DIGESTIBILITY, INTAKE AND MINERAL UTILIZATION OF COMBINATIONS OF GRASSES AND LEGUMES BY LAMBS

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ABSTRACT

Defined mixtures of two grass (orchardgrass, perennial ryegrass) and two legume (alfalfa, red clover) hays, harvested in two cuttings in 2 yr, were fed in digestibility, intake and mineral balance trials to wether lambs. Each mixture was fed in ad libitum amounts to six crossbred wether lambs weighing 35 to 45 kg. The effect of increasing proportion of legume in mixtures on dry matter digestibility (DMD), neutral detergent fiber digestibility (DNDF) and digestible dry matter intake (DDMI) differed with species combination. For all combinations, a quadratic regression (P<.05) for DMD and DNDF indicated a small, negative associative effect for mixtures of grasses and legumes compared with pure species. Dry matter intake (DMI) showed a quadratic increase with level of legume inclusion, indicating a positive associative effect. The observed increase of DMI was approximately 6 to 7% over predicted values with 25 or 50% legume in the mixture. Intake of NDF also showed a quadratic response to level of legume, but lambs tended to eat to a fairly constant intake of 42 to 43 g NDF/kg wt. Mineral utilization (apparent absorption, retention) differed (P<.05) with cutting but not species combination, and generally improved with increasing legume content in the mixture. With the exception of calcium retention (negative quadratic effect), there was little evidence for significant associative effects between grasses and legumes in mineral utilization.

(Key Words: Digestibility, Intake, Minerals, Grasses, Legumes, Lambs.)

Introduction

Associative effects of different components of a diet on digestibility, intake and performance are widely recorded. The inconsistent nature of the results is indicated in a review by Schneider and Flatt (1975). The majority of such studies have used combinations of forages or low quality roughage and a readily available energy source. Fewer experiments have examined the possibility of associative effects between different forages. It is, however, generally observed that the performance of ruminants on mixtures of grasses and legumes, either fed as harvested forage or grazed, is superior to that of animals on grass alone (Thomson, 1979; Ulyatt, 1981). It is not clear whether this is due simply to differences in intake. Moseley (1974) suggests differences in herbage morphology, utilization, carbohydrate composition and molar ratios of ruminal volatile fatty acids as possible causative factors.

The purpose of this study was to investigate effects of feeding two grass hays, perennial ryegrass (Lolium perenne L.) and orchardgrass (Dactylis glomerata L.), and two legumes, alfalfa (Medicago sativa L.) and red clover (Trifolium pratense L.), individually and in different combinations to growing lambs. Feeding conditions were designed to simulate an intensive grazing situation, in which feed would be available in ad libitum amounts and grass and legume components would be of approximately equal dry matter (DM) digestibility and adequate in all required nutrients.
Forages. Stands of perennial ryegrass (Reveille), orchardgrass (Pennlate), alfalfa (Saranac AR) and red clover (Arlington) were established in spring 1980 on a Hagerstown silt loam soil (fine, mixed mesic Typic Hapludalf). No fertilizer was applied at seeding. Nitrogen (as NH$_4$NO$_3$) was broadcast at a rate of 56 kg/ha on the ryegrass on August 12 and on orchardgrass on September 12. All forages were cut and removed from the plots on September 8 and 9.

Two hay cuttings per year were made of each species in 1981 and 1982. Fertilizer treatment of the grasses in 1981 consisted of applications of N in April and June at 75 kg/ha, and application of 560 kg 0-15-30/ha; in 1982, of applications of 75 and 112 kg N/ha in April and June, respectively, and of 336 kg/ha muriate of potash in April. Alfalfa was fertilized with 448 kg 0-15-30 (with boron)/ha in April 1981, and with 448 kg/ha muriate of potash in April 1982. No fertilizer was applied to red clover in 1981, but plots were treated with 336 kg 0-15-30/ha in April 1982. Plots were sprayed for the control of weeds and insects as required.

