among two-group comparisons between disturbed habitat types, very similar overall classification accuracies were evident regardless of disturbance type (range = 59-74%). This suggested that the type of disturbance was not as influential on rabbit condition as the fact a habitat was disturbed.

Unlike winter, overall classification accuracies for all two-group comparisons in summer were not significantly ($\underline{P} > 0.05$) different between early and late seral stages (Table 3). Classification accuracies for comparisons between unburned herbicide-altered and burned herbicide-altered habitats increased significantly ($\underline{P} < 0.05$) from early to late seral stages in winter. Classification accuracies for comparisons between undisturbed and disturbed habitats in winter declined significantly ($\underline{P} < 0.05$) from early to late seral stages. Other two-group comparisons showed no significant ($\underline{P} > 0.05$) change in classification accuracy with succession in winter.

The number of times each condition parameter was selected by stepwise discriminant analysis for inclusion into specific two-group comparisons (Table 3) was calculated to determine which parameters were consistent predictors of rabbit condition (Table 4). Relative spleen mass and kidney fat index were the most important condition parameters for classifying rabbits according to habitat type. Parasitism index, relative liver mass, and stomach digesta dry mass also were consistent predictors for classifying rabbits according to habitat type. Body mass was the least sensitive physical condition indicator for cottontails.

Because specific contrasts indicated that most differences in selected condition parameters were attributable to time post-disturbance, herbicide alteration, or burning of habitats, we used a five-group discriminant analysis to simultaneously compare overall condition of rabbits as influenced by habitat quality. Discrimination of individual cottontail rabbits, based on overall condition, to their original habitat type (undisturbed, herbicide-altered, or burned herbicide-altered) and seral stage, yielded an overall classification accuracy of 55% in summer and 63% in winter (Table 5). Similar to two-group comparisons, overall classification accuracy of rabbits to habitat type and seral stage in five-group comparisons was significantly ($\underline{P} < 0.05$) higher in winter than in summer. Discriminating variables selected by stepwise discriminant analysis for inclusion into our five-group classification model were relative stomach digesta wet and dry masses, body mass, body and hind foot length, and kidney fat and parasitism indexes in summer. In winter, selected parameters were relative masses of wet and dried stomach digesta, thymus, kidneys, liver, kidney fat index, and parasitism index.

Canonical discriminant analysis produced, centroid means (Fig. 2) clearly indicating that cottontail rabbit condition was influenced by habitat disturbance and seral stage (Table 6 lists weighted coefficients for each condition parameter in the canonical variates). Mahalanobis distances between centroid means of undisturbed habitats and either herbicide-altered (early and late seral stage) or burned herbicide-altered (late seral stage in summer, early and late seral stages in winter) habitats were significantly ($\underline{P} < 0.05$) separated in summer and winter. Mahalanobis distances between the two disturbed habitat types in early seral stages separated significantly ($\underline{P} < 0.05$) from those in late seral stages in winter; however, only herbicide-altered habitats in early seral stages separated from the two disturbed habitat types in late seral stages in summer. Mahalanobis distances between centroid means of burned and unburned disturbed habitats were only significant ($\underline{P} < 0.05$) in winter of late seral stage.

Canonical variate I in summer represented a gradient of increasing kidney fat index, and to a lesser degree increasing parasitism index, and decreasing relative spleen mass; canonical variate II represented a gradient of increasing physical condition and parasitism index and decreasing relative stomach digesta dry mass. In winter, canonical variate I represented an increasing gradient of kidney fat index, and to a much lesser degree relative stomach digesta dry mass, and decreasing relative kidney mass; canonical variate II represented a gradient of increasing relative masses of liver and spleen, and parasitism index. Seral stage groups separated along canonical variate I in winter and summer. Burned and unburned groups separated along canonical variate II in winter.

