Nutritional Effects on Thyroids, Ovaries, and Thymuses in White-tailed Deer

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Abstract: Forty-four adult (≥2.5 years) white-tailed deer (*Odocoileus virginianus*) does were separated into 9 groups. Each group was offered a paired combination diet of high, medium, or low protein; and high, medium, or low digestible energy (DE) for a 60-day period ending December 1983 (Study I). Forty-six adult does were maintained on a paired combination diet of high or low protein and high or low digestible energy for a 6-month period ending December 1984 (Study II). Thyroid gland activity data were collected only from deer in Study II. Thyroid activity based on cell height was greater at high than at low protein ($P \leq 0.05$). Ovarian activity and thymus weight data were collected from deer in Studies I and II. Number of corpora lutea in Study I was greater at medium protein than at high or low protein and greater at high and medium energy than at low energy ($P \leq 0.05$). Mean size of the 5 largest ovarian follicles in Study II was greater at high than at low protein ($P \leq 0.01$). Cervical thymus weights in Study I were greater at high than at low energy ($P < 0.05$). No protein x energy interactions were observed for variables investigated in either study ($P > 0.05$).

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Nutrition is an important and complex factor affecting growth and reproduction in white-tailed deer (Sadleir 1969, Moen 1973, Kirkpatrick 1975, Ozoga and Verme 1982). Nutritional levels have been related to physiological responses in deer (Verme 1969, Vogelsang 1977, Ozoga and Verme 1982). For example, cyclic an-

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nual variations in thyroid gland morphology and activity appear to coincide with annual cycles in food consumption, body weight, and metabolic rate (Silver et al. 1969). Also, Sadleir (1969) concluded that nutritional levels have significant effects upon reproductive response in white-tailed deer.

Our purpose was to examine effects of long and short term diets of differing protein and energy levels on thyroid and ovarian activity and thymus weights. Dietary effects on the thyroid are of value because of the importance this gland has in growth (Hoffman and Robinson 1966). Managers can benefit from an increased understanding of specific nutrient effects and timing of such effects on successful reproduction.

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Methods

Forty-four adult (≥2.5 years) doe white-tailed deer were captured on 19 October 1983 (Study I), and 46 adult does were captured on 15 June 1984 (Study II) near Rivera in Kleberg County, Texas. Does were captured using a helicopter-assisted drive net (Beasom et al. 1980). Does were separated randomly into 9 groups for Study I and 4 groups for Study II, and were placed in pens (1–4 ha) containing limited natural vegetation located 48 km north of Rivera.

Does were fed a pelleted ration composed of cottonseed meal and hulls, ground corn, calcium, phosphorus and trace mineral supplement, molasses, and apple flavoring for a 3-week period prior to placement on experimental diets. Following the adjustment period, groups in Study I were offered 1 of 9 rations, each differing in protein and/or digestible energy (DE) content. Cottonseed hulls were used as a dietary diluent. The 9 diets were composed of all combinations of high protein (18%), medium protein (13%), and low protein (7%) and high energy (3.0 kcal/g DE), medium energy (2.2 kcal/g DE), and low energy (1.5 kcal/g DE). Does were fed ad libitum for a 60-day period terminating 20 December 1983. Errors in high protein/high energy and high protein/medium energy diet formulation in 1983 necessitated repetition of these diets with 17 does under similar circumstances in 1984. Data from the 1983 deer on these diets were not included in the analysis.

Does in Study II were placed on 1 of 4 diets differing in protein and energy content following the period of dietary adjustment. Diets were composed of all combinations of high (18%) and low (7%) protein, and high (3 kcal/g DE) and low (1.5 kcal/g DE) energy. Does were fed ad libitum for a 6-month period terminating 3–4 December 1984.

Thyroid glands were collected only in Study II and were stored in 10% formalin. Histological examinations were made using 6–8 μm cross sections which had been stained with PAS-hematoxylin (Purves and Griesbach 1951). Thyroid sections were sampled systematically using a stage micrometer at 430x and 1,000x for follicle and cell height measurements, respectively (Demarais 1984). Four follicle

cells were measured from each of 25 follicles, one from each "side," for a total of 100 cell heights per deer. Colloid and follicle diameters were measured in 50 follicles.

Reproductive tracts of does in both studies were stored in 10% formalin. Fixed ovaries were sectioned longitudinally at 2–3 mm intervals. All corpora lutea (CL) and tertiary follicles 1 mm in diameter were counted, and diameters of the 5 largest tertiary follicles were measured (Kirkpatrick 1974).

Cervical and thoracic lobes of the thymus were removed separately and trimmed of fat. Fresh weights of each lobe were recorded for does in both studies (Ozoga and Verme 1978). The treatment's main effects were evaluated using two-way analysis of variance. Multiple comparisons were made using the LSD procedure when significant main effects were noted.

