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A NOVEL APPROACH TO GRASS-LEGUME MANAGEMENT

By

Juan Kevin Quamina Solomon

A Thesis
Submitted to the Faculty of
Mississippi State University
in Partial Fulfillment of the Requirements
for the Degree of Master of Science
in Agriculture-Agronomy
in the Department of Plant and Soil Sciences

Mississippi State, Mississippi

May 2010

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A 2-yr grazing study quantified pasture and animal responses of four forage systems (FS) grazed at two stocking rates (SR; 3 or 6 animals ha⁻¹). Using ‘Marshall’ annual ryegrass (*Lolium multiflorum* Lam.) and ‘Durana’ white clover (*Trifolium repens* L.), FS treatments included spatially separated grass and legumes within the same paddock (SS), monoculture grass (MG), monoculture legume (ML), and a binary grass and legume mixture (MIX). Annual herbage mass (HM) was similar among FS at high SR (1900 kg ha⁻¹), but at low SR, grass plots had greater HM (2900 vs. 2000 kg ha⁻¹) than plots of legume monocultures. Animals on SS (1.12 kg) had greater average daily gain (ADG) than ML (0.97 kg), but neither was different from MG (1.08 kg) or MIX (1.00 kg). Low SR animals had greater ADG than high SR (1.09 vs. 0.99 kg). These results indicate that SS grazing system can improve pasture productivity.

DEDICATION

The accomplishment thus far in my academic career is the backbone of my mother Elfreda V. C. Solomon and all my siblings for their never ending support throughout my childhood and academic pursuit.

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CHAPTER I

INTRODUCTION

Spatial patterns in pasture can enhance legume utilization and management while reducing dependence on energy demanding nitrogen (N) requirements in cattle systems. Inclusion of forage legumes in pastures has positive effects on pasture outputs as well as on the environment. Major benefits of forage legumes include contributing N to grasslands through N fixation and providing high-quality forage for animal production (Nelson and Burns, 2006). Ruminants that graze forage legumes, compared with grasses, generally display faster growth and better productivity (Mouriño et al., 2003). Legumes are able to provide a significant amount of N to the pasture system, which reduces the amount of fertilizer required (Mouriño et al., 2003). Nitrogen is of particular interest because it is usually the most limiting nutrient for forage production, and fertilizer N represents a major variable input cost. Currently, there is a serious need to maintain or increase forage supplies while simultaneously reducing input of N fertilizer, which is energy intensive and costly to manufacture and use. This need will continue in the foreseeable future because of the finite supply of fossil fuel, potential for an unstable supply chain and the increasing world population.

Legumes have been proposed as an integral component of forage agriculture throughout the world and the benefits of forage legumes in pastures are well documented

yet widespread adoption of legumes in mixed pastures remains elusive. Typically, legumes in pastures are grown in mixtures with grasses or as monocultures. Widespread adoption of grass-legume mixtures has been severely limited mainly by loss of legume stand. It is well established that in temperate systems animals exhibit a partial preference for legumes. Their intake on legume is greater than on grass and animals can adjust their daily grazing substantially (Chapman et al., 2007). In mixed pasture communities, spatial variability and selective grazing introduce inefficiencies in pasture-based production systems. These include increased expenditure of energy associated with an increase in foraging costs, and an increase in grazing duration required to meet animal requirements (Parsons et al., 1994). Selective grazing of the preferred component within a pasture mixture, often the legume, can decrease the presence of that component in the feed, since the preferred species must compete for growth resources against the other species that are not incurring the same defoliation costs. Persistence of legumes is recognized as a major limitation worldwide.

In legume monocultures, total annual yield generally is lower than yield of grass pastures for reasons that include energetic costs of maintaining N_2 fixation. There is evidence that on legume monocultures, early satiety could be a function of the release of ammonia from the soluble or rapidly degraded protein fraction and subsequent uptake by the blood. Rumen ammonia accumulation has been implicated, as a main reason why animals will not graze to maximize their daily intake to meet nutritional needs.

Additionally, bloating problems are common when animals graze pure clover diets. Further, pure legume pastures do not meet the requirement for optimal C:N ratio. The possible role of C:N balance in feedstuffs comes under scrutiny because of the large

impact that variability in the C:N ratio has on rumen digestion and metabolism (Dove, 1996; Cosgrove et al., 1999).

Achieving management goals in mixed legume-grass swards is not a trivial exercise, since the rates of gross herbage production vary greatly in time and space, and transition from vegetative to reproductive growth differs between species and is strongly related to seasonal conditions that are also highly variable. The common grazing behavior phenomenon of apparent selection for legume species in a mixture with grass species has been considered. It is well documented that animal intake of the legume is greater than that of grass (Kenny and Black, 1984), and that the nutritive value of legumes exceeds that of grass, often by a wide margin. This leads to the notion that a high proportion of legume is desirable in mixed pastures. Because of grazing selectivity, desired proportions of legumes in mixed pastures are difficult to maintain. Worldwide, this difficulty has been considered the premier reason for lack of widespread adoption of legume-grass mixtures in pastures, although the benefits that can be accrued from such systems are well known. In the conventional approach of intermingled species mixtures, interspecific competition for growth resources, active selection by grazing animals, and spatial variability of food resources in the pastures all interact in complex ways that are difficult to predict and control. An alternative approach is warranted.