DISCUSSION

Cottontail rabbits are habitat generalists and inhabit a variety of disturbed and transitional habitat types (Chapman et al. 1982). Lochmiller et al. (1991) found variable results in population response to specific habitat disturbances on our study area; however, rabbits showed a positive response to general habitat disturbance and a preference for burned habitats. Inconsistency in how and what individual condition indicators responded to type of habitat disturbance probably reflected the complex nutritional changes that occurred in the environment (not just simply altering energy or protein) and differing sensitivities of these indicators to specific nutrient limitations. This was supported by variations in the number of times each condition indicator was selected by stepwise discriminant analysis as an important parameter for inclusion into specific two-group comparisons. Several condition parameters were repeatedly selected during specific seasonal or seral stage comparisons.

Kidney fat and parasitism indices, and relative masses of spleen, liver, and dried stomach digesta were the most frequently selected predictors of habitat disturbance and seral stage. Our selection of these and other indices of condition for use in assessing how habitat disturbance influences condition in cottontail rabbits was made after review of a variety of literature on lagomorphs and other small mammalian herbivores. We know of no study which has explored the sensitivity of these collective indices in cottontail rabbits to changes in habitat quality.

Kidney fat index is a reflection of stored energy reserves (Flux 1971) and is routinely used to assess physical condition in a variety of animal species. Although captive feeding-trials have indicated that this fat index is sensitive to short-term nutritional stress (Henke and Demarais 1990), its use in field-assessments of lagomorph condition have been limited (Flux 1971). The parasitism index has been shown to vary with changes in habitat quality (Boggs et al. 1990). Spleen mass varies in response to different environmental stressors, including food shortages (Chapman et al. 1982; Henke and Demarais 1990), crowding (Conaway and Wight 1962), and hemoparasitism (Watkins et al. 1991). Liver mass is influenced positively by the rate of nutrient assimilation (Koong et al. 1985) and presence of secondary plant metabolites (Bergeron and Jodoin 1989). Digesta dry mass reflects dietary bulk intake, which decreases when crude protein in the diet of lagomorphs decreases (Bailey 1969; Bookhout 1965; Sinclair et al. 1982). Lack of a clear trend in univariate statistics and known variations in the response of condition parameters to habitat quality demonstrated a need for a multivariate approach in determining trends in physical condition of cottontail rabbits.

Discriminant analyses provided a multivariate perspective of how habitat disturbance influenced the overall condition of cottontail rabbits. These analyses indicated that disturbance, either from overstory removal with herbicides or burning, altered the overall condition of animals on our study area. Analyses also showed that post-disturbance effects were temporary due primarily to the rapid progression of vegetation towards a grass-dominated habitat, which more closely resemble those conditions prevailing on undisturbed environments. The majority of the observed differences and changes in condition are thought to be attributable to disturbance- and successional-induced effects on the nutritional quality of the habitat, as measured by herbaceous forage production and nutrient composition. One of the most dramatic disturbance-induced changes in the vegetation was herbaceous forb production. The importance of forbs in the diet of cottontail rabbits is due to their digestibility (Bailey 1969; Bogle et al. 1989) and protein content (Bogle et al. 1989).

Classification accuracy of individual cottontail rabbits into appropriate unburned and burned habitats was high (i.e., undisturbed habitats and burned-controls), suggesting that fire had a positive influence on populations. The effects of burning disturbed habitats also were more apparent in later than early seral stages as indicated by improved classification accuracies and significant separation between burned and unburned groups. As described by Engle et al. (1991b), burning was effective at prolonging positive effects of herbicide-induced disturbance with respect to forb production. Five-group comparisons using canonical analysis also indicated that influences of burning on condition were more prevalent in winter than summer (Fig. 2). Increased density of cottontail rabbit populations following prescribed burning has been reported (King et al. 1991) and attributed to improved nutritional conditions (Lochmiller et al. 1991). Prescribed burning has been repeatedly shown to improve nutritional quality (Allen et al. 1976; Hallisey and Wood 1976) and production of herbaceous forages in a variety of habitat types. Similar observations have been reported for our study area (Bogle et al. 1989; Engle et al. 1991b; Soper et al. 1993).

