EFFECTS OF SEASON AND PLANE OF NUTRITION UPON SERUM LIPIDS AND PROTEIN OF WHITE-TAILED DEER

by

Thomas Randall Porterfield

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APPROVED:

Dr. R. H. Giles, Jr.

Dr. James B. Whelan

Dr. P. T. Chandler

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INTRODUCTION

The white-tailed deer (Odocoileus virginianus, Boddaert) is one of the most important big game animals in the United States. Land area for hunting is steadily decreasing, and hunting pressure is increasing steadily. Throughout much of its home range, the white-tailed deer is the only big game animal present in numbers large enough to support intense hunting. Therefore, it appears likely that management of the white-tailed deer will become more intensive in the future, at least in areas where large huntable populations of other big game animals are not present. In order to wisely manage deer populations, the biologist of the future will need a large amount of information in a usable form. More information concerning all facets of deer management is needed.

At present, little is known about the physiology of the white-tailed deer. Nearly all management practices are directed towards improving the quality of entire populations, but the size, productivity, and well-being of individual animals are governed by the effects of the environment upon the individual animal. It would be of great advantage to the wildlife manager to be able to predict the effects of each of his management practices upon the physiology of individual deer. Given this predictive ability, a manager could select the specific management tools which would allow him to most effectively reach his goals for the total population.

Very little research has been done concerning the physiology of the blood of white-tailed deer. Blood analyses are often used with humans, laboratory animals, and domestic livestock to determine responses to

various treatments or to serve as an indicator of physical condition. Blood analyses have seldom been used as a research technique with white-tailed deer. Blood deteriorates quickly after death, and blood samples taken from hunter-killed deer are usually in a badly deteriorated condition. Good refrigeration facilities are needed to preserve blood collected in the field. In the past, many researchers have been unable to preserve blood long enough for analysis to be made because field storage facilities were unreliable. However, if the researcher is properly equipped, blood samples are easily obtained, easily analyzed, and can be collected without harm to the animal. Blood samples can be taken from the same animal several times a year without seriously impairing his health. It appears that blood analyses may become an important research tool in the future.

OBJECTIVES

A project was designed to study the effects of season and diet upon the blood physiology of white-tailed deer. The specific objectives of this study were:

(1) To determine the effect of plane of nutrition and season upon the serum lipids and serum proteins of white-tailed deer.

(2) To determine if any relationship exists between blood components and the physical condition of white-tailed deer.

(3) To determine if plane of nutrition can be predicted by analysis of serum lipids and proteins in captive white-tailed deer.

LITERATURE REVIEW

Several researchers (Matthews 1969; Fowler et al. 1967; Long et al. 1965; and McEwen et al. 1957) have reported that captive white-tailed deer restrict food consumption at certain times of the year. Weight losses in captive deer closely parallel observed weight losses in wild deer populations. Food consumption is restricted during certain periods regardless of the availability of food. Although some workers (Long et al. 1965) have reported reduced consumption for short periods during the summer, the most notable period of reduced feeding occurs during the fall and winter (Fowler et al. 1967; Long et al. 1965; Cowan and Long 1962; and McEwen et al. 1957). Reduced consumption is first noted at the beginning of the breeding season, and weight losses continue until early spring. Weight losses occur in both sexes, but generally bucks undergo the greatest losses.

Seasonal oscillations of growth were reported for both male and female Columbian black-tailed deer, even though a uniform ration was fed throughout the year (Wood et al. 1962).

McEwen at al. (1957) reported that protein levels as low as 7% were tolerated without apparent stress by captive white-tailed deer during periods of reduced feeding. During periods of weight recovery and new antler growth, however, at least 17% protein was required in the ration for maximum weight gains and antler growth.

Sexually mature deer were found to require less food per unit body weight to maintain themselves in winter than sexually immature deer (Fowler et al. 1967). Cowan and Long (1962) found that slightly

less food was required for three year-old deer to gain weight than for two year-old deer to gain weight at an equal rate. A significant increase in food consumption with age was also reported for deer fed a constant ration throughout two winters (Long et al. 1965). This could be attributed to a change in environmental factors, however, because the deer were not kept in a constant environment.

The level of food consumption by white-tailed deer seems to depend upon several factors including age, sexual maturation, and season. Therefore, the actual dietary requirement of an individual animal will vary depending upon an interaction of all these factors. Whether the variation in the nutritional requirement of individual animals is accompanied by changes in the serum lipids or proteins has not been reported.

In a comparison of the chemical composition of rumen contents from white-tailed deer collected in six southeastern states, Krkpatrick et al. (1969) reported significant seasonal differences in crude protein, nitrogen-free extract, and dry matter for all four seasons. Crude fiber content was significantly lower in spring and summer than in fall and winter indicating a change in diet from easily digestible plant parts to less digestible fibrous parts of plants.

Researchers in Oregon reported seasonal variations in protein content of plants preferred by black-tailed deer. Heaviest mortality generally coincided with the periods of lowest protein content of plants (Einarsen 1946).

Significant seasonal variations in total serum proteins were reported by Matthews (1969) in white-tailed deer fed a ration of constant

composition. Significant differences were also found between months for total serum proteins of captive deer fed a ration formulated to simulate wild forage. Because data were available for only 6 months in the latter case, no seasonal trends were noted. No significant differences in the total serum proteins were found between sexes in either study group.

No significant differences in esterified cholesterol levels in the serum were found in lactating dairy cows given a basal ration with no added fat, cows given the same ration plus 6% tallow, and cows given the same ration with a supplement of 6 percent cottonseed oil. Lactating dairy cows given a ration containing one percent roughage had significantly higher levels of some specific cholesterol esters than cows receiving 2.5 percent roughage (Brown and Stull 1967).

In feeding trials with Holstein calves, Chandler et al. (1968) compared the effects of various rations on blood lipids and blood proteins. Rations contained either 0, 2, 4, 6, or 8 percent corn oil, and either 0, 7, 14, 21, or 28 percent soy protein. After 18 weeks of feeding, significant ration effects were found in total serum proteins and all the protein constituents except alpha globulin. The effect of dietary protein was found to be dependent upon the level of corn oil fed with the greatest changes occurring when high levels of the oil were fed. Significant ration effects were found in all lipid constituents after 18 weeks of feeding, and in all constituents except cholesterol after 13 weeks of feeding. In both tests, blood lipids were higher in calves fed medium-oil rations than in calves fed high-oil rations.