We are proposing a system of spatial separation of monoculture grass and legume in a 50:50 ratio within the same paddock. In such a system, half of the same paddock is planted to a grass and the other half is planted to a legume, but not in mixture. Such a system offers opportunity for animals to select a diet to match their preference, overcome constraints (e.g., rumen ammonia accumulation, bloating) associated with pure legume

diets and has potential to support more animals per unit area than legume monoculture pastures. In such a system, if interspecific competition between grass and legume is eliminated, we can be confident that a stable pasture composition is met, and targeting of N fertilizer application to the grass component as well as herbicide for control of grass weeds and broadleaf weeds becomes easily manageable. Preliminary research in Europe, New Zealand, and Australia demonstrate that performance of animals grazing spatially separated monocultures of grass and legume within the same paddock was sometimes similar to and often better than that of animals grazing monoculture legumes, and generally was superior to performance of animals grazing mixed legumes-grass or sole grass pastures (Cosgrove et al., 2003; Rutter et al., 2003; Champion et al., 2004; Venning et al., 2004). There is evidence that suggests that the use of a 50:50 ratio is ideally suited for allowing animals to select for optimum dietary preferences and to maximize daily intakes (Chapman et al., 2007). Research of this nature has not been reported in the USA, so this study possibly represents groundbreaking research that may lead to a new phenomenon in pasture management. The null hypothesis of this study is that different forage systems or stocking rate will have no effect on animal performance or forage production. The objectives of this study were: (1) to quantify pasture productivity among monoculture grass, monoculture legume, a binary grass-legume mixture, or spatially separated monoculture grass and legume (50:50 ratio) within the same paddock under continuously stocked grazing management using two levels of stocking rate and (2) to measure performance and physiological responses of animals grazing such systems.

CHAPTER II

LITERATURE REVIEW

The Importance of Grass-Legume Mixtures in Pastures

It is established that forage and grazing lands forms the backbone of profitable forage-livestock systems and contribute substantially to the agricultural economy globally (Sanderson et al., 2004). In animal production systems that rely solely on forage for their daily nutritional needs, grass-legume mixtures are preferred due to several advantages over monocultures (Haynes, 1980). Greater total herbage yield may be obtained by growing a grass and a legume in association, rather than in individual swards, where no fertilizer nitrogen (N) is applied; the use of legumes in pastures may also result in increased N content and a high well-balance mineral content of herbage, all of which are of importance in animal nutrition (Haynes, 1980).

Forage legumes are used in many grassland farming areas of the world, their importance having arisen principally because of their ability to fix atmospheric N₂ biologically and secondly because of their nutritional value such as high protein concentration and digestibility (Iglesias and Lloveras, 1998; Rochon et al., 2004). The importance of pasture legumes for improving the N status of soils and for maintaining a high level of total sward production without N fertilizers has long been recognized (Ledgard and Steele, 1992). In addition to reducing N inputs costs and risk of N leaching

at the farm level, another agronomic advantage is better distribution of annual herbage production (Frame and Newbould, 1986).

During the past four decades, agricultural production has relied heavily on frequent application of N fertilizers (Ledgard and Steele, 1992). With increasing interest in low-input sustainable agriculture throughout the world and concern about possible environmental problems associated with high nitrogen fertilizer use, interest has rekindled in using pasture legumes in Europe and USA as a source of biologically-fixed N (Ledgard and Steele, 1992). Nelson and Burns (2006) suggested two significant changes that further enhanced interest in grass-legume mixtures. One was the rapid increase in grazing management technologies and the shift to intensively managed pastures where the goal was reducing harvesting costs and at the same time, maintaining high nutrient value. The other was the shift to an ecologically based management system (Nelson and Burns, 2006). Liu and Revell (2001) documented some key potential benefits of legume mixtures in pastures. Through differences in growth patterns and adaptabilities, legume species mixtures will better tolerate environmental variations (seasonal or spatial) and use environmental resources more efficiently. Also, they will be more productive; and with varying susceptibilities, pasture mixtures with legumes are expected to have greater tolerance to pests and diseases, which will help maintain high and stable legume proportion in the pasture (Lui and Revell, 2001). A key potential attraction of forage legumes is not simply a function of the aforementioned benefits but also animal products from legume based swards are also perceived by consumers as being more natural than equivalent products from intensively managed, high fertilizer input grass-based swards or concentrate supplemented diets (Rochon et al., 2004).

The nutritional value of forage legumes is in general superior to that of grasses and that grazed legume-based swards are applicable not only in low-input systems, but also in providing the dietary requirements of high-producing ruminant livestock (Rochon et al., 2004). Legumes generally have faster rates of particle breakdown in the rumen, thereby enabling a higher voluntary feed intake, compared with the high fiber concentration and bulkiness of grass (Rochon et al., 2004). The higher crude protein (CP) concentration of forage legumes and increased susceptibility of its fiber to degradation in the rumen are additional factors contributing to increased livestock productivity (Rochon et al., 2004).

While the benefits of growing legumes and grasses in combinations are known, there are still numerous difficulties to maintain a balance in swards of grass-legume mixtures at the interspecies and intraspecies level. This review is related to a plant animal interaction study evaluating a new management system for utilizing legumes in pastures using two forage species, white clover (*Trifolium repens* L.) and annual ryegrass (*Lolium multiflorum* Lam.). Thus, the review will focus on and discuss information related to these two species and their management factors relevant to this study.

