NUTRITIONAL VALUE OF FLAT PEA HAY FED TO SHEEP AT DIFFERENT LEVELS

by

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(ABSTRACT)

Two experiments were conducted with sheep to determine the nutritional value of 'Lathco' flatpea (Lathyrus sylvestris) hay. In experiment 1, a finishing trial was conducted with 50 group fed crossbred lambs (25 ewes and 25 wethers) fed diets consisting of 70% chopped forage and 30% ground corn grain for 70 d. Five pens of five wethers and five pens of five ewes were selffed diets in which the forage consisted of 100:0, 75:25, 50:50, 25:75, and 0:100 proportions of alfalfa (Medicago sativa L.) and flatpea hay. Feed efficiencies decreased linearly ($P<.05$) as flatpea increased in the diet. Linear decreases ($P<.05$) were obtained in kidney and pelvic fat, backfat, leg conformation, and yield grade with increased flatpea in the diet. Blood urea-N (BUN), ruminal pH and NH_3-N increased ($P<.005$) as proportion of flatpea increased. Total volatile fatty acid (VFA) and acetate concentration decreased linearly ($P<.005$) and propionate, isovalerate, and valerate levels increased linearly ($P<.01$) with increased proportions of flatpea hay.

In experiment 2, 30 wether lambs were fed diets consisting of 100:0, 75:25, 50:50, 25:75 and 0:100 proportions of chopped alfalfa and flatpea hays in a digestion trial. Neutral detergent fiber (NDF), acid detergent fiber (ADF), cellulose, hemicellulose and energy digestibilities decreased linearly ($P<.01$) with increased levels of flatpea hay. Nitrogen retention was not different between sheep fed the different diets. Apparent absorbtion and retention of Ca decreased linearly ($P<.05$) with increased level of flatpea hay. Ruminal pH and NH_3-N, and BUN increased linearly ($P<.001$) as flatpea was increased in the diet. Ruminal total VFA, acetate and acetate to propionate ratio decreased linearly ($P<.001$) and propionate, isobutyrate, isovalerate, and valerate levels increased linearly ($P<.001$) as level of flatpea increased.

(Key Words: Medicago sativa L., Lathyrus sylvestris, Digestibility, Performance, Lambs.)

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INTRODUCTION

The Appalachian region of the United States encompasses approximately 51 million hectares. Topographically, the region contains more mountains, hills, and plateaus than lowlands or plains. The soil types in this region are generally shallow and stony, with low fertility. Mining, logging and farming operations in this region can cause substantial soil losses through erosion. Methods of erosion prevention and reclamation of disturbed soil areas are important in this region. This is important as more and more cropland is being converted to non-agricultural uses and more marginal land is forced into production.

Establishment of plant cover in these areas is difficult due to soil characteristics and fertility constraints. Plant species which show both hardiness and productivity under marginal fertility conditions are needed to reclaim and convert these areas into productive land. A legume which has proven to be well adapted for establishment and growth in these areas is 'Lathco' flatpea (Lathyrus sylvestris L.). It has been studied sporadically since it was first introduced into the U.S. in the 1870's.

In addition to its erosion control properties and productivity under poor fertility conditions, flatpea has a number of characteristics which make it potentially valuable as a forage plant. It is a high yielding, palatable legume, high in protein. The use of flatpea as a forage crop has been questioned due to problems reported in animals fed the forage. Flatpea is known to contain a free amino acid, L-2,4 diaminobutyric acid (DABA), considered to be a neurolathyrogen.

The ability of ruminants to degrade amino acids in the rumen via microorganisms is thought to be one way of overcoming any possible toxic effects of compounds such as DABA. Because flatpea has potential as a forage for ruminants, the objective of this study was to investigate the nutritional value of flatpea for ruminants. Animal performance, nutrient utilization, and ruminal fluid and blood parameters were measured.

REVIEW OF LITERATURE

Soil Erosion

In the U.S. there is an estimated loss of approximately 40 million tons of soil per year to erosion (USDA, 1987). This loss is due to water erosion in the form of sheet and rill erosion, and wind erosion. Some of the causes for these losses include stripmining, deforestation due to logging and fire, improper cropping practices, unchecked runoff from developed areas and unregulated grazing practices (USDA,1978).

Erosion in Appalachia. In the Appalachian region of the U.S. major losses of soil are due to water runoff in the form of sheet and rill erosion. Combined, they can account for losses of 20.4 t/ha. Approximately 78% of this erosion occurs on crop, pasture, range and forest land (USDA, 1982). Estimates show only 15 to 20% of the farmland in this region is in land capability classes I to III.

Soils in the Appalachian region are generally stony, shallow, acid in nature, and medium to low in fertility (Appalachian Regional Commission, 1979). Annual rainfall in the region ranges from 50 cm in the eastern slopes of the Alleghenies to more than 200 cm in the Great Smokey mountains (Anonymous, 1959). The annual precipitation for the region is between 100 to 125 cm, but seasonal differences can be extreme (Jones et al., 1983).

Erosion Control. In order to prevent the erosion there are a number of steps which must be taken. First and foremost the water runoff which is causing erosion must be controlled (Jones et al., 1975). An important next step is the modification or elimination of practices or operations which led to the erosion problem. Once this step is implemented there is a need to return these land areas to a productive condition as range, pasture or to crop land, production, depending on characteristics of the site (Jones et al., 1979). The establishment of plant cover to protect and stabilize the soil surface and to reduce effects of runoff water is essential (Armiger et al., 1979).

Stripmined areas are difficult to repair due to extreme changes to the land being mined. Topsoil and subsequent subsoil layers are removed to reach the material being mined. Upon completion of the mining operation, overburden material is returned as a mixture of the original upper and lower soil layers (Power and Bennett, 1977). The resulting surface layer is one which is low in organic matter, pH, soil nutrients (N, P, K, Ca and Mg), and water holding capacity (Power et al., 1975). In order to return these areas to a productive system, such as a pasture, plant species must be utilized which can survive under these conditions. These plants must also provide enough cover to protect soil against erosion, and provide adequate forage for animal production (Bennett et al., 1976).

Numerous legume species have been studied under these situations to determine their ability to provide erosion control and nutritious forage. Some of these include: sericea lespedeza (Lespedeza cuneata, Dumont, G.Don), crownvetch (Coronilla varia L.), birdsfoot trefoil (Lotus corniculatus L.), bitterblue lupine (Lupinus angustifolius L.), alfalfa (Medicago sativa L.), hairy vetch (Vicia villosa Roth) and yellow sweetclover (Melilotus officinalis Lam.) (Bennett, 1971; Barker et al., 1977 and Miller and Zalunardo, 1979). Flatpea has been shown to control erosion and provide adequate forage yields for animal production.

Flatpea

Characteristics of Flatpea. Flatpea, a member of a genus which contains more than 100 species, is characterized as a long-lived perennial legume (Grunder and Dickson, 1948). It has a deep central tap root extending to depths of 7 to 10 m in established stands (Wagner, 1943). Long and slender weak stems to a length of 1.3 to 2 meters are radiated from this central tap root. These stems tend to form dense mats (Anonymous, 1972). The leaves are in single pairs with lanceoleate leaflets. The presence of tendrils has also been noted (Gulenkova, 1977). The flowers are pink to salmon, 3 to 10 occurring in a loose raceme (Grunder and Dickson, 1948).

Origin and Historical Development in the U.S.. Flatpea is found occurring naturally along the woodlands and forests of Central Europe and its range extends eastward to the Caucausas and northward to Scandinavia (Senn, 1938; Gulenkova, 1977). The plant was first brought under cultivation in 1862 by William Wagner in Wurtemberg, Germany (Wagner, 1943; Grunder and Dickson, 1948). Wagner was a plant breeder who was trying to develop a good forage plant which was also a source of nectar for honey production (Pellet, 1941). It was brought into the U.S. by Wagner in 1878 and planted in fields in western Pennsylvania (Wagner, 1943). This variety was named Lathyrus sylvestris Wagnerii. A number of experiment stations across the U.S., including Michigan, Virginia, California, Vermont, Connecticut and Pennsylvania began reporting on this new forage crop (Daniel et al., 1946). Michigan researchers reported that the forage was readily consumed by cattle (Kedzie, 1893). In Virginia

(Smyth, 1892) and Vermont (Piper, 1914) hay yields of 5.0 and 2.0 t/ha were achieved, respectively, from two cuttings. Researchers in Washington state reported yields of 4.5 to 5.6 t/ha (Daniel et al., 1946).

A number of problems restricted the widespread use of flatpea by farmers, despite the encouraging results reported. These included the price of the seeds, which in 1900 was $6 to 12/kg (Pellet, 1941). There was also some difficulty in establishment of a stand due to poor germination of seeds. This was due mainly to an extremely hard seedcoat, which required some scarification to improve germination rate (Wagner, 1943). Also, length of time needed to establish a strong stand of flatpea can range from 2 to 4 yr (Grunder and Dickson, 1948; Robertson, 1970).

Although flatpea did not gain popularity as a forage plant, its dense growth characteristics enabled it to find a niche as an erosion control plant. The first reported use of flatpea was on logged areas of western Washington state (Daniel et al., 1946; Robocker and Kerr, 1964). Flatpea was able to successfully compete with native species such as bracken fern (Pteridium aquilinum), and was able to stabilize the logged over slopes (Pellett, 1941; Daniel at al., 1946; Grunder and Dickson, 1948). Flatpea has been used primarily in this capacity and has been used extensively along highway slopes and rights-of-way (Robertson, 1970; Slayback and Dronen, 1974; Zak and Kasheski, 1974).

New Cultivar. Plant breeders at the USDA Plant Materials Center, Big Flats, NY and USDA-SCS in Harrisburg, PA released a new cultivar 'Lathco', of flatpea in the fall of 1972(Anonymous, 1972; McWilliams, 1973). In NY, Lathco flatpea has been shown to be adequate for erosion control (Slayback and Dronen, 1974). In Pennsylvania its ability to compete with native species and provide good cover was reported by Long et al. (1977). Lathco has also been used in New Jersey (Belcher and Sharp, 1983) and Massachusetts (Zak and Kasheski, 1974). Some advantages of this cultivar over earlier genotypes include greater seed production and quicker establishment (McWilliams, 1973). Seed yield was 200 to 225 kg/ha and stands established in 1 to 2 yr (Belcher and Sharp, 1983).

The availability of seed of the cultivar Lathco has prompted renewed interest in flatpea as a forage crop. Also, the ability of Lathco to produce forage under poor soil conditions such as low moisture and nutrient (N, P) availability make it a strong candidate for use on marginal land (Wright, et al., 1984).