Nelson and Watkins (1967) compared two groups of wethers given an adequate diet plus a cottonseed meal supplement. There were no significant differences in total serum proteins or any of the protein fractions between sheep given the supplement daily and sheep given the supplement every sixth day. The authors also found no significant changes with season.

Klosterman et al. (1950) reported, however, that serum albumin decreased significantly in pregnant ewes on a low protein ration, but no significant changes occurred in globulins or total serum proteins. A highly significant positive correlation between successive years was found for total serum proteins and serum globulins. This indicated that the albumin fraction was more likely to be affected by ration.

The total serum proteins concentration of captive white-tailed deer fed a ration of either pure aspen browse or pure white cedar browse declined steadily throughout the feeding trial, but there were no significant differences in total serum proteins or in any of the protein fractions between the two groups (Ullrey et al. 1964).

In further trials with white-tailed deer, Ullrey et al. (1968) found no significant differences in total serum proteins or in any of the serum protein fractions between deer given a diet of white cedar browse and deer fed exclusively balsam fir browse. Total serum proteins were greater at the end of the trial than at the beginning for both groups.

In feeding trials comparing diets of either pure white cedar browse or pure jack pine browse, total serum proteins of white-tailed deer increased significantly in both groups during the trial, but there

were no significant differences between the two groups (Ullrey et al. 1967).

Matthews (1969) found no correlation between food consumption and total plasma proteins in white-tailed deer fed a commercial ration of constant composition.

Increases in gamma globulin and total serum proteins and decreases in serum albumin were reported for a captive male white-tailed deer with chronic arthritus. A lactating doe decreased in total protein and gamma globulin during the lactation period (Sikes et al. 1969).

Total serum proteins of captive white-tailed does increased by 28 percent from day 1 to day 7 post partum, but relative proportions of the various fractions did not change significantly during the first 3 weeks after parturition. Fawns showed less variability in total serum proteins than their mothers, but the relative proportions of the protein fractions varied considerably during the first 3 weeks following birth. Albumin and beta globulin increased while alpha and gamma globulins decreased during the study (Youatt et al. 1965).

It appears that serum lipids and serum proteins do not change greatly in response to diet over a fairly wide range of feeding. Most significant changes in the serum proteins or lipids occur in response to an unusual situation such as pregnancy, severe dietary deficiency, disease, or wide variations in feeding (as in Chandler's (1968) trials where protein in the ration ranged from 0-28% and fat ranged from 0-8%).

The diet of white-tailed deer varies greatly throughout the year. Deer are seasonal breeders, and their sex glands are active only during the breeding season. Because of the two factors mentioned above, it

would be expected that endocrine activity (other than that of the sex glands) would fluctuate greatly in deer. Hoffman and Robinson (1966) reported seasonal fluctuations of several indicators of endocrine activity in white-tailed deer. Seasonal trends were noted in pituitary weight, adrenal weight, adrenal zona glomerulosa width, thyroid weight, thyroid epithelial cell height, and thyroid colloid storage.

In work with captive deer in very poor condition from malnutrition, Teiri et al. (1958) could find no apparent correlation between condition and either erythrocyte count, white cell count, or hemoglobin concentration. Statistical tests could not be used because the sample size was too small.

Blood samples taken during feeding trials with captive white-tailed deer indicated that neither red cell counts, white cell counts, hemoglobin concentration, packed cell volume, non-protein nitrogen concentration, nor phosphorous concentration could be used as indicators of the condition of the deer (Duvendeck 1962).

Red cell counts, hemoglobin concentration, and packed cell volume were determined from blood samples collected from wild California deer. No conclusive correlation could be found between any of these parameters and the general health and condition of the individual animal (Rosen and Bischoff 1952).

It appears that such blood characteristics as red cell count, white cell count, hemoglobin concentration, packed cell volume, non-protein nitrogen concentration, and phosphorous concentration do not change greatly in white-tailed deer in response to nutritional deficiency or deteriorating condition. Neither serum lipids nor serum proteins have

been investigated in white-tailed deer to determine if any serum lipid or protein fraction is affected significantly by either nutritional deficiency or deteriorating condition.

METHODS AND MATERIALS

Experimental Design

Wild deer were collected by members of the Southeastern Cooperative Wildlife Disease Study group during a study of parasitism in southeastern deer. Collections were made at A. P. Hill Military Reservation in Virginia, Ft. Stewart Military Reservation in Georgia, Forks Game Management Area in South Carolina, and Choccolocco Game Management Area in Alabama. Deer were taken without regard to sex, age, or size. It was originally planned to include areas from two other southeastern states in the sampling, but this proved to be impossible for political reasons. Five deer were taken from each of the four study areas during each season for a total of 80 deer. Each deer was shot and a blood sample was taken from the heart immediately after death. Blood samples were cooled, allowed to clot, and centrifuged to obtain the sera. All blood sera samples were frozen for subsequent lipid and protein analyses.

Blood samples were taken from captive deer at Pennsylvania State University by forcibly restraining the deer and strapping them to a platform. These deer were being used in a nutrition experiment and had received a ration of known composition at three levels of feeding. One group was fed ad libitum; a second group received 80 percent of the amount consumed by the first; and a third group received 60 percent of the amount consumed by the first. These three diets were designated 100 percent ad libitum, 80 percent ad libitum, and 60 percent ad libitum respectively.

Collection of blood samples was scheduled for all four seasons, but

several deer died during the winter, and it was considered unwise to disturb the replacements or the surviving deer. Therefore no winter collection could be made.

The volume of serum received in many cases was not sufficient to allow analysis of both serum lipids and serum proteins. Due to this limitation of sample volume, the serum from captive deer was analyzed only for proteins.