Results and Discussion

Thyroid activity was greater at high protein than at low protein ($P \leq 0.05$), but no energy relationship was observed ($P > 0.05$) (Table 1). It appears that thyroid activity is sensitive to variation in diet quality during July–November. Normally-occurring winter hypothyroidism (Silver et al. 1969, Watkins et al. 1983) can be exacerbated by decreased food intake (Seal et al. 1972).

No protein or energy effects were noted for the ratio of colloid diameter to follicle diameter and mean colloid diameter ($P > 0.05$) (Table 1). Hoffman and Robinson (1966), Kalisnik (1972), and Winston and Henderson (1981) reported that high degrees of colloid storage reflect relative inactivity of the thyroid.

Conception in white-tailed deer in south Texas begins in early December and largely terminates by 12 January (Harwell and Barron 1975). Therefore, most does in Study I had ovulated before their date of sacrifice on 20 December. Few does in Study II, however, had ovulated by their date of sacrifice on 3–4 December. There-

Table 1. Six-month dietary protein and energy effects on thyroid activity of adult female white-tailed deer in south Texas.

Diet[a]	*N*	Cell height (μ)[b] $\bar{x}$	Percent colloid[c] $\bar{x}$	Colloid diameter $\bar{x}$
HP-HE	13	8.8[A]	47.9	117.2
HP-LE	8	8.0[A]	41.8	119.9
LP-HE	8	7.1[B]	38.8	130.6
LP-LE	12	7.4[B]	43.4	121.2

[a]HP = high protein; HE = high energy; LP = low protein; LE = low energy.

[b]Dissimilar capital letters (A, B) in the same column indicate differences ($P \leq 0.05$) between protein treatments.

[c]Percent colloid = average colloid diameter/average follicle diameter × 100.

Table 2. Sixty-day dietary protein and energy effects on ovarian activity of adult female white-tailed deer in south Texas (Study I).

	Pre-ovulatory				Post-ovulatory	
	No. of follicles[b]		Size (mm) of follicles[c]		No. of corpora lutea[d,e]	
Diet[a]	N	$\bar{x}$	N	$\bar{x}$	N	$\bar{x}$
HP-HE	4	3.0	4	2.0	4	1.8^{BC}
-ME	2	3.5	2	3.3	7	2.0^{BC}
-LE	1	1.0	1	1.4	3	1.0^{BD}
MP-HE	2	1.5	2	2.1	2	2.5^{AC}
-ME	2	10.5	2	3.3	2	2.0^{AC}
-LE	1	2.0	1	3.2	0	
LP-HE	0		0		4	2.0^{BC}
-ME	0		0		3	2.0^{BC}
-LE	4	1.5	4	1.6	3	1.0^{BD}

[a]HP = high protein; HE = high energy; MP = medium protein; ME = medium energy; LP = low protein; LE = low energy.
[b]Number of follicles >1 mm in diameter.
[c]Mean size (mm) of 5 largest follicles.
[d]Dissimilar capital letters (A, B) in the same column indicate differences ($P \leq 0.05$) among protein treatments.
[e]Dissimilar capital letters (C, D) in the same column indicate differences ($P \leq 0.05$) among energy treatments.

Table 3. Six-month dietary protein and energy effects on ovarian activity of adult female white-tailed deer in south Texas (Study II).

	Pre-ovulatory				Post-ovulatory	
	No. of follicles		Size (mm) of follicles[b,c]		No. of corpora lutea	
Diet[a]	N	$\bar{x}$	N	$\bar{x}$	N	$\bar{x}$
HP-HE	8	4.0	8	2.7^{A}	4	1.8
-LE	10	3.2	10	2.3^{A}	1	2.0
LP-HE	8	2.6	8	2.0^{B}	0	
-LE	12	2.3	12	1.9^{B}	0	

[a]HP = high protein; HE = high energy; MP = medium protein; ME = medium energy; LP = low protein; LE = low energy.
[b]Mean size (mm) of 5 largest follicles.
[c]Dissimilar capital letters (A, B) in the same column indicate differences ($P \leq 0.01$) among protein treatments.

fore, results of luteal and follicular production are reported separately for ovulating and non-ovulating does.

Dietary protein affected mean CL counts in Study I ($P < 0.01$), with medium protein intake inducing greater ovulation rates than high and low protein intake ($P < 0.05$). Dietary energy also affected mean CL counts ($P < 0.001$) in Study I, with high and medium energy intake inducing greater ovulation rates than low energy intake ($P < 0.05$) (Table 2). Dietary protein levels positively influenced the number of follicles greater than 1 mm in diameter ($P = 0.056$), and the mean size of the 5 largest follicles ($P < 0.01$) in Study II (Table 3). No protein x energy interactions were observed ($P > 0.05$).

"Flushing" is the process of feeding high nutrition diets prior to breeding in order to increase reproductive response (Sadleir 1969). It has not been clearly demonstrated whether flushing is associated directly with dietary protein, energy, or a combination of factors (Smith 1985). Data from this study confirm that both protein and energy positively influence ovarian activity.