White Clover

The use of white clover swards in temperate grazing systems has been widespread because of its benefits to feed quality for the animals and inputs of N through fixation of atmospheric N₂ (Ledgard and Steele, 1992). White clover is also advantageous in low fertilizer N input systems because of the ability of its associated *Rhizobium* bacteria to fix N. Through fixation, white clover supplied much of the N needed for growth of itself and other species within the sward (Sanderson et al., 2003). Thus, there can be less

reliance on fertilizer N with benefits to cost, the environment and the drain in fossil fuel energy for N fertilizer manufacture (Gooding and Frame, 1997; Sanderson et al., 2003). White clover also compliments the growth pattern of the main grass species. This complementarity in resource capture in white clover-perennial ryegrass mixtures was examined using relative resource total (RRT) (Menchaca and Connolly, 1990). Over five harvests, RRT increased from 1, indicating no benefits from mixing to values greater than 3 implying great benefits (Menchaca and Connolly, 1990). Longevity, winter hardiness, plasticity, persistence under multi-cut systems and resistance to treading by livestock, as well as good regrowth are all attributes that make white clover a most suitable component in pastures (Adamovich, 2001). Schils et al. (1999) predicted that the prevailing management on grassland will be a gradual reduction in fertilizer N input, thus creating renewed interest in white clover.

Origin and Agronomic Description

White clover has its origin in the Mediterranean region where it has been found in great natural abundance (Caradus and Woodfield, 1997; Ball et al., 2002). Over the past 60 years there have been over 230 white clover cultivars, commercial ecotypes and lines developed worldwide for on-farm use (Caradus, 1986). It has been validated as one of the most agronomically important of the 250-300 species in the genus *Trifolium*, due to its extensive use in pastoral systems throughout the temperate zones of the world (Caradus et al., 1997).

Frame and Newbould (1986) described white clover as a stoloniferous perennial forage legume. Pederson (1995) posited that white clovers are distinguished based on their known morphological characteristics and as documented by Sheaffer (2007). They

are categorized as; (1) Small types: small leaflets, short peduncle and produce very little forage yield because of a prostrate growth habit but are very persistent under close defoliation, (2) Intermediate types: are intermediate between small and large white clovers and due to high seed production is persistent, and (3) Large types: have the largest petioles, peduncles, leaflets, flowers, stolons and therefore are highest yielding.

An intermediate type white clover cultivar 'Durana' was used in the study related to this review, thus, an agronomic description is warranted. Durana, an intermediate type white clover was developed by the University of Georgia Agricultural Experiment Station (USA) and AgResearch Ltd. (New Zealand), and its intended use is a renovation legume for grass pastures in the southeastern USA (Bouton et al., 2005). It is a persistent, low-growing, densely spreading, profuse flowering, and high stolon growing points cultivar which makes it more persistent than ladino cultivars (Bouton et al., 2005). The large white clovers, usually referred to as ladino, Lodi, Italian or giant white clover, originated from the Po Valley in Italy. They have a characteristic white "V" mark on their leaves and has all vegetative organs much larger than those of other registered varieties (Caradus et al., 1989). True ladino types or those with dominant ladino component originating primarily from Italy and USA are characterized by large leaves (23.2 mm leaflet width), low cyanogenesis ratings (7%) and long petioles (Caradus et al., 1989). The stolons are very thick and fleshy with long internodes and root readily at the nodes. The number of vascular bundles in the petiole is 6.7 to 8.7 compared with 5.0 to 5.3 in intermediate types and petioles are usually hollow (Caradus and Woodfield, 1997). Also, leaf to stem ratio of ladino clover typically is lower and they do not produce such a dense sward as intermediate types (Caradus and Woodfield, 1997).

Adaptation

Although a temperate species found where soil moisture is adequate for growth, white clover is widely adapted to regions from the Arctic to subtropics and has a wide altitudinal range, reportedly up to 6000 m in the Himalayan regions (Sareen, 2008). White clover, a cross-pollinated species, encompasses a wide range of diverse ecotypes and is an adaptable forage legume that will grow almost anywhere in the humid, temperate regions of the world (Pederson et al., 1999). It has been reported that white clover grows in locations with annual rainfall of 31 to 191 cm, average temperatures of 4.30 to 21.80°C, and a soil pH of 4.5 to 8.2 (Duke, 1981). White clover is adapted to a wide range of soil conditions but not acid, poorly-drained soils (Frame and Laidlaw, 2005). Brink (1995) reported that white clover thrives well under varying environmental conditions in the southeastern USA. In a comparative study of growth of U.S. and New Zealand white clover cultivars, similar reports were made by Pederson et al. (1999) that several varieties thrive well in this region of southeastern USA where the climatic extremes vary from season to season but routinely include droughty, hot, humid summers with complete leaf desiccation and cool, wet winters in saturated soils. Genotypic variability is not the only mechanisms by which white clover adapts to specific environments (Pederson et al., 1999). Its phenotypic plasticity is essential along with its high degree of genetic variability to allow white clover to grow and survive in highly variable environments (Woodfield and Caradus, 1994).

Growth and Persistence

The establishment of white clover from seed after germination is characterized by two distinct morphological growth phases, a seminal tap-rooted stage with radiating

stolon systems lasting 1 to 2 yr, followed by a clonal form of growth (Brock and Tilbrook, 2000). Transition of a plant from tap-rooted to clonal form occurs when the tap root and primary stem axis die, releasing a variable number of stolons as independent clonal plants (Brock and Tilbrook, 2000). Hay and Hunt (1989) described white clover as a guerilla-type species, extending into favorable niches by spread of its stolons.

Persistence of this important forage legume is dictated by two mechanisms each playing a role in the survival of the plant as local environmental condition dictates; their inherent traits of vegetative propagation and annual reseeding ability (Brink et al., 1999). Persistence of white clover depends on many environmental factors such as climatic conditions, soil type, slope aspect, water, frequency and extent of grazing and cutting, soil fertility, plant genetics and insects and pathogen infestation (Sanderson et al., 2003).