Antiquality Component. Flatpea has been fed to livestock with no apparent adverse effects (Anonymous, 1972). However, there have been reports of animal deaths when flatpea was fed to sheep (Daniel et al., 1946; Pavelka et al., 1985). The cause of deaths (three animals) in the former study was not found, while in the latter, one animal was euthanized. Necropsy results were inconclusive. Reports of some Lathyrus species, including flatpea, to contain known neurotoxins have been noted (Ressler, et al., 1961; Barrow, et al., 1974; and Bell, 1981). Neurotoxic effects have been reported when seeds and young seedlings were fed to rats and rabbits, respectively (Lewis et al., 1948). This was not the case when flatpea forage was fed to chicks (Miller et al., 1948). The toxic factor found to be fatal to rats was determined to be a nonprotein amino acid, L-2,4 diaminobutyric acid (DABA) (Huang et al., 1950; O'Neal, 1968). This amino acid is found in highest concentrations in the seeds and young seedlings rather than in mature plant parts (Ressler, 1975; Foster et al., 1984). Concentrations in the seeds and seedlings were 9.1 and 25.5 g/kg DM, respectively (Ressler, 1964). The concentration found in the mature plant was approximately 2.8 g/kg DM (Van Etten and Miller, 1963; Pavelka, 1985).

The most recent theory on the toxic effects of DABA was given by O'Neal et al.(1968) based on data obtained from rats given intraperitoneal doses of of DABA. These workers suggested that DABA causes inhibition of ornithine carbamoyl transferase, resulting in an ultimate NH_3 toxicity in the animal (Figure 1). The ability of ruminants to degrade amino acids in the rumen via microorganisms may be the reason that toxicity symptons do not occur when certain toxic amino acids are present in the diet (Allison, 1978). Results reported by Pavelka et al., (1985) support this hypothesis. These workers, using ruminal fluid from sheep adapted to flatpea for 3 wk, estimated the rate of microbial degradation of DABA to be 42 to 69 $\mu M \cdot h^{-1} \cdot liter^{-1}$ They suggested that at this rate the rumen microorganisms would be able to degrade all the DABA present in 22 to 36 h.

Previous Feeding Experiments. Only a few feeding and digestion trials have been reported. The first digestibility trial was conducted by Hodgson and Knott (1936). Their results showed apparent digestibilities for DM, crude protein, crude fiber, ether extract, and nitrogen free extract were 60.31%, 78.32%, 41.85%. 45.75% and 65.22% respectively. The forage used in that trial had been harvested at the full bloom stage with some pods which contained seeds.

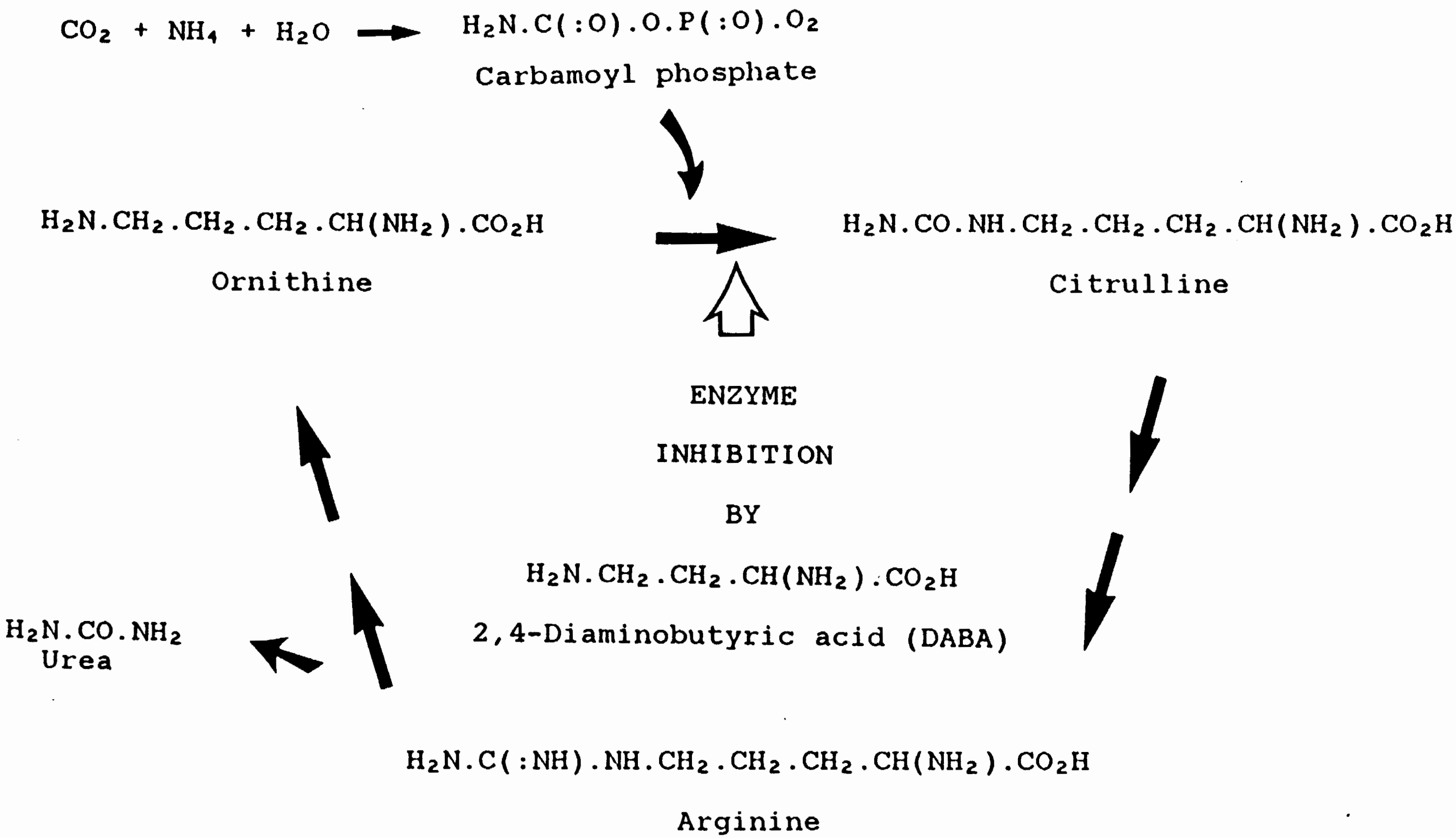

Figure 1. Suggested mode of action for DABA

Table 1. DIGESTION COEFFICIENTS FROM LAMBS FED FLATPEA HAY

Item	Maturity-fresh			Maturity-dehydrated		
	Pre bloom	Early bloom	Seed	Pre bloom	Early bloom	Seed
Dry matter	76.3	69.1	66.2	74.8	66.4	63.5
Crude protein	90.2	88.4	86.5	87.0	87.4	82.5
Ether extract	64.6	66.7	64.0	63.5	63.0	63.4
Crude fiber	57.6	46.5	42.5	55.9	46.9	42.7
Ash	61.1	56.1	61.3	56.5	56.9	61.4

Adapted from Daniel et al.,(1946)

Another series of digestion experiments was conducted by Daniel et al. (1946), in which forage harvested at three stages of maturity was fed. Flatpea was fed either as fresh or dehydrated forage. The stages of maturity at time of harvest were: pre-bloom, early bloom, and seed stage. The seed stage material was comparable to the material used by Hodgson and Knott (1936). The results of these trials (table 1) showed highest digestibility of DM, crude protein and crude fiber when pre-bloom maturity forage was fed. There was also a trend for higher digestibility of fresh forage as compared to dried forage. The workers also reported the death of three sheep which had been fed flatpea for periods of 7, 11, and 15 d. Autopsies revealed no insight as to the cause of death. The other sheep in the trials were kept on flatpea for 6 mo, with no ill effects.

Grunder and Dickson (1948) reported on grazing trials conducted in Puyallup, WA on fields which contained stands of flatpea seeded 6 yr earlier. The fields were grazed by grade dairy cows with calves for 2 yr, followed with Shorthorn steers and heifers for 2 more years. Grazing during early season growth (May/June) produced gains of 1.04 to 1.36 kg/d. Gains from animals grazing these areas from late June through August were .18 to .68 kg/d. The late grazing of these pastures into early September showed animal weight losses of .08 to .28 kg/d. Grunder and Dickson (1948) suggested that the pasture became less nutritive with age. They also speculated that, if animals were given an energy supplement, continued gains would be possible longer into the season.

The three previous trials were conducted using 'Wagnerii' flatpea. Since the release of 'Lathco' only one series of trials has been reported (Pavelka et al., 1985). In a performance trial with lambs, flatpea was fed at a maximum level of 20% in the diet for 42 d. Feed efficiency was not significantly different for animals fed diets containing 20% alfalfa, flatpea, or timothy (Phleum pratense L.) with values of 4.82, 5.5, and 4.75 kg feed/kg gain, respectively. Pavelka (1985) reported the results of two trials using three Hereford X Angus steers grazed for 60 d, and six Holstein steers grazed for 90 d. The Hereford x Angus animals gained .22 kg/d while the Holstein animals gained .52 kg/d.

Journal Article 1

FEEDLOT PERFORMANCE, CARCASS CHARACTERISTICS, AND RUMEN AND BLOOD PARAMETERS OF LAMBS FED DIFFERENT LEVELS OF FLATPEA HAY

Abstract

Fifty crossbred lambs (25 ewes and 25 wethers) were used in a 10 wk performance trial. Animals were self-fed diets consisting of 70% chopped forage and 30% ground corn grain. Forage consisted of the following proportions of alfalfa (Medicago sativa L.) and 'Lathco' flatpea (Lathyrus sylvestris L.) hay: 100:0; 75:25; 50:50; 25:75; and 0:100. Live weights were taken at 2 wk intervals. Ruminal fluid and blood samples were taken initially, after 5 wk and at the conclusion of the trial (10 wk). Animals were slaughtered at the conclusion of the trial and carcass data were obtained. Cumulative average daily gains were not different (P>.05), although gains of animals fed 100% flatpea hay tended to be lower, compared to gains of sheep fed the other diets. Quality grade and flank streaking increased when flatpea comprised up to 50% of the forage and decreased as flatpea increased in the ration (quadratic effect, P<.01). Kidney and pelvic fat, backfat, leg conformation, and yield

grade decreased linearly (P<.05), with level of flatpea. Linear increases in ruminal pH (P<.05), ruminal NH_3-N and blood urea-N (P<.01) were recorded with increased levels of flatpea hay. Total volatile fatty acid and acetate concentrations decreased linearly (P<.005) with increased flatpea in the diet. Linear increases in propionate, isovalerate, and valerate concentrations (P<.01) occurred with increased levels of flatpea. No differences (P>.05) were noted in serum concentrations of Ca, Mg, or inorganic P among lambs fed the different diets.
(Key Words: Medicago sativa, Lathyrus sylvestris, Lambs, Performance, Carcasses.)