Two-group and five-group comparisons of individual cottontail rabbits from undisturbed and disturbed upland hardwood forest-tallgrass prairie also suggested that habitat disturbance had a positive influence on overall condition of cottontail rabbits. Habitat disturbances on our study area using herbicides to remove woody overstory vegetation significantly improved nutritional quality and production of herbaceous (Bogle et al. 1989; Engle et al. 1991b) and woody forages (Stritzke et al. 1991a; Soper et al. 1993).

Initially, each herbicide removed the oak-dominated overstory (Stritzke et al. 1991a, 1991b), resulting in dramatic increases in the production of herbaceous forbs following release. Fast growing early-seral dominants such as these have been shown to contain greater tissue nitrogen concentrations but are gradually replaced by slower-growing plants as soil nitrogen resources become depleted (McLendon and Redente 1992). Similar successional changes occurred on our study area several years after herbicide disturbance; pioneer forbs were replaced by annual grasses on both herbicide-altered habitat types (Engle et al. 1991a, 1991b), and eastern red cedar has invaded. This successional change in vegetation was reflected in both two-group and five-group discriminant comparisons. Classification accuracy for comparisons between undisturbed and disturbed habitats declined from early to late seral stages. Five-group canonical analyses revealed significant separation (Mahalanobis distances) between early and late seral stages for both burned and unburned herbicide-disturbed habitats. These separations were most evident in winter.

The degree and similarity in separations of individuals between the various disturbed habitat types using two-group comparisons suggested that overall physical condition of cottontail rabbit was not as dependent on intensity of habitat disturbance as it was to disturbance itself. Classification accuracies were greater for comparisons between undisturbed vs disturbed or undisturbed and burned controls compared to all others (triclopyr vs tebuthiuron, burned herbicide- vs unburned herbicide-altered, burned vs unburned).

These results indicate that spatial and temporal changes in habitat quality does influence overall condition of cottontail rabbits. Univariate comparisons were not sufficiently discriminating to detect changes in cottontail condition under these experimental environments. The relationship of habitat-induced alterations in condition to intrinsic population attributes such as recruitment and survival rates were not addressed. However, changes in population density that we observed with habitat disturbance on the study area (Lochmiller et al. 1991) suggest that such a relationship existed. Only adult rabbits were monitored; condition among juveniles may be more disparete among disturbance types.

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Table 1. Physical condition parameters (mean with SE in parentheses) in summer of adult cottontail rabbits (*Sylvilagus floridanus*) as influenced by habitat disturbance and successional stage. Differences among habitat types (T) and successional stages (S) were tested by ANOVA.