Blood Serum Analyses

Lipids Analysis

Blood lipids were fractionated by thin layer chromatography. Lipids were extracted from one milliliter aliquots of raw serum with a mixture of 5 ml. petroleum ether and 5 ml. ethyl ether. One ml. of 95 percent ethanol was mixed with the serum prior to extraction. Two extractions were made on each sample using the ether mixture, and a final extraction was carried out using 10 ml. of petroleum ether. The clear liquid containing the dissolved lipids was decanted after each extraction and saved.

The solution of dissolved lipids was evaporated to dryness on a steam bath under a hooded exhaust fan. A continuous stream of nitrogen gas was directed over the liquid during evaporation. The nitrogen environment served to facilitate evaporation of the solvent, and to prevent oxidation of the lipids by excluding oxygen during the heating process.

After evaporation, the lipid residue was redissolved in 0.5 milliliter petroleum ether. This solution was spotted on Uniplate pre-coated glass chromatography plates supplied by Analtech, Inc. Six sample spots were

made on each plate, and a standard solution of known composition was spotted on each corner of the plate. The neutral lipids standard contained equal weights of cholesterol, palmitic acid, tripalmitin, and cholesterol stearate, and the polar lipids standard contained equal weights of lysolecithin, lecithin, phosphatidyl-ethanolamine, and cholesterol. Both standards were dissolved in chloroform.

Separation of the lipids was carried out using Gelman S1325 thin layer chromatography chambers with Gelman saturation pads. The upper trough of the chamber was filled with oven-dried dessicant crystals, and the lower trough was filled with the appropriate solvent system solution. Chambers were made completely airtight by sealing with masking tape.

Neutral lipids were separated in a solvent system composed of 150 parts petroleum ether, 30 parts ethyl ether, and 2 parts acetic acid.

Polar lipids were separated in a solvent system consisting of 120 parts chloroform, 80 parts methanol, and 20 parts distilled water. Prior to separation of the polar lipids, the plates were placed in the chambers with a solvent system of pure chloroform and no saturation pads. The solvent was allowed to run until the neutral lipids were moved to an area above the planned solvent front of the polar lipids system.

All plates were dried in an oven at 60° - 75°C. until all traces of solvent had evaporated. Plates were sprayed with a fine mist of 50 percent sulfuric acid and placed in an oven at 188°C for 15 min. to develop the spots.

Plates were scanned with a Photovolt densitometry system. A Photovolt Model 52-C Densitometer, equipped with an automatic TLC stage,

was connected to a Photovolt Model 520A Multiplier Photometer. The photometer was connected to a Photovolt Model 42-B Variable Response Recorder. The recorder was connected to a Photovolt Model 49 Automatic Integrator. The photomultiplier was set at 2 for scanning TLC plates. This apparatus produced a chart of the varying amounts of light passing through the plate. Use of the automatic integrator allowed rapid calculation of the area under any portion of the curve.

Proportional concentrations of lipid constituents were calculated by comparing the area under that portion of the curve representing a given constituent with the total area under the curve. Correction factors for each constituent were calculated by dividing the known concentration of a constituent in the standard by the apparent concentration as determined from the plate. The area under each portion of the curve was multiplied by the appropriate correction factor before relative concentrations were calculated. Neutral lipid and polar lipid constituents were calculated as percentage of the total lipids. The proportion of polar lipids in each sample was calculated from the neutral lipids plates by using a correction factor calculated from the polar lipids standard on those plates. This technique was demonstrated to the author by Dr. P. T. Chandler, and Chandler's technique was used without modification.

Protein Analysis

Serum proteins were separated by electrophoresis on cellulose acetate strips. The technique was described by Briere and Mull (1964). Some slight modifications were necessary to adapt the technique to the equipment available. Five microliter aliquots of raw serum were trans-

ferred by micropipette to a Gelman serum applicator. The serum was placed on Sepraphore III cellulose acetate strips using the applicator to apply a uniform band across the strip. Strips were placed in a Beckman Model R Electrophoresis Cell filled to the proper level with buffer solution. Electrophoresis occurred for 90 min. at a constant voltage of 210 V. Strips were removed immediately from the cell and stained for 5 min. in Ponceau S stain. Background stain was removed by immersion for 2 min. in each of three trays containing 5% acetic acid. Strips were mounted on glass plates and allowed to dry completely. Strips were immersed for 20 - 30 sec. in a 10% solution of acetic acid in ethanol. The strips became transparent as they dried.

Strips were left on the glass plates and were scanned with the same densitometry equipment used to scan TLC plates. A green 525 mμ filter was used on the densitometer, and the photomultiplier was set at 1.

A Beckman duostat was used to regulate voltage during electrophoresis. A tris-barbital-sodium-barbital buffer solution (pH 8.8) was used in the electrophoresis cell. Strips were soaked for 10 min. in the buffer solution before serum was applied. Reagents for mixing the buffer solution and the Ponceau S stain were obtained in pre-measured packages from Gelman Instrument Company of Ann Arbor, Michigan. Sepraphore III cellulose acetate strips were also obtained from Gelman Instrument Company.

DATA ANALYSES

Wild Deer

Data collected from wild deer were analyzed by computer using the BMD-01V program described in the Biomedical Computer Programs manual (Dixon 1967). BMD-01V is a packaged computer program developed by the UCLA Medical Center. This program produces a table containing sample size, mean, and standard deviation for each treatment group, and an analysis of variance table with F ratios.

Data from all study areas were pooled for each season. No statistical tests were made to determine if there were significant differences between areas because the four study areas were sampled in the same fixed sequence during each season. Only one area was sampled each week, and thus there was a 4 to 5 week interval between the initial and final sample collections for each season. Therefore, it was probable that any differences between areas which might occur could be due to seasonal changes. However, scatter diagrams of each blood factor plotted against area indicated that no relationship existed between any of the blood parameters and the area from which the deer were collected.

Data from all age classes were also grouped together for the analysis of seasonal variation. Scatter diagrams of blood parameters plotted against age were made, and no relationship could be found between age and any of the blood factors studied. Also only a few of the possible age classes were represented in any one season's sample, and many of the possible age classes were not present at all in the total sample. Therefore, it was impossible to make useful statistical comparisons between age classes.

