THESIS

EFFECTS OF NITROGEN FERTILIZATION ON THE NUTRITIONAL

QUALITY OF MULE DEER WINTER FORAGES

Submitted by

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WE HEREBY RECOMMEND THAT THE THESIS PREPARED

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ABSTRACT OF THESIS

EFFECTS OF NITROGEN FERTILIZATION ON THE NUTRITIONAL
QUALITY OF MULE DEER WINTER FORAGES

Winter mortality resulting from starvation has been a serious

problem on mule deer (Odocoileus hemionus) ranges throughout much

of the western United States. Such losses have occurred in Colorado

during recent years after snow concentrated animals on areas having

inadequate food supplies. Associated with their interest in reducing

starvation losses, the Colorado Game, Fish and Parks Division

began testing fertilizer and 2, 4-D treatments to improve forage

conditions on winter feeding sites used by mule deer in Middle Park,

Colorado. This study undertook to determine how nitrogen treatments

affected the nutritional quality of two forage plants on winter range.

Ammonium nitrate fertilizer (33% N) treatments supplying 30,

60, 90, and 120 pounds of elemental nitrogen per acre were applied

to three study sites during early November, 1969. An untreated plot

on each site served as a control. Bluebunch wheatgrass (Agropyron

spicatum Pursh) and big sagebrush (Artemisia tridentata Nutt.)

current annual growth collected from the fertilized plots during May,

June, September, and December of 1970 and March, 1971 were

analyzed for crude protein, cell-wall constituents, calcium, and

phosphorus. Separate analyses were performed on leaf and twig

portions of big sagebrush. The in vitro digestibilities of all forage samples were determined in duplicate and big sagebrush leaves were analyzed for volatile-oil content.

Nitrogen fertilization failed to increase significantly ($P > 0.05$) the concentration of crude protein in big sagebrush tissues during May and September but increased significantly ($P < 0.05$) the concentration of crude protein in big sagebrush tissues during June, December, and March. Nitrogen fertilization increased significantly ($P < 0.05$) the concentration of crude protein in bluebunch wheatgrass during June. This increase was retained throughout the non-growing season. Nitrogen treatments failed to influence significantly ($P > 0.05$) the concentrations of cell-wall constituents, calcium, phosphorus, or volatile oils in the tissues or the in vitro digestibility of the forages. Concentrations of all measured nutrients in the forages and the digestibilities of the forages varied among study sites and among collection dates.

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CHAPTER I

INTRODUCTION

Winter represents the period of greatest nutritional stress for big game animals in Colorado because (1) forage nutritional quality and abundance are lowest and (2) snow depth and crusting conditions often limit the amount of range available to the animals (Gilbert et al. 1970). When these conditions occur on marginal winter range, starvation losses can result. One such incident occured in Middle Park, Colorado during the winter of 1964-65 when an estimated 30 to 50 percent of the Middle Park mule deer (Odocoileus hemionus) herd was lost to starvation. Losses have also occurred in Middle Park during other years. These losses might be reduced if techniques were available for improving the quantity and quality of forage on critical winter feeding sites. One possible solution might be range fertilization.

Fertilizers alter the chemical composition of plants. Thomas et al. (1964) found nitrogen fertilization increased the percentage of nitrogen, calcium, and phosphorus in grasses. Bailey (1968) reported fertilization produced a 13 percent increase in the crude protein concentration of witchhobble (Viburnum alnifolium) browse. Similar reports showed fertilization increased browse production (Gibbens and

Pieper 1962) and the palatability of forages (Gibbens and Pieper 1962, Thomas et al. 1964). Inferences of these reports plus the adaptability of the Middle Park situation to range fertilization prompted this study.

This study was one phase of a larger project conducted cooperatively in Middle Park by the Colorado Game, Fish and Parks Division and the Rocky Mountain Forest and Range Experiment Station. The larger project was designed to measure the effects nitrogen and herbicide treatments had upon forage production and forage preferences of mule deer.

Problem Analysis

The objective for this segment of the project was to measure the effects nitrogen treatments only had upon the nutritional quality of selected forage plants used by mule deer in Middle Park. Specific questions to be answered were:

(1) What effect does nitrogen fertilization have upon the concentration of the following components in selected winter forage plants:

(a) Crude protein

(b) Cell-wall constituents

(c) Calcium

(d) Phosphorus

(e) Volatile oil in big sagebrush (Artemisia tridentata Nutt.) leaves?

(2)   How is the _in vitro_ digestibility of selected winter forage

plants influenced by nitrogen fertilization?

(3)   How are effects related to the amount of nitrogen applied

and season of the year?

Delimitations

1.   Study Area:

This study was conducted on native sagebrush winter

range in Middle Park, western Grand county, Colorado

near Kremmling and Hot Sulphur Springs.

2.   Forage Species Tested:

Plants routinely consumed by mule deer wintering in

Middle Park were selected for study.   These were:

(a) Big sagebrush current annual growth including

leaves and terminal woody twigs.

(b) Bluebunch wheatgrass (_Agropyron spicatum_ Pursh).

3.   Duration of the study:

September, 1969 to December, 1971.

4.   Sample collection dates:

(a) March, 1970

(b) June, 1970

(c) September, 1970

(d) December, 1970

(e) March, 1971

Definitions

(a) In vitro digestibility - the percentage of forage dry matter placed into solution by rumen microorganisms and their enzymes during a specified period of time while incubating in a test tube.

(b) Nutrient - any food component consumed by an animal supplying essential elements for metabolic functioning.

(c) Nutritional quality - A combination of the amounts of available nutrients in a food and the digestibilities of those nutrients.

(d) Twig - a terminal woody growth produced during the current growing season.

(e) Winter forage - any plant or plant part consumed by mule deer on winter range.

CHAPTER II

STUDY AREA

Field work for this study was conducted on experimental sites established in Middle Park during 1969 by the Colorado Game, Fish and Parks Division (Fig. 1). Middle Park is a large (about 800 square miles) topographic depression in northcentral Colorado within the drainage of the Colorado River. Mountain ranges varying in elevation from 9,000 to 13,000 feet encircle the Park. The lowest elevation of the valley floor is approximately 7,000 feet. Coniferous forests predominate above 9,000 feet whereas lower elevations are dominated by big sagebrush plant communities. Much of Middle Park is under-lain by alternating coarse sandstones and mudstones of the Middle Park Formation which were thrusted up forming cliffs and ledges separated by slopes (Izett 1968).

Izett (1968) reported average annual precipitation in Middle Park is 21 inches with most falling in April and July. Gilbert et al. (1970) stated snow depth in the Park varies with elevation, exposure, and gradient but seldom exceeds accumulations of 2 feet on the valley floor. They explained southerly and windswept slopes are seldom snow-covered throughout the winter and receive considerable use by feeding mule deer wintering in the Park.
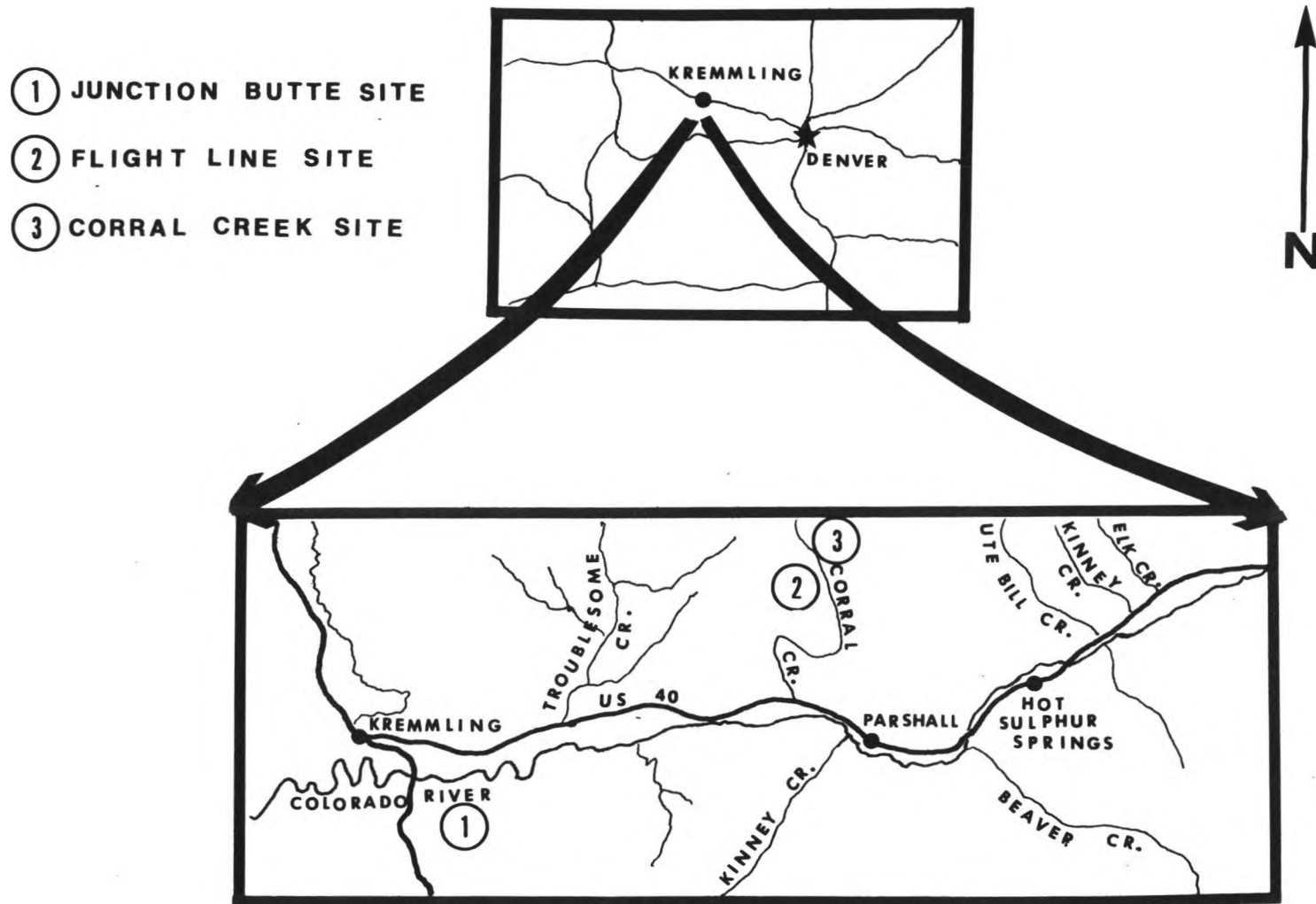
Fig. 1. Location of study sites, Middle Park, Colorado.
Scale: 1/3 inch equals 1 mile.

Study Sites

Three study sites were established in Middle Park by the Colorado Game, Fish and Parks (CGFP) Division, one on public land administered by the Division and two on lands administered by the Bureau of Land Management. Selection of individual sites was based on (1) accessibility, (2) uniformity of plant communities within sites and (3) site quality for mule-deer winter range. Sites were used as replicates of the big sagebrush vegetation type.

At each site, there were 10 treatment plots, each 100-by-100-feet (Fig. 2). A 10-foot strip separated fertilized plots from an 8-foot deer-proof woven-wire fence constructed around each site to prohibit use by mule deer and livestock. The fenced area was 520-by-220-feet.

Treatments applied to the 10 plots according to a randomized block design - randomized at each site were: 30, 60, 90, and 120 lbs of N per acre, each replicated with 2 lbs 2, 4-D per acre, one plot with 2, 4-D only and one plot as a control (Carpenter 1969). Fertilizer treatments were applied by Len H. Carpenter, CGFP Division, during November, 1969 prior to snow cover to make use of winter moisture and the 2, 4-D was applied in May, 1970.

Junction Butte Study Site

This study site, located approximately 2 miles southeast of Kremmling, is situated on a south-facing hillside below steep slopes

JUNCTION BUTTE SITE

120# N 2# 2,4-D	90# N 0# 2,4-D	0# N 2# 2,4-D	30# N 2# 2,4-D	30# N 0# 2,4-D
120# N 0# 2,4-D	0# N 0# 2,4-D	90# N 2# 2,4-D	60# N 2# 2,4-D	60# N 0# 2,4-D

N

FLIGHT LINE SITE

90# N 2# 2,4-D	30# N 0# 2,4-D	60# N 0# 2,4-D	120# N 2# 2,4-D	90# N 0# 2,4-D
0# N 0# 2,4-D	0# N 2# 2,4-D	30# N 2# 2,4-D	60# N 2# 2,4-D	120# N 0# 2,4-D

N

CORRAL CREEK SITE

120# N 0# 2,4-D	30# N 0# 2,4-D	90# N 0# 2,4-D	90# N 2# 2,4-D	120# N 2# 2,4-D
30# N 2# 2,4-D	60# N 0# 2,4-D	0# N 0# 2,4-D	0# N 2# 2,4-D	60# N 2# 2,4-D

N

Fig. 2. Randomized location of the ten treatments on each study area. Stipled plots were sampled during this study. (# equals pounds)

and prominent rock outcrops. Soils on the site are shallow sandy-loams and have an A horizon ranging from 2 to 6 inches in thickness and an organic-matter concentration of 1.3 percent in the top 6 inches Heil (1969).

Fletcher (1969) reported precipitation on the Junction Butte site averages about 11 inches annually of which half falls during the growing season. Snow rarely accumulates on the site owing to rapid snow melt.

Vegetation on Junction Butte was sparse prior to fertilizer treatment. Big sagebrush plants growing on the site were stunted, averaging 12 to 18 inches in height.

Flight Line Study Site

The Flight Line study site is located on gently sloping uplands approximately 13 miles northeast of Kremmling. Soils on the site are deep sandy loams having an A horizon up to 18 inches thick and an average concentration of organic matter of 2.5 percent in the top 6 inches (Heil 1969).

Fletcher (1969) reported precipitation on the Flight Line site averages about 18 inches annually with half falling during the growing season. Snow accumulations of 18 to 20 inches occurred on the site and snow cover often remained in some places until late April or early May.

Prolific plant growth characterized the Flight Line site. Big sagebrush plants ranged to 3 feet in height.

Corral Creek Study Site

This study site, located approximately 1 mile northeast of the Flight Line site, sits on a west-facing mountain exposure having an average slope of 8 percent (Fletcher 1969). Loamy soils possessing an A horizon of 6 to 10 inches and an average organic matter concentration of 2.6 percent in the top 6 inches occur on the site.

Vegetative growth on Corral Creek was judged intermediate between that on Junction Butte and Flight Line (R. Bruce Gill, personal communcation). Big sagebrush plants showed vigorous growth on Corral Creek prior to fertilizer treatment but the quantity of vegetation present was noticeably less than that at Flight Line. Snow depths on Corral Creek were judged intermediate between Junction Butte and Flight Line (O. C. Wallmo, personal communication).

CHAPTER III

METHODS AND MATERIALS

This chapter discusses sample collection, sample preparation, and laboratory procedures and reagents. Included also are discussions on statistical tests employed and statistical design of the experiment.

Sample Collection Dates

Plant samples were scheduled originally to be collected during March, June, September and December of 1970 and March, 1971 but this schedule was later modified. Snow conditions on the Flight Line and Corral Creek sites during March, 1970 prohibited the collection of plant samples, making it necessary to delay sampling these sites until the snow melted in early May, 1970. Consequently, only data from samples collected on the Flight Line and Corral Creek sites are presented for May, 1970.

Collection of Samples for Chemical Analyses

Plant samples used for chemical analyses were collected from treatment plots according to a prepared plan: (1) a 10-foot buffer zone was established on the interior borders of the plot being sampled to reduce bias resulting from fertilizer leaching between plots; (2) the remaining 80- x -100-foot area was partitioned into two

40-by-100-foot units, and; (3) one sample (approximately 100 g fresh-weight basis) of big sagebrush and bluebunch wheatgrass was clipped from each unit. A 100-foot steel tape was used to delineate plots. Only sagebrush plants having a height of 12- to -18 inches were sampled by clipping 10 terminal shoots (3-to-4 inches long) from 15 to 20 plants per sample. Plant size was standardized because most sagebrush plants growing on the Junction Butte site were within the 12- to -18 inch range but many plants growing on Flight Line and Corral Creek were taller and may have had different physiological responses to fertilizer treatments. Shoots (leaves and twigs) were clipped equally from the tops, bottoms, and sides of sagebrush plant crowns. No systematic plan was used to locate donor plants. Blue-bunch wheatgrass (culms, leaves and infloresences) was sampled by clipping the plants immediately above ground level. Once clipped, tissues were placed into paper sacks labelled according to plant type, study site, nitrogen treatment, and collection date and were trans-ported to Colorado State University for processing and chemical analysis.

On campus, plant tissues in paper sacks, were air-dried for approximately 2 weeks. Once air-dried, sacks containing sagebrush clippings were shaken vigorously to separate the leaves from woody twigs. Current-annual growth was removed next from woody twigs at the node. Leaves and current-growth twigs were ground and analyzed

separately. Air-dried tissues were ground in a Wiley mill with a 0.50 mm screen, placed into labelled glass containers, and stored in a cool, dry place until analyzed.

