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Authors: Henke, Scott E., and Demarais, Stephen

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EFFECT OF DIET ON CONDITION INDICES IN BLACK-TAILED JACKRABBITS

Scott E. Henke and Stephen Demarais
Department of Range and Wildlife Management, Texas Tech University, Lubbock, Texas 79409, USA

ABSTRACT: Changes in blood, urine and physical condition indices in 23 adult male black-tailed jackrabbits (*Lepus californicus*) with ad libitum feeding and 25% feed restriction were measured over a 2 wk period from 30 May to 12 June 1988. Feed restricted jackrabbits had (1) lower posttrial body weights and kidney fat indices, (2) higher femur marrow fat, serum bilirubin and cortisol concentrations, and adrenal cortex width, and (3) depressed immune function. No single index alone could best measure the nutritional status of these jackrabbits.

Key words: Condition, experimental study, black-tailed jackrabbit, *Lepus californicus*, nutritional index, stress, experimental study.

INTRODUCTION

Estimating the nutritional status of a wildlife population relative to the carrying capacity of its habitat by using condition indices has been, and continues to be, an important step in the wildlife management process. Quantification of baseline values and the effect of specific nutritional levels are needed to validate condition indices (LeResche et al., 1974). Although some controlled nutrition validations have been conducted on cottontail rabbits (*Sylvilagus floridanus*) (Kirkpatrick and Kibbe, 1971; Warren and Kirkpatrick, 1978), neither baseline data nor controlled evaluations on jackrabbits have been reported. The objective of this study was to quantify the response of selected blood, urine, and physical condition indices in black-tailed jackrabbits (*Lepus californicus*) to two levels of nutrition over a 2-wk period.

MATERIALS AND METHODS

Twenty-four adult male black-tailed jackrabbits were live-trapped in Lubbock, Lubbock County, Texas (USA; 33°35′N, 101°54′W) from 30 April to 8 May 1988. Individual jackrabbits were weighed to the nearest 0.04 kg (capture weight), and individually housed in stainless steel rabbit cages. A grid rack allowed urine and feces to fall through, ensuring a sanitary environment. Purina Rabbit Chow Complete Blend (14.3% crude protein; Ralston Purina Co., St. Louis, Missouri 63166, USA) and water were provided ad libitum during an acclimation period. Lettuce washed in water was provided daily for roughage but added negligible protein or energy to their diet (Harrison and Fowler, 1984). Jackrabbits were considered adapted when their daily feed consumption stabilized. Feed consumption was considered stable when it remained within 10% of the 7-day running mean for a 7-day period; a process which took 22 days. The mean daily feed consumption during this period was 148 ± 2 g (range = 137 to 160 g). One jackrabbit died during the acclimation period due to unknown causes.

Following the acclimation period, jackrabbits were reweighed to the nearest 0.04 kg (pretrial weight), and randomly assigned to one of two diets, ad libitum or 25% feed restriction for a 2-wk period (30 May to 12 June 1988). Jackrabbits were placed on 25% feed restriction based on the results of Warren and Kirkpatrick (1978). BUN concentrations can reflect moderate feed restrictions of protein and energy (Warren and Kirkpatrick, 1978); however, to be of value in estimating nutritional status, catabolism of body protein should not occur because it will mask the effects of the feed restriction. Therefore, a 2-wk feed restriction was chosen based upon pre-experimental time trials with jackrabbits to find the time period directly before body catabolism (S. E. Henke, unpubl. data). The 25% feed restriction for each jackrabbit consisted of 75% of the running mean for its last 7-day ad libitum consumption during the acclimation period.

Euthanasia was accomplished with a 1.5 ml intraperitoneal injection of T61 euthanasia solution (Taylor Pharmaceuticals, Decatur, Illinois 62525, USA). The time period between initial handling and death was recorded. Jackrabbits were aged using eye lens weights (Connolly et al., 1969; Rongstad, 1966). Jackrabbits were reweighed to the nearest 0.04 kg (posttrial weight), and total body length (head from hind legs) was measured to the nearest 1.0 mm. Weight-length relationships outlined by Bailey (1968) were determined.

Blood samples were obtained by cardiac

puncture with a 12 cc syringe and an 18 ga needle, allowed to clot for at least 1 hr on wet ice, and then centrifuged at 1,400 rpm for 15 min. Serum was collected and frozen at −23 C. Whole blood was drawn into heparinized microcapillary hematacrit tubes at the time of heart puncture and centrifuged at 1,400 rpm for 5 min. Packed cell volume (PCV) was read on a Damon Micro-capillary Reader (Needham Heights, Massachusetts 02161, USA). Thin blood smears were prepared at the time of collection and stained with a Wrights-Giemsa stain within 24 hr. Differential white blood cell counts were conducted by counting 200 leukocytes at 1,000× magnification.