In 1981, first cuttings were made of perennial ryegrass and orchardgrass on June 1, and of alfalfa and red clover on June 6, using a mower conditioner and field drying. Second cuttings of orchardgrass were made on July 13, and of ryegrass, alfalfa and red clover on July 14. In 1982, a first cutting of orchardgrass and alfalfa was made on June 4, and of ryegrass and red clover on June 17 and 18. Some heat-drying of the alfalfa, ryegrass and red clover was required after cutting due to rain damage. Second cuttings of alfalfa and orchardgrass were made on July 20. and of ryegrass and red clover on July 30.

Feeding Trials. For each cutting, baled hays of each forage species were weighed and milled and mixed in a commercial transit mixer, with a 2.5-cm screen, to provide five forage treatments for each grass-legume combination: 100% grass; 3:1 grass-legume; 1:1 grass-legume; 1:3 grass-legume; 100% legume. Each cutting was fed to 32 crossbred wether lambs, weighing 35 to 45 kg, in three replicated trials. Within each trial, two lambs were assigned at random to each forage species or mixture and animals were rerandomized between trials. After each trial, lambs were allowed a 14-d rest period and fed a low quality grass-legume hay. Lambs during the trials were maintained in individual stainless steel metabolism crates for a 21-d period, and were weighed without overnight fasting before and after entering the crates. The lambs were given ad libitum access to each forage treatment, with a 10 to 15% refusal level, throughout the trial. Each trial consisted of a 14-d preliminary period, followed by a 7-d total collection of feces and urine. Feces were mixed daily, subsampled, composited and stored at 5 C. Urine was collected in plastic bottles to which 100 ml 3 N HCl were added each day. The volume was adjusted daily to 2 liters with deionized water, the urine mixed and a 100-ml aliquot taken, composited and stored at 5 C. Samples of forage and refusals were taken from each lamb each day and composited.

At the conclusion of each trial, forage, refusal and fecal samples were dried at 60 C for 48 h in a forced-air oven and ground through a 1-mm stainless steel screen. Dried samples were analyzed for neutral detergent fiber (NDF; Goering and Van Soest, 1970), crude protein (AOAC, 1975) and minerals by emission spectrophotometry; S was determined by a Leco induction furnace procedure. Concentrations of Ca and Mg in urine were determined by atomic absorption analysis after diluting with acidic .5% lanthanum solution, and P by the Fiske and Subbarow (1925) procedure.

Levels of intake based on g/kg body weight (BW) and digestibility coefficients of DM and NDF were calculated. Apparent absorption values of major elements (Ca, Mg, P, K, S) and apparent retentions of Ca, Mg and P were determined. Effects of variables (year, cutting, trial, species combination, level of legume inclusion) on digestibility, mineral utilization and intake values were analyzed statistically by analysis of variance for a completely randomized design (SAS, 1982). Treatment sums of squares were partitioned to test for linear and quadratic effects of proportion of legume in the mixtures.

Results

Composition of Diets. Regression analyses of NDF concentration in four cuttings of four grass-legume mixtures (alfalfa-orchardgrass,
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alfalfa-ryegrass, red clover-orchardgrass and red clover-ryegrass) at different levels of legume inclusion are illustrated in figures 1 and 2. Results indicate that, in general, composition of the prepared grass-legume mixtures was close to theoretical values. Year, cutting and forage type (grass vs legume) influenced (P<.01) NDF concentration, with a consistently lower level in the legume species (51.9 vs 65.1% in legumes vs grasses). Concentrations of NDF in first-cutting mixtures in both years declined in a linear fashion with an increase in the level of legume in the diet. In regrowth cuttings, differences in NDF concentration between grass and legume components were smaller or nonsignificant.