Introduction

Flatpea (Lathyrus sylvestris L.) is well adapted for use on acid, infertile soils (McWilliams, 1973; Wright et al., 1984), and has a crude protein content of 22 to 30% (Daniel et al., 1946; Grunder and Dickson, 1948). Flatpea was introduced as a forage plant (Pellet, 1941) and forage quality was evaluated by a number of researchers (Hodgson and Knott, 1936; Daniel et al., 1946; Grunder and Dickson, 1948). A number of factors, including poor germination and low seed yield have hindered widespread use of flatpea (Robertson, 1970). Introduction of a new cultivar, 'Lathco', which had increased seedling vigor and higher seed yield presented the opportunity for increased use of flatpea as a potential forage crop. Feeding value of this cultivar was studied by Pavelka et al., (1985). The present study was conducted with sheep to evaluate the effect of feeding different levels of flatpea hay on feedlot performance, carcass characteristics, and blood and ruminal fluid parameters.

Materials and Methods

Allotment, Management, and Weights. A finishing trial was conducted with 25 crossbred ewe and 25 crossbred wether lambs. The average initial weight of the ewes and wethers was 27.3 kg and 24.3 kg, respectively. Animals were blocked by weight within sex and randomly allotted to five pens. All animals were wormed with Levamisol.[1] Initial and final weights were an average of two consecutive daily weights. During the 70 d trial animals were weighed at 2 wk intervals. The animals were group fed in 10 pens (2.4 x 11m) in a shed, open on one side. The pens had 4 m under cover, bedded with sawdust, and 7 m on sloped concrete without cover. Animals were fed ad libitum, with feed being added twice daily. Re-

[1] Pittman-Moore, Terrahaute, IN.

fusals were collected and weighed each morning. The amount fed was adjusted daily to obtain a 10% refusal rate. Animals were allowed iodized salt free choice, and water was supplied by nipple waterers.

At the conclusion of the trial animals were slaughtered over an 8-d period. Animals remained on their respective diets until slaughtered. Animals were transported approximately 1 km, and live weights were taken immediately prior to slaughter for determination of dressing percent. Carcasses were chilled at 2 to 4 C until internal temperature reached 5 C. All carcasses were evaluated at the same time and tissue samples were taken from selected animals after evaluation was completed.[2]

Feedstuffs. Corn grain used in the five diets was U.S. grade 2 yellow dent. Alfalfa hay was sun cured second cutting alfalfa harvested at less than 1/10 bloom, which was harvested on approximately the same date as the flatpea hay. Alfalfa hay was purchased from a commercial producer in Martinsville, VA. First cutting flatpea hay was cut with a drum mower on June 11, 1984 and allowed to sun-cure for 3 d prior to baling with a square baler. During this period the

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[2] Carcass evaluations were performed by Dr. R. F. Kelly, Professor, Food Science and Technology Department.

hay was tedded twice to facilitate drying. Second cutting hay (regrowth after first cutting) was prepared similarly, initially cut on August 14, 1984. First cutting flatpea hay was at the early bud stage of growth while second cutting flatpea hay was cut at the vegetative stage from plots at the Virginia Tech Agronomy Research Farm, Blacksburg.

Diets. Five diets, consisting of 70% forage and 30% corn grain, were allotted at random to the five pens of each sex. The forage portion consisted of the following proportions of alfalfa and flatpea hay: 100:0; 75:25; 50:50; 25:75; 0:100. Forage was ground through a 3.8 cm screen, and corn was ground through a 2.5 cm screen. Samples of hay and corn were taken prior to grinding and mixing. A sample of each bale of hay was obtained using a core sampler. Samples from all bales of each type of hay for each diet were pooled to obtain a sample for analysis (table 2). Corn samples were taken for each diet prior to grinding (table 2). Samples from each bag (30 kg) of hay-grain mixture were taken and composited.

Analysis of Feeds. All feed samples were ground in a Wiley mill, through a 2 mm screen. Dry matter and ash determinations were according to AOAC (1980) procedures. Crude protein of feed samples was determined using an automated macro-Kjeldahl apparatus (AOAC, 1980). Neutral detergent

TABLE 2. CHEMICAL COMPOSITION OF ALFALFA, FLATPEA AND CORN-PERFORMANCE TRIAL

Component	Forages: Alfalfa	Forages: Flatpea	Corn grain
	-------------%-------------		
Dry matter	91.30	91.15	92.68
Crude protein[a]	17.30	23.80	9.64
NDF[a b]	44.68	45.39	---
ADF[c]	32.43	38.78	4.65
Lignin	8.32	12.58	1.5
Cellulose	25.92	21.79	3.43
Hemicellulose	11.74	4.84	---
Ash[a]	7.35	6.52	7.73
Calcium[a]	.95	.58	---
Magnesium[a]	.26	.23	.10
Phosphorous[a]	.28	.29	.30

[a] Dry basis
[b] Neutral detergent fiber
[c] Acid detergent fiber

fiber (NDF)(Van Soest, 1963), acid detergent fiber (ADF)(Goering and Van Soest, 1970), permanganate lignin and cellulose (Van Soest and Wine, 1968) determinations were also made. Feed samples were digested with a 3 to 1 mixture (v/v) of HNO_3 and $HClO_4$ (Hern, 1979) for mineral determinations. Calcium and Mg determinations were made by atomic absorption spectrophotometry. Lanthanum chloride was used in dilution to prevent P interference. Inorganic P content was determined colorimetrically by the method of Fiske and Subarrow (1925). Samples for DABA analysis were taken from a random selection of bales. Determination of DABA content was performed after derivatization by passing through fluorescence detector equipped with a 9 μl flow cell and excitation filters (Joyce Foster, personal communication). Procedures are outlined in further detail in Appendix F.

Ruminal Samples. Ruminal samples were taken 2 h after feeding, initially, after 5 wk and at the conclusion of the feeding trial. Samples were obtained using a stomach tube and filter attached to a vacuum pump, were strained through four layers of cheesecloth, after which pH was determined. A 5 ml aliquot was added to prepared tubes for ruminal NH_3-N determination (Chaney and Marbach, 1962). Another 5 ml aliquot was added to tubes containing 1 ml metaphosphoric acid and 4 ml internal standard for use in volatile fatty acid (VFA) determinations (Erwin et al., 1961) by gas chromatography.

Blood Samples. Blood samples were taken, 6 h post feeding via jugular puncture, initially, after 5 wk, and at the conclusion of the feeding trial. Blood urea-N was determined by the method of Coulombe and Favreau (1963). Serum samples for Ca and Mg were diluted with $LaCl_2$ and minerals were determined by atomic absorption spectrophotometry. Serum inorganic P was determined by the method of Fiske and Subarrow (1925) after deproteinization with 10% (w/v) trichloroacetic acid solution. (w/v).

Statistical Analysis. The data were tested by analysis of variance by the general linear model (GLM) procedure(SAS, 1982). Linear, quadratic, cubic, and quartic contrasts were made. Harvey's Least Squares procedure as adapted to SAS (SAS, 1977) was used, due to missing values, to determine least squares means for rumen and blood data. All values given in the tables are least square means unless noted otherwise.

Results and Discussion

Feed Composition. The dry matter, NDF, ADF, lignin, cellulose, hemicellulose, and ash contents of the five diets (table 3) were not significantly different (P>.05). The crude protein content of the diets increased linearly (P<.05) with increased flatpea. Calcium, Mg and P content of these diets were not significantly different, but Ca tended to be lower in the diet containing flatpea hay as 75% of the roughage. Diaminobutyric content of the flatpea hay was 12.7 g/kg of dry matter. This was higher than concentrations previously reported (Van Etten and Miller, 1963; Pavelka et al., 1985).

Animal Performance. During the trial eight lambs deaths were recorded. One animal was euthanized due to a compound fracture of the rear leg. Another animal was diagnosed as having died of enterotoxemia. The remaining six animals had shown some sign of respiratory problems either before death or upon examination at necropsy. A summary of animal mortalities is given in Appendix C, with a description of each in Appendix D. The results of the necropsy reports, combined with the poor health of a number of the remaining lambs showed a respiratory problem did exist in this group of lambs, regardless of diet. Even though clinical symptoms were not seen in all lambs, the possibility of subclinical respiratory problems is strongly suggested. Treatment histories for all sick lambs are given in Appendix B.

TABLE 3. CHEMICAL COMPOSITION OF DIETS FED IN PERFORMANCE TRIAL

Component	Proportion of alfalfa:flatpea hay[a] 100:0	75:25	50:50	25:75	0:100	SE[b]
	---%---					
Dry matter	91.30	91.25	91.05	91.09	91.15	.08
Crude protein[c]	14.58	15.92	17.06	17.91	19.95	.6
Neutral detergent fiber[c]	38.70	40.51	39.29	39.87	39.67	1.46
Acid detergent fiber[c]	23.86	25.39	24.75	25.21	25.86	5.09
Lignin[c]	6.29	6.82	7.36	6.23	6.61	3.2
Cellulose[c]	19.17	21.10	20.73	20.05	21.25	6.16
Hemicellulose[c]	15.44	15.11	14.54	14.90	13.83	3.0
Ash[c]	5.41	5.33	5.40	5.31	5.33	.12
Calcium[c]	.68	.61	.54	.47	.58	.04
Magnesium[c]	.20	.20	.20	.20	.19	.01
Phosphorous[c]	.28	.29	.30	.30	.30	.08

[a] All diets contained forage mixtures shown (70% of total) plus 30% corn grain.
[b] Standard error of means.
[c] Dry basis

Daily gain of lambs tended to decrease (P>.05) with increased levels of flatpea hay during the 10 wk trial (table 4). However, the only large difference was when the level of flatpea hay increased from 75 to 100% of the roughage portion. Lowest feed intake was for animals fed 100% alfalfa hay, but differences among treatments were not significant. The intake level for all diets was greater than was reported by Kromann et al. (1975) for animals of similar size consuming a 70% dehydrated alfalfa 30% corn diet. Feed to gain ratio increased linearly (P<.05), with increased level of flatpea hay.

The carcass characteristics (table 5) showed no differences among diets for carcass conformation, while leg conformation decreased linearly (P<.05) with level of flatpea hay. Firmness values followed a quadratic pattern (P<.005), increasing from 6.75 to 7.72 when 50:50 was fed, then declining to 5.75 when 0:100 was fed. Quality grade was not significantly different (P>.05) among diets. Backfat and kidney and pelvic fat values decreased linearly (P<.02) as level of flatpea increased. Yield grade values showed a significant (P<.02) cubic pattern with no differences between lambs fed the mixed hay diets but a difference of almost one full grade between those fed 100% alfalfa and 100% flatpea

TABLE 4. PERFORMANCE OF LAMBS FED DIFFERENT PROPORTIONS OF ALFALFA AND FLATPEA HAY

	Proportion of alfalfa:flatpea hay[a]					
Item	100:0	75:25	50:50	25:75	0:100	SE[b]
No. of Animals	10	8	8	10	6	
Initial wt., kg	25.31	25.74	26.70	26.05	28.51	1.44
Final wt., kg	35.15	35.31	35.80	35.29	36.05	1.94
Daily gain,g	140	137	130	132	108	20.0
Kg feed/d	1.20	1.32	1.27	1.36	1.51	.08
Kg feed/kg gain[c]	8.68	9.65	9.75	10.31	14.89	1.44

[a]All diets contained forage mixtures shown (70% of total) plus 30% corn grain.