Urine samples were obtained with a 12 cc syringe and an 18 ga needle and tested for glucose, blood, ketone, leukocytes, nitrite, bilirubin, urobilinogen, specific gravity, protein, and pH using a Multistix test (Miles Laboratory, Elkhart, Indiana 46515, USA). Kidney fat index (KFI) (Riney, 1955) was calculated using both kidneys and their surrounding fat weighed to the nearest 0.01 g. Both adrenals were cleaned of fat and connective tissue, fixed in 10% buffered formalin, and weighed to the nearest 0.01 mg. Adrenal cross sections (transverse and medial) were evaluated for adrenal radius and cortex width using 0.025 mm calipers at 10× magnification. Liver tissue slices of approximately 2 to 4 mm thickness were fixed in 10% buffered formalin. Liver pathology was evaluated using 4 μm cross sections embedded in paraffin blocks and stained with hematoxylin-eosin.

Right femurs were frozen at −23 C. After slight thawing the femurs were cracked and the marrow forced from the cavity with a wood applicator. Fat was estimated for a 0.5 to 1.0 g sample of marrow using a reagent-dry assay (Verme and Holland, 1973).

Serum samples, frozen for 1 wk, were thawed and analyzed for bilirubin and immunoglobulin G (IgG) using the Multi-Stat III-plus fluorescence light scattering microcentrifugal analyzer (Instrumentation Laboratory, Lexington, Massachusetts 02173, USA). Reagents and standards were obtained from the manufacturer. Controls analyzed with each trial consisted of two commercial human samples (Fisher Scientific, Inc., Springfield, New Jersey 07081, USA) and a pooled jackrabbit serum sample. Serum cortisol was determined using radioimmunoassay (Abrahams, 1974).

Data were analyzed using an unbalanced, completely randomized design with the two feeding regimens as treatment levels. Distributions of appropriate residuals were tested using the Shapiro-Wilk test at $P < 0.05$. Analyses were performed on log-transformed data for adrenal weights and KFI due to nonnormal distributions of residuals. The effects of diet on posttrial body weight, adrenal weight, femur marrow fat, and kidney fat and adrenal cortex width were tested with a general linear models analysis of covariance using the pretrial weight and the adrenal radius, respectively, as the covariates. Means expressed for posttrial weight, adrenal weight, adrenal cortex width, femur marrow fat, and kidney fat were corrected for the covariate. Effects of diet on the remaining physical characteristics, and blood and urine condition indices were tested with a general linear models analysis of variance.

RESULTS

Capture and pretrial body weights and the time period for death to occur were similar ($P > 0.418$) for the two diet groups. The feed-restricted group exhibited a lower posttrial body weight ($P = 0.069$) (Table 1). The weight-length relationships using capture, pretrial or posttrial body weights were not affected ($P > 0.27$) by diet (Table 1). Adrenal weights and radius were not affected ($P > 0.411$) by diet; however, adrenal cortex width increased ($P = 0.016$) with the 25% feed restriction. Spleens appeared enlarged in the feed-restricted group, although they were not measured. The feed-restricted group had a lower ($P = 0.001$) kidney fat index, but had more ($P = 0.0001$) femur marrow fat (Table 1).

The effects of feed restriction on liver histology were very mild. Architecturally there were changes noted in terms of chord differentiation, with some minimal loss of cellular integrity in the central chords of livers of feed-restricted animals. In only four of the feed-restricted animals there was a mild and patchy fatty infiltration in the liver.

Feed restriction lowered PCV ($P = 0.05$), IgG concentrations ($P = 0.0001$), and relative percentages of lymphocytes, basophils, and eosinophils ($P < 0.0001$); conversely, feed restriction raised relative percentages of juvenile neutrophils ($P = 0.05$) and neutrophils ($P = 0.0001$), bilirubin concentrations ($P = 0.01$), cortisol concentrations ($P = 0.0001$), and neutrophil : lymphocyte ratios ($P < 0.0001$) (Table 2). Relative percentages of monocytes were

TABLE 1. The effect of ad libitum and 25% feed-restricted diets on mean physical characteristics of 23 adult male black-tailed jackrabbits from Lubbock, Texas, June 1988.