The climatic factor temperature has a critical effect on clover growth because in the field clover grows most rapidly during spring and autumn, when moderate temperatures prevail (Bienhart, 1963). Brink (1995) reported increased dry matter (DM) yield of three cultivars of white clover during the spring and summer seasons and significantly lower DM yields of all the cultivars during the autumn season. Bienhart (1963) reported that total plant dry weight for white clover was greatest at 16.7 and 23.3°C and was reduced at both 10 and 30°C. Brock et al. (1989) suggested that temperature has very important effects on clover growth and that the optimum temperature for growth of white clover was 24°C. The ability of white clover to survive high temperature is very dependent on soil moisture levels and clover survival is reduced as temperature increases (Brock et al., 1989). The major constraint to white clover persistence is mainly moisture stress and this has been a continuing problem in some

areas worldwide (Chapman, 1986; Sheath and Hay, 1989). Turner (1990) observed that white clover experienced rapid wilting of leaves, petioles and a reduction in stolon length when water supply is restricted, all of which have significant negative impact on biomass yield. Turner (1990) suggested that clover has poor stomatal control of leaf hydration and water loss, and stomatal closure may be incomplete even when turgor is low, thus verifying white clover susceptibility to water deficits.

Bailey and Laidlaw (1999) reported that soil pH < 6.0 and adverse effects of phosphorous (P) deficiency on young plants resulted immediately in a large reduction in stolon branch numbers. Thus, survival of white clover in swards at establishment is critically dependent on P supply, and that one of the main benefits of liming is the resultant improvement in P availability. Singh and Sale (2000) reported that a deficiency in P interacts with water stress to limit clover persistence, increased P nutrition for white clover improved soil-plant water relations through an increase in coarse and fine roots and an overall increase in root length density. The resulting effects of this trend is greater extraction of soil water from drying soil, enhancing white clover ability to persist in water deficit areas (Singh and Sale, 2000). Singh and Sale (2000) concluded that increased drought tolerance of frequently defoliated high P white clover plants was apparently related to their greater root growth, particularly coarseness, length, density, and increased xylem diameter in the primary roots. Consequently, an increased root conductivity of these plants enhances the water uptake and leaf area expansion, even under dry conditions, compared with low P plants (Singh and Sale, 2000).

Bailey and Laidlaw (1998) reported the following observations: (1) at most harvests of white clover, the application of P and potassium (K) each caused increases in

DM yield, the effects of K becoming more pronounced at successive harvests., (2) by the fifth harvest, yield from their zero K treatment was less than 20% of that under the 400 mg K per pot treatment, but there was a significant interaction between the effects of P and K owing to complete lack of response to P under the zero K treatment and relatively poor response to K under the zero P treatment. Bailey and Laidlaw (1998) suggested that provided white clover can withstand moderate P stress during establishment, its persistence in sward is likely to be curtailed more by K deficiency than by low or inadequate P supplies.

Griffith et al. (2000) reported that use of mineral N on white clover swards have all generally showed negative consequences unlike its use on grass swards. For all clover cultivars studied, N assimilation rates, whole plant C:N ratios and root: shoot ratios were independent of mineral N availability (Griffith et al., 2000). Further, clover growth rates were also independent of mineral N availability except for a slight (< 10%) reduction at low N availability levels (Griffith et al., 2000). Johnson and Morrison (1997) in a study of spring fertilizer N on ryegrass-white clover swards grazed by beef cattle reported that there was no effect of spring fertilizer N on white clover proportion in the sward over the season as a whole and the use of extra N did not have a significant effect on white clover plants leaf area. Further, white clover dry weight and total shoot and root proportion did not respond to an application of N (Castle et al., 2002). Further evidence to support these claims on a general negative correlation of mineral N use on clover swards has been documented. Harris and Clark (1996) reported that for mixtures of perennial ryegrass and white clover treatments that did not receive N fertilizer at low and high stocking rates had a mean clover proportion of 16.5% during their trial with maximum clover

proportion in late summer. Mineral N application in their study caused a large decrease in clover proportion on low stocking rate treatments 10.6 and 2.2% that received 200 or 400 kg N ha⁻¹ respectively and clover yield was lower on all treatments that had received N fertilizer (Harris and Clark, 1996).

Several studies have shown that mineral N application on clover swards has a negative impact on the N₂ fixing capabilities of white clover (Harris and Clark, 1996; Høgh-Jensen and Schjoerring 1997; Griffith et al., 2000). Griffith et al. (2000) reported that a linear inverse relationship was found between nitrate uptake and N₂ fixation rates in white clover. These authors suggested that there is a strong indication that N₂ fixation was regulated in order to keep specific N assimilation rate constant and concluded that N₂-fixing activity is regulated by the internal N status of plants and therefore, it is highly unlikely that white clover growth is driven by mineral N availability. Harris and Clark (1996) documented several factors that contributed to reduced N fixation activity under N fertilizer. They indicated decline in clover proportion in pastures will result in less N fixed per unit area. Also, prolonged application of N fertilizer reduces infections and resultant nodule formation by *Rhizobia* in the soil as well as restricting nodule development. These authors further reported that the presence of readily available soil N favors uptake of mineral N by clover since this is an energetically less expensive process than fixing atmospheric N, thus clover substituted fixed N for mineral N. In this regard, Ledgard et al. (1994) concluded that the negative effects of mineral N on N₂ fixation were greater than the effects on clover growth and that for every 1 kg N applied, clover will fixed approximately 0.5 kg less N. High soil N levels can lead to reduce persistence of white clover in pasture systems.