[b] Standard error of means.

[c] Linear effect (P<.05)

TABLE 5. CARCASS CHARACTERISTICS OF LAMBS FED DIFFERENT PROPORTIONS OF ALFALFA AND FLATPEA HAY-PERFORMANCE TRIAL

Item	Proportion of alfalfa:flatpea hay[a]					
	100:0	75:25	50:50	25:75	0:100	SE[b]
No. of Animals	9	8	8	10	6	
Carcass conformation[c]	11.42	10.70	10.72	10.90	10.47	.42
Leg conformation[c,d]	11.51	11.00	11.06	10.92	10.53	.38
Flank streaking[e,f]	3.89	4.10	4.68	3.93	3.23	.31
Firmness[f,g]	6.75	6.90	7.72	6.62	5.75	.40
USDA quality grade[h,i]	8.92	8.60	9.46	8.70	7.68	.40
Backfat[d], mm	4.41	2.54	2.45	3.32	1.61	.64
KP[d,j] fat, %	2.70	2.25	2.62	2.47	1.57	.28
Yield grade[k]	2.91	2.31	2.38	2.58	1.98	.25
Dressing percentage	49.47	49.87	51.13	48.76	47.23	1.33

[a] All diets contained forage mixtures shown (70% of total) plus 30% corn grain.
[b] Standard error of means.
[c] Code: 10- low choice; 11- average choice; 12- high choice, etc.
[d] Linear effect ($P<.05$)
[e] Code: 3-traces; 4-slight; 5-small; etc.
[f] Quadratic effect ($P<.005$)
[g] Code: 5-slightly soft; 6-tends to be soft; 7-tends to be slightly firm; 8-slightly firm, etc.
[h] Code: 7- good+; 8- choice-; 9-choice; 10- choice+, etc.
[i] Quadratic effect ($P<.05$)
[j] Kidney,Pelvic.
[k] Cubic effect ($P<.02$)

diets. All lambs were below accepted market slaughter weight.

Ruminal Fluid and Blood. The pH of initial ruminal fluid samples were similar (table 6). At the midpoint of the trial no significant differences in pH were obtained, but values tended to be higher for lambs fed higher proportions of flatpea hay. Final samples showed a linear increase ($P<.05$) with level of flatpea hay, however, maximum values were reached with the 75% flatpea diet. Initial ruminal fluid NH_3-N levels were not significantly different ($P>.05$). At the midpoint and final samplings ruminal NH_3-N levels increased linearly ($P<.01$) with level of flatpea hay. The levels were highest at the conclusion of the test. This increase followed the level of crude protein in the diet. This increase is similar to that reported by other researchers when high levels of crude protein were fed (Bartley et al., 1976, 1981; Fenderson and Bergen, 1976).

Blood urea-N (BUN) levels were not different ($P>.05$) initially. At the midpoint and end of the trial a linear increase ($P<.01$) occurred with increasing amounts of flatpea hay. Highest BUN levels were obtained at the conclusion of the trial for all diets. The BUN level increased as the ruminal NH_3-N level increased. The increase in BUN with in-

TABLE 6. RUMINAL FLUID pH AND AMMONIA-N, AND BLOOD UREA-N OF LAMBS FED DIFFERENT PROPORTIONS OF ALFALFA AND FLATPEA HAY-PERFORMANCE TRIAL

		Proportion of alfalfa:flatpea hay[a]					
Item	Period	100:0	75:25	50:50	25:75	0:100	SE[b]
Ruminal pH							
	Initial	6.37	6.32	6.41	6.50	6.45	.12
	Midpoint[c]	6.08	6.31	6.53	6.56	6.47	.15
	Final[d]	6.28	6.51	6.72	6.94	6.81	.08
Ruminal Ammonia-N[e]							
	Initial	11.48	10.01	13.61	13.11	13.82	1.72
	Midpoint[c]	12.99	15.48	21.34	21.12	24.32	2.07
	Final[c]	17.29	25.20	23.08	26.71	34.21	2.00
Blood urea-N[e]							
	Initial	14.53	12.74	14.84	14.02	13.80	.74
	Midpoint[c]	15.05	14.69	17.18	21.22	21.58	1.18
	Final[c]	25.44	26.18	29.20	34.40	32.53	1.97

[a] All diets contained forage mixtures shown (70% of total) plus 30% corn grain.
[b] Standard error of means.
[c] Linear effect (P<.01)
[d] Linear effect (P<.05)
[e] Values are in mg/dl.

crease in protein level is in agreement with results reported by Preston et al. (1965) and Young et al. (1973).

Initial VFA concentrations were not different (table 7). At the midpoint total VFA concentrations increased with the 25% flatpea then decreased with higher levels of flatpea (quadratic effect, $P<.05$). No differences ($P>.05$) were obtained in acetate, propionate, or butyrate levels or in the acetate to propionate ratio. Isobutyrate and isovalerate increased linearly ($P<.001$) as proportion of flatpea hay used in the diet increased. Valerate increased with increase of flatpea hay up to 50% of forage, then decreased (quadratic effect, $P<.005$). At the conclusion of the trial total VFA, acetate, and acetate to propionate ratio decreased linearly ($P<.005$), and propionate, isovalerate, and valerate levels increased linearly ($P<.01$) with increased flatpea in the diet (table 8).

Serum Ca, Mg, and inorganic P, (table 9) among lambs, were not different ($P>.05$) initially. At the midpoint, there was a linear decrease ($P<.001$) in serum Ca as flatpea increased. At the end of the trial there were no significant differences ($P>.05$) among treatment groups, but there tended to be an increase in serum minerals with increased flatpea in the diet.

The chemical composition of flatpea hay was comparable to alfalfa hay (table 2). Animal performance was not af-

TABLE 7. RUMINAL VOLATILE FATTY ACID (VFA) LEVEL OF LAMBS FED DIFFERENT PROPORTIONS OF ALFALFA AND FLATPEA HAY-PERFORMANCE TRIAL

	Proportion of alfalfa:flatpea hay[a]					
Item	100:0	75:25	50:50	25:75	0:100	SE[b]
Initial						
Total VFA, umol/ml	81.77	85.57	89.03	74.94	82.75	6.26
Moles/100 moles						
Acetate	64.11	63.88	64.85	65.10	65.63	.91
Propionate	20.39	20.29	19.51	20.17	19.34	1.22
Isobutyrate	.80	.66	.72	.74	.66	.11
Butyrate	12.54	12.89	12.71	11.65	12.41	.93
Isovalerate	.99	.96	1.02	1.11	.88	.15
Valerate	1.17	1.33	1.18	1.19	2.05	.38
Acetate:propionate ratio	3.20	3.22	3.42	3.28	3.40	.23
Midpoint						
Total VFA, umole/ml	81.60	89.21	88.77	80.19	71.19	5.73
Moles/100 moles						
Acetate	66.17	66.44	65.44	64.75	64.86	.76
Propionate	21.36	20.64	19.64	21.09	21.63	.96
Isobutyrate[c]	.70	.81	1.11	1.11	1.11	.08
Butyrate	9.83	9.96	11.16	10.36	9.96	.61
Isovalerate[c]	.85	.99	1.32	1.40	1.34	.13
Valerate[d]	1.09	1.14	1.33	1.29	1.09	.07
Acetate:propionate ratio	3.16	3.24	3.35	3.10	3.03	.16

[a] All diets contained forage mixtures shown (70% of total) plus 30% corn grain.

[b] Standard error of means.

[c] Linear effect (P<.001)

[d] Quadratic effect (P<.005)

TABLE 8. FINAL RUMINAL VOLATILE FATTY ACID (VFA) LEVEL OF LAMBS FED DIFFERENT PROPORTIONS OF ALFALFA AND FLATPEA HAY-PERFORMANCE TRIAL

	Proportion of alfalfa:flatpea hay[a]					
Item	100:0	75:25	50:50	25:75	0:100	SE[b]
Final						
Total VFA,[c] umol/ml	101.39	94.70	91.81	82.06	90.94	3.75
Moles/100 moles						
Acetate[d]	65.86	65.89	64.28	63.50	61.68	.91
Propionate[d]	20.02	20.12	22.36	23.19	23.50	.87
Isobutyrate	1.58	1.02	.92	.91	1.13	.62
Butyrate	10.38	10.66	9.80	9.55	10.60	.46
Isovalerate[d]	.91	.96	1.22	1.44	1.56	.10
Valerate[e]	1.26	1.36	1.41	1.40	1.53	.07
Acetate:propionate ratio[d]	3.33	3.31	2.89	2.75	2.62	.16

[a] All diets contain forage mixtures shown (70% of total) plus 30% corn grain.
[b] Standard error of means.
[c] Linear effect (P<.005)
[d] Linear effect (P<.001)
[e] Linear effect (P<.01)

TABLE 9. CALCIUM, MAGNESIUM, AND INORGANIC PHOSPHOROUS CONCENTRATION IN SERUM OF LAMBS FED DIFFERENT PROPORTIONS OF ALFALFA AND FLATPEA HAY-PERFORMANCE TRIAL

	Proportion of alfalfa:flatpea hay[a]					
Item	100:0	75:25	50:50	25:75	0:100	SE[b]
	mg/dl					
Initial						
Calcium	9.40	9.12	9.25	9.40	9.83	.35
Magnesium	2.04	1.97	1.94	2.31	2.14	.11
Inorganic phosphorous	7.61	7.87	7.48	6.98	6.30	.67
Midpoint						
Calcium[c]	9.82	9.53	9.03	9.36	8.96	.19
Magnesium	2.16	2.07	2.04	2.22	1.90	.11
Inorganic phosphorous	7.21	7.57	7.09	7.40	7.05	.46
Final						
Calcium	10.40	10.07	9.98	9.95	9.66	.27
Magnesium	2.22	2.17	2.23	2.31	2.34	.09
Inorganic phosphorous	7.47	7.62	7.81	8.81	8.30	.53

[a] All diets contained forage mixtures shown (70% of total) plus 30% corn grain.
[b] Standard error of means.
[c] Linear effect (P<.001)

fected by addition of flatpea hay to the diet up to the 75% level. Due to the high protein content of this hay, it should be used with a feed relatively low in protein to meet and not exceed the protein requirement. Results from this trial show that flatpea hay can replace up to 75% of the alfalfa in a diet. No documented evidence of toxicity was observed in the animals fed different proportions of alfalfa and flatpea hay, even though concentration of DABA was higher than previously reported.