Physical characteristic	Ad libitum (n = 12)		Feed restricted (n = 11)	
	$\bar{x}$	±SE	$\bar{x}$	±SE
Capture body weight (kg)	2.5	0.1	2.4	0.1
Pretrial body weight (kg)	2.5	0.1	2.5	0.1
Posttrial body weight (kg)[a,b]	2.5	0.0	2.3	0.1
Capture body weight/body length (g/dm)	5.4	0.2	5.4	0.1
Pretrial body weight/body length (g/dm)	5.4	0.1	5.4	0.1
Posttrial body weight/body length (g/dm)	5.4	0.2	5.1	0.1
Adrenal weight (mg)[b]	277.4	1.9	278.8	2.0
Adrenal radius (mm)	1.6	0.1	1.5	0.1
Adrenal cortex width (mm)[c,d]	0.3	0.1	0.5	0.1
Femur marrow fat (%)[b,e]	42.6	2.2	62.2	2.3
Kidney fat (%)[b,e]	1.2	0.1	1.1	0.0

[a] Diet effect (P = 0.069).
[b] Covariant-adjusted with pretrial body weight as covariate.
[c] Diet effect (P = 0.016).
[d] Covariant-adjusted with adrenal radius as covariate.
[e] Diet effect (P < 0.001).

similar (P = 0.874) between the two diet groups (Table 2). Red blood cells of the feed-restricted group were irregularly shaped and had spurs.

Linear relationships were observed between differential white blood cell percentages and adrenal weights for the control group (Table 3). Linear relationships were observed between differential white blood cell percentages and cortisol concentration, adrenal weights, and cortex width, and between cortisol concentration and adrenal weights and cortex widths for the feed-restricted group (Table 3).

Diet did not affect (P > 0.50) levels of glucose, bilirubin, ketone, blood, nitrite, leukocytes, specific gravity, pH, protein and urobilinogen in the urine. Urine glucose, ketone, urobilinogen, nitrite, leukocytes, bilirubin and blood were not present and urine specific gravity was 1.0 for the two diet groups. Urine pH and protein were 8.5 ± 0.0 and 658.3 ± 235.0 mg/dl, respectively, for the control group, and 8.4 ± 0.1 and 625.2 ± 268.0 mg/dl, respectively, for the feed-restricted group. The sensitivity of the pH, specific gravity and protein tests, respectively, were ±0.5 pH units, ±0.010 mg/ml, and ±25 mg/dl.

DISCUSSION

The lower posttrial body weights in the feed-restricted group suggested a loss in fat reserves and/or muscle catabolism. However, if catabolism did occur, it probably was in an early stage of development since ketones did not occur in the urine. Ketones are produced during catabolism (Weinstein and Schwartz, 1985). In mammalian biochemistry, hepatic tissue will oxidize ketones as energy substrates until saturated, then further increases in ketones will cause a rise in blood and urinary concentrations (Sodeman, 1985).

The weight-length relationships were not a sensitive indicator of short-term nutritional stress since no differences were evident between the two diets. Perhaps a nutritional stress longer than 2 wk is needed to induce a difference in the weight-length relationship in lagomorphs.

The KFI was a good indicator of short-term nutritional stress in jackrabbits. However, caution may be needed in using the KFI alone to determine nutritional stress in jackrabbits. Flux (1971), in a study conducted in New Zealand, East Africa and Scotland, determined that (1) hares accu-

TABLE 2. The effect of ad libitum and 25% feed-restricted diets on blood characteristics of 23 adult male black-tailed jackrabbits from Lubbock, Texas, June 1988.

Component	Ad libitum (n = 12)		Feed restricted (n = 11)	
	$\bar{x}$	±SE	$\bar{x}$	±SE
PCV (%)[c]	77.7[a]	2.6	66.2[b]	4.1
Cortisol (μg/dl)[f]	60.0	7.5	126.4	5.0
Bilirubin (mg/dl)[d]	0.8	0.0	0.9	0.0
Immunoglobulin G (g/dl)[f]	2.5	0.1	1.6	0.2
Lymphocytes (%)[f]	69.0	1.9	46.8	2.1
Neutrophils (%)[f]	22.2	1.5	47.4	2.1
Monocytes (%)	2.6	0.1	2.6	0.2
Basophils (%)[f]	1.5	0.1	1.0	0.1
Juvenile neutrophils (%)[c]	0.7	0.1	0.9	0.1
Eosinophils (%)[e]	4.0	0.6	1.3	0.1
Neutrophil : Lymphocyte[f]	0.3	0.0	1.1	0.1

[a] Sample size = 11.
[b] Sample size = 10.
[c] Diet effect ($P < 0.05$).
[d] Diet effect ($P < 0.01$).
[e] Diet effect ($P < 0.001$).
[f] Diet effect ($P < 0.0001$).

mulate fat only during winter as a short-term energy source, (2) no direct correlation existed between food supply and fat deposition for hares, and (3) hares, being subject to predation, are unlikely to carry unnecessary fat at any time. The kidney fat accumulated by jackrabbits of this study and the lack of kidney fat in jackrabbits captured in the fall (S. E. Henke, pers. obs.) is consistent with the conclusions of Flux (1971).