Concentrations of crude protein in the pure species, expressed as means for all cuttings, were 17.6% for alfalfa, 16.2% for red clover, 14.0% for perennial ryegrass, and 13.4% for orchardgrass (table 1). These concentrations are adequate to meet the requirements of 40-kg lambs (NRC, 1985). Concentrations of protein in the legumes were higher (P<.05) than in the grasses. Concentrations of macro- and micro-elements appeared generally adequate to meet requirements, with the possible exceptions of S, Cu and Zn in both grasses and legumes. The legumes contained higher (P<.05) concentrations of Ca and Mg, and lower (P<.05) concentrations of S and Mn than the grasses, with no significant differences in P, K, Fe, Cu or Zn. First-cutting forages contained lower (P<.05) concentrations of P, Mg, S, Mn and Zn, and higher (P<.05) concentrations of Fe, than second-cutting forages.

Digestibility and Intake. Means by cutting, species combination and level of legume inclusion for dry matter digestibility (DMD) and digestible NDF (DNDF) are given in table 2 and for DM intake (DMI), digestible dry matter intake (DDMI) and NDF intake (NDFI) in table 3. There were differences (P<.05) between years in DMD, DNDF and NDFI values, but interactions with cut and treatment were not significant and data for 1981 and 1982 were therefore combined. The species mixture x legume interaction for DMD and DNDF was significant. Dry matter intake and DDMI were

Figure 1. Concentration of NDF in first cuttings of grass-legume mixtures in two years.

Figure 2. Concentration of NDF in second cuttings of grass-legume mixtures in two years.
TABLE 1. CONCENTRATIONS OF CRUDE PROTEIN AND MINERALS IN PURE GRASS AND LEGUME SPECIES IN TWO CUTTINGS (YEARS COMBINED)*

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<th>Item</th>
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<th>P</th>
<th>Mg</th>
<th>K</th>
<th>S</th>
<th>Fe</th>
<th>Mn</th>
<th>Cu</th>
<th>Zn</th>
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<td>.24</td>
<td>.25</td>
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<td>134</td>
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</table>

*Expressed on a dry matter basis.
TABLE 2. DRY MATTER AND NEUTRAL DETERGENT FIBER DIGESTIBILITY OF GRASS-LEGUME COMBINATIONS IN TWO CUTTINGS (YEARS COMBINED)

<table>
<thead>
<tr>
<th>Treatment a</th>
<th>Cut 1 DMD bcd</th>
<th>Cut 2 DMD bcd</th>
<th>CV b</th>
<th>Cut 1 DNDF bcd</th>
<th>Cut 2 DNDF bcd</th>
<th>CV</th>
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<td>55.3</td>
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<td>58.8</td>
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<td>66.5</td>
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<td>60.1</td>
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<tr>
<td>Red clover</td>
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<td>4.2</td>
<td>56.8</td>
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<td>60.0</td>
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a A, alfalfa; O, orchardgrass; PR, perennial ryegrass; RC, red clover.
b DMD, dry matter digestibility; DNDF, neutral detergent fiber digestibility.
c Linear and quadratic contrast effects of legume (P<.01).
d Species mixture x legume interaction (P<.05).
e Coefficient of variation.

(P<.05), of percentage legume in the mixtures on DMD. Digestible dry matter did not change (P>.05) with the ratio of red clover to perennial ryegrass. Regressions differed with species combination, as indicated by a significant species mixture x legume interaction. Averaged over all combinations, a quadratic relationship (P<.05) for DMD indicated a negative associative effect for mixtures of grass and legume when compared to pure species. The effect, however, was small, i.e., a depression of approximately one digestibility unit for observed values compared with values predicted from arithmetic means of pure species. There was a linear decline (P<.05) in DNDF with level of legume inclusion for alfalfa-orchardgrass, red clover-orchardgrass and red clover-ryegrass mixtures, and a quadratic effect (P<.05) for alfalfa-ryegrass and for all species combinations. The species mixture x legume interaction was significant, with a greater decline for alfalfa than for red clover combinations. The quadratic regression (P<.05) for all forage combinations indicated a negative associative effect but the magnitude of the effect was, again, small (1 to 2 digestibility units).