	Habitat disturbance						Successional stage (S)		ANOVA (*P* <)		
Condition parameter	Tebuthiuron	Tebuthiuron w/fire	Triclopyr	Triclopyr w/fire	Undisturbed	Control w/fire	1987-88	1990-92	T	S	T*S
Body mass (Kg)	1.00(0.03)^{ac}	1.09(0.03)^{b}	0.97(0.04)^{ac}	0.93(0.04)^{a}	1.04(0.03)^{bc}	0.91(0.06)^{ac}	0.98(0.02)	1.01(0.02)	0.01	N	N^{d}
Length (mm)											
hind foot	85.0(0.83)	86.4(0.70)	84.9(0.83)	83.5(1.01)	85.6(0.90)	80.7(1.65)	86.9(0.67)^{a}	83.4(0.47)^{b}	N	0.01	N
total body	389.6(5.57)^{ab}	402.8(3.83)^{b}	388.0(5.02)^{a}	380.4(6.52)^{a}	389.7(4.46)^{a}	374.9(9.83)^{a}	384.7(3.76)^{a}	391.4(2.90)^{b}	0.03	0.02	N
Kidney fat index^{e}(%)	8.24(0.71)	8.46(0.83)	7.75(0.89)	9.76(1.04)	5.91(0.49)	5.84(0.61)	8.66(0.64)	7.36(0.39)	N	N	N
Condition index^{f}	12.05(0.39)	12.73(0.32)	11.79(0.35)	11.63(0.49)	12.90(0.39)	11.52(0.86)	12.13(0.27)	12.19(0.23)	N	N	N
Parasitism index	37.8(4.74)	52.3(9.79)	54.5(7.05)	42.3(5.63)	50.0(6.3)	46.9(15.0)	59.2(5.61)^{a}	40.3(3.62)^{b}	N	0.01	N
Relative mass (%)											
Adrenal^{g}	14.3(0.60)	14.1(0.50)	15.3(0.60)	14.9(0.60)	15.4(0.40)	14.1(0.60)	14.4(0.40)	15.0(0.30)	N	N	N
Liver^{h}	2.21(0.04)	2.18(0.04)	2.27(0.04)	2.28(0.06)	2.19(0.04)	2.32(0.06)	2.24(0.03)	2.23(0.02)	N	N	N
Kidney^{h}	0.56(0.01)	0.55(0.01)	0.58(0.01)	0.60(0.02)	0.57(0.01)	0.63(0.03)	0.56(0.01)	0.58(0.01)	N	N	N
Spleen^{g}	52.7(4.60)^{a}	61.0(6.10)^{ab}	58.9(5.00)^{ab}	79.3(8.80)^{b}	54.7(5.70)^{a}	108.5(17.00)^{b}	62.0(4.90)	67.5(4.00)	0.03	N	N
Thymus^{g}	35.5(3.80)^{a}	26.4(2.20)^{a}	36.0(3.60)^{ac}	46.4(4.10)^{bc}	29.6(3.50)^{a}	35.6(4.90)^{a}	37.1(2.50)^{a}	33.1(1.90)^{b}	0.01	0.03	N
Stomach digesta^{h}											
wet	3.07(0.15)	3.14(0.14)	3.42(0.13)	3.29(0.17)	3.14(0.18)	3.01(0.23)	3.74(0.09)^{a}	2.87(0.08)^{b}	N	0.01	0.01
dry	0.49(0.03)	0.49(0.02)	0.55(0.03)	0.49(0.03)	0.46(0.03)	0.50(0.04)	0.51(0.02)	0.49(0.01)	N	N	0.02

Table 1. continued pg. 2.

[a-c] Means within habitat disturbance or successional stage with the same superscript were not statistically different ($P > 0.05$).

[d] Not significant.

[e] Kidney fat index = (kidney fat mass / kidney mass) x 100.

[f] Condition index = (body mass - 251.09) / body length^3.

[g] [parameter mass (mg) / body mass (g)] x 100.

[h] [parameter mass (g) / body mass (g)] x 100.

Table 2. Physical condition parameters (mean with SE in parentheses) in winter of adult cottontail rabbits (<u>Sylvilagus floridanus</u>) as influenced by habitat disturbance and successional stage. Differences among habitat types (T) and successional stages (S) were tested by ANOVA.