Plant samples were collected during May, June and September of 1970 only from the 10-foot border surrounding each study site by employing the sampling scheme described above. Samples were collected from these borders so as not to interfere with a browse-production study being conducted simultaneously on the three sites. However, plant materials in these borders became exhausted making it necessary to collect samples from the 100-by-100-foot treatment plots during December, 1970 and March, 1971.

Collection of Samples for Volatile-Oil Analyses

Terminal shoots (leaves and twigs) used for volatile-oil determinations were clipped from big sagebrush plants according to the sampling scheme described above. Again, only sagebrush plants 12-to-18-inches in height were sampled. Standardization of plant size was necessary because Powell (1968) found volatile-oil production by big sagebrush plants to be related to plant size and plant vigor. After clipping, samples were placed into paper bags and transported immediately to Kremmling and stored in a freezer. Elapsed time between clipping in the field and storage in the freezer varied to $1\frac{1}{2}$ hours. Samples were clipped at approximately the same time each day to control possible hourly variations in volatile-oil content.

Frozen samples were packed in dry ice, transported to Colorado State University, and stored in a freezer until analyzed for volatile-oil content.

Chemical Analyses of Samples

Separate portions of ground plant tissue were used for each chemical analysis with the exception of calcium and phosphorus. Ground tissues were mixed thoroughly prior to removing them from storage containers.

Crude Protein Determinations

Crude protein concentration (N concentration times 6.25) was determined by the micro-Kjeldahl procedure (Streeter 1969). Two hundred milligrams of ground plant tissue were placed into 100 ml Kjeldahl digestion flasks containing 3 ml of concentrated sulfuric acid and approximately 2 g of potassium sulfate. Two selenized Hengar granules were added next to catalyze the digestion process. Mercuric oxide, another catalyst, was tested during preliminary trials but gave consistently lower values for total nitrogen content than did the selenized Hengar granules.

Samples were digested for approximately two hours (at least 30 minutes after the sample turned clear). Flasks were rotated every 15 to 20 minutes to insure complete digestion of all plant tissues. After digesting, samples were removed from the digestor and allowed to cool.

A 50 ml Erlenmeyer flask containing 10 ml of 2 percent boric acid solution was placed beneath the condenser tip of the distillation apparatus to trap ammonia distilled from the digested plant tissues. The boric acid contained stock indicator solution, prepared by dissolving 1.25 g of methyl red and 0.825 g of methylene blue in one liter of 90 percent ethanol. Seven milliliters of stock indicator solution were added to each liter of boric acid solution.

Samples were titrated to a blue-gray color with 0.098 N sulfuric acid and nitrogen concentration (air-dry basis) calculated with the formula:

$$\% \ N \ = \ \frac{\text{Acid Normality X Ml. Acid X 1400}}{\text{Mg tissue digested}}$$

Cell-Wall-Constituent Determinations

Fiber content of the plant samples was determined using the procedure for cell-wall-constituent analyses proposed by Van Soest (1967). This procedure fractionated plant tissues into cellular contents (neutral-detergent solubles) and cell-wall constituents (CWC). Cellular contents contained proteins, carbohydrates, lipids and minerals. Short and Reagor (1970) stated CWC divides plant tissues into portions digestible and non-digestible by ruminants.

Five hundred milligrams of ground plant tissue were placed into 500 ml Erlenmeyer flasks. Then, added in sequence were 100 ml

of room-temperature neutral-detergent solution, 2 ml of decalin, and 0.5 g of sodium sulfite. Decalin was added to control foaming by the mixture during boiling. Next, the flasks were placed on a heating mantle and their contents boiled gently for one hour and then cooled.

After cooling, the solutions were filtered through fritted glass crucibles using a low vacuum. The residues were washed twice each with acetone and hot water to remove any residual cellular contents. Filtered samples were then placed into an electric drying oven and dried overnight at 100 C.

Oven-dried crucibles plus residue were weighed next and then ashed in a muffle furnace for 4 hours at 550 C and cooled. Crucibles plus ash were weighed and the percent CWC (dry-matter basis) calculated with the formula:

$$\% \text{ CWC } = \frac{Wg - Wt}{Wa - Wm} \times 100$$

Where:

Wg = gross dry weight of the crucible plus neutral-detergent residue.

Wt = gross dry weight of the crucible plus ash.

Wa = air-dry weight of plant tissue tested.

Wm = moisture content of plant tissue tested.

Digestion of Samples for Mineral Determinations

Calcium and phosphorus determinations were made on two aliquots of plant tissue digested in perchloric and sulfuric acids.

One-gram portions of ground plant tissue were placed into 125 ml polyethylene bottles. Two ml of concentrated perchloric acid and 3 ml of concentrated sulfuric acid were then added to each bottle. Bottle caps were not tightened to allow gas to escape. Samples were stored next in a fume hood and left to pre-digest for 24 hours.

After pre-digesting, bottle caps were tightened and the bottles placed into a tray beneath a hot water tap. Hot water (about 70 C) was allowed to flow slowly over the bottles for 4 to 6 hours thereby completing the digestion process. Bottles were placed next in a fume hood to cool and the bottle caps loosened to permit gas to escape. After cooling, samples were poured through filter paper into 50 ml volumetric flasks and diluted to volume with distilled water. A 25 ml aliquot of the solution was then tested for calcium concentration and the remaining 25 ml tested for phosphorus concentration.

Calcium Determinations

Calcium was measured in the samples with a Perkin-Elmer model 303 atomic-absorption spectrophotometer equipped with a hollow-cathode lamp.

Twenty-five ml aliquots of the samples were placed into 50 ml volumetric flasks and diluted to volume with a solution containing

2 percent lanthanum and 10 percent hydrochloric acid. Lanthanum and hydrochloric acid were added to prohibit phosphorus in the samples from interferring with calcium measurements.

Five-ml portions of the dilutions were pipetted next into 50 ml volumetric flasks, diluted to volume with distilled water, and portions (3 to 5 ml) of the dilution were tested for calcium concentration.

Phosphorus Determinations

Phosphorus was measured in the plant tissues with the molybdo-vanadate procedure described by the Association of Official Agri-cultural Chemists (1955). With this method, phosphorus was con-verted to phospho-molybdovanadate and then quantitated with a spectrophotometer. Slight modifications were made in the recommended procedure during the present investigation.

Twenty-five ml aliquots of the digested plant samples were poured into 50 ml volumetric flasks and diluted to volume with distilled water. Next, five ml portions of the solutions were pipetted into 50 ml volumetric flasks, 20 ml of molybdovanadate reagent were added, and the mixture diluted to volume with distilled water.

After standing for 30 minutes, each sample was read twice for percent absorbance (i.e. optical density) on a Beckman spectrophoto-meter set at a wavelength of 400 millimicrons. Standard solutions containing 0 ppm phosphorus and 2 ppm phosphorus were used to calibrate the spectrophotometer. Percent absorbance for each sample

was next converted to phosphorus concentration (air-dry basis) using standard curves prepared with sodium phosphate.

Volatile-Oil Determinations

Volatile-oil determinations were made using the steam-distillation procedure reported by Nagy (1966).

One hundred g of sagebrush leaves were taken from the freezer and placed into a 5-liter, round-bottom distillation flask equipped with a 24 cm condenser and an oil trap. Next, the distillation flask was half-filled with water and the contents boiled vigorously for 6 hours with heat supplied by an electric heating mantle.

After distillation, the oil was drained from the trap into a separatory funnel, separated into glass vials and weighed. Volatile oil concentration (fresh-weight basis) was obtained from the oil weight per 100 g of leaves.

Digestibility of Plant Samples

The procedure for determining in vitro digestibility of forages described by Pearson (1970) was used in this investigation. Five digestion trials were completed with the plant samples collected during any given collection period comprising a digestion trial. Individual plant samples were digested in duplicate. Samples of alfalfa hay were digested in each trial to test for variability in rumen fluid among digestion trials.

Rumen fluid used for digestion trials was obtained by stomach pumping captive mule deer housed on the Colorado State University campus. Experimental animals were fed a ration (Table 1) of cottonseed hulls (70%) and protein supplement (30%). The protein supplement contained 32 percent protein. A vitamin A, D, and E complex was added also to the ration (200, 000 I. U. per ton of ration). Animals were placed on the ration 2 weeks prior to the first digestion trial and remained on the ration until all digestion trials were completed,

Stomach pumping was accomplished by inserting one-inch half polyethylene tubing down the animal's esophagus and into the rumen. A vacuum pump was used next to induce siphoning of rumen contents (rumen fluid plus food particles) into a pre-warmed thermos. Animals were taken off feed 24 hours prior to pumping to reduce the quantity of undigested food in the rumen at the time of pumping. Once collected, rumen contents were transported immediately to the laboratory for processing.

In the laboratory, rumen contents were strained twice through two layers of cheesecloth to remove the larger particulate matter. The resulting fluid was then mixed with buffer solution. Carbon dioxide was bubbled next through the mixture for 15 to 20 minutes to lower the solution pH below 7.0. The solution was then allowed to stand until most particulate matter in the solution had settled to the bottom of the container (about 20 minutes).

Next, 50 ml of the rumen fluid-buffer mixture were pipetted into 100 ml digestion tubes containing 0.5 g of oven-dried forage. The digestion tubes were then flooded with carbon dioxide to create anaerobic conditions, stoppered, and incubated in a water bath at 39 C for 48 hours. Empty digestion tubes were included in each trial to determine the amount of foreign particulate matter remaining in the rumen fluid-buffer mixture.

After 48 hours, microbial digestion was terminated and enzymatic digestion initiated by adding hydrochloric acid and pepsin. Hydrochloric acid lowered the pH of the digestion medium to about 2.0, killing all microorganisms and activating the pepsin. The digestion tubes were then stoppered and placed back into the water bath at 39 C for an additional 48 hours.

Table 1. Nutrient concentration (oven-dry basis) of the ration fed mule deer used as rumen-fluid donors (after Schoonveld 1971).

	Percent Nutrient Content*						
	CP	EE	M	Ash	CWC	ADF	Lignin
	12.1	2.5	8.8	6.1	73.1	46.7	15

*CP   Crude protein
 EE   Ether extract
 M   Moisture
 CWC Cell-wall constituents
 ADF Acid-detergent fiber

Following enzymatic digestion, 12 ml of 0.65 M sodium carbonate were added to each digestion tube to terminate pepsin activity. The samples were then vacuum-filtered, oven-dried, and weighed.

Percent in vitro digestibility (oven-dry basis) of each forage sample was calculated with the formula:

$$\% \text{ Digestibility} = \frac{Wf - Wr - C}{Wf} \times 100$$

Where:

Wf = Oven-dry weight of forage sample tested

Wr = Oven-dry weight of undigested residue

C = Correction factor based upon the amount of sediment in the digestion-tube blanks.

Statistical Analyses of Data

Statistical analyses of data were performed with a 5x3x5 factorial analysis of variance design to test for statistically significant ($P < 0.05$) differences in nutrient concentration and in vitro digestibility of forages resulting from nitrogen treatment (5 treatments including control) study site (3 sites), and sample-collection date (5 dates). The three main variables were treated as fixed (systematic) effects.

Sources of variability in significant ($P < 0.05$) analyses of variances were located with the Least Significant Range (LSR) test. Bartlett's test for homogenity of variance was computed prior to comparing treatment means (Sokal and Rohlf 1969).

Data analyses were performed on a CDC-6400 computer at the
Colorado State University Computer Center and on an Olivetti-
Underwood Programma 101 desk calculator.

CHAPTER IV

RESULTS

This chapter discusses the results of statistical analyses of data. The nutrient concentration and digestibility of the forages varied considerably among collection sites and among collection periods. The nature of these variations and the physiological and/or environmental conditions which might have produced them are also discussed.

Crude Protein Concentration

The crude protein concentrations of bluebunch wheatgrass, sagebrush leaves, and sagebrush twigs are presented in Appendix I according to nitrogen treatment, study site, and collection date. Crude protein concentrations resulting from nitrogen treatment differed considerably among collection periods (Figs. 3, 4 and 5). Therefore, results obtained during collection periods were discussed individually.