Statistical comparisons between sexes were not made because there were not enough bucks in the sample to allow meaningful comparisons.

A multiple regression analysis was also performed with the data collected from wild deer. The BMD-02R program described in Biomedical Computer Programs (Dixon 1967) was used for this analysis. The BMD-02R program computes a series of multiple regression equations to describe the data at hand. It consists of a series of steps, and at each step a new variable is entered into the regression equation. The variable entered at any given step is that one which causes the greatest reduction in the error sum of squares of the regression, and which is most highly correlated with the dependent variable assuming previously selected variables in the equation are held constant.

Body weight was selected as the dependent variable, and the various lipid fractions and protein fractions, the albumin/globulin ratio, sex, age, condition index, and season were independent variables. Because season is a discreet variable, dummy variables were used to indicate season. Dummy variables were used to overcome linear dependency which would occur if season were represented by a single variable. Three dummy variables were used to indicate season in this analysis.

Condition index was a subjective rating given to each deer by the researchers who performed necropsy. Each deer was rated as being either in poor, fair, good, or excellent condition. Condition index was a discreet variable with four classifications, and it was handled in the same manner as season. Three dummy variables were used to indicate condition.

Discreet variables such as season were handled by substituting dummy variables in the following manner. Both season and condition index were discreet variables with four classifications, and both were represented by three dummy variables.

Classification	X_1	X_2	X_3
1	+1	0	0
2	0	+1	0
3	0	0	+1
4	-1	-1	-1

Seasons listed in the order of spring, summer, fall, winter; and condition was listed in the order poor, fair, good, and excellent. The X's are listed in the thesis as $Season^1$, $Season^2$, etc.; the superscript of the variable corresponds to the subscript of the dummy variables used to represent it.

Captive Deer

Data from captive deer were analyzed by the Virginia Polytechnic Institute Statistics Laboratory. Means and standard deviations were estimated by computer for each outcome group and for each treatment group. An outcome group consisted of three deer of the same sex and approximately equal pre-trial weights. One of the three deer was given each of the three experimental diets. The weekly food allotment of the deer given restricted diets (80% and 60% ad libitum) was calculated from the previous week's consumption by the unrestricted animal in the same outcome group. A treatment group consisted of all the animals fed a given experimental diet.

An analysis of variance table was produced for each blood factor during each season, and F ratios were calculated to determine if significant differences existed between treatments.

Data were incomplete for the fall sampling period, and analysis was not attempted with the fall sample. Also, because no samples were taken during the winter, and because the fall sample was not large enough to be analyzed by this technique, no comparisons between seasons were made.

Serum lipids analyses were carried out with a limited number of the blood samples from captive deer, but these data were not included in the analysis because the sample size was too small.

Data from the captive deer were analyzed with the BMD-02R program. Mean weekly food consumption prior to the collection of blood was chosen for the dependent variable. Age, sex, body weight, season, and the serum protein fractions were the independent variables. Season was treated the same in this case as for the wild deer data, except that only three seasons were considered. Only two dummy variables were used to represent season.

Classification	X_1	X_2
1	+1	0
2	0	+1
3	-1	-1

Seasons were listed in the order of spring, summer, and fall.

A second stepwise regression analysis with the BMD-02R program was performed with age, sex, season, and the serum protein fractions as the

independent variables. Digestible protein intake per week per unit metabolic size was calculated for each animal, and the mean weekly intake over a two month period immediately prior to collection of the blood sample was used as the independent variable. Body weight was not used as an independent variable because metabolic size was determined by the formula:

$$\text{Metabolic Size} = (\text{Body Weight in Kilograms})^{3/4}.$$

The proportion of crude protein in the ration and the digestibility coefficient of the protein in the ration were obtained from Croyle (1969), who used a nutritionally identical ration in deer nutrition experiments at Pennsylvania State University.

RESULTS

Wild Deer

A summary of the data by season is given in Tables 1 and 2. These tables contain the mean and the standard deviation in each season for each blood factor studied.

Significant seasonal differences were found for only three of the blood parameters studied. Fatty acids were significantly higher at the 0.01 level in spring and summer than in fall and winter. Lecithins were significantly lower in summer than in fall and winter. Alpha globulins were significantly lower in spring than in summer; significantly higher in summer than in fall; and significantly lower in fall than in winter. In the latter two cases, the level of significance was 0.05.

Although F ratios suggested that significant differences were approached in the cases of beta globulins, triglycerides, cholesterol, and polar lipids, significant seasonal differences were not found for any of the remaining blood parameters.

Spot X was the name given to an unknown compound which was found in the serum lipids analyses of blood samples from wild deer. Spot X migrated to a position between phosphatidyl-ethanolamine and cholesterol on polar lipids plates. No further identification of the compound was attempted.

As shown in Table 3, the first two variables selected by the BMD-02R program as most influential were dummy variables used to indicate condition index. With only these two variables in the equation, the

Table 1. Seasonal differences in the means and standard deviations of serum lipids of 80 deer from 4 areas in the southeastern United States.

Season		LIPID FRACTIONS (%) Neut.[1]	(%) Polar	(%) Chol.[2]	(%) F. A.[3]	(%) TG.[4]	(%) Chol. E.[5]	(%) Lys.[6]	(%) Lec.[7]	(%) PE.[8]	(%) Spot X
Spring	Mean	78.2	21.9	10.9	13.9[a]	17.6	35.8	1.6	13.5	0.2	6.5
	S. D.	10.8	10.9	5.9	5.0	9.2	12.6	2.1	9.0	0.7	3.7
Summer	Mean	82.7	17.3	8.0	14.3[a]	23.6	36.9	1.1	11.4[c]	0.1	4.7
	S. D.	5.5	5.4	2.3	5.6	13.1	13.9	1.6	5.1	0.3	2.6
Fall	Mean	77.5	22.5	8.0	9.8[b]	20.3	39.4	1.0	15.6[d]	0.5	5.3
	S. D.	6.4	6.4	2.3	4.7	7.4	9.1	1.3	5.6	0.9	3.3
Winter	Mean	76.2	23.8	10.4	9.8[b]	16.4	39.7	1.6	17.2[d]	0.4	4.6
	S. D.	8.7	8.7	4.5	5.1	3.3	15.2	1.5	6.4	0.6	3.4

a,b Means with different superscripts are significantly different at the 0.01 level.

c,d Means with different superscripts are significantly different at the 0.05 level.