	Habitat disturbance						Successional stage (S)		ANOVA ($\underline{P}$ <)		
Condition parameter	Tebuthiuron	Tebuthiuron w/fire	Triclopyr	Triclopyr w/fire	Undisturbed	Control w/fire	1987-88	1990-92	T	S	T*S
Body mass (Kg)	1.01(0.02)	1.05(0.02)	1.03(0.02)	1.05(0.02)	1.05(0.02)	1.06(0.02)	1.03(0.01)	1.05(0.01)	N	N	N^{e}
Length (mm)											
hind foot	86.1(0.62)	86.6(0.66)	86.7(0.55)	86.0(0.49)	87.0(0.69)	86.2(0.51)	86.6(0.46)	86.3(0.24)	N	N	N
total body	400.6(3.18)	404.2(2.99)	400.1(2.90)	406.0(2.41)	406.1(2.76)	401.7(4.93)	407.5(1.84)^{a}	399.4(1.59)^{b}	N	0.01	N
Kidney fat index^{f}(%)	79.38(9.02)	74.51(7.29)	82.55(10.15)	75.48(7.63)	82.48(9.76)	107.34(12.76)	38.02(2.98)^{a}	119.86(4.16)^{b}	N	0.01	N
Condition index^{g}	11.83(0.27)	12.01(0.23)	12.12(0.26)	11.93(0.23)	11.96(0.25)	12.56(0.31)	11.51(0.15)^{a}	12.48(0.14)^{b}	N	0.01	N
Parasitism index	14.5(3.71)	13.3(5.84)	19.3(8.31)	11.3(2.98)	11.1(3.50)	2.4(1.26)	22.7(4.24)^{a}	4.1(0.65)^{b}	N	0.01	N
Relative mass (%)											
Adrenal^{h}	15.2(0.70)	14.7(0.60)	16.0(0.60)	14.6(0.70)	14.9(0.50)	15.3(0.90)	15.2(0.50)	15.0(0.30)	N	N	N
Liver^{i}	2.78(0.07)^{a}	2.60(0.05)^{c}	2.57(0.06)^{bc}	2.43(0.07)^{bd}	2.51(0.06)^{bc}	2.29(0.08)^{d}	2.48(0.04)^{a}	2.62(0.04)^{b}	0.01	0.01	0.02
Kidney^{i}	0.56(0.01)^{a}	0.54(0.01)^{ac}	0.52(0.01)^{bc}	0.54(0.02)^{ab}	0.51(0.01)^{b}	0.52(0.01)^{ab}	0.53(0.01)	0.53(0.01)	0.01	N	0.01
Spleen^{h}	51.0(7.20)^{a}	40.9(3.50)^{a}	41.1(3.60)^{a}	39.1(2.80)^{a}	30.8(2.40)^{b}	34.9(3.50)^{ab}	41.6(3.40)	38.7(1.70)	0.02	N	0.02
Thymus^{h}	18.2(1.90)^{a}	17.8(1.80)^{a}	14.0(1.40)^{a}	18.2(2.70)^{a}	15.3(1.70)^{a}	13.7(1.80)^{a}	11.7(0.90)^{a}	19.4(1.10)^{b}	0.04	0.01	N
Stomach digesta^{i}											
wet	2.27(0.14)	2.42(0.14)	2.44(0.16)	2.24(0.12)	2.60(0.21)	1.97(0.23)	2.87(0.09)^{a}	1.90(0.08)^{b}	N	0.01	0.01
dry	0.40(0.04)^{a}	0.37(0.02)^{ab}	0.40(0.04)^{a}	0.31(0.02)^{b}	0.44(0.03)^{a}	0.35(0.04)^{ab}	0.39(0.02)	0.37(0.02)	0.04	N	0.01

Table 2. continued pg. 2.

[a-d] Means within habitat disturbance or successional stage with the same superscript were not statistically different ($P > 0.05$).

[e] Not significant.

[f] Kidney fat index = (kidney fat mass / kidney mass) x 100.

[g] Condition index = (body mass - 251.09) / body length^3.

[h] [parameter mass (mg) / body mass (g)] x 100.

[i] [parameter mass (g) / body mass (g)] x 100.

Table 3. Classification accuracies (%) by season, for two-group comparisons of cottontail rabbits (Sylvilagus floridanus) from undisturbed and disturbed habitats undergoing secondary succession. Classification accuracy was determined by discriminant analysis of habitat classes based on their influence on condition parameters (selected by stepwise discriminant analysis).

Habitat type comparisons	Early seral stage 1987-1988		Late seral stage 1990-1992	
	Summer	Winter	Summer	Winter
Herbicide-altered	59.0	63.3	64.4	67.7
vs				
Burned herbicide-altered	64.1	57.1	53.5	81.1
Overall	61.5	60.2	58.9	74.4
Burned	64.1	61.9	51.8	79.7
vs				
Unburned	61.7	63.8	60.7	64.4
Overall	62.9	62.9	56.2	72.0
Undisturbed	66.7	81.8	67.9	60.0
vs				
Disturbed	68.8	73.2	70.7	63.7
Overall	67.7	77.5	69.3	61.6
Tebuthiuron-altered	80.5	62.8	85.0	74.4
vs				
Triclopyr-altered	40.0	69.2	34.0	65.9
Overall	60.2	66.0	59.5	70.1
Undisturbed	--	--	64.3	75.0
vs				
Burned-control	--	--	73.9	79.0
Overall			69.1	77.0

Table 4. Number of times each condition parameter was a significant discriminator variable (determined by stepwise discriminant analysis) in our two-group comparisons (Table 3) of overall condition of cottontail rabbits (<u>Sylvilagus floridanus</u>) from different habitat types.