May 1970

The crude protein concentration of bluebunch wheatgrass, sagebrush leaves, and sagebrush twigs showed no significant (P> 0.05)
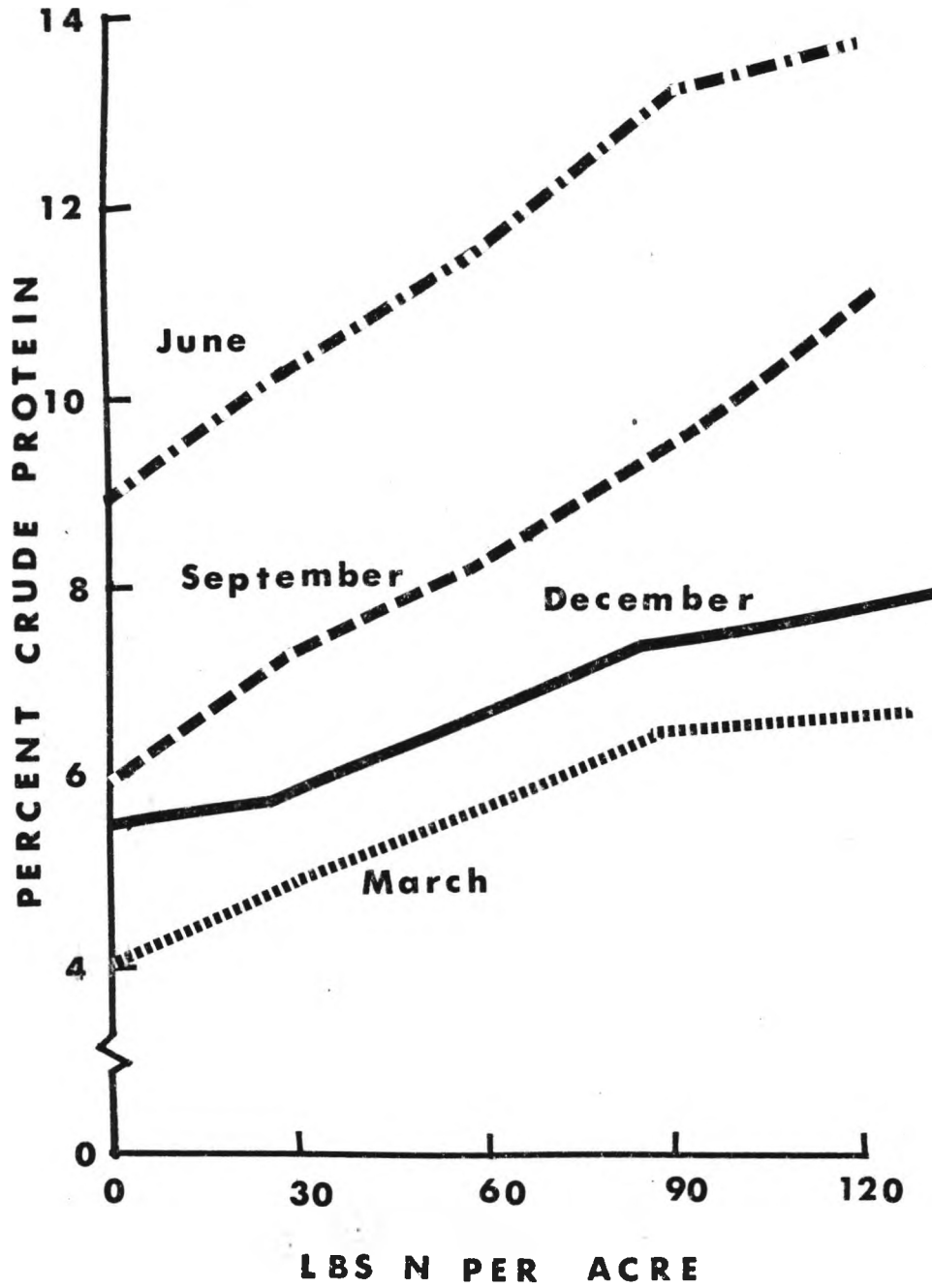
Fig. 3. Crude protein concentration (air-dry basis) of
bluebunch wheatgrass according to collection
date and nitrogen treatment (mean of all sites).
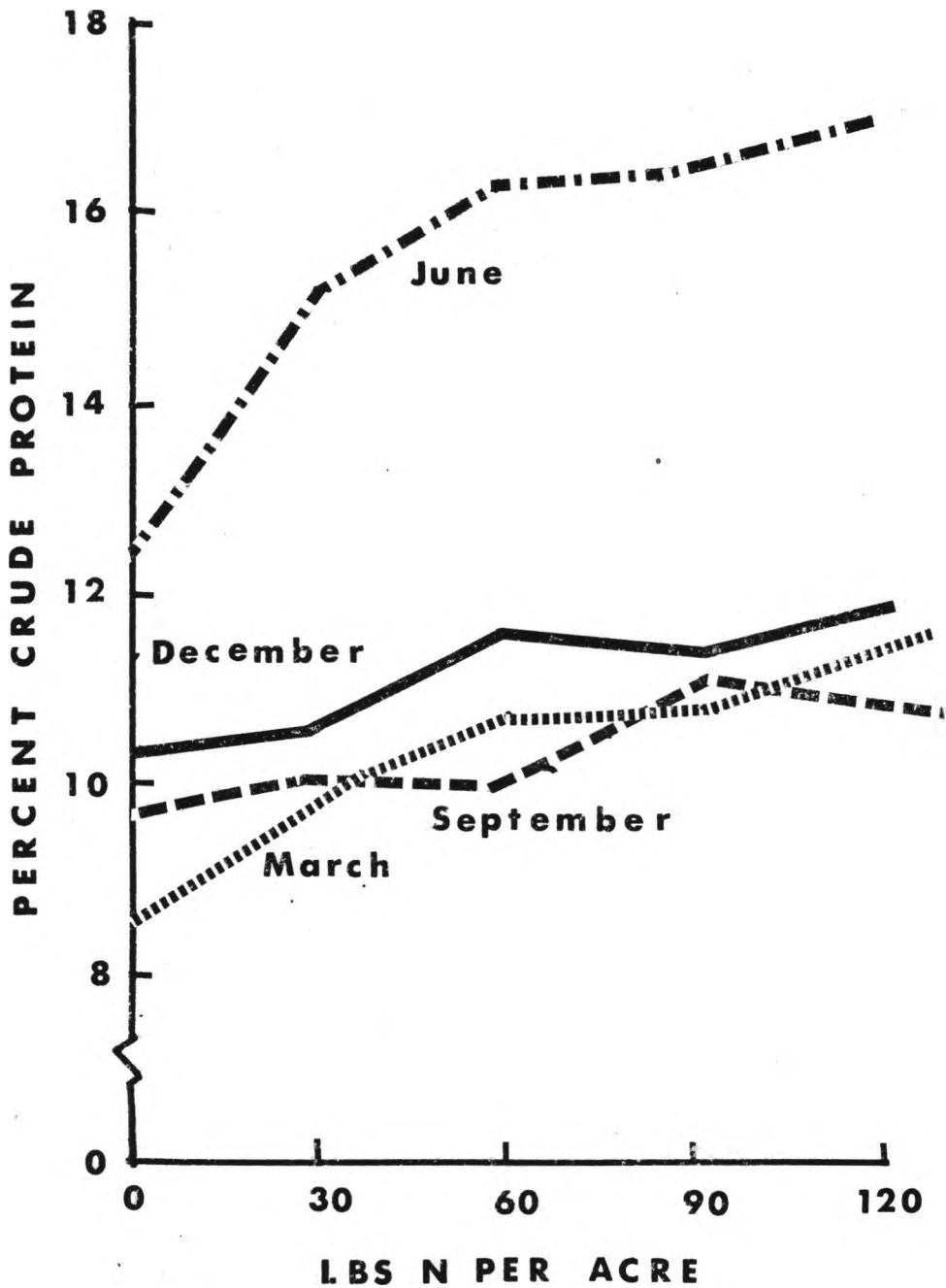
Fig. 4. Crude protein concentration (air-dry basis) of big sagebrush leaves according to collection date and nitrogen treatment (mean of all sites).
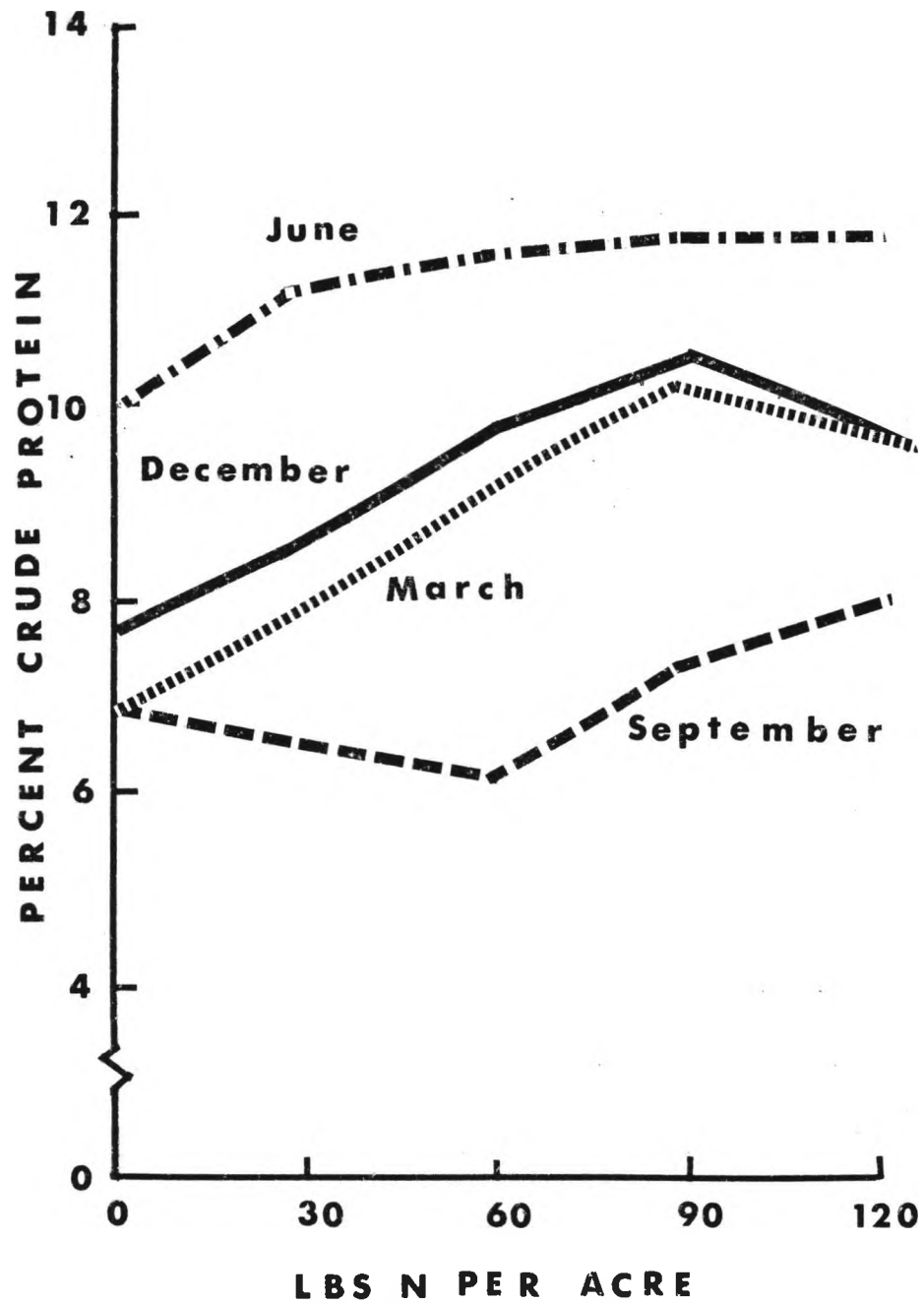
Fig. 5. Crude protein concentration (air-dry basis) of big
sagebrush twigs according to collection date and
nitrogen treatment (mean of all sites).

variation during May among nitrogen treatments. Sagebrush leaves and twigs harvested on unfertilized plots during May averaged 11.7 and 8.7 percent crude protein (air-dry basis) respectively while similar tissues from fertilized plots (all levels of nitrogen combined) averaged 12.1 and 9.1 percent crude protein respectively. Bluebunch wheatgrass clipped on unfertilized plots contained 4.0 percent crude protein compared to 4.9 percent crude protein for that harvested from fertilized plots during May (all levels of nitrogen combined).

The May plant collection was made immediately after a 2-foot snow cover melted from the experimental plots and before new growth by the plants was visibly evident. Some treatment effect was indicated by the results but, because most of the tissues collected were formed during the 1969 growing season before nitrogen was applied, data obtained from the sample were deleted from discussions and figures describing annual trends in nutrient concentration.

June 1970

Nitrogen treatment increased significantly ($P < 0.01$) the crude protein concentration of all test forages during June. This period was characterized by early plant growth and abundant soil moisture from snow melt. Generally, increased crude protein concentration resulting from nitrogen treatment was similar at the three study sites for all forages. Big sagebrush leaves and bluebunch wheatgrass increased more in crude protein concentration than sagebrush twigs

as a result of nitrogen treatment. Maximum increases in crude protein concentration for bluebunch wheatgrass, sagebrush leaves, and sagebrush twigs during June were 50 percent, 30 percent, and 18 percent (air-dry basis), respectively, greater than the protein concentration of similar tissues harvested from unfertilized plots. All levels of nitrogen application increased significantly ($P < 0.05$) the crude protein concentration over the controls.

The findings were explained best by metabolic processes operating within plant tissues. Plants absorb nitrogen from the soil as nitrates and then reduce nitrates to nitrites prior to synthesizing amino acids and proteins. Plant leaves are important sites of nitrate reduction and protein synthesis (Meyer et al. 1965). Thus, bluebunch wheatgrass and sagebrush leaves possibly increased more in crude protein concentration than sagebrush twigs during June because of greater rates of nitrate reduction and protein synthesis.

September 1970

All levels of nitrogen treatment did not increase significantly ($P > 0.05$) the crude protein concentration of the forages during September. Only the two heaviest applications (i.e. 90 and 120 pounds N per acre) increased the percentage of crude protein in sagebrush leaves and twigs. The reduced protein concentration in sagebrush leaves and twigs between June and September was perhaps the result of (1) leaching of nitrogen from the tissues by rain and/or (2) a

reduction in metabolic activity by sagebrush plants during the fall. Laycock and Price (1970) reported exposure to rain decreased the protein concentration of mature plant tissues. Therefore, leaching was suspected since August and September were the months of 1970 receiving the heaviest amounts of precipitation in Kremmling (Table 2). However, Dietz et al. (1962) reported similar decreases in the crude protein concentration of unfertilized big sagebrush plants from summer to mid winter, which suggested metabolic phenomena also may have been involved.

Crude protein concentration of bluebunch wheatgrass during September showed a significant (P <0.01), linear relationship with nitrogen treatment. However, wheatgrass in Idaho was believed to have terminated active growth by July (Blaisdell et al. 1952). Perhaps the protein present in wheatgrass tissues during September was stored from the growing season (June-July). The only seasonal changes in crude protein concentration of bluebunch wheatgrass were uniform decreases at each level of nitrogen treatment. The largest seasonal decrease in protein concentration occurred between June and September when the wheatgrass presumably became dormant and leaching of nitrogen by rain might have occurred.

December 1970

Nitrogen treatment increased significantly (P <0.01) the crude protein concentration of sagebrush leaves and twigs collected during

Table 2. Monthly precipitation (in inches) at Kremmling, Colorado, 1969-70 (taken from USDC Weather Service).

Month	Year	
	1969	1970
January	1.84	0.55
February	0.31	0.15
March	0.06	0.80
April	0.76	0.94
May	0.44	0.03
June	4.32	1.50
July	0.92	0.71
August	1.18	2.84
September	1.18	1.64
October	3.77	1.34
November	0.32	1.08
December	0.90	0.55
Total	16.00	12.13

December. Leaves from sagebrush plants growing on plots fertilized with 120 pounds N per acre increased 15 percent in crude protein concentration over leaves from sagebrush plants growing on unfertilized plots. Additionally, sagebrush twigs clipped on fertilized plots increased protein concentration by as much as 30 percent over twigs taken from unfertilized plots. All levels of nitrogen application increased significantly ($P < 0.05$) the concentration of crude protein over the controls.

March 1971

The crude protein concentration of sagebrush leaves in March was increased by fertilizer by as much as 32 percent over leaves from plants on unfertilized plots, while twigs from sagebrush plants on fertilized plots increased to 50 percent in crude protein concentration over twigs harvested from sagebrush plants on unfertilized plots.

Increased crude protein concentration of sagebrush tissues during December and March resulting from nitrogen treatment was unexplained. Dietz et al. (1962) reported higher crude protein concentration in unfertilized sagebrush annual growth during early April over early January. Similar findings in the two studies suggested big sagebrush plants maintained a low level of metabolic activity (e.g. protein synthesis) throughout fall and early winter but increased metabolic activity and growth during late winter. Intermittent snow cover on the sites provided soil moisture during late winter but I

found no information on the plant's ability to use soil moisture at that time. Thus, the failure of nitrogen treatments to increase crude protein levels in big sagebrush tissues during May, 1970 possibly indicated applied nitrogen had not penetrated to the root depth of the plants by that time.

Cell-Wall Constituents (CWC)

Cell-wall-constituent concentration of the forages showed no significant ($P > 0.05$) variations among nitrogen treatments but varied significantly ($P < 0.01$) among collection periods and among collection sites (Figs. 6, 7, and 8).

The CWC concentration of bluebunch wheatgrass was significantly ($P < 0.05$) lower during June and September than during December and March. Bluebunch wheatgrass averaged 57.1 and 56.4 percent CWC (oven-dry basis) during June and September, respectively, but increased to 62.4 and 61.7 percent CWC (oven-dry basis) during December and March, respectively.

Big sagebrush tissues varied also in CWC concentration among collection periods. Sagebrush leaves and twigs were highest in CWC concentration during September, decreased significantly ($P < 0.01$) by December, but increased significantly ($P < 0.05$) by March.

Seasonal trends in CWC concentration were thought to be due partly to annual growth patterns and partly to moisture conditions during the time periods concerned. Laycock and Price (1970) listed
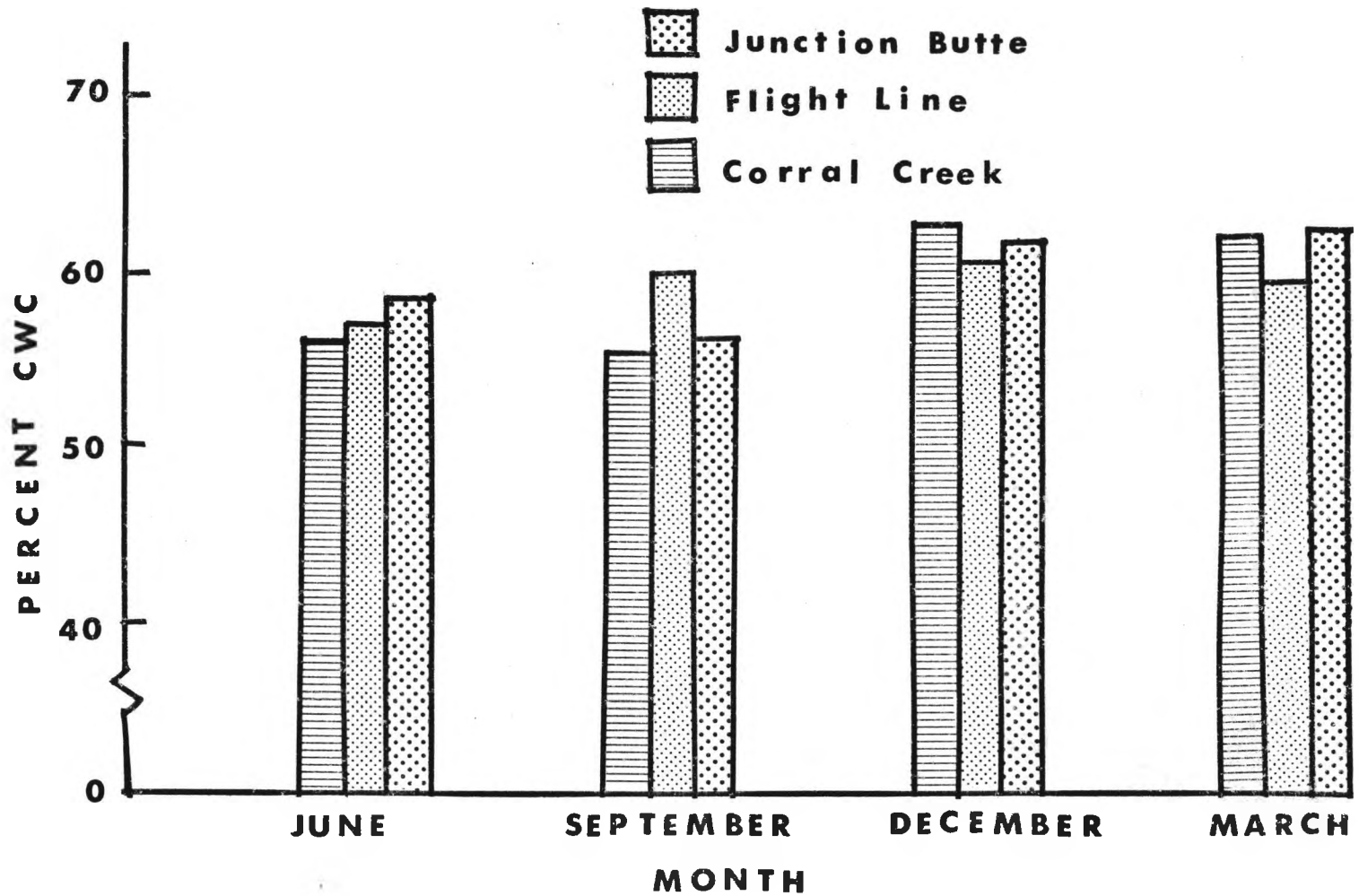
Fig. 6. Cell-wall-constituent concentration (oven-dry basis) of bluebunch wheatgrass according to collection date and study site (mean of N levels plus control).