1. Neutral lipids
2. Cholesterol
3. Fatty Acids
4. Triglycerides
5. Cholesterol Esters
6. Lysolecithins
7. Lecithins
8. Phosphatidyl-ethanolamine

Table 2. Seasonal differences in the means and standard deviations of serum proteins of 80 deer from 4 areas of the southeastern United States.

		PROTEIN FRACTIONS				
Season		(%) Albumin	(%) Globulin	(%) Alpha G.	(%) Beta G.	(%) Gamma G.
Spring	Mean	63.6	36.4	7.1[a]	11.3	18.0
	S. D.	7.9	7.9	3.1	3.1	5.1
Summer	Mean	59.6	40.6	9.1[b]	13.0	18.5
	S. D.	8.5	8.4	3.6	3.1	6.8
Fall	Mean	56.7	43.3	7.6[a]	14.1	21.6
	S. D.	12.6	12.6	3.2	3.9	10.7
Winter	Mean	59.1	40.9	9.7[b]	13.0	18.2
	S. D.	9.5	9.6	2.6	3.8	5.9

a,b Means with different superscripts are significantly different at the 0.05 level.

Table 3. Results of the multiple regression analysis of data from 80 wild deer.

Step	Variable Added	Multiple R	Multiple R^2	Increase in R^2
1	Condition Index[1]	0.4741	0.2248	0.2248
2	Condition Index[2]	0.5943	0.3532	0.1284
3	Age	0.6854	0.4698	0.1166
4	Season[1]	0.7233	0.5232	0.0534
5	Polar Lipids	0.7436	0.5529	0.0297
6	Cholesterol	0.7574	0.5737	0.0208
7	Lecithins	0.7651	0.5854	0.0117
8	Season[2]	0.7682	0.5901	0.0047
9	Sex	0.7705	0.5937	0.0036
10	Beta Globulin	0.7725	0.5968	0.0031
11	Fatty Acids	0.7736	0.5985	0.0017
12	Spot X	0.7740	0.5991	0.0006
13	Neutral Lipids	0.7743	0.5996	0.0005
14	Albumin	0.7745	0.5998	0.0003
15	Globulin	0.7812	0.6103	0.0106
16	Alb./Glob. Ratio	0.7828	0.6128	0.0026
17	Triglycerides	0.7840	0.6146	0.0018
18	Cholesterol Esters	0.7905	0.6249	0.0103
19	Phosphatidyl-ethanolamine	0.7907	0.6252	0.0002
20	Lysolecithins	0.7911	0.6258	0.0007
21	Season[3]	0.7911	0.6259	0.0001
22	Gamma Globulin	0.7912	0.6259	0.0001
23	Alpha Globulin	0.7946	0.6314	0.0054
24	Condition Index[3]	0.7948	0.6317	0.0003

multiple R^2 (the coefficient of determination) was 0.35.

One of the dummy variables representing season was the next variable entered in the equation. The multiple R^2 was increased to 0.52, an increase of 0.05 due to the addition of the season variable.

Percent polar lipids was the next variable selected. The multiple R^2 was increased 0.03 when polar lipids was added to the equation.

Percent cholesterol was the final variable entered which caused a significant decrease in the error sum of squares when added to the regression equation. With six significant variables in the equation, multiple R^2 was 0.57. The regression equation for the data from wild deer was:

$$y = 35.3 - 7.0x_1 - 12.6x_2 + 1.5x_3 + 3.6x_4 + 0.3x_5 - 0.4x_6$$

where

y = body weight in kg.

x_1 = condition index[1] (dummy variable)

x_2 = condition index[2] (dummy variable)

x_3 = age in years

x_4 = season[1] (dummy variable)

x_5 = polar lipids (percentage of total lipids)

x_6 = cholesterol (percentage of total lipids)

Captive Deer

A summary of the serum proteins data is given in Table 4. No significant differences between diet groups were found for any of the serum protein fractions. Significant differences between outcome groups were found for beta

Table 4. Differences in the means and standard deviation of serum proteins of 50 captive deer fed 3 different diets.

Diet		PROTEIN FRACTIONS (%) Albumin	(%) Globulin	(%) Alpha G.	(%) Beta G.	(%) Gamma G.
Spring Collection						
100%	Means	55.9	44.1	9.0	15.9	19.3
	S. D.	13.1	13.1	2.9	8.2	6.7
80%	Means	61.0	39.0	11.0	13.7	14.4
	S. D.	5.9	5.9	3.6	2.0	4.6
60%	Means	58.9	41.2	10.0	15.6	15.6
	S. D.	4.4	4.4	1.2	4.1	5.8
Summer Collection						
100%	Means	54.8	45.2	12.1	15.2	15.6
	S. D.	8.6	8.6	2.8	5.6	5.8
80%	Means	59.5	40.5	9.7	15.7	15.1
	S. D.	14.9	14.9	3.4	5.2	7.4
60%	Means	57.7	42.4	9.5	16.8	16.1
	S. D.	17.8	17.8	2.2	7.3	9.7
Fall Collection						
100%	Means	67.3	32.7	6.8	12.7	13.1
	S. D.	8.5	8.5	3.3	1.3	4.4
80%	Means	58.5	41.6	12.4	12.3	16.8
	S. D.	1.8	1.7	1.2	1.9	0.3
60%	Means	61.2	38.8	10.9	13.2	14.7
	S. D.	3.2	3.2	1.0	2.6	2.5

globulins in summer, but differences between diet groups were not significant.

Two BMD-02R multiple regression analyses were performed on the data from captive deer. In the first, mean weekly dry matter intake over a 2 month period prior to blood collection was chosen as the dependent variable.