In undisturbed white clover, the prevailing light and temperature conditions control the number and size of leaves, size of stolons and their rate of development in pure clover and mixed swards (Brock et al., 1989). Stolon elongation was greater in high temperatures but branches increased as temperature declined (Brock et al., 1989). The most marked response of white clover to reduced light intensity is a reduction in the formation of stolons from axillary buds (Bienhart, 1963). Dry matter production, stolon elongation, petiole elongation and leaf lamina size were all enhanced by long photoperiod (Junttila et al., 1990).

The rate of appearance of white clover leaves were slower in winter and increased later in spring than companion grass in the hill country of New Zealand (Chapman et al., 1983). Butcher et al. (1996) suggested that leaf and stolon senescence have an important impact on the persistence of the legume in pasture. Winter growth of white clover is slow and in late winter-early spring new growth commences, forming new nodal roots, with older roots and stolons beginning to die and decay (Hay et al., 1983). During the spring season, up to 70% of the total stolon senesced mainly from the basal ends of the stolons (Butcher et al., 1996). Clover growth rate is lower than most temperate grasses in early to mid spring and in late-summer the plants that have survived grow quickly and recreate equilibrium (Brock et al., 1989). Stolon growth is important in the production and persistence of white clover. Brock et al. (1996) suggested, therefore, that the key to persistence is a high growing density, but this varies with season.

White clover is vulnerable to both root and shoot competition and seedlings even compete with each other and can be more susceptible to this kind of competition than with some weed seedlings (Wardle and Nicholson, 1994). The longevity of white clover

leaves and petioles ranged from 21 to 86 d (mean = 59 d), of main stolons sections from 111 to over 677 d (mean = 411 d) and roots from 27 to 621 d (mean = 290 d) (Sturite et al., 2007). About 60% of the leaves produced had turned over by the end of the growing season and another 30% had died or disappeared by subsequent spring (Sturite et al., 2007). Sturite et al. (2007) suggested that the leaves were the most dynamic parts of white clover plants and substantially more ephemeral than stolons and roots. The inherently short life span probably added to winter stress as an important cause of leaf death during the cold season, which may result in a substantial pool of N at risk to off-season losses (Sturite et al., 2007).

Grazing Management

Brink and Pederson (1993) documented that the use of appropriate grazing management is a major factor influencing white clover growth. Thus, grazing management systems and/or variables such as rotational versus continuous stocking, grazing pressure and stocking density greatly influence white clover persistence through their effect on propagation. Kang et al. (1995) reported that defoliation during early stages of seedling development can influence white clover growth. Shoot dry weight increased linearly as defoliation was delayed from unifoliate leaf stage to the eight trifoliate leaves stage. Kang et al. (1995) concluded that regardless of cultivar leaf size classification, permitting seedlings to develop at least four trifoliate leaves before initial defoliation will provide the greatest opportunity for seedling growth and potential survival. Harris et al. (1999) reported that deferred grazing of pasture increased clover proportion in pasture in subsequent seasons. Williams et al. (2003b) reported that under rotational stocking with sheep, white clover can give reliably high yield over a 10-yr

period. Brink and Pederson (1993) made several important observations on white clover response to grazing method.

These include: (1) mean single leaf area was greater under rotational stocking than under continuous stocking, (2) mean single leaf area was similar under both grazing methods when precipitation was 59% above normal, (3) mean petiole length of all cultivars was always greater under rotational than continuous stocking because defoliation interval was shorter under continuous stocking, (4) the response of stolon growth to grazing method was similar under the two systems with adequate moisture but in dry conditions stolon dry weight was reduced by 70% under continuous stocking compared to rotational stocking, (5) stolon branching was greater under rotational stocking than continuous stocking, (6) similar and contrasting observations for stolon growing point density. Brink (1995), based on results obtained on plant morphology, suggested that white clover could withstand frequent defoliation during the spring and early summer and less frequent defoliation during late summer and autumn without stolon loss associated with season long frequent defoliation.

Nitrogen Fixation by White Clover

A key asset of white clover in pastoral systems is their ability to convert atmospheric N into N available for plant use (Ledgard and Steele, 1992). Several studies over an extensive period under varying agro-ecological conditions have reported on the quantity of N fixed by white clover annually (Table 1).

Table 1. Nitrogen realized from N₂ fixation by white clover pastures.

Investigator (s)	N fixed <u>kg N ha⁻¹ yr⁻¹</u>	Country
Grant and Lambert, 1979	17	New Zealand
Rumball, 1979	380	New Zealand
Lane, 1985	138-212	Australia
Crush et al., 1987	600-700	New Zealand
Brock et al., 1989	100-300	New Zealand
Ledgard and Steel, 1992	82-283	New Zealand
Watson and Goss, 1997	168	United Kingdom
Elgersma et al., 1998	217-445	Netherlands
Elgersma et al., 2000	142-337	Netherlands
Ledgard et al., 2001	39-154	New Zealand
Abbasi and Khan, 2004	45-86	Pakistan

There is wide variation in N fixed by white clover as reported in Table 1. One suggested possible reason for this trend is that annual biological N fixation is related to differences in the white clover proportion of the herbage in the pasture (Kristensen et al., 1995). Elgersma et al. (1998) supported this, indicating that the high N fixing values were associated with high white clover proportion in the mixtures. Ledgard et al. (2001) pointed to weather for low annual N fixation. In their study, low N fixation coincided with poor clover herbage accumulation in spring and summer, and was associated with cooler spring temperatures and early onset of dry conditions than in other years. Ledgard et al. (1992) documented that high intensity grazing, especially frequent defoliation during spring, of white clover sward produces annual increases of 10 to 33% in biological N fixation. Thus environmental as well as management conditions are principally responsible for variations in N fixed by clover and these conditions will vary in different geographical zones (Ledgard et al., 1992).