Fig. 7. Cell-wall-constituent concentration (oven-dry basis) of big sagebrush leaves according to collection date and study site (mean of N levels plus control).

Fig. 8. Cell-wall-constituent concentration (oven-dry basis) of big sagebrush twigs according to collection date and study site (mean of N levels plus control).

15 elements and inorganic compounds, 8 carbohydrates, 23 amino acids, and 15 organic acids often leached from plant tissues by rain. They also stated young plant tissues are less susceptible to leaching than mature tissues. Cellulose, hemicellulose, and lignin (components of CWC) are not leached from plants by rain (Guilbert et al. 1931). Perhaps during September the plants matured and rain leached many of the protoplasmic components (i.e. neutral-detergent solubles) from the tissues making it appear the plants increased in CWC concentration.

December, 1970 was a dry month in Middle Park. Moveover, the effect of snowfall on leaching, while unknown, probably is negligible. However, big sagebrush tissues decreased sharply in CWC concentration by December. These findings possibly resulted from (1) the sagebrush plants becoming metabolically active and producing new growth and (2) leaching of nutrients being minimal. Bluebunch wheatgrass remained constant in CWC concentration during December and March. Assuming no new growth by bluebunch wheatgrass after July (Blaisdell et al. 1952), these findings possibly indicated most nutrients in the wheatgrass susceptible to leaching were removed by December.

Variations in the CWC concentration of plants growing on different sites were not explained ecologically but might have resulted from variations in soil-moisture conditions on the sites. Meyer et al.

(1965) reported plants growing in moisture-deficient areas grow slowly, have poor cell division, and develop thick cell walls.

Calcium Concentration

The average calcium concentration in bluebunch wheatgrass, sagebrush leaves, and sagebrush twigs is shown in Figs. 9, 10, and 11 respectively and Appendix I. Nitrogen treatments failed to alter significantly ($P > 0.05$) the calcium concentration of any forage. Therefore, the 10 calcium determinations made on each forage from each site were averaged and the means compared statistically among collection periods and among study sites.

Bluebunch wheatgrass and sagebrush leaves were highest in calcium concentration during September, when the tissues presumably matured, but decreased in calcium concentration by December and March. Big sagebrush twigs were lowest in calcium concentration during June but increased in calcium concentration by September. Unlike bluebunch wheatgrass and sagebrush leaves, however, calcium levels in sagebrush twigs remained constant September through March. These findings agreed with Dietz (1965) who reported the stems of four deciduous shrubs contained highest percentages of nutrients during late winter.

Seasonal trends in the calcium concentration of the forages were explained best by the role of calcium in plant cell physiology. Meyer et al. (1965) reported 70 percent of the calcium in plant tissues is
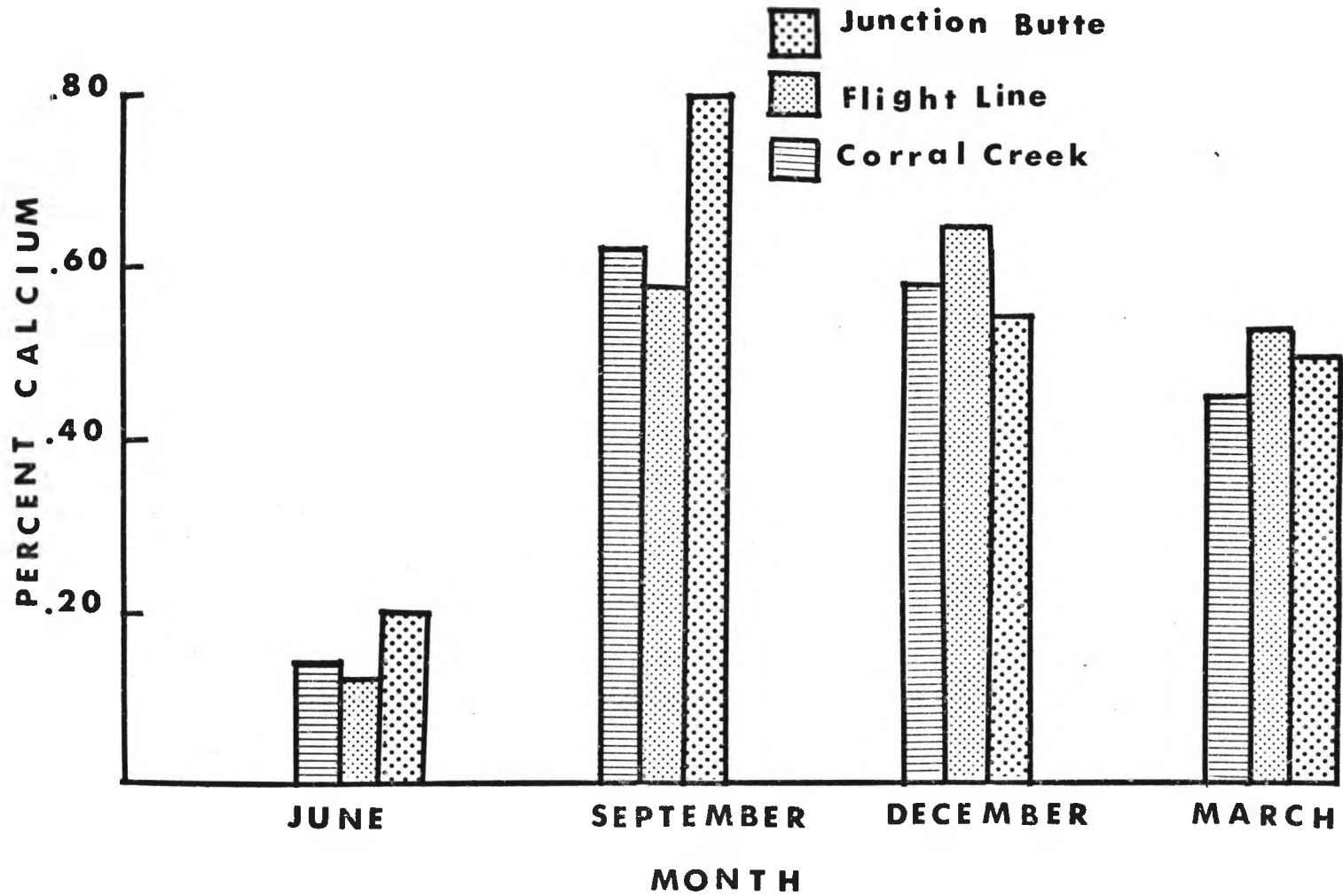
Fig. 9. Calcium concentration (air-dry basis) of bluebunch wheatgrass according to collection date and study site (mean of N levels plus control).
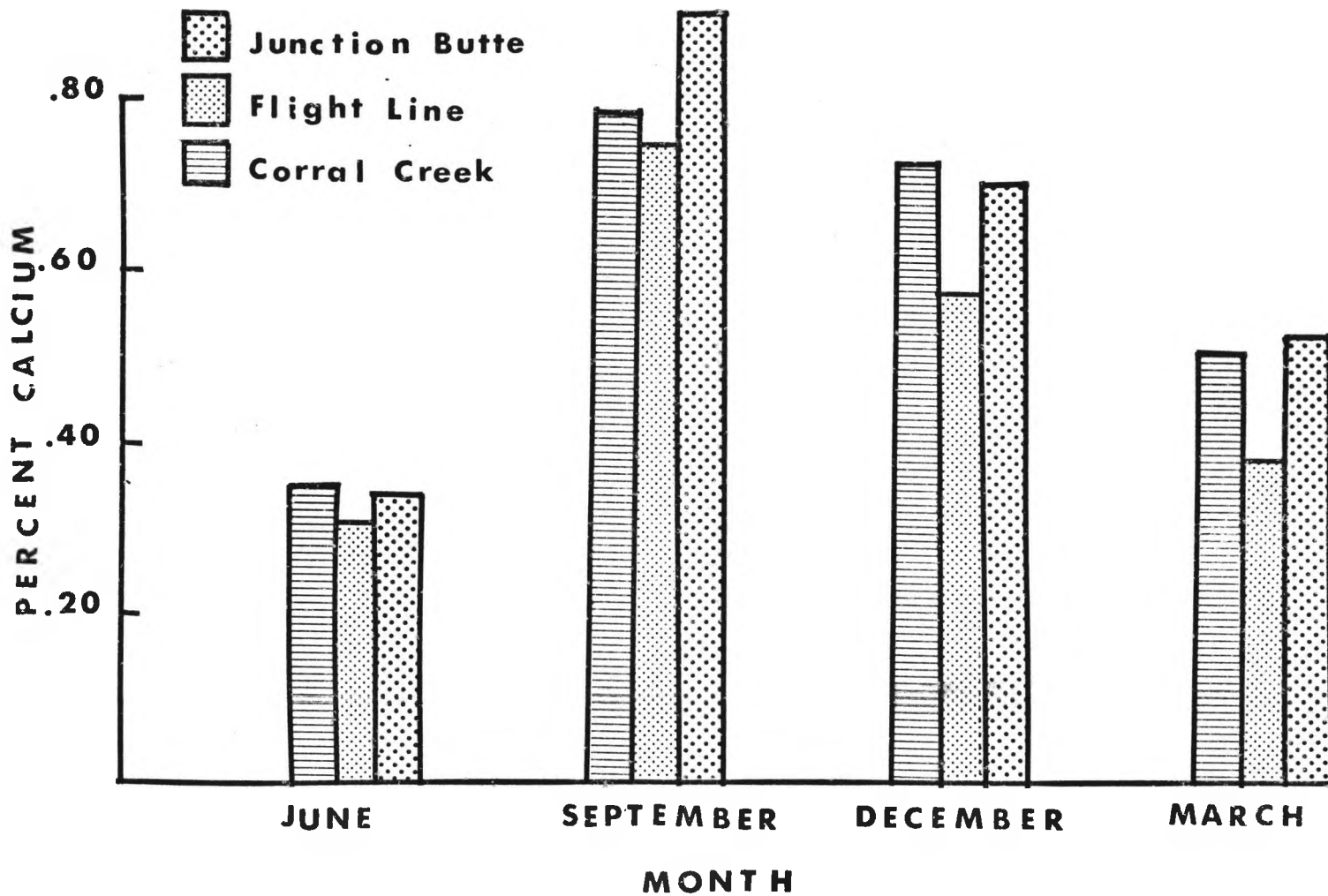
Fig. 10. Calcium concentration (air-dry basis) of big sagebrush leaves according to collection date and study site (mean of N treatments plus control).
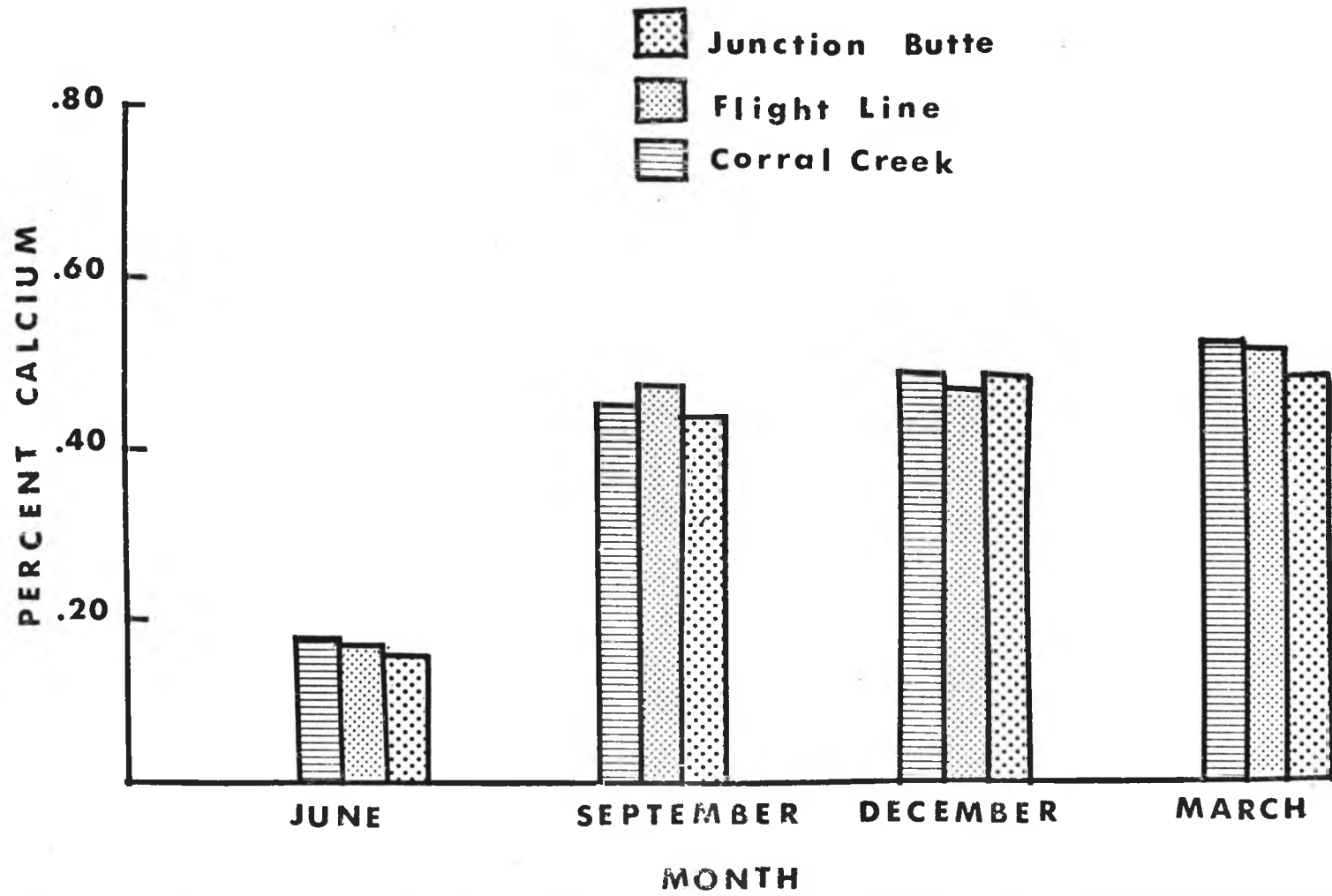
Fig. 11. Calcium concentration (air-dry basis) of big sagebrush twigs according to collection date and study site (mean of N levels plus control).

fixed as calcium pectate in the middle lamella of plant cells walls and that the remaining calcium is associated with cellular protoplasts in meristematic tissues. Calcium in the form of calcium pectate is immobile within plant tissues whereas ionic calcium associated with cellular protoplasts is transient. Calcium levels in the three forages then, may have been lowest during June because of low quantities of cell-wall material in young growth.

As the tissues matured, and developed more cell-wall material, their calcium concentration would be expected to increase. Decreased calcium concentration in sagebrush leaves by December and March might be the result of (1) ionic calcium transferring from leaves to twigs and/or (2) the formation of new sagebrush leaves inherently low in calcium concentration. Leaching resulting from snow melt might have reduced the calcium concentration in bluebunch wheatgrass by December and March.