The possible inability of a low protein diet to meet threshold protein requirements for ovulation has been noted in white-tailed deer (Murphy and Coates 1966, Ransom 1967). Low protein diet accounted for some of the lowest CL values in Study I, and for smaller follicle counts and sizes in Study II. Low dietary protein reduced ovarian activity. However, a consistent relationship involving dietary protein and ovarian response was not observed.

The reason medium protein levels accounted for higher CL production compared to high protein levels in Study I is not clear. Data suggest an optimum protein level for ovulation rates with a decreased response at low and high protein values. No such relationship has been documented for wild cervids. However, Smith (1985) and Verme (1969) considered the possibility of a decreased ovulation response to increasing protein levels due to an excessive body weight effect. A test of this hypothesis in this study was hindered by lack of intake and weight gain data. Crude protein percentages greater than 16% have been associated with lowered fertility, as indicated by lower plasma progesterone levels and services per conception in dairy cattle (Jordan and Swanson 1979, Folman et al. 1981).

High and medium dietary energy were associated with highest numbers of CL produced. Similar results have been reported for white-tailed deer (Vogelsang 1977). That CL production did not differ between high and medium energy diets may have been due to the ability of the does to regulate intake to adjust total energy consumption. Ammann et al. (1973) reported that does consuming a ration containing an energy content greater than 2.17 kcal/g decreased food intake. Both high (3.0 kcal/g) and medium (2.2 kcal/g) energy diets in the present study were above this level. Low energy does probably were unable to consume energy in sufficient quantity due to lower energy densities of low energy diets formulated with cottonseed hulls as a diluent. Studies have indicated that rumen-fill and rate of passage ultimately limit voluntary intake in sheep fed high levels of sawdust in their diets (Weston 1966). Lack of intake data in our study prevents firm conclusions regarding energy effects on ovarian activity.

Ovarian follicle size was responsive to higher protein levels in Study II. Howland et al. (1966) reported that follicular size and development were positively related to subsequent reproduction in domestic sheep. Follicular size, and to a lesser extent, number of follicles >1 mm in diameter, have been suggested as indices of dietary protein and of reproduction (Bellows et al. 1963). Kirkpatrick (1974) expressed reservations that follicular measurements related directly to productivity. Follicular data, therefore, should be interpreted with caution.

The cervical lobes of the thymus gland were heavier in does maintained on high than on low energy diets in Study I ($P \leq 0.05$) (Table 4). No protein or energy effects on thymus weight were observed in Study II ($P > 0.05$). Deer maintained

Table 4. Sixty-day dietary protein and energy effects on thymus gland weight (g) of adult female white-tailed deer in south Texas.

Diet[a]	N[b]	Cervical lobe[c] $\bar{x}$	Thoracic lobe $\bar{x}$	Thymus $\bar{x}$
HP-HE	6	6.9^{A}	3.4	8.5
-ME	9	3.1^{AB}	2.2	5.3
-LE	3	1.3^{B}	1.0	1.8
MP-HE	4	4.5^{A}	3.5	8.0
-ME	4	6.7^{AB}	3.4	10.2
-LE	1	3.5^{B}	1.4	4.8
LP-HE	2	10.1^{A}	3.4	12.7
-ME	3	4.4^{AB}	2.6	7.0
-LE	7	2.1^{B}	1.3	3.5

[a]HP = high protein; HE = high energy; MP = medium protein; ME = medium energy; LP = low protein; LE = low energy.
[b]N = 39 due to damage of 5 thymuses during collection.
[c]Dissimilar capital letters (A, B) in the same column indicate differences ($P \leq 0.05$) among energy treatments.

on high or moderate nutritional diets of pelleted ration (Ullrey et al. 1971) exhibited heavier thymus glands than deer on low nutritional diets in Michigan's upper peninsula (Ozoga and Verme 1978).

The use of thymus glands as a nutritional index was suggested by Ozoga and Verme (1978). However, they limited its usefulness to fawns, due to thymus regression with maturity (Anderson et al. 1974, Ozoga and Verme 1978). Data from Study I indicate that the thymus in older deer retains some sensitivity to nutritional change. However, thymus response by our does to dietary energy was much less marked than that observed in fawns (Ozoga and Verme 1978).

Conclusions

Dietary protein levels positively influenced thyroid activity, while no relationship between follicle size or percent colloid and thyroid inactivity was observed. The "flushing" effect on ovulatory response, prior to breeding, appears to be associated with higher protein and energy levels in forages. CL counts and follicular measurements indicated a positive protein and energy influence on ovarian activity. Additional studies employing dietary component breakdowns, similar to those used in Study II, and rate of intake data could help to illuminate this phenomenon.

The cervical lobe of the thymus gland is a sensitive indicator to diet quality in fawns (Ozoga and Verme 1978). The present study indicates that thymus weight data from adult does also could be used in management decisions. It is evident that future efforts to quantify reproductive and other physiological relationships are essential. Correlation with seasonal events (i.e. ovulation), and consideration of factors such as intake and weight gain data will be helpful in improving interpretation of dietary effects.

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