Dry Matter Production

White clover dry matter (DM) yield is of great importance for sustained animal production. Sheldrick et al. (1993) in a field evaluation of two white clover cultivars selected for winter hardiness at the North Wyke Research Station in the UK, reported annual DM yield ranging from 5000 to 7600 kg ha⁻¹ in eight cuts per annum. Zimková and Smajstrla (1993), in a field trial on production and persistence of white clover varieties under different climatic conditions at Nitra and Banská Bystrica in Slovakia, reported mean DM yield for white clover varieties under different climatic conditions ranged from 850 to 3980 kg ha⁻¹ yr⁻¹ at four cuts. Mean DM yield reported in southeastern USA for regal white clover during a consecutive spring-summer-spring period was 3980, 3060, 3680 kg ha⁻¹ respectively (Brink, 1995). In addition, cutting at a 2.5-cm stubble height and harvest intervals of 7, 28, and 49 d, average DM yield across the spring-summer-spring seasons was 3530, 4270, and 5170 kg ha⁻¹ for the three harvest intervals respectively. At the same harvest intervals but cutting at 10.0-cm stubble height, average DM yield was 1690, 2330, and 2200 kg ha⁻¹ for the three harvest intervals respectively (Brink, 1995). Adamovich (2001), in a study on productivity and coexistence of white clover in Latvia recorded DM yield ranging between 4610 to 6260 kg ha⁻¹ in three cuts. Marshall et al. (2003) in a clipping study at Aberystwyth in the UK reported a range of 4556 to 5928 kg ha⁻¹ DM yield from data of 5 cuts and 4909 kg ha⁻¹ DM from data of 6 cuts per annum across 3 yr. Tekeli and Ates (2005), in a European study with treatments of white clover and tall fescue harvested three times per year, reported that mean annual DM yield was 6200, 6230, 5840 kg ha⁻¹ during a 3-yr period. Rutter et al. (2002), studying ingestive behavior of heifers grazing monocultures of

ryegrass or white clover at North Wyke in the UK, reported mean herbage mass of white clover to be 4061 kg DM ha⁻¹ in their study. Williams et al. (2003a), in a grazing study of sheep and cattle at Aberystwyth in the UK, reported white clover mean herbage mass measured at monthly intervals across 2-yr among varieties to be 4500 to 4900 kg DM ha⁻¹ yr⁻¹.

Based on results from a study done in Spain, Iglesias and Lloveras (1998) suggested that differences in climatic conditions/weather differences could have influenced the forage DM yields and the quality of winter legumes. Iglesias and Lloveras (1998) further reported that significant interaction occurred between winter legume and location and between winter legume and year. In their study, there was less DM yield of crimson clover (*Trifolium incarnatum* L.) at Mabengondo (3000 kg ha⁻¹) than at Puebla de Brollón (6200 kg ha⁻¹) during a 2-yr study. At Mabengondo average DM in the first year (5000 kg ha⁻¹) was greater than the second year (1000 kg ha⁻¹).

Animal Feeding Value and Nutrient Composition

The essential qualities of this important forage crop white clover are its protein and mineral rich constituents, and ability to retain high digestibility since there is continual generation of new leaves from stolons, which is partially compensating for advance in maturity of the existing foliage (Frame, 1993). Stypiński (1993) reported that in addition to those qualities of white clover another important characteristic of this forage is its high palatability compared to many grass species. Ayres et al. (1998) points to low retention time in the rumen owing to low fiber and hence higher voluntary intake at equivalent digestibility.

Table 2. Chemical concentration of forage quality variables of white clover from several studies reported.

CP	Constituents†				Investigator (s)
	NDF	ADF	ADL	IVOMD	
g kg ⁻¹ DM					
170.6	397.0	238.0	190.0	792.0	Søgaard, 1993
232.0	359.0	239.0	58.0	-	Berardo, 1997
217.2	413.0	271.8	50.0	715.8	Ayres et al., 1998
215.0	376.0	278.0	-	747.0	Harris et al., 1998
168.0	450.0	268.0	-	-	Kunelius et al., 2006

† CP-Crude protein, NDF- Neutral detergent fiber, ADF – Acid detergent fiber, ADL- Acid detergent lignin, IVOMD- In vitro organic matter digestibility.

Stypiński (1993) reported the following nutritive values for white clover in g kg⁻¹ DM, CP = 266, pure protein = 196.0, crude fiber = 199.0, ash = 85.0, P = 4.9 g, K = 26.0, calcium (Ca) = 9.0, magnesium (Mg) = 2.0 and sodium (Na) = 3.0. The nutritive value of white clover is generally high for leaves and petioles but as the proportion of inflorescence increases the digestibility of white clover decrease (Søgaard, 1993). Harris et al. (1998) documented that the value of a diet depends on the proportion of nutrients digested and on the efficiency with which these nutrients are absorbed and utilized within the animal tissues. Inclusion of white clover in the diet of cattle leads to greater milk production (Harris et al., 1998) and increased liveweight gain of grazing cattle and sheep (Beever et al., 1986; Bax and Schils, 1993).