Phosphorus Concentration

Phosphorus concentration of the forages did not vary significantly ($P > 0.05$) among nitrogen treatments on any collection date but varied significantly ($P < 0.01$) among study sites and among collection dates (Figs. 12, 13, and 14). Big sagebrush leaves were lowest in phosphorus concentration during June but increased significantly ($P < 0.01$) in phosphorus concentration during September. Phosphorus levels in sagebrush leaves did not vary significantly ($P > 0.05$)
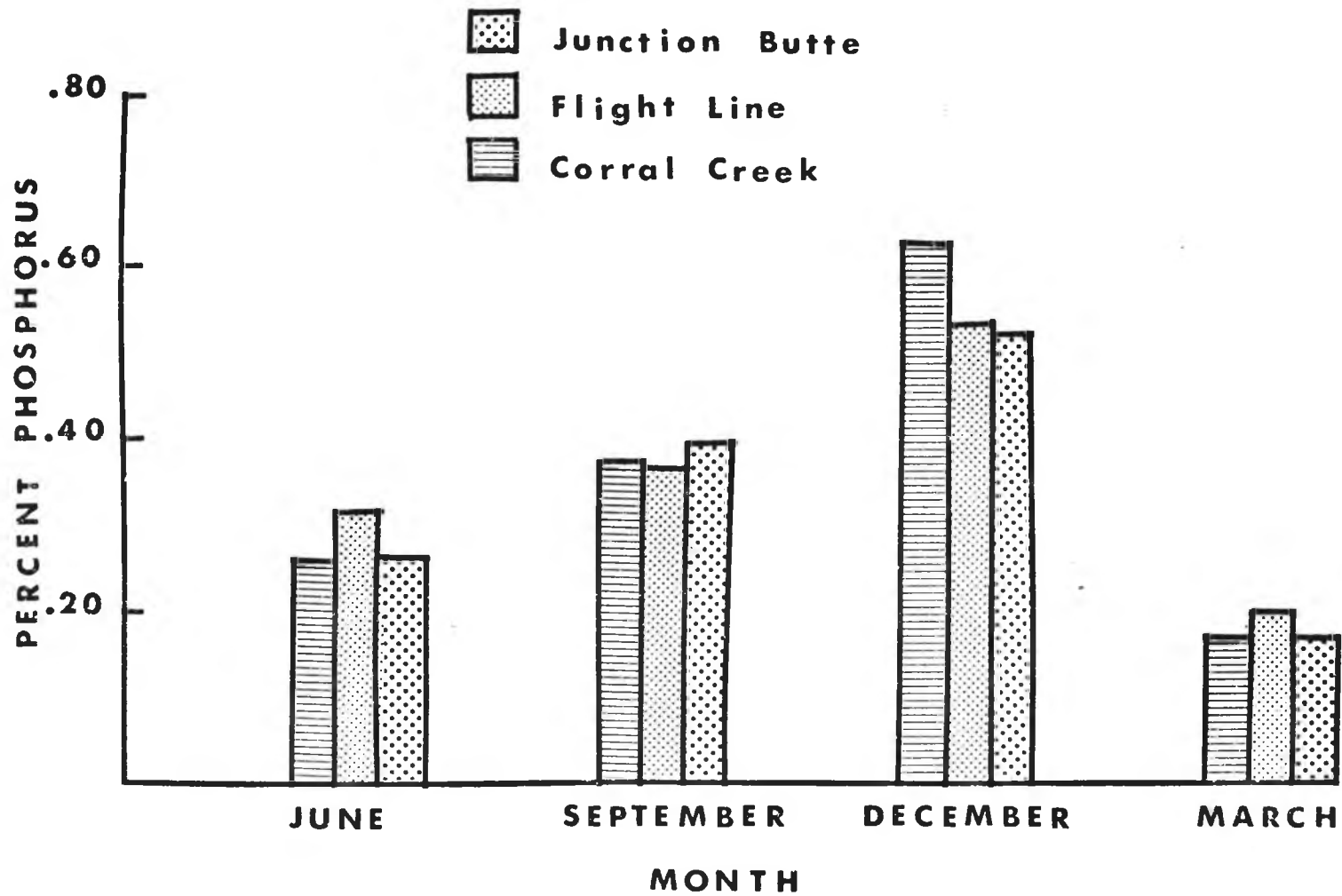
Fig. 12. Phosphorus concentration (air-dry basis) of bluebunch wheatgrass according to collection date and study site (mean of N levels plus control).
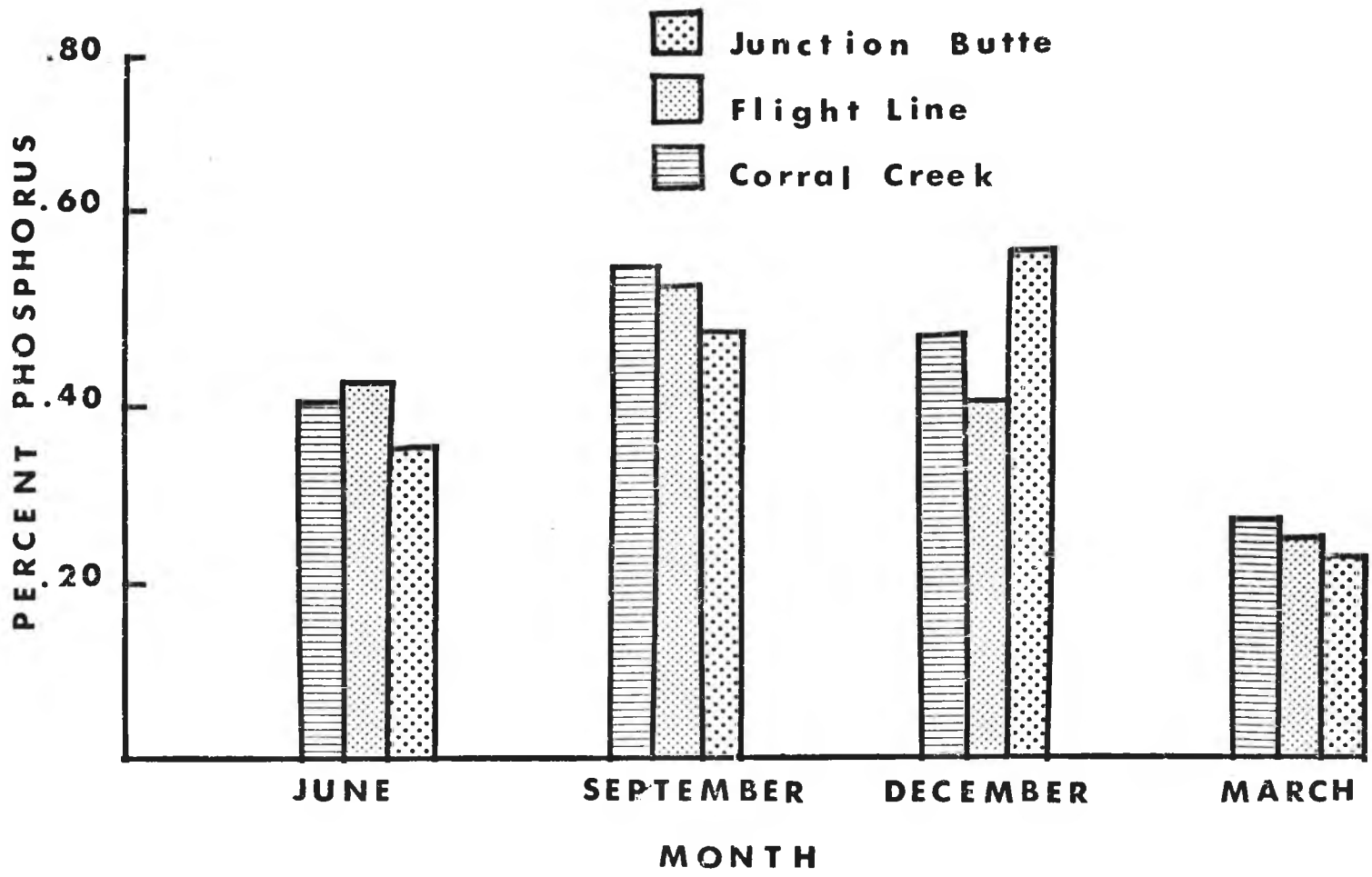
Fig. 13. Phosphorus concentration (air-dry basis) of big sagebrush leaves according to collection date and study site (mean of N levels plus control).
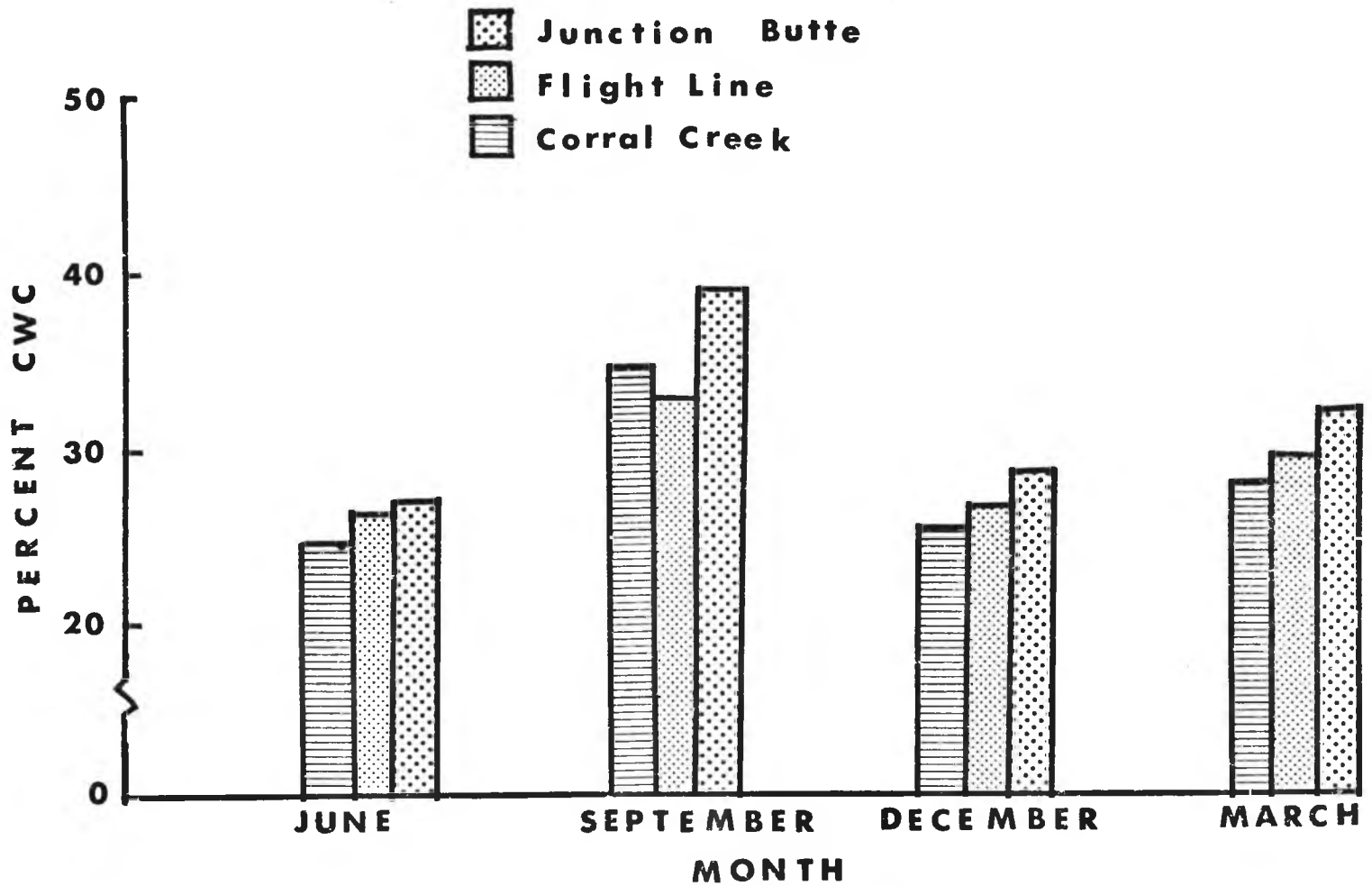
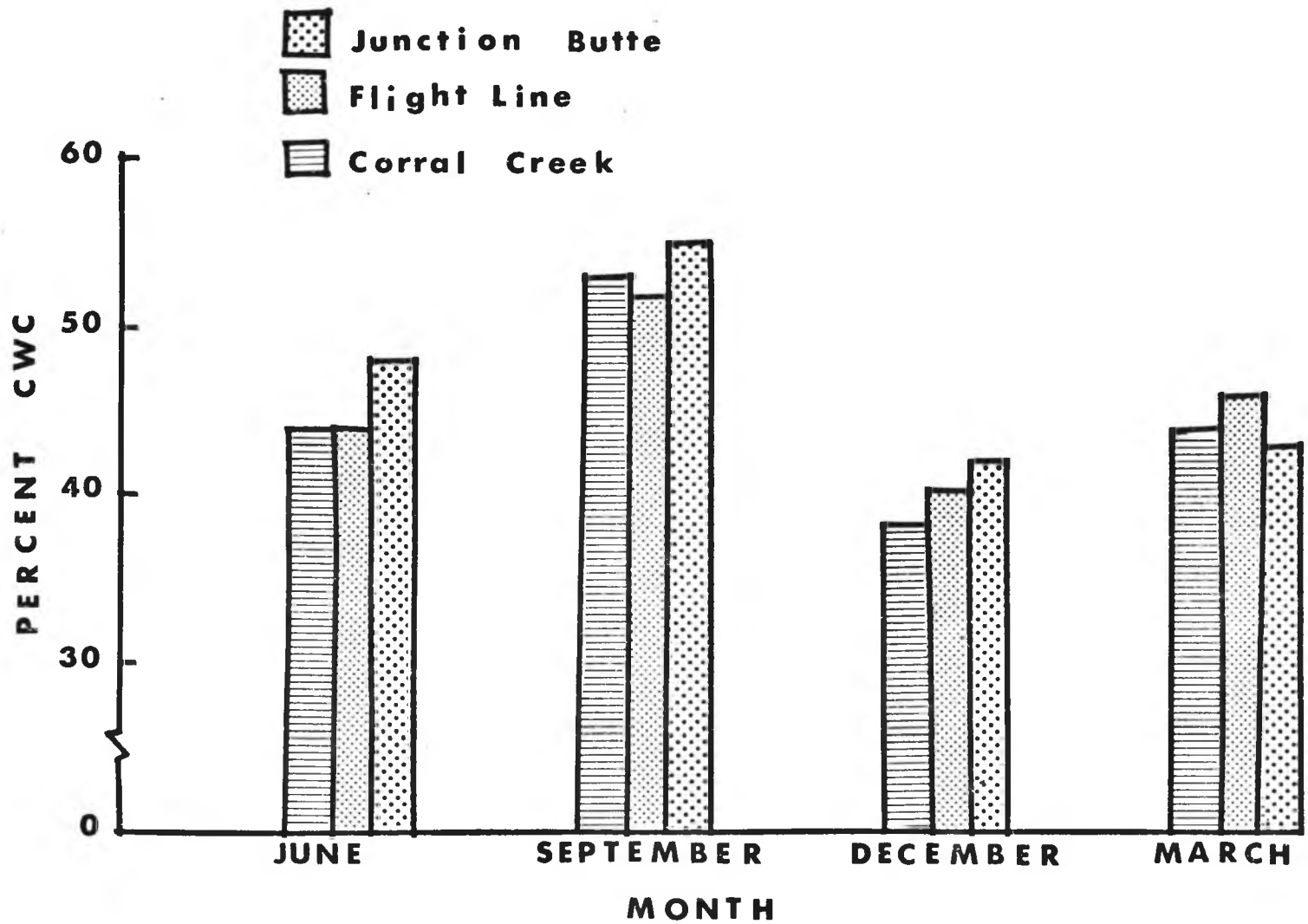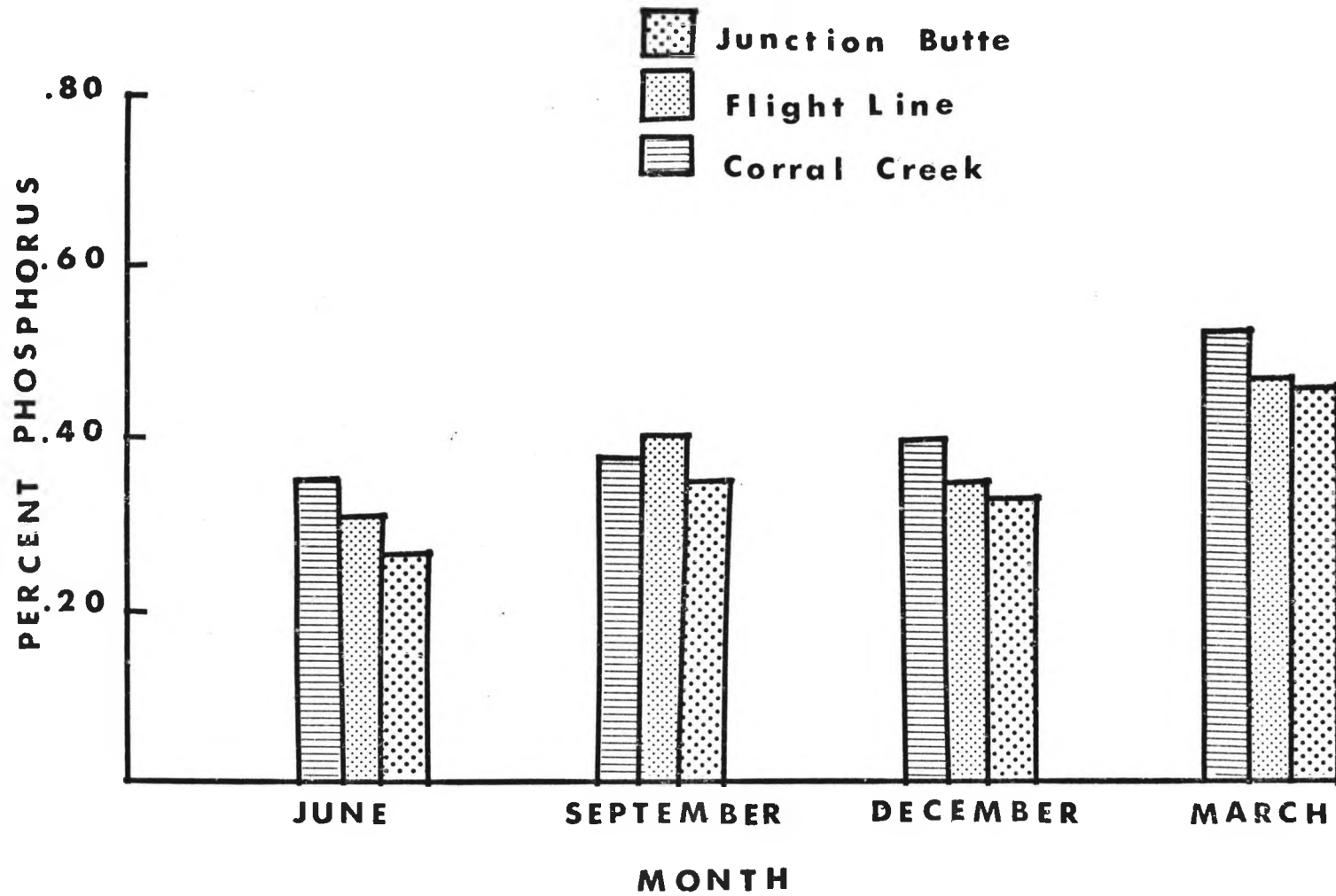Fig. 14. Phosphorus concentration (air-dry basis) of big sagebrush twigs according to collection date and study site (mean of N levels plus control).

between September and December but decreased significantly
(P <0.01) between December and March. Also sagebrush twigs were
lowest in phosphorus concentration during June but, phosphorus
concentration increased progressively from September to March.