Condition parameters	Early seral stage (1987-1988)		Late seral stage (1990-1992)			
	Summer	Winter	Summer	Winter	Total	Mean
Body mass	0	2	0	0	2	0.50
Hind foot length	2	2	1	0	5	1.25
Total body length	0	4	0	1	5	1.25
Kidney fat index	0	2	2	4	8	2.00
Condition index	0	2	3	0	5	1.25
Parasitism index	0	3	2	2	7	1.75
Relative masses						
Adrenal	3	2	1	0	6	1.50
Liver	1	2	0	4	7	1.75
Kidney	0	2	0	2	4	1.00
Spleen	2	4	1	1	8	2.00
Thymus	0	2	0	1	3	0.75
Stomach digesta						
wet	1	2	0	1	4	1.00
dry	0	3	2	2	7	1.75

Table 5. Classification accuracies (%) by season, for five-group comparisons of cottontail rabbits (Sylvilagus floridanus) from undisturbed habitats and disturbed (burned and unburned herbicide-altered) habitats undergoing secondary succession. Classification accuracy was determined by discriminant analysis of habitat classes based on their influence on condition parameters (selected by stepwise discriminant analysis for each season).

Habitat type (seral stage)	Summer	Winter
Herbicide-altered (early, 1987-1988)	53.3	54.8
Burned herbicide-altered (early, 1987-1988)	64.7	78.3
Herbicide-altered (late, 1990-1992)	58.5	60.0
Burned herbicide-altered (late, 1990-1992)	59.3	73.7
Undisturbed	39.5	48.7
Overall	55.1	63.1

Table 6. Coefficients for condition parameters used to separate by season five-group comparisons of cottontail rabbits (<u>Sylvilagus</u> <u>floridanus</u>) from undisturbed and disturbed (burned and unburned herbicide-altered) habitats undergoing secondary succession in canonical discriminant analysis.

	Summer canonical variate		Winter canonical variate	
Condition parameter[a]	I	II	I	II
Condition index	-0.14	0.79	0.06	0.22
Relative spleen mass (%)	-0.35	-0.07	-0.09	0.52
Relative stomach digesta dry mass (%)	0.26	-0.45	0.33	0.31
Relative liver mass (%)	0.02	-0.38	0.21	0.74
Stomach worm (<u>Obeliscoides cuniculi</u>) count	0.39	0.61	-0.17	0.62
Kidney fat index (%)	1.04	-0.01	1.20	-0.01
Relative kidney mass (%)	0.02	0.06	-0.36	-0.47

[a] See Table 1 for description of condition parameters.

Figure 1. Average grass and forb production on undisturbed and disturbed (burned and unburned herbicide-altered) upland hardwood forest-tallgrass prairie, by early (1987-1988) and late (1990-1992) seral stages.