With increasing levels of legume, DMI of alfalfa-orchardgrass, red clover-orchardgrass and red clover-ryegrass combinations increased linearly, while intake increased in a quadratic fashion for alfalfa-ryegrass and for all species combinations (figure 4). The species mixture x legume interaction was not significant. The curvilinear response in DM intake indicates a
### TABLE 3. LEVELS OF INTAKE OF DRY MATTER, DIGESTIBLE DRY MATTER AND NEUTRAL DETERGENT FIBER IN GRASS-LEGUME COMBINATIONS IN TWO CUTTINGS (YEARS COMBINED)

<table>
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<th>Treatment</th>
<th>DM&lt;sub&gt;cde&lt;/sub&gt;</th>
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<th>Cut 2</th>
<th>CV&lt;sup&gt;h&lt;/sup&gt;</th>
<th>DDM&lt;sub&gt;bcf&lt;/sub&gt;</th>
<th>Cut 1</th>
<th>Cut 2</th>
<th>CV</th>
<th>NDFI&lt;sub&gt;bg&lt;/sub&gt;</th>
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<th>Cut 2</th>
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<sup>a</sup>A, alfalfa; O, orchardgrass; PR, perennial ryegrass; RC, red clover.

<sup>b</sup>DMI, dry matter intake; DDMI, digestible dry matter intake; NDFI, intake of neutral detergent fiber.

<sup>c</sup>Effect of cut (P<.05).

<sup>d</sup>Linear and quadratic contrast effects of legume (P<.01).

<sup>e</sup>Linear contrast effect of legume (P<.01).

<sup>f</sup>Species mixture × legume interaction (P<.05).

<sup>g</sup>Quadratic contrast effect of legume (P<.01).

<sup>h</sup>Coefficient of variation.
GRASS AND LEGUME MIXTURES FOR LAMBS

Figure 4. Responses to percentage of legume in grass-legume mixtures on DM and NDF intake (g/kg BW-75) for combined years and cuttings, based on significant regression coefficients. X--X, all combinations; ▲--▲, alfalfa-orchardgrass; ○--○, alfalfa-perennial ryegrass; ●--●, red clover-orchardgrass; ■--■, red clover-perennial ryegrass.

positive associative effect, evidenced mainly at the lower levels of legume inclusion. An increase of 4 to 5 g/kg BW-75 of observed above predicted DM intake values with inclusion of 25% or 50% legume in the diet represents a 6 to 7% increase in DM intake. There was a significant linear increase in DDMI with increasing level of legume for all species combinations, with a species mixture x legume interaction (P<.05).

Intake of NDF by lambs showed a quadratic response to level of legume in the mixture for the red clover-orchardgrass group of forages and for all species combinations (figure 4). Regressions for the other mixtures were not significant. The species mixture x legume interaction was not significant. Seventy-five percent of observed NDF intake values were in the range of 40 to 46 g/kg BW-75, with an overall mean of 41.8 g/kg BW-75 for first-cutting and 42.6 g/kg BW-75 for second-cutting forages. The small positive deviation (approximately 2 g/kg BW-75) of actual from predicted intake values, and the nature of the regressions, indicated that lambs on ad libitum feeding consumed different grasses, legumes and mixtures with a fairly constant intake value of 42 to 43 g NDF per kg BW-75.

Mineral Utilization. Apparent absorption and apparent retention of elements were generally higher for second-cutting compared with first-cutting forages, with differences (P<.05) for the apparent absorption of Ca (−5.4 vs −9.8%), Mg (10.9 vs 7.5%), and S (49.5 vs 40.0%), and for retention of Ca (−.30 vs −.81 g/d), and Mg (.06 vs −.08 g/d). There were no differences (P>.05) in apparent absorption or retention of elements between different grass-legume mixtures.