With respect to phosphorus concentration, bluebunch wheatgrass
exhibited a seasonal trend similar to sagebrush leaves except that
bluebunch wheatgrass increased significantly (P <0.01) between
September and December. This implied phosphorus uptake by wheat-
grass between September and December. However, Blaisdell et al.
(1952) reported bluebunch wheatgrass in Idaho terminated growth by
July. Possibly the phosphorus levels of bluebunch wheatgrass during
September were abnormally low as a result of leaching. Leaching
resulting from snow melt might also have accounted for the reduced
phosphorus concentration in bluebunch wheatgrass during March.

Variations in the phosphorus concentration of plants growing on
different sites were not explained by mineral concentrations of the
soils. Phosphorus levels for soils on Junction Butte, Flight Line,
and Corral Creek were 19, 23, and 27 pounds P_2O_5 per acre
respectively (Heil 1969). Thus, other unexplained factors were
involved.

Calcium-Phosphorus Ratio

Gilbert (1957) reported proper calcium-phosphorus nutrition
for animals to be a function of (1) the percentage of calcium and

phosphorus in the diet, (2) the ratio between calcium and phosphorus in the diet, and (3) the presence of vitamin D in the diet. According to Gilbert, desirable calcium-phosphorus ratios for animal nutrition lie somewhere between 2:1 and 1:2 if sufficient vitamin D is available.

The forages tested were within the desired range (Table 3). Only bluebunch wheatgrass during March, 1971 had a calcium-phosphorus ratio outside the desired range (2.53:1).

Table 3. Calcium-phosphorus ratios for bluebunch wheatgrass, sage-brush leaves, and sagebrush terminal twigs according to collection date (all study sites combined).

Forage	Collection Date	% Ca*	% P*	Ca: P Ratio
Bluebunch Wheatgrass	May	.12	.23	1 : 1.92
	June	.15	.28	1 : 1.87
	September	.67	.38	1.76: 1
	December	.58	.56	1.03: 1
	March	.48	.19	2.53: 1
Sagebrush Leaves	May	.26	.38	1 : 1.46
	June	.32	.39	1 : 1.22
	September	.81	.51	1.59: 1
	December	.66	.49	1.35: 1
	March	.47	.26	1.35: 1
Sagebrush Twigs	May	.15	.28	1 : 1.87
	June	.18	.31	1 : 1.72
	September	.43	.37	1.16: 1
	December	.48	.38	1.26: 1
	March	.49	.49	1 : 1

*Air-dry basis

The mineral requirements of most wild ungulates are unknown but Mc Ewen et al. (1957) found white-tailed deer (Odocoileus virginianus) fawns maintained on a diet containing 0.30 percent each of calcium and phosphorus showed no signs of nutritional stress during the winter providing they entered the winter in good physical condition. However, fawns maintained on diets of 0.09 percent phosphorus failed to survive the winter. Assuming similar mineral requirements for mule deer, the forages tested here contained adequate quantities of calcium and phosphorus during the winter.

Dry-Matter Digestibility

Dry-matter digestibility of the plant tissues showed no significant ($P > 0.05$) variation among nitrogen treatments (Appendix I) but varied significantly ($P < 0.05$) among collection dates.

Bluebunch wheatgrass decreased steadily in dry-matter digestibility during the collection periods (Fig. 15). The digestibility of bluebunch wheatgrass did not vary significantly ($P > 0.05$) among study sites during June and September but varied significantly ($P < 0.05$) among collection sites during December and March. During December and March, bluebunch wheatgrass from the Flight Line site was more digestible than that from the Corral Creek or Junction Butte sites. This might be explained by the lower CWC concentration of bluebunch wheatgrass from the Flight Line site in December and March.
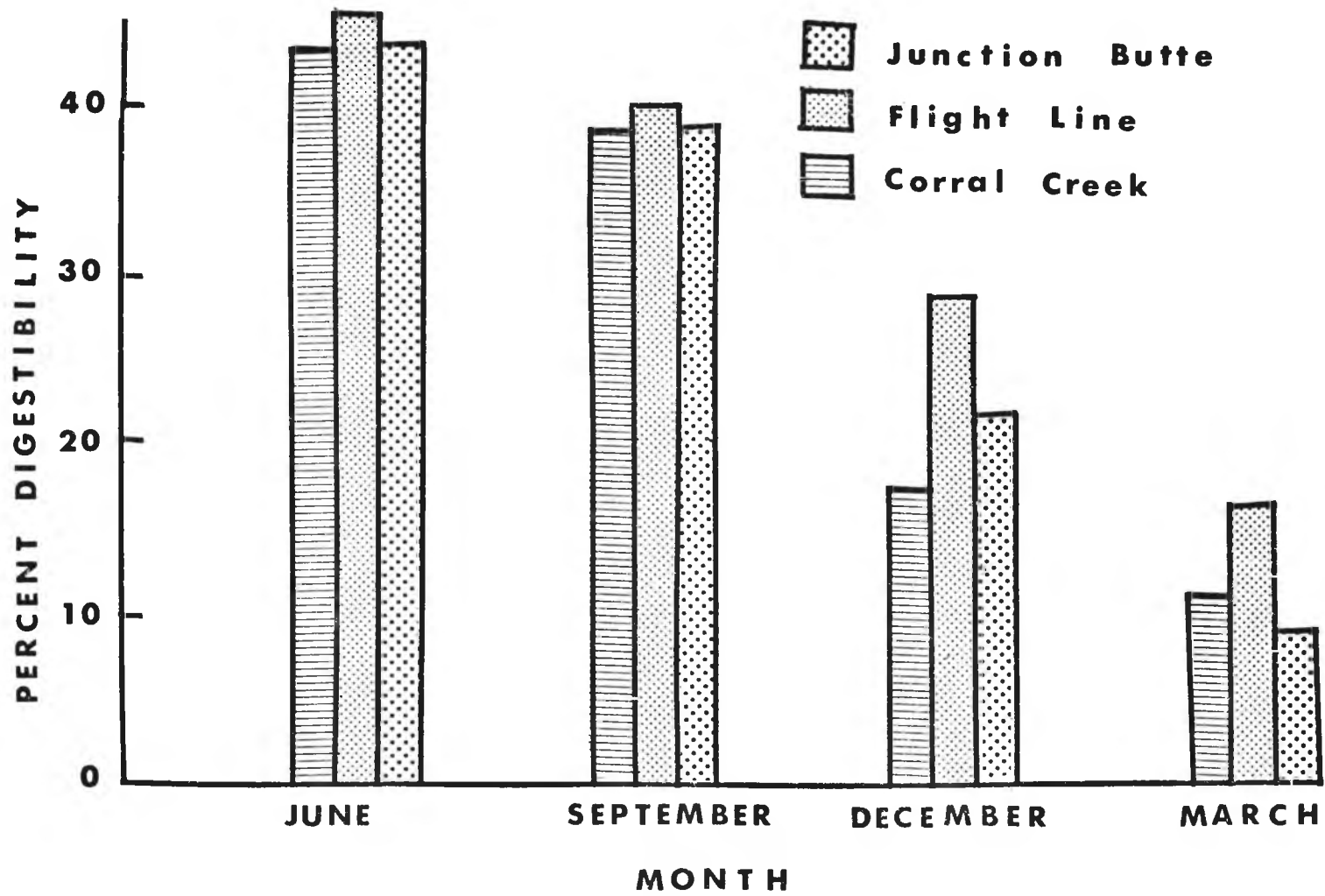
Fig. 15. Dry-matter digestibility of bluebunch wheatgrass according to collection date and study site (mean of N levels plus control).

Big sagebrush leaves decreased also in dry-matter digestibility during successive collection periods (Fig. 16) but the samples did not vary significantly (P >0.05) among study sites.

The pattern was somewhat different with sagebrush twigs in that digestibility of the December sample was higher than that of September or March samples (Fig. 17).

Results of in vitro digestion trials on big sagebrush tissues might have been in error because (1) the digestibility of sagebrush twigs was nearly that of sagebrush leaves and (2) the digestibility of sagebrush leaves and twigs were not correlated with the CWC concentration of the respective tissues. The dry-matter digestibility of blue-bunch wheatgrass was significantly (P <0.05) correlated with CWC concentration (r = -0.90).

In the forage-grinding process, sagebrush leaves and twigs formed small, compact clumps which might have decreased microbial digestion by reducing the forage surface exposed to the micro-organisms. Volatile oils in the sagebrush tissues were not thought to have interferred with microbial digestion because oven-dried sage-brush tissues contained only trace quantities of volatile oils.

Variations in dry-matter digestibility of the forages among collection dates (i.e. among digestion trials) could not be attributed to variations in rumen fluid used in the respective trials. Samples of alfalfa included in each digestion trial were 45.1, 47.4, 52.6, 45.6
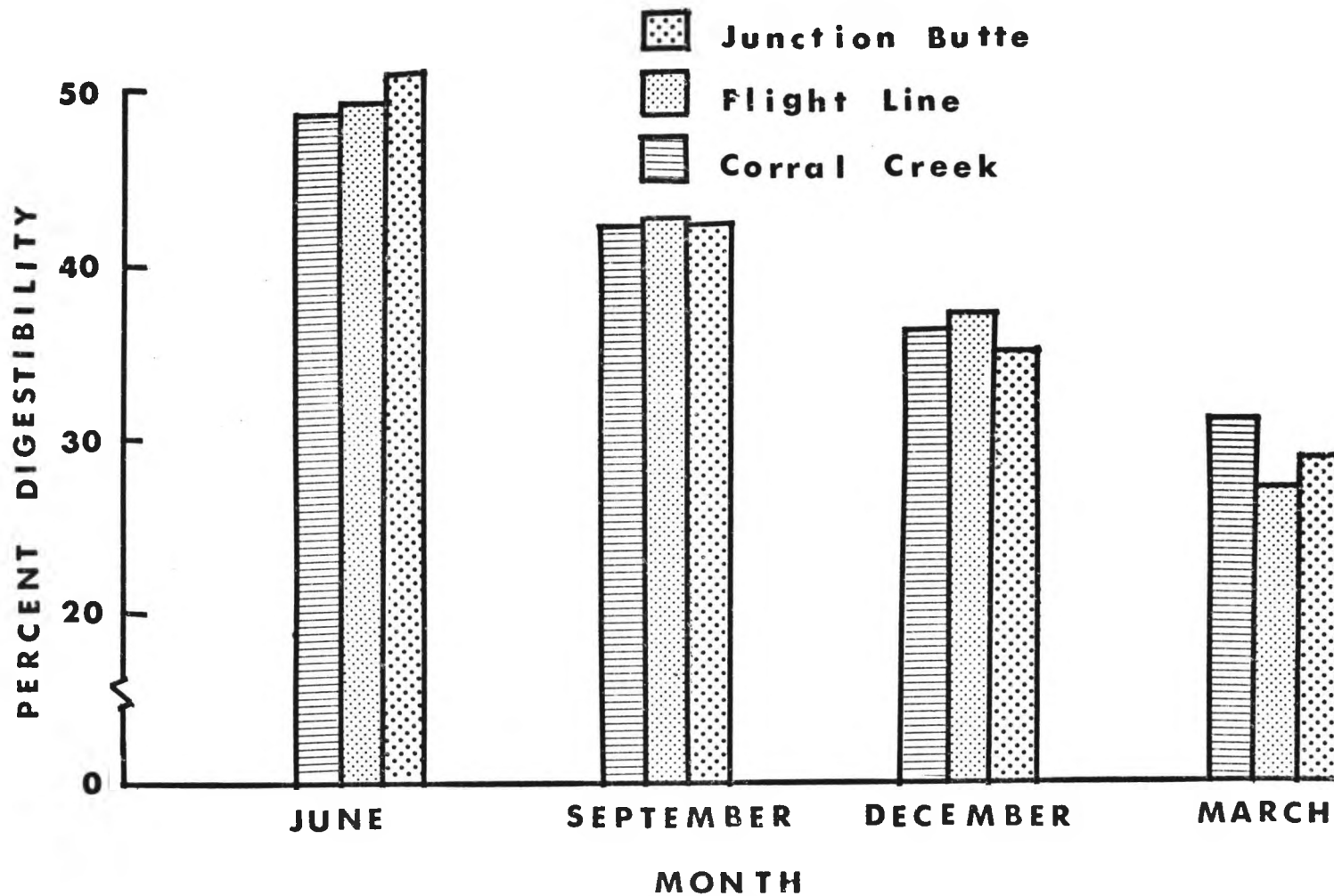
Fig. 16. Dry-matter digestibility of big sagebrush leaves according to collection date and study site (mean of N levels plus control).
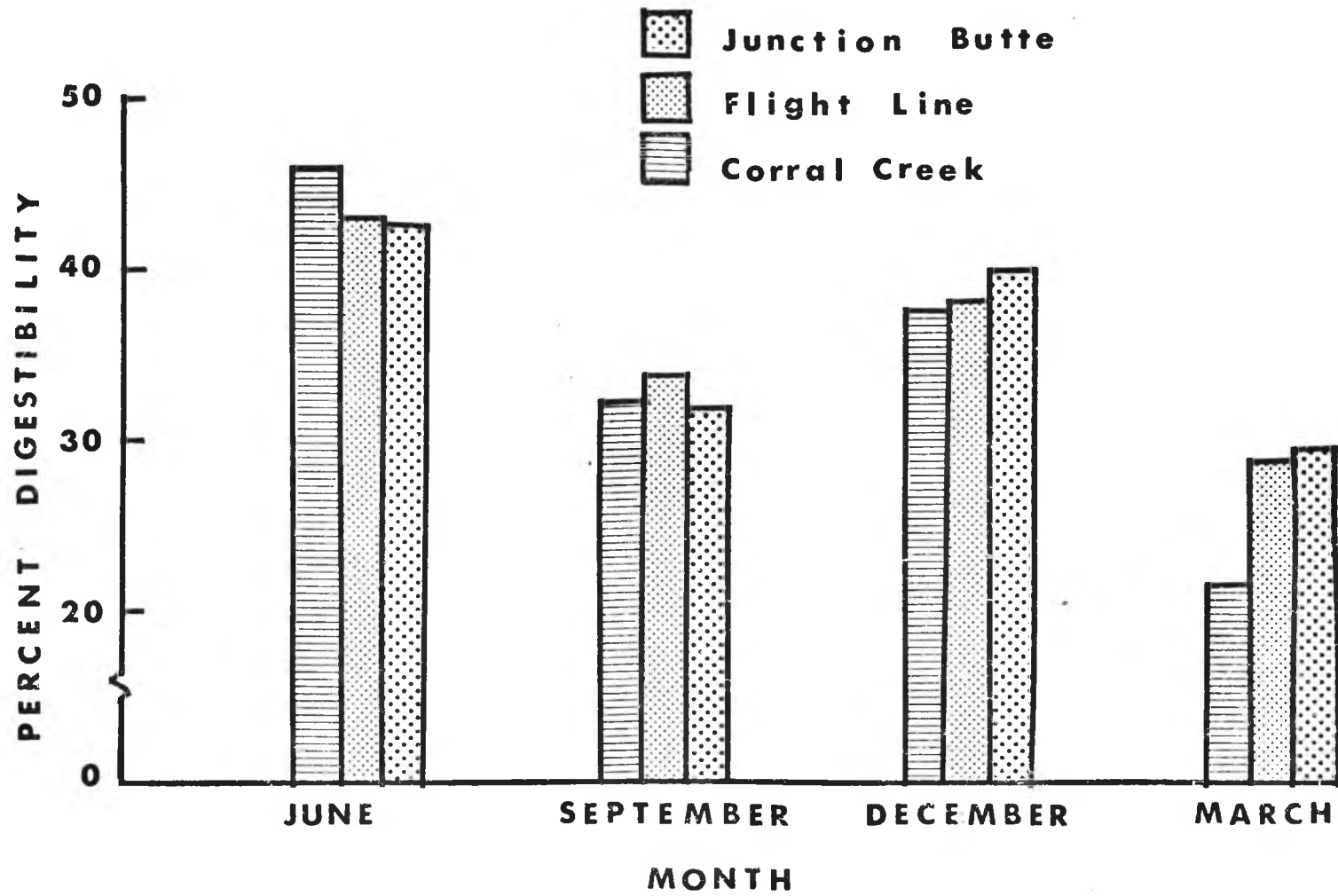
Fig. 17. Dry-matter digestibility of big sagebrush twigs according to collection date and study site (mean of N levels plus control).

and 47.9 percent digestible (oven-dry basis) which were consistent with Morrison's (1950) figure of 50.3 percent for alfalfa digested by cattle and sheep. The variations in alfalfa digestibility were not sufficient to explain the variations in sample digestibilities.