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Journal Article II

APPARENT DIGESTIBILITY AND NUTRIENT BALANCE IN LAMBS FED DIFFERENT LEVELS OF FLAT PEA HAY

Abstract

Thirty crossbred wether lambs with an average weight of 31.7 kg were used in a balance trial. Lambs were blocked by weight and randomly allotted to five diets with the following proportions of alfalfa (Medicago sativa L.) and 'Lathco' flatpea (Lathyrus sylvestris L.) hays: 100:0; 75:25; 50:50; 25:75 and 0:100. Ruminal fluid and blood samples were taken initially and at the end of the collection period. Dry matter digestibility decreased linearly (P<.01) as level of flatpea hay increased. Apparent digestibilities of crude protein and lignin were not different (P>.05) among diets. Digestibility of neutral detergent fiber (NDF), acid detergent fiber (ADF), cellulose, hemicellulose, and energy decreased linearly (P<.01) as level of flatpea increased. Nitrogen retention was not significantly affected by level of flatpea hay. Calcium excretion, apparent absorption, and retention decreased linearly (P<.01) with increased proportions of flatpea hay and was related to dietary intake of Ca. Apparent absorption (%) and retention of Mg were not

significantly affected by diet. Ruminal pH and NH_3-N, and blood urea-N increased linearly (P<.001) as flatpea increased in the diet, apparently a reflection of dietary N level. Ruminal total volatile fatty acids (VFA) and acetate concentrations decreased linearly (P<.001) and propionate, isobutyrate, isovalerate, and valerate concentrations increased linearly (P<.001) as the percentage of flatpea in the diet increased. Serum Ca, Mg, and inorganic P were not affected by diet.
(Key Words: Medicago sativa, Lathyrus sylvestris, Lambs, Metabolism, Digestibility.)

Introduction

The use of flatpea (Lathyrus sylvestris L.) as an erosion control plant on land areas with fertility problems has been well established. (Pellett, 1941; Robocker and Kerr, 1964). These areas have potential for productive use as pasture areas. Flatpea has a number of characteristics which make it potentially valuable as a forage plant. It is a high yielding, palatable legume, which is high in protein (Daniel et al., 1946; Grunder and Dickson, 1948). Although flatpea has been studied sporadically over the last 100 yr since its introduction into the U.S. in the 1870's, it has not gained acceptance from producers (Pellet, 1941; Long et al., 1977).

The use of Flatpea for use as a forage crop has been limited due to reported problems (Daniel et al., 1946; Pavelka, 1985). Flatpea is known to contain a free amino acid, L-2,4-diaminobutyric acid (DABA), considered to be a neurolathyrogen (Huang et al., 1950, Ressler, 1964). The ability of ruminants to break down amino acids in the rumen via microorganisms has been suggested as one way of overcoming possible effects of toxic amino acids (Allison, 1978). The introduction of a new cultivar 'Lathco' has renewed interest in flatpea as a forage crop (Slayback and Dronen, 1974, Long et al., 1977). This study was conducted to evaluate the nutritional value of this cultivar. The effect of proportion of flatpea hay on digestibility and nutrient balance in lambs was studied.

Materials and Methods

Thirty crossbred wethers averaging 31.7 kg were assigned to six blocks of five animals, based on live weight. Wethers within each block were randomly allotted to five diets consisting of the following proportions of alfalfa and flatpea hay: 100:0; 75:25; 50:50; 25:75; 0:100. Hay was ground through a 2.5 cm screen. Wethers were placed in metabolism stalls similar to those described by Briggs and Gallup (1949) which allow for separate collection of urine and feces. A

5-d adaptation period to the stalls was followed by a 15-d transition period to experimental diets. This extended transition period was allowed for animals to recover from rumen sampling prior to feeding experimental diets. Experimental diets were introduced into the basal ration gradually over this 15-d period. Test diets were then fed for a 10-d preliminary period followed by a 10-d collection period. Animals were fed twice daily at 0600 and 1800 h. Animals recieved 400 g of ground hay plus 4.5 g iodized salt at each feeding. Feed was added to individual feed boxes and mixed with 80 ml water to control dust. Water was provided at all times except during feeding.

Alfalfa hay was sun cured second cutting alfalfa harvested at less than 1/10 bloom, which was harvested on approximately the same date as the flatpea hay. Alfalfa hay was purchased from a commercial producer in Martinsville, VA. Second cutting flatpea hay was cut at the vegetative stage from plots at the Virginia Tech Agronomy Research Farm, Blacksburg, with a drum mower on August 14, 1984 and allowed to sun-cure for 3 d prior to baling with a square baler. During this period the hay was tedded twice to facilitate drying.

Samples of feed were collected at each feeding 2 d prior to the beginning until 2 d prior to the end of the excretion collection period, and were composited and subsampled. Subsamples were ground in a Wiley mill through a 2 mm screen.

Feces were collected each morning and dried in a forced air oven at a maximum temperature of 60 C for a minimum of 24 h. For each animal the dried feces were composited in metal cans double lined with polyethylene bags and loosely covered to allow moisture equilibration. At the conclusion of the trial, composites were weighed, mixed, subsampled, and ground in a Wiley mill through a 2 mm screen. Urine was collected in 3.8 liter polyethylene jars containing 25 ml of a 1:1 (v/v) solution of concentrated H_2SO_4 and deionized H_2O, diluted with approximately 500 ml deionized H_2O. The urine was collected daily and diluted to a constant volume (5 liter) with deionized H_2O, mixed, checked for acidity and a 2% sample by volume was taken and stored in tightly closed polypropylene containers under refrigeration. These samples were filtered through No. 42 Whatman filter paper and subsampled. Samples for N analysis were kept refrigerated while samples for mineral analysis were frozen. Urine samples for Ca and Mg were diluted with $LaCl_2$ and mineral level was determined by atomic absorption spectrophotometry.

Ruminal samples were taken 2 h post feeding prior to transition period and at the conclusion of the collection period. Samples were obtained using a stomach tube and filter attached to a vaccuum pump. Samples were strained through four layers of cheesecloth, after which pH was determined (pH meter). A 5 ml aliquot was added to prepared tubes for ruminal NH_3-N determination (Chaney and Marbach, 1962). Another 5 ml aliquot was added to tubes containing 1 ml metaphosphoric acid and 4 ml internal standard for use in VFA determinations by gas chromatography (Erwin et al., 1961).

Blood samples were taken 6 h post feeding via jugular puncture prior to the transition period and at the end of the collection period. Packed cell volume was determined using an Adams micro-hematocrit II,[3] and blood urea-N (BUN) was determined by the method of Coulombe and Favreau (1963). For Ca and Mg determinations samples were diluted with $LaCl_2$ prior to analysis by atomic absorption spectrophotometry. Serum inorganic P was determined by the method of Fiske and Subarrow (1925), after deproteinization with 10% trichloroacetic acid.

Dry matter, ash, and gross energy determinations were made according to AOAC (1980) procedures. Crude protein of feed, feces, and urine samples was determined using an auto-

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[3] Clay Adams-Division of Becton Dickson and Company, Parsippany, NJ.

mated macro-Kjeldahl apparatus (AOAC, 1980). Neutral detergent fiber (Van Soest, 1963), ADF (Goering and Van Soest, 1970), permanganate lignin (Van Soest and Wine, 1968), and cellulose (Van Soest and Wine, 1968) determinations were also made. For mineral determination, feed and feces samples were digested with a 3 to 1 (v/v) mixture of HNO_3 and $HClO_4$ (Hern, 1979). Calcium and Mg determinations were made by atomic absorption spectrophotometry. Inorganic P content was determined colorimetrically by the method of Fiske and Subarrow (1925). Determination of DABA content in forage was performed after derivatization by passing through fluorescence detector equipped with a 9 μl flow cell and excitation filters (Joyce Foster, personal communication). Procedures are outlined in further detail in Appendix F.

Statistical Analysis. The data were tested by analysis of variance by the general linear model (GLM) procedure of SAS (SAS, 1982). Linear, quadratic, cubic, and quartic contrasts were made. All values used in tables are least squares means.

Results and Discussion

Feed Composition. The dry matter, NDF, ADF, lignin, cellulose, hemicellulose, gross energy and ash content of the two hays were not significantly different ($P>.05$) (table 10). The crude protein content for flatpea hay was similar to that reported by Hodgson and Knott (1936). Calcium and P contents were lower for flatpea than for alfalfa. Calcium content was higher while inorganic P content of flatpea hay was lower than that reported by Grunder and Dickson (1948). Diaminobutyric concentration was 12.02 g/kg dry matter. This was higher than values reported by other researchers (Van Etten and Miller, 1963; Pavelka et al., 1985).

Apparent Digestibility. Dry matter digestibility of the diets decreased linearly ($P<.01$) as proportion of flatpea hay increased (table 11). The apparent digestibility of crude protein and lignin were not different among the diets. The crude protein digestibility was lower than was reported for 'Wagnerii' flatpea by Hodgson and Knott (1936). Digestibility of NDF, ADF, cellulose, hemicellulose and energy decreased linearly ($P<.01$) as flatpea increased in the diet.

Nutrient Balance. The nitrogen intake of animals on 100% flatpea hay was 30.97% higher than for animals fed 100% alfalfa (table 12). Nitrogen excretion in both the feces and urine increased linearly ($P<.01$) as intake of flatpea increased. There was a linear increase ($P<.01$) in grams of N absorbed, but apparent N absorption, as a percent of intake,

TABLE 10. CHEMICAL COMPOSITION OF DIETS FED IN METABOLISM TRIAL

	Proportion of alfalfa:flatpea hay				
Component	100:0	75:25	50:50	25:75	0:100
Dry matter %	89.49	89.56	89.63	89.68	89.75
Crude protein*%	17.00	18.30	19.60	20.90	22.20
Neutral detergent fiber*%	42.25	42.49	42.71	42.94	43.17
Acid detergent fiber*%	33.91	33.73	33.56	33.38	33.21
Lignin*%	10.27	10.03	9.80	9.56	9.33
Cellulose*%	23.50	23.51	23.51	23.52	23.52
Hemicellulose*%	9.26	9.30	9.32	9.35	9.38
Ash*%	7.73	7.63	7.53	7.43	7.33
Calcium*%	.98	.92	.82	.79	.74
Magnesium*%	.17	.16	.16	.16	.15
Phosphorous%*	.29	.28	.26	.25	.24
Gross energy* kcal/g	4.51	4.50	4.50	4.50	4.50

* Dry basis

TABLE 11. APPARENT DIGESTIBILITY OF DRY MATTER, CRUDE PROTEIN, FIBER COMPONENTS, AND ENERGY BY LAMBS FED DIFFERENT PROPORTIONS OF ALFALFA AND FLATPEA HAY

	Proportion of alfalfa:flat pea hay					
Component	100:0	75:25	50:50	25:75	0:100	SE[a]
	%					
Dry matter[b]	59.74	58.53	56.16	55.45	53.30	.55
Crude protein	70.89	71.08	69.73	70.88	71.37	.48
NDF[b,c]	36.94	34.46	33.73	36.38	32.37	1.09
ADF[d,e]	45.97	44.25	41.53	40.58	39.85	.85
Lignin	25.63	23.84	23.44	24.49	24.75	1.55
Cellulose[e]	56.05	53.39	49.31	47.91	46.03	.97
Hemicellulose[e]	49.02	45.38	41.18	35.90	40.51	2.50
Energy[e]	58.17	56.71	53.98	52.04	52.45	.73

[a] Standard error of mean
[b] Linear effect (P<.01)
[c] Neutral detergent fiber
[d] Acid detergent fiber
[e] Linear effect (P<.001)

TABLE 12. NITROGEN BALANCE OF LAMBS FED DIFFERENT PROPORTIONS OF ALFALFA AND FLATPEA HAY

	Proportion of alfalfa:flatpea hay					
N	100:0	75:25	50:50	25:75	0:100	SE[a]
Intake[b] g/d	19.47	20.97	22.48	23.99	25.50	
Excreted						
Feces[b] g/d	5.67	6.08	6.80	6.98	7.30	.11
Urine[b] g/d	11.82	11.93	13.58	14.59	16.14	.33
Total[b] g/d	17.49	18.01	20.39	21.58	23.45	.37
Apparent absorption						
g/d[b]	13.80	14.89	15.68	17.00	18.20	1.1
% intake	70.88	71.00	69.73	70.88	71.37	.10
Retention						
g/d	1.97	2.99	2.10	2.41	2.05	.37
% intake	10.16	14.13	9.31	10.06	8.05	1.61

[a] Standard error of the mean
[b] Linear effect (P<.01)

was not different among diets. No differences ($P>.05$) were obtained in N retention among the diets.