Nutritional Implication for Grazing Animals on White Clover

Burggraaf et al. (2008) posited that protein in white clover is poorly utilized by ruminants because of its extensive degradation to ammonia in the rumen. Beever et al. (1986) in a study of forage species and season on nutrient digestion and supply in grazing

cattle reported rumen fermentation indices of white clover diets, showing NH_3 (rumen ammonia-N) concentrations ranging from 200 to 240 mg l^{-1} in early season and in excess of 350 mg l^{-1} during the late season. Beever et al. (1986) further reported that the six clover diets had dietary N/OM (organic matter) concentration ranging from 43 to 49 g kg^{-1} , and non-ammonia N (NAN) flows/N intake varied between 0.54 and 0.75 g g^{-1} , indicating substantial losses of dietary N before the small intestine (35% N intake). These losses, in turn, were associated with elevated rumen NH_3 concentrations (230 to 390 mg l^{-1}). Harris et al. (1998) reported that blood urea levels of dairy cows in New Zealand grazing on 200, 500, and 800 g kg^{-1} DM of white clover proportion in pasture was 3.21, 5.51, and 6.60 mmol l^{-1} respectively indicating that as the proportion of white clover increases the concentration of blood urea levels increased. These authors suggested that the high blood and milk urea levels measured, in one of their experiment was evidence that protein was not fully utilized and was therefore wasted. This resulted in a decrease in the Casein:TN (total N) ratio in milk from cows on a diet with a high proportion of clover (Harris et al., 1998).

Wolfe and Lazenby (1972) reported that of the 289 moderate and severe cases of bloat observed during their experiment, 221 occurred on pastures with a high proportion of clover (60 to 80% white clover) compared to 58 on pastures with medium (20 to 50%) and 10 animals on pastures with a low proportion of clover (15 to 25%). These authors also reported that liveweight gain on the highest proportion of clover were 20 to 30% lower than on the other two types in both years. This reduced liveweight gains were attributed to depressive effects of bloat on herbage intake.

Burggraaf et al. (2008) made several observations in their study and reported that the presence of high levels of condense tannins (CT) in white clover aid in the reduction of bloat and protein degradation. They reported that white clover flowers contain CT and as the proportion of flowers increased, CT levels increased 0 no flowers = 0.0 CT, 100% flowering = 52.4 g CT kg⁻¹ DM. They suggested that as the proportion of flowers increased, there is a decreasing amount of plant N appearing as ammonia in the rumen. Net ammonia released after 24 h incubation ranged from 120 to 290 mM M⁻¹ of forage N, with highest value for no-flower treatment.

Annual Ryegrass

Annual ryegrass, also referred to as Italian ryegrass, has emerged as one of the main forage species for cattle production in southeastern USA for winter and spring grazing season (Redfearn et al., 2002). Cultivation of annual ryegrass in the Southeast accounts for 1.1 million hectares annually (Evers, 1995). The main use of annual ryegrass is for production of high nutritive value forage for stocker cattle, replacement heifer and lactating dairy cows during the winter and spring seasons (Balasko et al., 1995; Kallenbach et al., 2003; Lippke et al., 2006).

Origin and Agronomic Characteristics

Annual ryegrass is indigenous to southern Europe, northern Africa and western Asia and it is a bunch grass that usually behaves as an annual or winter annual but under favorable conditions can act as a short-lived perennial (Nelson et al., 1997; Casler and Kallenbach, 2007). There are several important agronomic characteristics that account for the widespread use and popularity of this forage including high herbage yield, a long

growing season, tolerance to a wide range of environmental conditions and grazing practices, rapid seedling establishment, weed suppression, excellent persistence under close grazing, compatibility with several forage legumes and excellent forage quality and palatability (Jung et al., 1996; Franca et al., 1998). Optimum growth of annual ryegrass is attained on soils of $\text{pH} \geq 5.7$ with lower forage production on more acid soils (Evers and Nelson, 2000).

Since 'Marshall' annual ryegrass is being used in this study, agronomic description will focus mainly on this cultivar. It is a tall, erect-growing, wide-leaf cultivar with good seedling vigor (Arnold et al., 1981). Marshall annual ryegrass is a late maturing, diploid ($2n = 14$) annual and as a result of late maturity it will produce longer than other diploid varieties in the spring (Arnold et al., 1981). Redfearn et al. (2002) reported that Marshall annual ryegrass on average yielded 449 kg ha^{-1} more late season forage than 'Gulf' and other cultivars of ryegrass. Another key characteristic for its choice is cold tolerance because it will survive where winter temperatures are below freezing for several consecutive days or weeks (Arnold et al., 1981; Redfearn et al., 2002). One negative characteristics of Marshall annual ryegrass is its susceptibility to crown rust (*Puccinia coronata* Pers.), which can result in severe lost of forage yield (Hafley, 1996).

Fertilizer Management

Annual ryegrass is responsive to fertilizer, especially N. Lippke et al. (2006) reported that economically optimal levels of applied N were predicted to range from 250 to 315 kg ha^{-1} and for applied P was 31 to 41 kg ha^{-1} . Fertilizing vegetative ryegrass to

maintain N in leaf tissue $\geq 32 \text{ g kg}^{-1}$ provides economically optimal growth for both the crop and the young cattle grazing it (Lippke et al., 2006).

Herbage Yield

Herbage production from annual ryegrass is well documented and in Mississippi, many ryegrass variety trials with Marshall annual ryegrass have been done in several locations in the state. Lang and Johnson (2006) reported average yield of 7802 kg ha^{-1} with a fertilizer regime of 560 kg ha^{-1} of 15-15-10 in split applications and an additional 67 kg N ha^{-1} at two locations in Mississippi. Annual DM yield of Marshall annual ryegrass at the Starkville location was 8887 kg ha^{-1} and at Raymond was 10532 kg ha^{-1} . In Italy, Franca et al. (1998) reported DM yield of 4600 kg to 6000 kg ha^{-1} . In a 12-yr evaluation of annual ryegrass cultivars, Redfearn et al. (2005) reported that mean early season yield ranged from 2300 kg ha^{-1} to 4600 kg ha^{-1} and mean late season yield of 5100 kg ha^{-1} to 7100 kg ha^{-1} . These authors concluded that the yield of annual ryegrass cultivars was highly variable, indicating that responses of individual cultivars in terms of absolute yield and relative performances to each other were highly variable from year to year. Given the large yearly fluctuation in yield, lack of yield stability is a characteristic of many annual ryegrass cultivars (Redfearn et al., 2005). In a study in Canada where a single end-of-season harvest was done, yields of 4300 and $6700 \text{ kg DM ha}^{-1}$ in different years was reported (McCartney et al., 2007).