The greater femur marrow fat level in the nutritionally stressed animals cannot be explained readily. Femur marrow fat stores of deer are metabolized only after subcutaneous and abdominal cavity fat (Riney, 1955). Our femur marrow fat results must have been an artifact of pre-capture nutritional conditions which were not accounted for by the random assignment to treatments and the use of pretrial body weight as a covariate in the analysis.

TABLE 3. Linear correlation matrix between differential white blood cell percentages and cortisol concentrations and log-transformed adrenal weights and cortex widths in 23 adult male black-tailed jackrabbits on two levels of nutrition from Lubbock, Texas, June 1988.

	Feed restricted (n = 11)						Ad lib (n = 12)	
	Cortisol[a]		Adrenal cortex[b]		Adrenal weights[c]		Adrenal weights[c]	
	r^2	P	r^2	P	r^2	P	r^2	P
Cortisol[a]	—	—	0.57	≤0.01	0.46	≤0.02	—	—
% Lymphocytes	0.52	≤0.01	0.45	≤0.03	—	—	0.41	≤0.03
% Neutrophils	0.59	≤0.01	0.46	≤0.03	—	—	—	—
% Monocytes	0.43	≤0.01	—	—	—	—	0.28	≤0.08
% Eosinophils	0.53	≤0.01	0.47	≤0.02	—	—	0.36	≤0.04
Neutrophil : Lympocyte	0.56	≤0.01	0.53	≤0.01	0.32	≤0.07	0.27	≤0.08
% Juvenile neutrophils	—	—	—	—	—	—	0.50	≤0.01

[a] Serum cortisol concentration.
[b] Adrenal cortex width.
[c] Log-transformed combined adrenal weights.

Jackrabbits possibly may mobilize fat in a different order than deer. A 25% feed restriction for 3 wk reduced the fat content of bone marrow in cottontails (Warren and Kirkpatrick, 1978).

The PCV values of jackrabbits of this study were generally higher than the 42 to 55% reported for wild black-tailed jackrabbits in California (Harrison and Fowler, 1984). Centrifugation for 10 min and at 5,000 rpm did not affect the PCV results (S. E. Henke, unpubl. data). The lower PCV in the feed-restricted group can be related to nutritional deprivation. Splenic sequestration of abnormal red blood cells caused by a depletion of iron stores and lipid monolayers could result in lowered PCV (Moya et al., 1985). It is possible also that the feed-restricted jackrabbits drank more water to reach satiety, and thus lowered their PCV.

The increased serum bilirubin and mild fatty infiltration of the liver in the feed-restricted animals indicated some liver stress with microdamage which can be caused by nutritional stress (Iber and Latham, 1985). However, since only four of 11 (36%) feed-restricted jackrabbits had a fatty infiltration of the liver and all cases observed were mild, this suggests that the nutritional stress was in its early developmental stages.

Increased cortisol concentrations and depressed immune function indicated a stress response in the feed-restricted jackrabbits. Nutritional stress has been known to cause increased cortisol concentration, neutrophil and juvenile neutrophil percentages, and neutrophil : lymphocyte ratio, while depressing IgG levels, and lymphocyte, basophil and eosinophil percentages (Kelley, 1980; Henry and Stephen-Larson, 1985).

A relationship between the immune system and endocrine system has been reported (Kelley, 1989). The relationships observed in this study among adrenal weights, adrenal cortex widths, cortisol concentrations and differential white blood cell percentages support this conclusion. Prolonged periods of exposure to a stressor have been shown to cause an enlargement of the adrenal cortex (Kirkpatrick, 1980).

Short-term feed restriction had no effect on the urine parameters of jackrabbits. The urine parameters can be used as baseline values for jackrabbits.

In summary, the feed-restricted jackrabbits appeared to be in early nutritional distress. They had lower post-trial body weights and KFI values, mild liver damage, increased serum bilirubin and cortisol levels, increased adrenal cortex width, and depressed immune function. No single index was capable of indicating nutritional stress in this study.

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