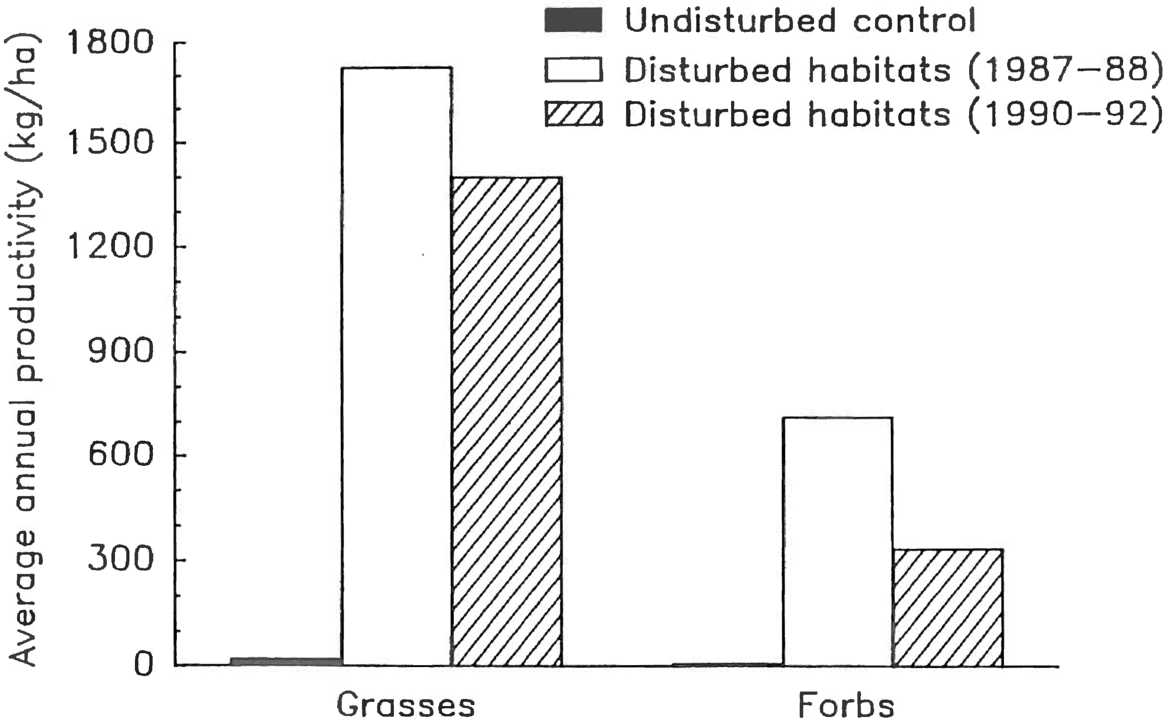
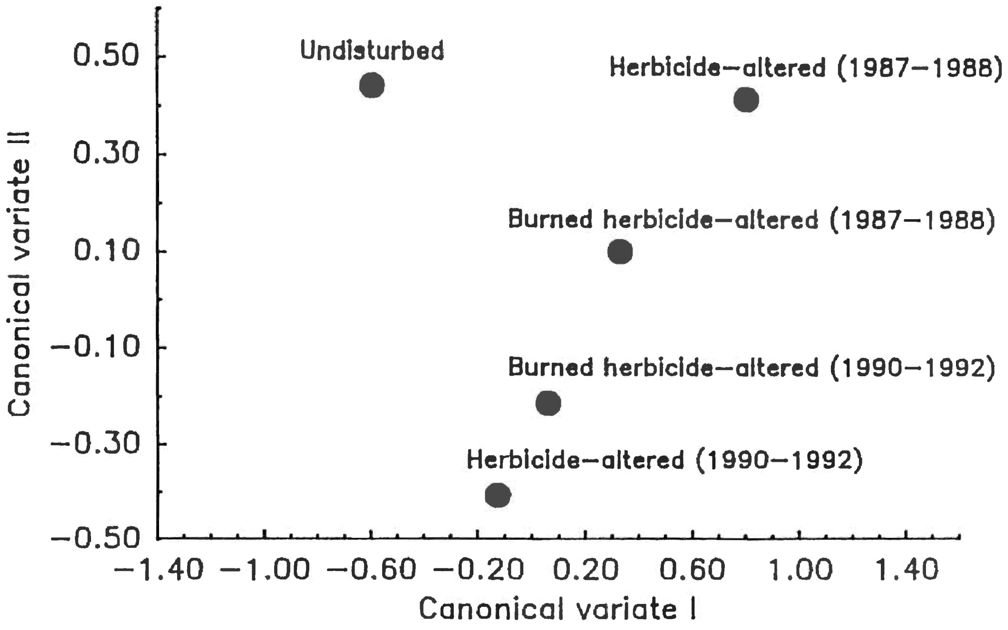
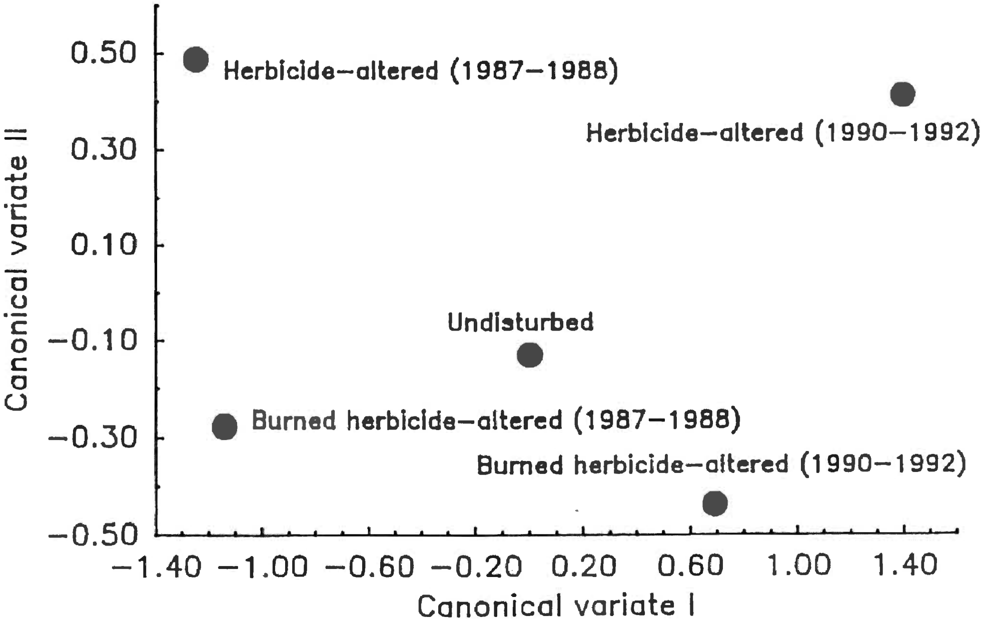