The effect of level of legume inclusion in the diets on mineral utilization was examined by regression analysis for combined years, cuttings and species mixtures (figure 5). Except for the apparent absorption of S, the species x legume

Figure 5. Responses to percentage of legume in grass-legume mixtures on apparent absorption (APP. ABS.; %) and retention (APP. RET.; g/d) of elements for combined years, cuttings and species combinations based on significant regression coefficients.
interaction was not significant. Apparent absorption of S increased with increasing proportion of legume in the alfalfa-orchardgrass and alfalfa-ryegrass mixtures, but did not change, or decrease, in the red clover-grass combinations. Apparent absorption of Ca, P and Mg increased linearly as the proportion of legume in the mixtures increased. There was little difference in the apparent absorption of S between grass and legume components of the mixtures, but a quadratic response was noted for the apparent absorption of K, indicating a small positive associative effect. Estimated retentions of Ca on all forage combinations were negative, with a significant quadratic effect of level of legume in the mixture, indicating a negative associative response. With the exception of the 100% grass diets, lambs were in positive P balance and there was a linear increase in P retention as the percentage of legume in the mixtures increased. Lambs remained in approximate Mg equilibrium on all forage combinations, with a small linear increase in apparent retention with increasing proportions of legume in the diet.

Discussion

Previous studies to examine the occurrence of interactions between grasses and legumes in feeding trials have given conflicting results (Forbes et al., 1943; Minson and Milford, 1967; Monson and Reid, 1968; Rattray and Joyce, 1969; Moseley, 1974; Moseley and Jones, 1979; Hunt et al., 1985). Responses seem to be related to the nature of the dietary components and their composition in terms of nutritional adequacy and, possibly, presence of toxic or inhibitory compounds. Minson and Milford (1967), for example, examined the effects of including different proportions of alfalfa and white clover (Trifolium repens L.) in a diet of mature Pangolagrass (Digitaria decumbens Stent) fed to sheep. Increasing the level of legume increased intake and DM and crude protein digestibility, and reduced body weight losses. The authors considered that the effects were due to the legume overcoming a protein deficiency (3.6%) in the grass. Al-ani et al. (1985) noted either positive or negative associative effects of alfalfa in wheat straw diets on DMD and DNDF depending on whether the straw was ammoniated or not treated. Ndlovu and Buchanan-Smith (1985) related the beneficial effects of alfalfa supplementation in diets of poor quality roughages (barley straw, brome-grass hay, corncobs) fed to sheep to increased ruminal concentrations of ammonia and volatile fatty acids, resulting in increased rates of fiber digestion and rates of passage of nondigested material.

The present trials were designed to examine the occurrence of associative effects between different combinations of grasses and legumes of similar digestibility and adequate in concentrations of protein and essential elements for growing lambs. The objectives in terms of nutrient concentration in the forages were, in general, met. There was evidence that the effect on DMD of combining a legume with a grass differed somewhat with the species mixture employed, as indicated by a significant mixture x legume interaction. Monson and Reid (1968) determined the in vitro dry matter digestibility (IVDMD) of mixtures of alfalfa and birdsfoot trefoil (Lotus corniculatus L.) with smooth bromegrass (Bromus inermis Leyss.), timothy (Phleum pratense L.), orchardgrass and reed canarygrass (Phalaris arundinacea L.). They found that, while IVDMD values of the grass-legume mixtures were generally similar to weighted means of the mixture components, species and cutting date affected the IVDMD of certain mixtures by as much as +3.5 to -4.0%. The overall negative associative effect of legume inclusion on DMD and DNDF noted in the present study is at variance with reports by Moseley (1974) and Hunt et al. (1985) of a positive associative effect of the legume on digestibility. In the latter report, however, there was a marked difference in the N content of alfalfa and tall fescue (Festuca arundinacea Schreb) hays combined in the mixtures, although digestibilities of the hays were similar. The overall effect of legume incorporation in the diet on DMD and DNDF of mixtures in the present trials was small and tends to support the conclusion of Thomson (1979), based on British and New Zealand studies with ryegrass and white clover (Trifolium repens L.) of equal digestibility fed in mixtures, that there do not appear to be associative effects on digestibility.