Calcium intake decreased linearly as amount of flatpea hay increased (table 13). This was due to the lower Ca content of the flatpea. Calcium excretion in feces and urine decreased linearly ($P<.01$) with proportion of flatpea, reflecting the difference in Ca intake. Apparent absorption and retention of Ca increased when the diet contained 25% flatpea hay, decreased up through 75% then increased again with the 100% flatpea diet (cubic effect, $P<.05$). No explanation for this observation can be offered.

Magnesium intake tended to decrease as level of flatpea increased (table 14). Fecal excretion of Mg did not differ. Magnesium apparent absorption (g/d) decreased linearly ($P<.001$) as level of flatpea hay increased, probably a reflection of decreased intake. Urinary Mg excretion decreased linearly ($P<.05$) as proportion of flatpea increased in the diet probably reflecting differences in absorbed Mg. Chicco et al., (1972) reported that absorbed and urinary Mg were highly correlated. Retention of Mg was not significantly influenced by diets, expressed either as g/d or as percent of intake.

TABLE 13. CALCIUM BALANCE IN LAMBS FED DIFFERENT PROPORTIONS OF ALFALFA AND FLATPEA HAY

Item	Proportion of alfalfa:flatpea hay					
	100:0	75:25	50:50	25:75	0:100	SE[a]
Intake[b] g/d	6.86	6.42	5.98	5.54	5.09	
Excreted						
Feces[c] g/d	6.35	5.75	5.69	5.70	4.88	.16
Urine[c] g/d	.097	.035	.030	.019	.020	.02
Total[cd] g/d	6.38	5.77	5.70	5.72	4.90	.16
Apparent absorption						
g/d[bd]	.50	.67	.29	-.16	.21	.12
% intake[bd]	7.33	10.42	4.8	-2.97	4.14	2.92
Retention						
g/d[bd]	.48	.64	.27	-.18	.19	.17
% intake[bd]	6.97	10.04	4.51	-3.31	3.74	2.94

[a] Standard error of means.
[b] Linear effect (P<.05)
[c] Linear effect (P<.01)
[d] Cubic effect (P<.05)

TABLE 14. MAGNESIUM BALANCE IN LAMBS FED DIFFERENT PROPORTIONS OF ALFALFA AND FLATPEA HAY

	Proportion of alfalfa:flatpea hay					
Item	100:0	75:25	50:50	25:75	0:100	SE[a]
Intake g/d	1.62	1.60	1.58	1.56	1.54	
Excreted						
Feces g/d	.247	.248	.263	.260	.240	.01
Urine[b] g/d	.414	.363	.329	.320	.335	.02
Total[c] g/d	.661	.612	.592	.580	.575	.02
Apparent absorption						
g/d[c]	1.37	1.35	1.32	1.30	1.30	.08
% intake	84.72	84.46	83.35	83.37	84.47	.50
Retention						
g/d	.96	.99	.99	.98	.97	.02
% intake	59.13	61.17	62.55	62.85	62.74	1.36

[a] Standard error of means.
[b] Linear effect (P<.05)
[c] Linear effect (P<.01)

Phosphorous intake tended to decrease as proportion of flatpea hay increased (table 15), due to the lower concentration of P in the flatpea. Fecal excretion of P decreased linearly ($P<.01$) with increased flatpea hay in the diet. Inorganic P in the urine was not detectable. Apparent P absorption, expressed as g/d, decreased linearly ($P<.001$) as level of flatpea hay increased.

Ruminal Fluid and Blood. Initially, ruminal pH, NH_3-N, and BUN were similar for lambs fed the five diets ($P>.05$). At the end of the trial, ruminal pH and NH_3-N, and BUN increased linearly ($P<.001$) as level of flatpea hay increased (table 16). Nitrogen intake increased as proportion of flatpea increased. These results agree with those obtained in the feeding trial (Journal Paper No. 1) and with results of other researchers (Bartley et al., 1976, 1981; Fenderson and Bergen, 1974; Preston et al., 1965). Ruminal total VFA, acetate, and acetate to propionate ratio decreased linearly ($P<.001$) and propionate, isobutyrate, isovalerate and valerate levels increased linearly ($P<.001$) as proportion of flatpea hay increased (table 17) Serum Ca, Mg, and inorganic P were not affected by diet (Table 18).

Flatpea hay was lower in digestibity than alfalfa but not low enough to disregard flatpea as a potential forage crop. No detrimental effects were seen from feeding flatpea

TABLE 15. PHOSPHOROUS BALANCE IN LAMBS FED DIFFERENT PROPORTIONS OF ALFALFA AND FLATPEA HAY

Item	Proportion of alfalfa:flatpea hay 100:0	75:25	50:50	25:75	0:100	SE[a]
Intake g/d	2.07	1.97	1.87	1.78	1.69	
Excreted						
Feces[b] g/d	.92	.87	.86	.87	.78	.03
Urine[c] g/d	--	--	--	--	--	
Apparent absorption						
g/d[d]	1.14	1.10	1.01	.91	.90	.03
% intake	55.32	55.63	54.01	51.12	53.48	1.57

[a] Standard error of means.
[b] Linear effect (P<.01)
[c] Not detectable
[d] Linear effect (P<.001)

TABLE 16. RUMEN pH AND AMMONIA-N, BLOOD UREA-N, AND PACKED-CELL VOLUME OF LAMBS FED DIFFERENT PROPORTIONS OF ALFALFA AND FLATPEA HAY-METABOLISM TRIAL

	Proportion of alfalfa:flatpea hay					
Item	100:0	75:25	50:50	25:75	0:100	SE[a]
Initial						
Ruminal pH	7.18	7.09	7.01	7.34	7.36	.07
Ruminal NH_3-N mg/dl	11.16	9.64	8.99	8.87	9.65	.83
Blood urea-N mg/dl	11.55	11.23	10.22	10.57	10.72	.47
PCV,[b]%	36.35	33.77	35.30	36.57	34.05	.93
Final						
Ruminal pH[c]	6.58	6.65	7.01	7.02	7.00	.08
Ruminal NH_3-N mg/dl[c]	27.69	27.85	31.28	34.55	36.34	2.26
Blood urea-N mg/dl[c]	27.02	28.47	30.47	32.29	34.53	1.48
PCV,[b]%	36.20	34.48	35.31	36.67	34.41	.68

[a] Standard error of means.
[b] Packed-cell volume.
[c] Linear effect (P<.001)

TABLE 17. RUMINAL VOLATILE FATTY ACID (VFA) LEVEL OF LAMBS FED DIFFERENT PROPORTIONS OF ALFALFA AND FLATPEA HAY-METABOLISM TRIAL

	Proportion of alfalfa:flatpea hay					
Item	100:0	75:25	50:50	25:75	0:100	SE[a]
Initial						
Total VFA, umol/ml	73.30	76.52	72.87	63.75	62.14	4.79
Moles/100 moles						
Acetate	69.38	66.77	70.62	69.93	70.17	1.91
Propionate	19.63	22.91	18.61	18.71	19.14	2.21
Isobutyrate	1.08	1.00	1.03	1.05	1.17	.07
Butyrate	8.06	7.67	7.88	8.47	7.64	.49
Isovalerate	1.06	.96	1.04	1.05	1.17	.07
Valerate	.80	.69	.82	.79	.78	.04
Acetate:propionate ratio	3.55	3.31	3.77	3.74	3.66	.25
Final						
Total VFA, umole/ml[b]	100.43	94.13	79.77	81.19	78.82	5.11
Moles/100 moles						
Acetate[b]	66.81	65.64	65.13	61.58	61.35	.53
Propionate[b]	20.28	21.57	22.24	24.85	24.31	.81
Isobutyrate[b]	1.22	1.32	1.54	1.62	1.94	.06
Butyrate	8.30	7.89	7.11	7.75	7.59	.43
Isovalerate[b]	1.54	1.64	1.97	2.01	2.48	.08
Valerate[b]	1.85	1.94	2.00	2.18	2.32	.09
Acetate:propionate ratio[b]	3.34	3.05	2.95	2.50	2.53	.14

[a] Standard error of means.
[b] Linear effect ($P<.001$)

TABLE 18. CALCIUM, MAGNESIUM, AND INORGANIC PHOSPHOROUS CONCENTRATIONS IN SERUM OF LAMBS FED DIFFERENT PROPORTIONS OF ALFALFA AND FLATPEA HAY-METABOLISM TRIAL

	Proportion of alfalfa:flatpea hay					
Item	100:0	75:25	50:50	25:75	0:100	SE[a]
	mg/dl					
Initial						
Calcium	10.50	10.42	10.60	10.64	10.61	.20
Magnesium	1.97	1.94	1.95	2.00	2.06	.06
Inorganic phosphorous	6.48	6.44	6.34	5.77	6.58	.41
Final						
Calcium	10.71	10.64	10.72	10.63	10.70	.20
Magnesium	2.18	2.26	2.14	2.17	2.28	.06
Inorganic phosphorous	6.67	6.00	6.46	5.85	5.89	.41

[a] Standard error of means.

hay. Flatpea should probably be used in a ration which is low in crude protein since this hay more than meets the protein recommendations for most sheep rations.

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GENERAL DISCUSSION

The NDF, ADF, ash, and gross energy content of flatpea hay was similar to that of alfalfa hay. Comparison of individual fiber components (cellulose, hemicellulose, permanganate lignin) also showed no significant differences between the two hays. Crude protein content of flatpea was higher than that of alfalfa. Flatpea was lower in Ca content (.74%) compared to alfalfa (.98%). No differences were seen in Mg or inorganic P content.