Undisturbed control
Disturbed habitats (1987–88)
Disturbed habitats (1990–92)
Average annual productivity (kg/ha)
1800
1500
1200
900
600
300
0
Grasses
Forbs

Figure 2. Graphical representation of separation of canonical variate means for undisturbed and disturbed (burned and unburned herbicide-altered) habitats undergoing secondary succession. Significance of each condition parameter used in a canonical variate are listed elsewhere (see Table 6).



Summer



Winter

VITA

David G. Peitz

Candidate for the Degree of

Master of Science

Thesis: ESSENTIAL AMINO ACID NUTRITIONAL ECOLOGY OF COTTONTAIL RABBITS (*Sylvilagus floridanus*)

Major Field: Wildlife and Fisheries Ecology

Biographical:

Personal Data: Born in Ft. Madison, Iowa, December 4, 1961, the son of Richard and Myrtle Peitz.

Education: Graduated from Ft. Madison Senior High School, Ft. Madison, Iowa, in May, 1980; received Associate of Applied Science Degree in Mechanical Engineering Technology from Southeastern Community College at West Burlington, Iowa in June, 1982; received Bachelor of Science Degree in Fisheries and Wildlife Biology from Iowa State University at Ames in May, 1990; completed requirements for the Master of Science degree Oklahoma State University in May, 1993.

Professional Experience: Wildlife Aide, Iowa Department of Natural Resources, Tama, Iowa, May, 1989, to August, 1989; Undergraduate Research Assistant, Department of Wildlife Biology, Iowa State University, August, 1989, to May, 1990; Research Assistant, Department of Zoology, Oklahoma State University, June, 1990, to December, 1992.

Professional Organizations: Ducks Unlimited, and 1989 recipient of the Dennis Healy Memorial Scholarship; The Wildlife Society; Phi Kappa Phi Honor Society; Gamma Sigma Delta Honor Society.