Thomson (1979) proposed a similar lack of associative effects on voluntary feed intake of grass-legume mixtures. Evidence on this response is, however, also inconsistent. Minson (1981) attributed the large increases in intake resulting from the inclusion of a legume in tropical grass diets to the low protein content of the grass. This would not appear to be a reason for the positive associative effects on
intake noted for temperate grass-legume mixtures by Moseley (1974) and Moseley and Jones (1979); and Hunt et al. (1985) observed a simple linear increase in DM intake with increments of legume in alfalfa-tall fescue mixtures, even though the protein content of the fescue was low (6.9%). Lambs in the present study increased DMI as the proportion of legume in the mixture increased. For some mixtures the increase was linear; overall, the response was quadratic and represented a significant positive associative effect on intake at lower levels of legume inclusion in the diet. This increased intake, resulting presumably from an increased rate of passage of forage through the intestinal tract, may have caused the negative effect on DMD and DNDF through a decreased retention time of fiber in the reticulo-rumen. The combination of a positive associative effect on DMI and a negative effect on DMD resulted in a linear increase in DDMI with increasing legume inclusion for all grass-legume mixtures, although the effect differed between species combinations. If the level of intake of digestible dry matter or energy represents a principal component of live weight gain or milk production, as is generally accepted, the results do not support a marked associative effect between grasses and legumes of similar quality on animal response. The improvement in performance would result directly from the increase in intake of digestible dry matter, probably relating to the lower NDF content of the legume. Lambs in these trials, in fact, appeared to regulate their consumption of grass-legume mixtures to a reasonably constant NDF intake, although regression analysis did indicate a significant positive quadratic response. This supports the concept of Mertens (1973) and Van Soest (1982) of cell wall limiting ruminal capacity. These authors demonstrated a similar quadratic relationship between forage cell wall content and cell wall intake for a large population of temperate forages fed to sheep. Hunt et al. (1985) also found no differences in NDF intake of alfalfa-fescue mixtures fed ad lib to sheep.

Little is known of possible interactions between grasses and legumes in mineral utilization, although there are marked differences in concentrations of certain elements between the forage classes, as well as in apparent absorption and retention (Reid et al., 1970, 1978). It has been suggested that one reason for the improved performance of animals on grass-legume as compared with all grass diets may be the higher content or release of certain elements at the reticulo-ruminal level, which might influence the rate or extent of fiber degradation and(or) N utilization. Results of the balance trials indicated that, while apparent absorption and retention of the elements of Ca, Mg and P increased with increasing proportions of legume in the mixtures, the changes were linear with the exception of the apparent retention of Ca. An unexpected result was the consistent negative retention of Ca by lambs on grass and grass-legume mixtures. Only on all-legume diets did lambs approach Ca equilibrium. Joyce and Rattray (1970) determined mineral balances on sheep fed perennial ryegrass, white clover and 50:50 mixtures of the two species. At ad libitum feeding levels, animals showed highly positive Ca retentions, with no differences between diets in apparent absorption or retention. However, concentrations of Ca in the ryegrass were markedly higher than in these trials. Gueguen and Demarquilly (1965) obtained consistently positive Ca balances in sheep fed alfalfa, variable balances with perennial ryegrass and negative retentions with timothy. Reid et al. (1978) reported significantly higher Ca, Mg and P retentions for lambs fed a range of legume hays than for grass hays.

Results of this study indicate that, in general, where grasses and legumes of similar nutritive quality are fed ad libitum in specified mixtures to lambs, associative effects on digestibility, intake and mineral utilization do occur, but that in practical terms of animal response the effects may be of relatively minor significance. The main response to inclusion of a legume appears to be the increase in intake of digestible dry matter. Lambs on different cuttings and combinations of grass and legume species regulated their feed consumption to a fairly constant intake of plant cell wall material.

**Literature Cited**


Forbes, E. B., R. W. Swift, J. W. Bratzler, Alex Black,