The first variable entered by the BMD-02R program was body weight. With only one variable entered, R^2 was 0.68. This was highly significant at the 0.005 level.

One of the dummy variables representing season of collection was the next variable entered in the equation. Multiple R^2 increased to 0.81 when the season variable was entered.

After the first two variables had been entered, none of the remaining variables caused a significant decrease in the error sum of squares when added to the regression. The regression equation of dry matter intake on body weight and season for captive deer was:

$$y = 987.1 + 210.4x_1 - 1549.8x_2$$

where

y = mean weekly dry matter intake prior to sampling in grams

x_1 = body weight in kg.

x_2 = season[1] (dummy variable)

A complete summary of the results of this regression analysis is given in Table 5.

The second multiple regression analysis was performed with mean digestible protein intake per unit metabolic size per week as the dependent variable. Only one of the independent variables caused a

Table 5. Results from the multiple regression of mean weekly food consumption of captive deer on 11 parameters.

Step	Variable Added	Multiple R	Multiple R^2	Increase in R^2
1	Weight	0.8274	0.6847	0.6847
2	Season[1]	0.9047	0.8184	0.1338
3	Alb./Glob. Ratio	0.9080	0.8245	0.0061
4	Season[2]	0.9099	0.8279	0.0034
5	Sex	0.9118	0.8313	0.0070
6	Age	0.9156	0.8383	0.0011
7	Globulin	0.9162	0.8394	0.0002
8	Gamma Globulin	0.9163	0.8395	0.0002
9	Albumin	0.9164	0.8397	0.0000
10[a]	Globulin	0.9164	0.8398	

a The eleventh parameter, alpha globulin, is not included in the regression because the F ratio was too low for inclusion by the BMD-02R program.

significant reduction of the error sum of squares when selected by the BMD-02R program. One of the dummy variables representing season was the only significant variable entered in the regression equation. The coefficeint of determination for the regression was 0.27. The regression equation of digestible protein intake on season was:

$$y = 50.8 - 7.3x_1$$

where

y = grams of mean weekly digestible protein intake during a 2 month period prior to blood collection

$x_1 = \text{season}^1$ (dummy variable)

A summary of the food consumption data for captive deer is given in Table 6, and a complete summary of the digestible protein intake analysis is given in Table 7.

Table 6. Mean weekly consumption of dry matter and digestible protein in the ration of 50 captive deer.

Diet		Weight	Weight$^{3/4}$	Intake[1]	Intake[2]	DPI[3]
Spring Collection						
100%	Means	48.2	18.3	9063.6	9851.0	1055.5
	S. D.	6.8	1.9	2024.2	2983.2	319.4
80%	Means	43.1	16.8	6187.4	6143.4	658.0
	S. D.	7.6	2.2	901.1	738.9	79.1
60%	Means	38.6	15.5	4909.6	5553.4	597.6
	S. D.	5.4	1.6	1026.6	1006.7	107.1
Summer Collection						
100%	Means	53.1	19.7	10689.6	10411.9	1116.2
	S. D.	7.2	2.0	1108.3	1233.9	132.2
80%	Means	43.1	16.8	7718.8	7428.8	795.6
	S. D.	6.0	1.7	505.0	575.6	61.6
60%	Means	40.2	16.0	6510.5	6073.8	650.5
	S. D.	4.8	1.5	832.2	722.0	77.3
Fall Collection						
100%	Means	63.5	22.5	14352.8	13215.4	1415.4
	S. D.	7.5	2.0	1813.6	1379.2	147.7
80%	Means	46.3	17.7	10271.3	9557.9	1023.6
	S. D.	0.9	0.2	----[4]	----[4]	----[4]
60%	Means	41.2	16.2	9313.3	8558.4	916.6
	S. D.	7.4	2.2	1548.9	1770.1	189.6

1 Mean weekly dry matter intake in grams for one month priot to sampling.

2 Mean weekly dry matter intake in grams for two months prior to sampling.

3 Mean digestible protein intake in grams per week for two months prior to sampling.

4 No standard deviations given because all observations were the same in these cells.

Table 7. Results from the multiple regression analysis of mean weekly digestible protein intake of 50 captive deer on 10 parameters.

Step	Variable Added	Multiple R	Multiple R^2	Increase in R^2
1	Season[1]	0.5227	0.2732	0.2732
2	Beta globulin	0.5473	0.2995	0.0263
3	Season[2]	0.5685	0.3232	0.0237
4	Age	0.5841	0.3411	0.0179
5	Sex	0.5896	0.3477	0.0065
6	Alb./Glob. Ratio	0.5929	0.3515	0.0038
7	Albumin	0.6114	0.3738	0.0222
8	Gamma globulin	0.6191	0.3833	0.0096
9[a]	Alpha globulin	0.6192	0.3833	0.0000

a The tenth variable, total globulins, was not included in the regression because the F ratio was not high enough for inclusion by the BMD-02R program.

DISCUSSION

Concentrations varied greatly among individual animals for all of the blood components studied both in wild and captive deer. Significant differences between seasons were found for only three (fatty acids, lecithins, and alpha globulin) of the sixteen parameters studied in wild deer. However the F ratios for beta globulin, cholesterol, triglycerides, and polar lipids were only slightly below the critical level. Significant seasonal differences might have been found for these four serum fractions if a larger number of blood samples had been taken.

Some possible seasonal trends were observed for both serum lipids and serum proteins in wild deer (Tables 1 and 2). Beta globulin, cholesterol, triglycerides, and polar lipids change in relation to season. This may be due to seasonal changes in diet, or perhaps to internal biological rhythms. Since data are available for only 1 year, no definite conclusions can be made concerning seasonal changes in blood lipid and blood protein concentrations. At least 2 years of study would be required to determine if seasonal changes in blood composition in deer follow a pattern of annual cycles or fluctuate randomly.