Volatile Oils

The volatile-oil concentration of big sagebrush leaves failed to vary significantly (P > 0.05) among nitrogen treatments but varied significantly (P < 0.01) among study sites and among collection dates (Table 4).

Nagy (1966) reported decreased volatile-oil concentration in big sagebrush annual growth from fall to spring. In the present study, volatile oil concentration was highest during September and decreased significantly (P < 0.05) from September to December. It did not decrease from December to March. Since volatile oil concentration was measured on a fresh-weight basis, variations in leaf moisture content may have produced errors in seasonal comparisons. This would not be a problem within seasons.

Volatile-oil concentration of sagebrush leaves varied most between study sites in June and September when leaves from the Junction Butte site were higher in volatile-oil concentration than leaves from the Flight Line or Corral Creek sites. Because of its southern exposure and lower annual-precipitation profile, the Junction Butte site probably has a lower moisture economy. Powell (1968)

suggested moisture stress during mid-summer might cause increased oil production in vigorously growing sagebrush plants.

Powell also reported a highly significant (P <0.01) correlation between volatile-oil production by big sagebrush and percent nitrogen in the 6 to 12-inch soil layer. Findings of the present study did not appear to support Powell's conclusions.

Table 4. Volatile-oil concentration (fresh-weight basis) of big sage-brush leaves during various months of the year, all nitrogen levels combined.

Month	Study Site			Mean ±S. D.
	Junction Butte	Flight Line	Corral Creek	
June	1.8	1.3	1.2	1.43 ± .32
September	1.9	1.7	1.8	1.80 ± .10
December	1.7	1.7	1.7	1.70 ± .00
March	1.8	1.7	1.5	1.73 ± .12

CHAPTER V

DISCUSSION

Research should supply the needs of management. This study was prompted by a management problem and the results should be discussed in terms of that problem. Therefore, this chapter discusses selected results of this research in terms of mule deer nutrition and their possible management significance for the Middle Park mule deer herd.

Protein Consumption and Animal Reproduction

Protein consumption has been linked closely with animal reproduction. Maynard and Loosli (1965) reported rations containing quantities of protein adequate for animal maintenance and growth should be adequate also during the periods of conception and early fetal development. They additionally reported the protein requirements of pregnant females increased during the last half of gestation when fetal development accelerates.

Murphy and Coates (1966) conducted experiments on white-tailed deer in Missouri during the winter and found mature does fed rations containing 7 and 10 percent crude protein produced a higher percentage of stillbirths than does fed rations containing 13 percent crude protein. They summarized their findings by stating

"---protein content of forage may be the critical nutrient on some ranges and may account for the low productivity and poor physical development which has been observed."

Significance of Findings for the Middle Park Herd

Gill (1971) reported that conception of the Middle Park mule deer herd occurs mostly during November and December with parturition occurring mostly during mid-June. Therefore, if fawn production is below normal in the Middle Park herd it might be desirable to provide additional protein in the diets of pregnant does from mid-January until parturition. Nitrogen fertilization of winter range appears to be an effective method of accomplishing that goal. It should be pointed out, however, that factors such as disease, population density, and behavioral patterns may also exert influences on reproduction.

Findings of this study should be considered preliminary in solving the nutritional problems operating on the Middle Park herd. The merit of the study in solving the problem of winter starvation losses can be judged only after considering also information on increased forage production resulting from nitrogen fertilization.

Suggestions for Additional Study

Limitations on man-power and time required this study to answer a few questions relating to the fertilization of winter range.

Several other interesting questions exist and deserve consideration in present or future research endeavors. These might include but not be limited to:

(1)   How long do treatment effects last?

(2)   How do forage plants other than big sagebrush and bluebunch wheatgrass respond to fertilizer?

(3)   How would spring or summer application of fertilizer influence the responses occurring in plants?

(4)   Could the concentration of crude protein be increased more by a second nitrogen application?

(5)   How do soil-moisture conditions influence plant responses to fertilizer?

CHAPTER VI

SUMMARY

The effect of nitrogen fertilizer on certain nutritional components of two forage species used by mule deer on winter range in western Colorado was tested. Ammonium nitrate fertilizer (33% N) was applied by Len H. Carpenter, Colorado Game, Fish and Parks Division, to three study sites in Middle Park, Colorado during early November, 1969. Fertilizer treatments providing 30, 60, 90, and 120 pounds of elemental nitrogen per acre were applied randomly to 100- x 100-foot treatment plots on each study site. One plot on each site was not fertilized and served as a control.

Big sagebrush (Artemisia tridentata) current annual growth and bluebunch wheatgrass (Agropyron spicatum) harvested from fertilized plots during May, June, September, and December of 1970 and March, 1971 were analyzed for crude protein, cell-wall constituents, calcium, phosphorus and in vitro digestibility. Leaves and terminal twigs of big sagebrush current annual growth were anlyzed separately. The volatile-oil concentration of sagebrush leaves was determined also by steam distillation. Results of analyses were compared statistically with analysis of variance procedures according to nitrogen treatment, study site, and collection date.

The percentage of crude protein in forage samples collected during June increased significantly ($P < 0.05$) as a result of nitrogen treatment. Generally, each level of nitrogen application increased the percentage of crude protein in the forages over that of lesser nitrogen applications. Bluebunch wheatgrass and big sagebrush leaves increased more in crude protein concentration during June as a result of nitrogen treatment than sagebrush twigs. Maximum increases in crude protein concentration resulting from nitrogen treatment for bluebunch wheatgrass, sagebrush leaves, and sagebrush twigs during June were 50 percent, 30 percent, and 18 percent (air-dry basis), respectively, greater than the protein concentration of similar tissues harvested from unfertilized plots.

Nitrogen treatments failed to increase significantly ($P > 0.05$) the percentage of crude protein in sagebrush leaves or twigs during September but increased significantly ($P < 0.05$) the percentage of crude protein in both tissues during December and March. The percentage of crude protein in bluebunch wheatgrass showed a linear relationship with nitrogen application during all collection periods except May, 1970. The increased percentage of crude protein in bluebunch wheatgrass during the non-growing season (September through March) might have been the result of protein storage within the wheatgrass tissues during the growing season (June-July).

Nitrogen fertilization failed to alter significantly ($P > 0.05$) the concentration of CWC in the forages during any collection period.

However, the CWC concentration of the forages varied significantly (P <0.01) among collection dates and among study sites. The percentage of CWC in bluebunch wheatgrass was lower during June and September than during December and March. Sagebrush leaves and twigs were lowest in CWC during June, contained their highest percentage of CWC during September, and decreased in CWC by December and March. Other studies indicate leaching of cell contents by rain might account for increased CWC in the plant tissues during September.

The calcium and phosphorus concentration of the forages did not vary significantly (P >0.05) among nitrogen treatments but varied significantly (P <0.05) among collection periods and among study sites. The forages were low in calcium and phosphorus concentration during June but increased in calcium and phosphorus concentration by September. Bluebunch wheatgrass and sagebrush leaves decreased in calcium and phosphorus concentration by December and March but sagebrush twigs were consistent in calcium and phosphorus concentration during December and March. Calcium-phosphorus ratios for the forages were within limits recommended for proper animal nutrition except for bluebunch wheatgrass in March. Additionally, the forages contained adequate quantities of calcium and phosphorus during the winter for proper mule deer nutrition.

The volatile-oil concentration of sagebrush leaves did not vary significantly (P >0.05) among nitrogen treatments but varied

significantly (P <0.01) among study sites and among collection dates.

The in-vitro digestibility of the plant tissues showed no significant (P >0.05) variation among nitrogen treatments but varied significantly (P <0.01) among collection dates. The digestibility of bluebunch wheatgrass was correlated (r = -0.90) with the CWC concentration of the wheatgrass but the digestibilities of sagebrush leaves and twigs were not correlated with their concentration of CWC. This was speculatively attributed to the clumping of sagebrush tissues after grinding so that their physical form in the digestion medium was not compatable to that of bluebunch wheatgrass.

Previous workers have pointed out the importance of adequate protein consumption by pregnant does for fawn production and fawn survival. Nitrogen fertilization of big sagebrush winter range appears to be an effective method of increasing protein available to does during gestation which may influence indirectly reproduction occurring within the herd. Hopefully, associated studies will shed some light on the feasibility of the practice.

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Appendix Table I-A

Nutrient concentration and in vitro digestibility of bluebunch
wheatgrass according to collection site and nitrogen
treatment, May, 1970.

Study Site	Lbs. N Per Acre	Nutrient					DIG
		CP	M	CWC	Ca	P	
Junction Butte	0						
	30						
	60			Data not available			
	90						
	120						
Flight Line	0	5.1	5.9	60.0	.16	.24	17.0
	30	6.7	6.6	56.2	.14	.21	20.9
	60	5.7	6.2	60.4	.18	.22	18.8
	90	5.1	5.1	60.8	.14	.26	16.8
	120	5.9	5.6	61.3	.11	.23	15.3
Corral Creek	0	2.9	6.5	63.2	.12	.22	13.8
	30	4.5	6.2	64.6	.09	.23	8.8
	60	3.6	6.4	62.8	.07	.25	11.6
	90	3.8	6.2	62.8	.10	.27	11.6
	120	4.2	5.6	62.5	.09	.19	10.3

CP - Crude Protein
M   - Moisture
CWC - Cell-Wall Constituents
P   - Phosphorus
DIG - Dry-Matter Digestibility

Appendix Table I-B

Nutrient concentration and in vitro digestibility of big sagebrush
leaves according to collection site and nitrogen
treatment, May, 1970.

Study Site	Lbs. N Per Acre	Nutrient						
		CP	M	CWC	VO	Ca	P	DiG
Junction Butte	0							
	30							
	60	Data not available						
	90							
	120							
Flight Line	0	10.8	5.4	27.8	1.4	.24	.45	32.1
	30	13.2	9.9	28.6	1.1	.27	.37	31.9
	60	11.3	6.3	30.3	1.2	.24	.38	32.2
	90	11.6	5.2	31.3	1.2	.25	.35	28.9
	120	11.6	5.2	28.1	1.2	.17	.40	32.8
Corral Creek	0	12.6	5.2	26.6	1.0	.32	.34	37.6
	30	11.4	4.9	30.1	1.1	.29	.36	34.5
	60	13.3	9.9	27.1	1.1	.28	.36	29.1
	90	11.7	5.3	30.9	1.1	.27	.39	35.4
	120	12.5	5.8	28.7	1.2	.30	.39	36.5

CP   - Crude Protein
M   - Moisture
CWC - Cell-Wall Constituents
Ca   - Calcium
P   - Phosphorus
DiG - Dry-Matter Digestibility
VO   - Volatile Oil

Appendix Table I-C

Nutrient concentration and _in vitro_ digestibility of big sagebrush
twigs according to collection site and nitrogen
treatment, May, 1970.

Study Site	Lbs. N Per Acre	Nutrient					DiG
		CP	M	CWC	Ca	P	
Junction Butte	0						
	30						
	60		Data not available				
	90						
	120						
Flight Line	0	7.4	8.5	42.6	.14	.31	26.7
	30	8.8	8.2	45.3	.15	.29	27.8
	60	8.2	9.1	43.5	.15	.27	25.7
	90	9.6	9.2	41.9	.14	.28	22.6
	120	8.3	8.9	43.2	.13	.30	31.8
Corral Creek	0	10.1	4.9	42.9	.12	.27	28.1
	30	8.1	9.8	43.2	.19	.25	28.2
	60	10.6	5.1	43.2	.11	.29	32.8
	90	9.6	9.3	41.6	.16	.28	33.8
	120	9.9	9.4	41.7	.16	.26	32.6

CP   - Crude Protein
M   - Moisture
CWC - Cell-Wall Constituents
P   - Phosphorus
DiG - Dry-Matter Digestibility

Appendix Table I-D

Nutrient concentration and in vitro digestibility of bluebunch
wheatgrass according to study site and nitrogen
treatment, June, 1970.

Study Site	Lbs. N Per Acre	Nutrient					DiG
		CP	M	CWC	Ca	P	
Junction Butte	0	8.3	10.8	58.9	.12	.27	42.9
	30	8.6	10.6	60.5	.13	.27	42.6
	60	10.1	10.8	59.4	.15	.30	48.3
	90	9.9	10.0	59.9	.10	.23	42.9
	120	10.9	10.6	57.5	.12	.28	42.3
Flight Line	0	9.2	7.2	60.7	.12	.31	47.2
	30	10.0	7.3	58.5	.11	.28	43.0
	60	11.7	7.4	54.0	.14	.34	45.3
	90	15.3	7.6	53.1	.13	.29	48.0
	120	14.4	7.2	55.5	.12	.30	45.3
Corral Creek	0	9.4	8.4	56.2	.14	.26	40.5
	30	12.9	9.4	56.6	.13	.24	45.8
	60	13.0	10.6	56.8	.11	.29	41.6
	90	14.6	9.5	55.1	.15	.29	46.4
	120	16.3	9.0	54.4	.13	.29	43.8

CP   - Crude Protein
M   - Moisture
CWC - Cell-Wall Constituents
P   - Phosphorus
DiG - Dry-Matter Digestibility

Appendix Table I-E

Nutrient concentration and in vitro digestibility of big sagebrush
leaves according to collection site and nitrogen
treatment, June, 1970.

Study	Lbs. N	Nutrient						
Site	Per Acre	CP	M	CWC	VO	Ca	P	DiG
	0	12.0	8.2	29.6	1.6	.29	.34	52.9
	30	15.6	8.3	26.1	1.8	.32	.32	49.8
Junction Butte	60	16.2	9.0	28.6	1.8	.34	.38	48.6
	90	15.2	11.3	30.2	1.9	.30	.34	51.3
	120	17.5	10.3	28.2	1.7	.32	.37	51.1
	0	11.9	9.7	27.8	1.2	.26	.45	47.8
	30	13.5	11.0	30.0	1.5	.33	.34	46.0
Flight Line	60	14.9	11.0	27.7	1.2	.29	.41	52.1
	90	16.5	9.6	27.2	1.5	.34	.45	51.8
	120	16.4	9.4	27.2	1.2	.31	.46	49.1
	0	13.5	11.2	27.8	1.2	.34	.38	44.8
	30	16.8	9.6	27.8	1.1	.29	.45	43.4
Corral Creek	60	18.0	9.7	26.8	1.3	.32	.44	51.9
	90	17.3	8.4	26.8	1.2	.30	.38	50.1
	120	17.2	9.1	28.4	1.1	.37	.39	53.6

CP   - Crude Protein
M   - Moisture
CWC - Cell-Wall Constituents
Ca   - Calcium
P   - Phosphorus
DiG - Dry-Matter Digestibility
VO   - Volatile Oil

Appendix Table I-F

Nutrient concentration and in vitro digestibility of big sagebrush
twigs according to study site and nitrogen
treatment, June, 1970

Study Site	Lbs. N Per Acre	Nutrient					DiG
		CP	M	CWC	Ca	P	
	0	8.8	8.4	46.2	.18	.26	41.8
	30	9.3	9.6	48.9	.17	.25	44.8
Junction Butte	60	10.1	10.1	48.7	.16	.23	44.6
	90	9.6	9.6	51.0	.16	.22	44.6
	120	10.4	9.4	48.5	.16	.28	43.3
	0	10.3	8.6	43.1	.16	.32	40.8
	30	12.3	8.8	41.6	.21	.28	43.4
Flight Line	60	12.1	8.3	42.6	.16	.33	44.3
	90	12.8	9.8	42.1	.18	.32	46.1
	120	13.0	8.2	39.7	.18	.35	45.3
	0	10.9	8.9	42.9	.19	.32	46.0
	30	12.7	8.6	39.8	.20	.37	48.5
Corral Creek	60	12.2	10.6	43.1	.18	.35	46.0
	90	13.0	10.8	40.0	.19	.31	47.8
	120	11.9	9.5	43.1	.18	.39	46.4

CP   - Crude Protein
M   - Moisture
CWC - Cell-Wall Constituents
P   - Phosphorus
DiG - Dry-Matter Digestibility

Appendix Table I-G

Nutrient concentration and in vitro digestibility of bluebunch
wheatgrass according to collection site and nitrogen
treatment, September, 1970.