In reviewing previous work concerning feeding of flatpea to livestock, there is a question of toxicity (Daniel et al., 1946; Grunder and Dickson, 1948). According to these trials toxicity is unsubstantiated. The toxic symptoms previously reported with flatpea when fed to rabbits and rats, were not seen in sheep in the present performance or metabolism trials. The toxicity of the neurolathyrogen, DABA, was not seen when flatpea was fed to lambs. Animal deaths during the performance trial could not be linked to the flatpea or alfalfa used in the diets, and were probably due to an underlying respiratory problem throughout the lambs used. A number of respiratory distress cases were observed and treated in lambs other than those which died. No animal deaths occurred during the metabolism trial in which flatpea was fed in proportions of up to 100% of the ration. Also no respiratory, footrot, contagious echthyma, or rectal prolapse problems were seen in this trial, as were encountered in the performance trial.

Animal performance as measured by feed efficiency and carcass parameters did show a linear decrease as higher levels of flatpea were fed. These decreases can be related to the digestibility of the flatpea hay as determined in the metabolism trial. Flatpea had a lower cellulose and hemicellulose digestibility resulting in a decrease in dry matter and energy digestibilities. Daily feed intake tended to increase although not significantly ($P<.1$), as proportion of flatpea increased. The lower digestibility was probably responsible for the lower feed efficiency for animals fed increasing proportions of flatpea hay in the performance trial.

The performance trial was used to evaluate flatpea hay in comparison to alfalfa hay. The hay portion of the diets, and subsequently the crude protein levels were much higher than are commonly found in finishing rations as described by NRC (1985). Recommendations from NRC (1985) for protein range from 12 to 14% for lambs similar to those used in the performance trial. All rations in that trial exceeded these values. Also the grain content of the rations was substantially less than normal finishing rations for lambs. The NRC (1985) recommendations for finishing rations indicate a concentrate to forage ratio of 60:40 while the ratios used in this trial were 30:70. The lower values obtained for leg conformation, firmness, backfat, and kidney, pelvic fat are indicative of the lower energy availability of the diets with higher proportions of flatpea.

No significant differences were seen between diets due to sex, except for the 100% flatpea ration. The pen of wethers fed 100% flatpea had substantially lower gains, feed efficiencies, and carcass performance than wethers fed the other diets. Ewe lambs fed this diet had performance values similar to that of ewes fed the other four diets.

Rumen and blood parameters showed distinct changes according to proportion of flatpea in the diet. A significant rise occurred in ruminal NH_3-N from a low of 10 and 14 mg/dl initially to a high of 17.3 and 34.2 mg/dl at the conclusion of the performance trial for the 100% alfalfa and 100% flatpea diets, respectively, (linear effect, $P<.05$). This same trend was seen in the metabolism trial with initial NH_3-N levels of approximately 10 mg/dl increasing to 27.7 and

36.4 mg/dl for 100% alfalfa and 100% flatpea diets at the conclusion. The rise in ruminal NH_3-N as crude protein in the diet increased is similar to results reported by Bartley et al. (1976, 1981) and Fenderson and Bergen (1976). The increase in NH_3-N was accompanied by a similar linear increase in ruminal pH, as proportion of flatpea in the diet increased. This increase was seen at both the midpoint and conclusion of the performance trial. A linear increase in ruminal pH was seen again at the conclusion of the metabolism trial. The increase in ruminal pH has been shown to be related to an increase in ruminal NH_3-N (Bartley et al., 1976).

Blood samples taken at both the midpoint and end of the performance trial showed the level of blood urea-N increased linearly as level of flatpea increased in the diet. The increase from 25.44 to 32.53 mg/dl at the conclusion of the performance trial was nearly identical to the increase observed at the conclusion of the metabolism trial with urea-N increasing linearly from 27.02 to 34.53 mg/dl as flatpea increased in the diet. The blood urea-N level has been shown to be related to level of crude protein in the diet (Preston et al., 1965 and Young et al., 1973).

Ruminal VFA levels showed a number of responses to increasing the proportion of flatpea in the diet. Ruminal fluid samples taken at the midpoint of the performance trial showed a linear increase in isovalerate with an increase in flatpea in the diet. Isobutyrate increased from .70 moles/100 moles on 100% alfalfa to 1.11 moles/100 moles with the 50:50 diet and remained at that level with higher proportions of flatpea (quadratic effect). Valerate increased from 1.09 to 1.33 moles/100 moles as proportion of flatpea increased up to the 50% diet and then decreased to 1.09 moles/100 moles as flatpea increased. Samples obtained at the conclusion of the performance trial showed isovalerate, valerate, and propionate increased increased linearly with increased flatpea in the diet. The increases in isobutyrate, isovalerate, and valerate concentration can be associated with the increase in crude protein, since isobutyric, isovaleric and valeric are all end products of microbial degradation of protein as described by Hungate (1966) and Church (1988). These were accompanied by linear decreases in acetate, and acetate to propionate ratios. Total VFA level followed a quadratic pattern, decreasing from the level for lambs fed 100% alfalfa to that of lambs fed the 25:75 mixture, then increasing when 100% flatpea was fed. The changes in acetate, propionate, isovaleric, valeric, and acetate to propionate ratios, were the same as those obtained at the end of the metabolism trial. In the metabolism trial however there was a linear decrease in total VFA and a significant linear increase in isobutyrate as proportion of flatpea increased in the diet.

Serum Ca levels decreased linearly with increased flatpea in the ration at both the midpoint and end of the performance trial. This may have been a reflection of Ca intake as indicated by the decrease in Ca excreted. No significant differences for Mg or inorganic P were seen in the performance or metabolism trial.

Nitrogen intake increased linearly as flatpea increased in the diet. Fecal and urinary N excretion increased with increases in crude protein. These increases occurred with no significant change in N retention between the five diets. These results are similar to those described by Weston (1970) and Lu et al. (1982) when excess levels of crude protein were fed.

Some suggestions for further studies are as follows: A) further performance trials with both sheep and cattle using rations which are isonitrogenous and composed primarily of flatpea hay; B) a broader analysis of flatpea hay not only for DABA, but also for microminerals to check for any possible deficiencies or toxic levels; C) an examination of in vitro and in vivo effects of DABA on rumen microbial populations, if any; D) a controlled evaluation of the grazing potential of flatpea, both alone and in combination with other forage crops; E) an examination of the feasibility of producing haylage from flatpea due to difficulties involved in harvesting it for hay.

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Appendix A
Animal Handling and Sampling Schedule-Performance Trial

6/13/85	Lambs weighed
6/18/85	Lambs treated for worms and parasites
6/28/85	Lambs given Ovine ecthyma vaccine also individuals treated for footrot
7/10/85	Lambs weighed and sorted
7/11/85	Lambs weighed and allocated to pens
7/16/85	Feet clipped, pens 6-10
7/17/85	Feet clipped, pens 2-5
7/18/85	Feet clipped, pen 1
7/22/85	Lambs weighed and allotted to pens
7/23/85	Lambs weighed, ruminal fluid and blood sampled
8/06/85	Lambs weighed
8/20/85	Lambs weighed
8/27/85	Ruminal fluid and blood sampled
9/03/85	Lambs weighed
9/17/85	Lambs weighed
10/01/85	Lambs weighed, ruminal fluid and blood sampled
10/02/85	Slaughtering lambs started by block
10/09/85	Slaughtering lambs completed

Appendix B

Health Problems and Treatment-Performance Trial

Date	Animal	Diet	Problem and treatment
7/30	723	50:50	Possible pneumonia - 2 cc Gentocin 2x/d given for 3 d
8/02	2511	100:0	Off feed, lethargic - 1 cc Vit. B_1, 2 cc LA200, .25 cc BoSe, 1x/d and 1 cc Tylan 200. (Tylan 200 until 8/6)
8/03	2627	0:100	Uncoordinated, off feed, diagnosed as polioencephalomalacia - 250 mg Vit. B_1 given 125 mg IV, 125 mg IM
8/05	2511	100:0	Same - additional 2 cc LA200
8/21	2558	50:50	Lethargic, unthrifty - Penicillin 2 cc, Vit. B_1 1 ml, Gentocin .6 ml all were given 2x/d until 8/24
8/26	684	100:0	Respiratory distress - .5 cc Gentocin, 1 mg Vit. B_1(.5 cc), 2x/d until 8/29
8/29	710	25:75	Rapid respiration pulse, 106 F temp. - .5 cc Gentocin, .5 cc Vit. B_1, both given IV 2x/d until 8/31.
	684	100:0	Pulse 200, respiration 80, 105 F temp. - 2.5 cc LA200 1x/d thru 8/31
8/30	2294	0:100	Lethargic, off feed, unthrifty - given .5 cc Gentocin, 2x/d and .5 cc Vit. B_1, 1x/d thru 9/1 ,
9/07	715	0:100	Prolapsed rectum - pursestring sutures applied, epidural lidocaine given to stop animal from straining
9/08	715	0:100	Some swelling, sutures holding
9/10	715	0:100	Reprolapsed- Lidocaine epidural given and 19 mm pvc pipe sutured in.
9/16	2294	0:100	Lethargic, no appetite, .6 cc gentocin and 6 cc penicillin give 2x/d thru 9/19
9/17	684	100:0	Lethargic, still not doing well - given .4 cc Gentocin and 4 ml penicillin 2x/d thru 9/20
9/25	709	0:100	Lying in water, foaming at mouth, seems incognizant - 1 cc Vit. B_1, 1x/d given thru 9/30
9/26	709	0:100	Looking better, chewing cud
9/27	709	0:100	Animal on side, spastic, gnashing teeth, died in morning
10/1	684	100:0	Wool falling out - slaughtered carcass condemned

Appendix C

Animal Mortalities-Performance Trial

Date	Animal	Sex	Diet	Diagnosis
7/29	716	F	50:50	Enterotoxemia
8/11	723	M	50:50	No necropsy performed
8/19	2630	M	0:100	pulmonary congestion and edema, pulmonary abcess
8/20	686	M	0:100	pulmonary congestion and edema, possible signs polioencephalomalacia
8/24	2543	M	25:75	Broken leg (2x)
9/02	710	F	75:25	Cause of death not found
9/04	2627	M	0:100	Cause of death not found
9/27	709	F	0:100	Acute to chronic bronchopneumonia

APPENDIX D

Animal mortalities occurred throughout the trial starting at d 6 and continuing until d 66 (Appendix C). The first animal which died was 716, which was on the 50:50 diet. The diagnosis developed from the necropsy reported the cause of death as Enterotoxemia. Animal 723 died on d 19 and was also on the 50:50 diet. No necropsy was performed due to the deteriorated condition of the carcass. The lamb had apparently died early the previous evening. It should be noted that this lamb had been treated 10 d previously for possible pneumonia.