Fatty acid concentrations were significantly higher in spring and summer than in fall and winter. This difference between hot and cold periods could be due to a change of diet between these two periods. In spring and summer, green forbs, grass, and green leaves are a large part of the diet of deer. Mast, dry vegetation, dead leaves, and woody twigs are available in fall and winter, and these form a

major part of the diet (Kirkpatrick et al. 1969). Kirkpatrick et al. (1969) found no significant differences in crude fat concentrations of rumen contents among the seasons in his studies of southeastern deer, but significant differences among seasons were found for crude protein, crude fiber, and nitrogen-free extract. Apparently, then, if the seasonal variation in fatty acids was due to seasonal changes in diet, the diet effect is caused by either a change in the composition of the fat in the diet or by a change in some dietary component other than crude fat.

Concentrations of lecithin in summer were significantly different than the amounts found in fall and winter. In general, lecithin and fatty acids seem to be inversely related in these deer. While fatty acids were higher in spring and summer, lecithins are lower at this time. This relationship was not significant, however, and it is possibly due to chance. Seasonal changes in both lecithin and fatty acids may be caused by changes in the chemical composition of the diet.

The fact that fatty acids and lecithins concentrations changed significantly between summer and fall may indicate a hormonal effect due to the beginning of the breeding season. Sex hormones may cause increased removal of fatty acids from the blood and decreased removal of lecithins, or the syntheses of these compounds may be affected. It is probable that hormonal effects would differ between males and females especially during the breeding season. It is probable that the observed seasonal differences in concentrations of the two compounds in both sexes were caused by an interaction of hormonal and dietary changes.

The regular low-high-low-high fluctuation of alpha globulin cannot be explained by hormonal changes during the breeding season. The changes from lower values in spring and fall to higher values in summer and winter were of similar magnitude. Large seasonal changes in the protein content of forage plants were reported by Hundley (1956). An endogenous biological rhythm may be responsible for the changes. Because none of the other serum protein fractions were affected, the author believes that the observed changes in alpha globulin were probably due to chance. It seems reasonable that changes in diet or hormonal changes due to an endogenous rhythm would affect the other serum protein fractions as well as alpha globulin.

The unknown compound, Spot X, was found to a varying degree in all of the blood samples analyzed for serum lipids. It was present in all of the samples taken from captive deer on which lipids analyses were performed, and in all of the samples taken from wild deer. A similar compound is found in cattle, and high levels of the lipid-like substance are often associated with stress (Chandler pers comm.). It appears that Spot X may be a normal component of the blood of white-tailed deer. Whether the presence of Spot X results from the normally easily excitable nature of white-tailed deer cannot be postulated from the data at hand. Although the concentration of Spot X in the blood may change in response to various stress situations, Spot X does not seem to be a very sensitive indicator of stress in white-tailed deer. In this study, the concentration of Spot X did not change significantly from season to season, although it is probable that the level of stress

to which the deer were subjected did change considerable during the study period. It is possible, however, that a wide normal range of concentrations of this blood component exists in deer. A small sample in which there was a large amount of individual variation would result in a large standard deviation, and this would tend to mask the effects of stress upon the concentration of Spot X. The author does feel, however, that more work is justified to determine the identity of Spot X and the physiological role of the compound in white-tailed deer.

In captive deer, no significant differences due to diet were found for any of the serum protein fractions. The concentrations of the various fractions varied over a wide range, but analysis of variance and scatter diagrams indicated that the variation was not due to diet. Significant variation within treatments was found for beta globulins in the summer, but these differences indicate variation due to individual differences rather than diet effects. In most animals there is a homeostatic mechanism which tends to maintain serum proteins at a constant level unless the animal is fed a nutritionally inadequate diet which eventually produces a state of severe malnutrition (Cowan pers. comm.). These data suggest that such a mechanism is present in deer. No significant variation in the factors studied was found between deer fed ad libitum and deer restricted to 80 percent and 60 percent of the amount consumed by the ad libitum group. It should be noted, however, that the composition of the ration of all groups was the same, and only the level of consumption was varied. Possibly significant treatment effects would have been found if the deer were restricted to a diet which was deficient in some essential nutrient.

Chandler et al. (1968) fed dairy calves a ration varying from 0 to 28 percent in protein and determined the concentrations of serum protein fractions of these animals by electrophoresis. Total serum proteins and some of the serum protein fractions were significantly affected by the ration. Chandler's trials lasted for 18 weeks, and the ration effects did not disappear, but deer in this study were given the experimental ration for at least 7 months before blood samples were collected. That no significant diet effects on serum proteins were found in captive deer might be explained by assuming that the deer had become adjusted to the diet over the long period of feeding, and any ration effects could possibly have been overcome by the homeostatic mechanism of the animals. Since the percentage protein content of the ration did not change between diet groups, large diet effects upon the serum proteins would not be expected.

The regression equation for the relation of dry matter intake of captive deer, body weight, and season explained 81 percent of the variation when all significant variables were in the equation. The regression of digestible protein intake on season explained only 27% of the total variation. None of the serum protein fractions significantly improved the value of the regression for prediction in either case, and these variables were not included in the equations.

The multiple regression equation for the data from wild deer would explain only 57 percent of the variation when all significant variables had been entered. Even if the level of significance had been reduced from 95 percent to 80 percent, less than 60 percent of the variation could be explained, and only one new variable was entered.

Of all the serum lipid fractions and serum protein fractions studied in wild deer, only polar lipids and cholesterol caused a significant reduction of the error of squares when added to the multiple regression equation. The selection of these two lipid fractions and lecithins, which was not significant, prior to selection of any of the serum protein fractions in the BMD-02R analysis (See Table 4) indicates that serum lipids are more closely related to diet in the white-tailed deer than serum proteins. While most of the serum proteins are synthesized by the body, a large proportion of the blood lipids are either absorbed directly from the intestine or are produced in the liver from absorbed lipids soon after feeding. Therefore serum lipids, as expected, were more closely related to diet than serum proteins.