Study Site	Lbs. N Per Acre	Nutrient					DiG
		CP	M	CWC	Ca	P	
	0	6.9	9.1	57.4	.80	.40	35.8
	30	6.9	9.7	55.4	.71	.38	37.1
Junction Butte	60	9.3	10.0	54.9	.80	.41	37.4
	90	10.9	9.6	54.1	.90	.39	40.6
	120	10.4	9.3	56.0	.79	.40	38.9
	0	6.1	9.6	60.4	.56	.37	29.7
	30	8.1	10.9	58.2	.60	.36	38.7
Flight Line	60	7.4	12.5	59.2	.56	.36	41.2
	90	8.4	10.2	57.8	.54	.38	37.2
	120	12.1	11.2	54.8	.66	.36	47.8
	0	5.2	10.0	57.7	.58	.38	32.3
	30	7.1	10.8	57.4	.59	.34	36.6
Corral Creek	60	7.9	9.8	55.3	.62	.38	35.8
	90	9.8	11.8	54.4	.63	.39	43.8
	120	10.6	10.2	53.9	.66	.40	42.6

CP   - Crude Protein
M   - Moisture
CWC - Cell-Wall Constituents
P   - Phosphorus
DiG - Dry-Matter Digestibility

Appendix Table I-H

Nutrient concentration and in vitro digestibility of big sagebrush
leaves according to collection site and nitrogen
treatment, September, 1970.

Study Site	Lbs. N Per Acre	Nutrient						
		CP	M	CWC	VO	Ca	P	DiG
Junction Butte	0	9.6	14.4	34.0	1.8	.96	.46	43.6
	30	9.1	16.0	34.8	2.1	.79	.46	41.4
	60	10.2	15.2	32.5	2.1	.88	.51	42.2
	90	11.4	15.4	31.8	1.7	.94	.48	46.8
	120	11.8	15.8	31.6	2.0	.91	.48	44.6
Flight Line	0	8.9	14.0	31.6	1.8	.80	.55	45.4
	30	10.4	14.2	32.3	1.4	.80	.56	45.8
	60	8.9	14.1	33.6	1.8	.65	.52	44.6
	90	11.8	14.6	34.0	1.7	.88	.48	42.9
	120	9.8	14.1	33.0	1.7	.61	.52	40.8
Corral Creek	0	10.8	12.9	32.6	1.6	.68	.52	44.1
	30	10.5	14.8	32.7	1.9	.82	.57	44.8
	60	10.9	13.4	34.4	1.6	.91	.54	42.3
	90	10.4	14.3	34.5	1.8	.77	.51	43.6
	120	11.1	13.1	32.2	1.8	.76	.55	43.0

CP   - Crude Protein
M   - Moisture
CWC - Cell-Wall Constituents
Ca   - Calcium
P   - Phosphorus
DiG   - Dry-Matter Digestibility
VO   - Volatile Oil

Appendix Table I-I

Nutrient concentration and in vitro digestibility of big sagebrush
twigs according to collection site and nitrogen
treatment, September, 1970.

Study Site	Lbs. N Per Acre	Nutrient					DiG
		CP	M	CWC	Ca	P	
Junction Butte	0	7.5	12.8	53.8	.46	.39	35.0
	30	6.6	13.4	54.8	.40	.34	32.1
	60	6.6	12.4	56.8	.39	.36	28.8
	90	7.7	14.4	54.8	.42	.33	30.4
	120	8.9	13.4	51.8	.38	.33	35.7
Flight Line	0	6.4	11.8	50.6	.48	.44	36.0
	30	6.3	10.9	57.0	.42	.42	25.2
	60	5.3	12.2	53.6	.44	.39	36.4
	90	8.1	11.9	51.8	.42	.38	33.8
	120	6.9	11.9	46.4	.46	.38	40.7
Corral Creek	0	6.7	11.2	52.5	.43	.38	31.8
	30	6.3	11.4	52.7	.44	.38	33.1
	60	6.6	10.6	54.5	.42	.33	28.7
	90	6.6	13.2	50.6	.42	.34	34.9
	120	8.1	12.2	50.6	.42	.38	36.8

CP - Crude Protein
M   - Moisture
CWC - Cell-Wall Constituents
P   - Phosphorus
DiG - Dry-Matter Digestibility

Appendix Table I-J

Nutrient concentration and in vitro digestibility of bluebunch
wheatgrass according to collection site and nitrogen
treatment, December, 1970.

Study Site	Lbs. N Per Acre	Nutrient					
		CP	M	CWC	Ca	P	DiG
Junction Butte	0	4.5	9.2	63.4	.51	.53	18.2
	30	4.2	8.6	64.7	.52	.52	17.5
	60	4.8	8.8	62.8	.56	.47	20.4
	90	7.2	8.5	60.2	.56	.51	23.8
	120	6.8	8.4	63.2	.52	.58	22.3
Flight Line	0	7.7	8.8	60.8	.63	.52	26.6
	30	7.1	7.9	59.0	.63	.53	25.6
	60	8.8	6.2	60.3	.66	.48	26.4
	90	8.8	8.4	56.5	.76	.56	31.0
	120	12.5	10.9	28.3	.74	.60	38.0
Corral Creek	0	4.6	8.6	67.0	.62	.59	16.7
	30	5.9	7.4	64.6	.54	.69	14.8
	60	6.9	6.6	60.9	.53	.61	15.3
	90	6.6	6.8	64.2	.56	.61	16.4
	120	8.7	10.0	62.5	.54	.65	15.8

CP   - Crude Protein
M   - Moisture
CWC - Cell-Wall Constituents
P   - Phosphorus
DiG - Dry-Matter Digestibility

Appendix Table I-K

Nutrient concentration and in vitro digestibility of big sagebrush
leaves according to collection site and nitrogen
treatment, December, 1970.

Study	Lbs. N	Nutrient						
Site	Per Acre	CP	M	CWC	VO	Ca	P	DiG
	0	10.3	7.8	28.7	1.6	.60	.60	34.8
	30	9.9	9.4	30.7	2.0	.70	.58	32.6
Junction Butte	60	10.5	8.2	27.6	1.7	.68	.55	35.6
	90	13.6	9.8	27.6	1.6	.78	.55	35.2
	120	12.5	10.9	28.3	1.6	.74	.60	38.0
	0	9.4	8.6	27.6	1.6	.62	.52	38.1
	30	11.1	9.8	29.4	1.6	.64	.36	37.4
Flight Line	60	12.0	9.1	25.1	1.7	.48	.35	36.9
	90	11.4	10.2	29.3	1.6	.56	.40	37.8
	120	10.6	9.2	24.8	1.8	.48	.43	37.2
	0	11.1	7.6	26.6	1.8	.76	.54	34.8
	30	10.6	7.5	27.1	1.6	.69	.54	36.1
Corral Creek	60	12.6	9.0	24.1	1.7	.74	.44	35.5
	90	9.3	9.1	29.9	1.7	.71	.44	36.7
	120	12.5	8.9	27.8	1.5	.69	.46	39.4

CP   - Crude Protein
M   - Moisture
CWC - Cell-Wall Constituents
Ca   - Calcium
P   - Phosphorus
Dig - Dry-Matter Digestibility
VO   - Volatile Oil

Appendix Table I-L

Nutrient concentration and _in vitro_ digestibility of big sagebrush
twigs according to collection site and nitrogen
treatment, December, 1970.

Study Site	Lbs. N Per Acre	Nutrient					DiG
		CP	M	CWC	Ca	P	
	0	8.2	7.8	40.3	.52	.34	40.6
	30	9.2	8.0	43.2	.50	.33	39.1
Junction Butte	60	9.4	7.8	42.0	.46	.34	38.6
	90	10.0	11.2	42.8	.47	.36	41.8
	120	11.0	10.8	38.7	.49	.34	40.2
	0	7.6	7.4	42.3	.43	.36	35.5
	30	8.7	8.3	43.3	.50	.34	35.0
Flight Line	60	9.6	8.1	38.8	.46	.34	41.3
	90	10.3	10.1	42.2	.46	.33	40.4
	120	8.1	10.3	35.4	.48	.46	35.8
	0	7.8	6.7	40.8	.52	.40	32.5
	30	8.1	9.6	40.4	.45	.42	35.2
Corral Creek	60	10.2	11.2	36.1	.48	.43	38.9
	90	10.9	10.6	37.4	.49	.44	38.8
	120	10.7	9.0	39.1	.50	.42	39.8

CP   - Crude Protein
M   - Moisture
CWC - Cell-Wall Constituents
P   - Phosphorus
DiG - Dry-Matter Digestibility

Appendix Table I-M

Nutrient concentration and in vitro digestibility of bluebunch
wheatgrass according to collection site and nitrogen
treatment, March, 1971.

Study Site	Lbs. N Per Acre	Nutrient					DiG
		CP	M	CWC	Ca	P	
Junction Butte	0	3.3	8.6	62.0	.51	.15	8.6
	30	2.5	7.9	67.4	.48	.18	7.5
	60	4.0	8.2	64.4	.44	.16	7.6
	90	5.2	8.2	60.4	.50	.17	10.9
	120	6.2	8.2	61.3	.45	.20	8.2
Flight Line	0	6.2	8.1	60.7	.64	.19	16.3
	30	8.2	7.7	59.4	.58	.21	12.7
	60	7.8	7.7	56.6	.44	.18	19.8
	90	7.4	6.4	58.8	.48	.20	16.0
	120	7.4	6.9	59.7	.52	.24	23.8
Corral Creek	0	2.8	7.2	64.2	.40	.19	4.8
	30	4.4	8.2	64.0	.47	.17	9.8
	60	6.0	6.2	61.7	.48	.18	12.8
	90	6.5	6.8	61.2	.48	.18	14.0
	120	5.9	8.8	63.2	.41	.19	13.8

CP   - Crude Protein
M   - Moisture
CWC - Cell-Wall Constituents
P   - Phosphorus
DiG - Dry-Matter Digestibility

Appendix Table I-N

Nutrient concentration and in vitro digestibility of big sagebrush
leaves according to collection site and nitrogen
treatment, March, 1971

Study Site	Lbs. N Per Acre	Nutrient						DiG
		CP	M	CWC	VO	Ca	P	
Junction Butte	0	8.7	8.8	32.6	1.9	.68	.24	27.6
	30	9.2	9.8	31.3	2.0	.61	.24	28.2
	60	10.2	9.9	29.2	2.0	.48	.24	29.8
	90	10.6	9.2	31.9	1.7	.52	.22	28.1
	120	12.4	9.7	28.9	1.6	.33	.24	29.2
Flight Line	0	8.2	7.4	31.9	1.8	.42	.28	22.6
	30	10.7	7.6	30.9	1.4	.41	.24	30.3
	60	11.3	8.6	28.9	1.8	.34	.28	28.4
	90	11.3	9.8	29.7	1.9	.38	.24	25.0
	120	10.6	6.8	28.2	1.7	.35	.27	25.6
Corral Creek	0	8.8	8.6	29.9	1.5	.36	.24	26.7
	30	9.6	8.7	28.7	1.4	.28	.22	31.6
	60	11.0	9.6	27.6	1.2	.66	.26	33.2
	90	10.8	8.1	29.2	1.5	.58	.30	24.9
	120	11.3	7.1	25.2	1.6	.64	.37	32.0

CP   - Crude Protein
M   - Moisture
CWC - Cell-Wall Constituents
Ca   - Calcium
P   - Phosphorus
DiG - Dry-Matter Digestibility
VO   - Volatile Oil

Appendix Table I-O

Nutrient concentration and in vitro digestibility of big sagebrush
twigs according to collection site and nitrogen
treatment, March, 1971.

Study Site	Lbs. N Per Acre	Nutrient					DiG
		CP	M	CWC	Ca	P	
Junction Butte	0	7.1	6.2	47.0	.52	.40	28.1
	30	8.4	6.3	42.9	.46	.40	29.1
	60	9.2	6.9	44.3	.46	.48	22.9
	90	11.4	6.3	41.2	.50	.50	34.5
	120	11.3	5.8	42.8	.46	.51	31.5
Flight Line	0	5.8	5.8	50.0	.50	.41	22.6
	30	8.2	6.1	46.5	.52	.54	28.0
	60	9.0	6.1	46.4	.49	.54	25.9
	90	9.8	6.8	45.0	.49	.53	32.3
	120	8.8	7.6	41.6	.50	.31	31.6
Corral Creek	0	7.5	5.8	48.3	.53	.52	21.6
	30	7.6	5.9	47.1	.51	.51	20.6
	60	10.0	6.2	42.8	.40	.53	23.6
	90	9.7	6.2	39.4	.54	.60	23.6
	120	9.7	5.8	41.1	.54	.49	22.6

CP   - Crude Protein
M   - Moisture
CWC - Cell-Wall Constituents
P   - Phosphorus
DiG - Dry-Matter Digestibility

Appendix Table II-A

Factorial analysis of variance for the crude protein
concentration of test forage May, 1970.

Source	D.F.	Sum of Squares	Mean Square	F Value
Nitrogen	4	3.77	0.94	.96
Site	1	0.02	0.02	.02
Forage	2	532.89	266.45	700.28**
Nitrogen X Site	4	11.47	2.87	1.88
Nitrogen X Forage	8	7.98	.99	2.62*
Site X Forage	2	27.79	13.89	36.52**
Nitrogen X Site X Forage	8	7.53	.94	2.18
Residual	30	19.20		
TOTAL	59	610.65		

*Significant P <0.05
**Significant P <0.01

Appendix Table II-B

Factorial analysis of variance for the crude protein
concentration of test forages June, 1970.

Source	D.F.	Sum of Squares	Mean Square	F Value
Nitrogen	4	155.16	38.79	82.1**
Site	2	92.60	46.30	84.4**
Forage	2	327.08	163.54	202.9**
Nitrogen X Site	8	19.23	2.40	4.4*
Nitrogen X Forage	8	31.23	3.90	4.8**
Site X Forage	44	38.01	9.50	11.8**
Nitrogen X Site X Forage	16	19.36	1.21	1.5
Residual	45	31.62		
TOTAL	89	714.29		

*Significant P < 0.05
**Significant P < 0.01

Appendix Table II-C

Factorial analysis of variance for the crude protein
concentration of test forages September, 1970.

Source	D.F.	Sum of Squares	Mean Square	F Value
Nitrogen	4	78.48	19.62	13.9*
Site	2	5.52	2.76	4.6*
Forage	2	172.68	86.34	183.3**
Nitrogen X Site	8	11.93	1.49	2.5
Nitrogen X Forage	8	35.72	4.46	9.5**
Site X Forage	4	4.72	1.18	2.5
Nitrogen X Site X Forage	16	23.36	1.46	3.1**
Residual	45	30.63		
TOTAL	89	358.32		

*Significant P <0.05
**Significant P <0.01

Appendix Table II-D

Factorial analysis of variance for the crude protein
concentration of the forages December, 1970.

Source	D.F.	Sum of Squares	Mean Square	F Value
Nitrogen	4	56.31	14.08	18.2**
Site	2	3.11	1.55	1.8
Forage	2	304.17	152.08	311.4**
Nitrogen X Site	8	32.61	4.08	4.8*
Nitrogen X Forage	8	4.36	.55	1.1
Site X Forage	4	33.44	8.36	17.1**
Nitrogen X Site X Forage	16	23.57	1.47	3.0**
Residual	45	59.91		
TOTAL	89	484.04		

*Significant P <0.05
**Significant P <0.01

Appendix Table II-E

Factorial analysis of variance for the crude protein
concentration of the forages March, 1971

Source	D.F.	Sum of Squares	Mean Square	F Value
Nitrogen	4	102.16	25.54	77.3**
Site	2	9.35	4.67	4.3*
Forage	2	354.32	177.16	354.9**
Nitrogen X Site	8	22.06	2.75	2.6
Nitrogen X Forage	8	3.82	.48	.9
Site X Forage	4	51.08	12.77	25.4**
Nitrogen X Site X Forage	16	9.65	.60	1.2
Residual	45	27.23		
TOTAL	89	579.67		

*Significant $P < 0.05$
**Significant $P < 0.01$