Animal 2630 died 27 d into the trial. This lamb was being fed the 0:100 diet. Necropsy results showed pulmonary congestion and edema. Pulmonary abcesses showing heavy growth of Corynebacterium pyogenes were also present. The following day lamb 686 succumbed to similar symptons. This lamb was from the same pen and fed the same ration as the previous lamb. Clinical examination revealed the same pulmonary congestion and edema, lung abcesses, and edema of the mitral valve and chordae tendonae. Examination of the cerebral cortex and caudate nucleus revealed some possible cortical necrosis, a sign of polioencephalomalacia (PEM). A number of animals had recently been diagnosed as suffering from PEM at the research farms where these lambs originated.

Lamb 2543 was euthanized on 8/24 after being diagnosed as having a compound fracture of the femur. This animal was recovering from a minor fracture of the same leg. This animal had been bullied by its penmates for sometime and this was probably how the leg was refractured. Since this fracture would reqire extensive treatment and the lamb would be removed from the study, the animal was sacrificed and samples taken. No necropsy was performed. The lamb had been on the 25:75 diet.

Lamb 710 died 6 wk into the trial. This animal had recently been treated for respiratory problems and general poor health. The lungs showed a marked congestion and were pale indicating fatty deposits. An examination of sections of cerecrum showed possible early cortical necrosis. Clinician's diagnosis was inconclusive. This lamb had been fed the 75:25 diet.

Lamb 2627 died 44 d into the trial. Although the cause of death was not found, this animal also showed some signs of respiratory distress with a pneumonic right lung. This lamb had been treated 30 d previous for PEM. The animal responded to treatment slowly and was back on feed, but had only gained about 1 kg in 4 wk. This lamb had been on the 0:100 diet.

The last lamb to die did so only 4 d before the end of the trial. Lamb 709 had been given vitamin B_1 because it showed signs similar to lamb 2627, who had responded to this treatment. These signs included gnashing of teeth, foaming at the mouth, and a general look of disorientation. No prescription drugs were used due to the proximity of the conclusion of the trial. Necropsy results were similar to the other lambs. An acute pulmonary edema and acute to chronic bronchopneumonia were reported. This lamb had been fed the 0:100 diet.

The results of the necropsy reports combined with the poor health record of a number of the remaining animals show a respiratory problem did exist in this group of lambs. Even though clinical symptoms were not seen in all the lambs the possibility of subclinical respiratory problems is strongly suggested. The performance data of this study should be reviewed with this in mind.

Appendix E
Samples Taken After Necropsy and/or Slaughter

Liver
Kidney
Brain
Spinal column - last 3 thoracic vertebra
Loin eye muscle - last 3 thoracic vertebra
Long bone - front leg
Bone joint - front leg
Tendon - front leg
Blood
Rumen wall
Rumen contents - strained through four layers of cheesecloth
Small intestine - .33 m long from bile duct caudally
Lung
Heart
Spleen
Urine - not able to obtain from all animals
Bile - not able to obtain from all animals

APPENDIX F

For analysis of free amino acids, aproximately .5 g of lyophilized tissue was extracted with 90 ml of 50% (v/v) aqueous ethanol for 90 min, using Soxhlet extractors. Two ml of 15 mM S-(4-pyridylethyl)- DL-penicillamine (Pierce Chemical Co., Rockford, IL) was added as an internal standard. Sample extracts were concentrated under N at 40 C; and then resulting residue was resuspended in 10 ml of extraction medium. A 2.5 ml aliquot of this suspension was centrifuged at 3,000 x g for 10 min. The pellet was resuspended in 2.5 ml of extraction medium and recentrifuged two times. The three supernatants were combined and brought to a total volume of 7.5 ml with extraction medium; .25 ml of this preparation was loaded into a Sep-Pak C_{18} column (Waters Associates, Milford, MA) and eluted successively with .5 ml of water and 1.0 ml of methanol. The combined eluates were adjusted to a final volume of 2.0 ml with water.

Free amino acids in extracts were derivatized with o-phthalaldehyde (OPA, Pierce Chemical Co.) prepared by dissolving 50 mg of OPA in 1 ml of HPLC-grade methanol, adding 50 μl of 2-mercaptoethanol (Bio-Rad Laboratories, Richmond, CA) and bringing the solution to a final volume of 10 ml with .40 M sodium borate-KOH pH 9.5 containing .1% (v/v) Brij 35 (polyoxyethylene lauryl ether, Fisher Scientific, Pittsburgh, PA). Freshly prepared OPA stock solution was stored overnight at 0 to 5 C before use and was used for 2 d. A .1 ml aliquot of the solution was mixed with .02 ml of extract 90 s before injection onto the HPLC column. Derivatized samples were analyzed using a Beckman model 344 binary gradient HPLC system equipped with an Altex 4.6 x 45 mm, 5μm Ultrasphere-ODS octadecylsilane precolumn and an Altex 4.6 x 250 mm, 5 μm Ultrasphere-ODS octadecylsilane analytical column maintained at 45 C following the protocol of Jones et al., (1981, J. Liquid Chrom. 4:4565). Amino acid derivatives were detected using a Gilson model 121 fluorescence detector equipped with a 9 μl flow cell and filters for excitation at 305 and 395 nm and emission at 430 and 470 nm. Detector range and time constant settings were .02 relative fluorescence units and .5 s respectively. Amino acids were identified by comparing their retention times to those of pure amino acid standards (Sigma Chemical Co.) and by coinjection of known amino acids. Peak areas were determined using a Nelson Analytical (Cupertino, CA) model 4416X chromatography data system. Amino acids were quantified using standard curves for each amino acid generated over the concentration range found to occur in tissues examined. Yields of individual amino acids in extracts were adjusted, based on recoveries of the internal standard.

APPENDIX G

Mortality and Morbidity-Performance Trial

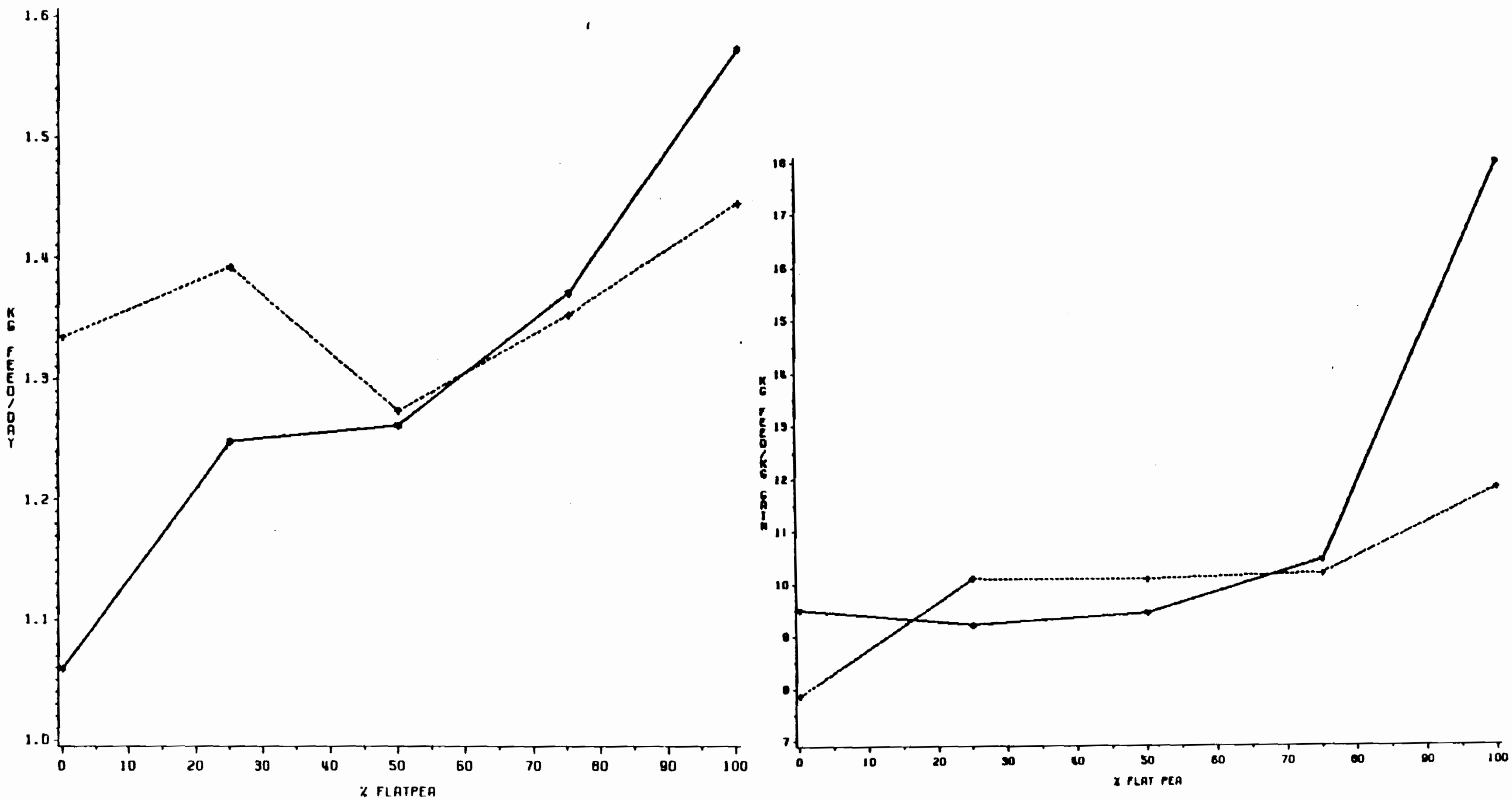
Item	Sex	Proportion alfalfa:flatpea hay 100:0	75:25	50:50	25:75	0:100
Morbid	wether	2	---	2	---	3
	ewe	---	---	---	---	1
Death	wether	---	---	1	1	3
	ewe	---	1	1	---	1

APPENDIX H

Feed Intake of Lambs by Period-Performance Trial

Days	Sex	Proportion of alfalfa:flat pea hay[a]				
		100:0	75:25	50:50	25:75	0:100
		kg/d[b]				
1-14	wether	.80	1.07	.79	.93	.77
	ewe	1.09	1.20	.86	.83	.94
15-28	wether	.98	1.31	1.00	1.16	.86
	ewe	1.34	1.47	1.28	1.15	1.17
29-42	wether	1.00	1.249	1.22	1.07	.99
	ewe	1.27	1.47	1.28	1.15	1.17
43-56	wether	1.14	1.26	1.38	1.37	.96
	ewe	1.42	1.41	1.29	1.37	1.18
57-70	wether	1.42	1.41	1.56	1.54	1.52
	ewe	1.62	1.54	1.43	1.52	1.34
average,	wether	1.05	1.24	1.26	1.37	1.57
1-70	ewe	1.33	1.39	1.39	1.35	1.44

[a] All diets contained forage mixtures shown (70% of total) plus 30% corn grain.
[b] Raw means.



Appendix I. Cumulative Daily Feed Intake and Feed Efficiency of Lambs- Performance Trial. ‡—‡—‡ = wether, +-+-+ = ewe.

VITA

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