The condition index was a subjective measure of the general health of the animal. Although it was largely a subjective measurement, condition index was determined after necropsy by competent veterinarians with extensive experience in working with white-tailed deer. Therefore the condition index was as good a measure of the general health and physical condition of the animal as could be obtained. Condition index was more closely related to body weight in the wild deer than either either age, sex, or any of the blood serum components. This substantiates the widely accepted opinion that condition is closely related to nutrition in the white-tailed deer. Correlation matrices produced by the BMD-02R program show that condition was not highly correlated with any of the serum protein fractions or serum lipid fractions. This supports the conclusion that there is a homeostatic

mechanism in deer which tends to maintain the concentrations of serum proteins and serum lipids at fairly constant levels, regardless of the condition of the animal. Although some of the deer in this study were in poor condition, none was emaciated or suffering from severe nutritional deprivation. It is probable that the composition of the blood would change significantly if the animal was in extremely poor condition, but no data are available to test this assumption.

Sex was not an important factor in any of the data analyses. None of the blood components studied was correlated closely with sex, and sex was not correlated closely with body weight in the wild deer. The addition of sex to the multiple regression equation for wild deer did not significatly reduce the error sum of squares. Because of the small numbers of males in the wild samples, analysis of variance could not be used to determine whether significant differences existed between sexes for any of the blood components. Sex was one of the parameters used to select outcome groups in captive deer, and therefore no sex effects were tested. Matthews (1969) found no significant differences in total serum proteins between sexes in two groups of captive deer. Even though there may be differences in the level of nutrition between sexes, these differences would probably not be reflected in the composition of the blood. More work is needed to determine if differences exist between the sexes for any of the lipids or proteins of the blood. If hormone levels significantly affect the concentrations of blood components, large differences should be found during the breeding season. Large differences in food consumption between sexes have been reported during the breeding season

and any diet effects upon the blood components should be accentuated during this period.

At this stage it does not appear likely that analyses of serum lipids and serum proteins can be used alone to predict the condition or nutritional status of white-tailed deer. Neither serum lipids analyses nor serum proteins analyses explained enough of the total variation in body weight or nutritional status of white-tailed deer to be useful in predicting either of these two parameters.

However, this author feels that more research in this area is justified. Large variations in food consumption were reported for captive deer, and these variations in food intake were generally attributed to causes that would affect deer in the natural state (Fowler et al. 1967; Long et al. 1965; Cowan and Long 1962; and McEwen et al. 1957). Seasonal changes in the chemical composition of rumen contents of white-tailed deer were reported by Kirkpatrick et al. (1969). Seasonal fluctuations in various indicators of endocrine activity have also been found in white-tailed deer (Hoffman and Robinson 1966). It is highly improbable that none of these factors has any effect upon the composition of the blood of white-tailed deer. Both the endocrine function of an animal and the nutritional well-being of the animal are intimately related to the blood. Further research is needed to determine the effect of each dietary component upon blood composition, the effects of various combinations of dietary components upon each blood constituent, and the relationship of each blood constituent to the physiology of the animal.

This author believes that even though the results of his study are

not a solid basis for prediction of condition or nutritional status by blood analysis, this study has provided some groundwork upon which further research can build. The author also believes that with further research to answer the questions raised by this work, blood analyses will be useful research tools for deer biologists in future years.

SUMMARY

Eighty blood serum samples collected from wild deer in four southeastern states were analyzed for serum lipids and serum proteins. Fifty blood serum samples collected from captive deer on three levels of diet were analyzed for serum proteins.

Fatty acids were significantly higher in spring and summer than in fall and winter, and lecithin was significantly lower in summer than in fall and winter. Alpha globulins changed significantly in all four seasons. No seasonal analyses were made for the captive deer, and there were no significant differences between diets for any of the serum protein fractions.

In stepwise multiple regression analyses, condition index, age, season, percent polar lipids, and percent cholesterol were found to be significantly related to body weight in wild deer. Body weight and season were significantly related to food consumption in captive deer, and season was significantly related to digestible protein intake.

Several conclusions were postulated from this study:

(1) Seasonal differences in fatty acids and lecithin concentrations were probably caused by an interaction of hormonal and dietary changes associated with the change in season.

(2) A homeostatic mechanism is present in white-tailed deer which resists changes in the concentrations of serum lipids and serum proteins.

(3) Although the general condition of deer is closely related to the nutritional status of the animal, no relationship exists between

either condition or nutritional status and any of the serum lipid fractions or serum protein fractions.

(4) Neither serum lipids analyses nor serum proteins analyses can be used to predict the condition or the nutritional status of white-tailed deer.

(5) Serum protein analyses cannot be used to predict food consumption or digestible protein intake in white-tailed deer.

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EFFECTS OF SEASON AND PLANE OF NUTRITION UPON SERUM LIPIDS AND PROTEINS OF WHITE-TAILED DEER

by

THOMAS RANDALL PORTERFIELD

ABSTRACT

Blood samples were collected from 80 wild deer in four areas of the southeastern United States, and from 50 captive deer on three levels of nutrition at Pennsylvania State University.

Blood lipids were fractionated by thin layer chromatography; serum proteins were separated by electrophoresis on cellulose acetate strips; and proportional concentrations of lipid and protein fractions were determined by densitometry.

Significant differences between seasons were found for fatty acids, lecithins, and alpha globulin in the wild deer. No tests for seasonal differences were made for captive deer.

Three regression equations were developed to describe the data. The following equations were obtained by computer using the BMD-02R packaged program.

$$y = 35.3 - 7.0X_1 - 12.6X_2 + 1.5X_3 + 3.6X_4 + 0.3X_5 - 0.4X_6$$

where:

y = body weight in kg. of wild deer

X_1= condition index[1] (a dummy variable)

X_2= condition index[2] (a dummy variable)

X_3= age in years

X_4= season[1] (a dummy variable)

X_5= polar lipids (percentage of total lipids)

X_6 cholesterol (percentage of total lipids)

The multiple R^2 for the equation above was 0.57.

$$y = -987.1 + 210.4X_1 - 1549X_2$$

where:

y = mean weekly food intake by captive deer for a 2 month period prior to blood collection. (grams dry matter intake)

X_1= body weight in kg. of captive deer

X_2= season[1] (a dummy variable)

The multiple R^2 for the second regression was 0.81.

$$y = 50.8 - 7.3X_1$$

where:

y = mean weekly digestible protein intake in grams by captive deer over a 2 month period prior to blood collection.

X_1= season[1] (a dummy variable).