Hickey and Hume (1994) reported that ryegrass herbage accumulation under sheep grazing ranged from 8000 to $10000 \text{ kg DM ha}^{-1} \text{ yr}^{-1}$ in New Zealand. In a study in Louisiana, average forage mass under continuous stocking for Marshall annual ryegrass was 5030 kg ha^{-1} and 7340 kg ha^{-1} per annum respectively (Hafley, 1996).

Nutritive Value

In a New Zealand study that measured chemical composition of annual ryegrass every 3 to 4 wk, nutritive value remained very high up until 180 d then declined rapidly after (Thom and Prestidge, 1996). Redfearn et al. (2002) reported a general decline of nutritive value at the different sample periods for various fractions tested, in a study with different annual ryegrass cultivars. This study found that the increase in CP observed between the January and February harvest was mostly due to the application of fertilizer N (83 kg ha^{-1}) following the January harvest. Another N application was made (83 kg ha^{-1}) following the March harvest and in this instance CP concentration did not increase. They suggested that this was due to dilution effect caused by greater forage mass (Redfearn et al., 2002).

Table 3. Crude protein, neutral detergent fiber (NDF), in vitro true digestibility (IVTD) and digestible neutral detergent fiber (DNDF) concentration of Marshall ryegrass averaged across two years (1997-1998 and 1998-1999) and four locations (Redfearn et al., 2002).

Chemical fractions	Dec	Jan	Feb	March	April	May
	g kg ⁻¹					
CP	245	232	262	181	178	131
NDF	371	382	395	400	510	557
IVTD	846	843	853	835	777	722
DNDF	574	588	623	588	565	504

Hafley (1996) reported in a grazing study, the chemical composition of Marshall annual ryegrass under continuous stocking was CP = 160 g kg^{-1} , NDF = 490 g kg^{-1} and IVTD = 690 g kg^{-1} . Genetic variation exists for many forage nutritive characteristics in

ryegrass such as digestibility, non-structural carbohydrates and N concentration (Jung et al., 1996). Thus, growing the late maturing variety such as Marshall annual ryegrass will allow producers to extend the production of high quality forage into late spring (Redfearn et al., 2002).

Table 4. Chemical composition and rumen ammoniacal-N concentration ($\text{NH}_3\text{-N}$) of Italian ryegrass fertilized with different levels of N across 2 yr (de Villiers and van Ryssen, 2001).

Chemical fractions†	N application rate		
	Low (100 or 200 kg ha ⁻¹)	Medium (400 kg ha ⁻¹)	High (600 or 800 kg ha ⁻¹)
CP (g kg ⁻¹)	199.0	214.0	242.0
TNC (g kg ⁻¹)	150.0	130.0	105.0
IVDOM (g kg ⁻¹)	704.0	704.0	695.0
$\text{NH}_3\text{-N}$ (mg 100 ml ⁻¹)	19.4	25.4	31.9

† CP = crude protein, TNC = total non-structural carbohydrates, and IVDOM = in vitro digestible organic matter.

Increasing the levels of N fertilizer application caused an increase in mean $\text{NO}_3\text{-N}$ concentration of the herbage (de Villiers and van Ryssen, 2001). This, however, did not surpass the safe limit blood levels of 57.0 to 60.0 mg 100 ml⁻¹ for ruminants (de Villiers and van Ryssen, 2001).

Grazing Management

Continuous stocking is a common grazing management practice used for annual ryegrass (Casler and Kallenbach, 2007). Average daily gain (ADG) from Marshall ryegrass utilizing continuous stocking was 1.42 kg d⁻¹ in the first year (71 d grazing) and 1.19 kg d⁻¹ in the second year (84 d grazing) but with rotational stocking, ADG was 0.96

kg d⁻¹ and 0.94 kg d⁻¹ for Years 1 and 2, respectively (Hafley, 1996). Zaragoza-Ramírez et al. (2008) reported ADG ranged from 1.0 to 1.2 kg and decreased as stocking rate increased in a study of stocker cattle grazed on Marshall annual ryegrass.

White Clover-Ryegrass Mixtures

Binary White Clover-Annual Ryegrass Sward Dynamics

Grass-legume swards have been considered by farmers difficult to establish satisfactorily and difficult to manage so as to ensure a sufficient legume component, especially under grazing (Rochon et al., 2004). Kemp and King (2001) in a discussion on competition in pastures posited that different forage species differ in resources they require to grow, develop and reproduce and this explains one of the problems with growing grasses and legumes in mixtures. If each species requires a completely different set of resources from every other species, the only “resource” they would compete for would be physical space (Kemp and King, 2001). This situation generally tends to be problematic in white clover-ryegrass binary mixtures, thus a fuller understanding of the dynamics of grass/clover swards would enable us to improve their reliability (Caradus et al., 1995).

The compatibility/competitive interactions between white clover and perennial ryegrass (*Lolium perenne* L.) and their implications for agronomic performance in mixtures have been explored and reported for many years (Annicchiarico and Piano, 1994). The relative abundance of white clover and its use in grassland agriculture is due to its growth characteristics (Rochon et al., 2004). Once white clover has become established, vegetative reproduction occurs through stolon development and this

mechanism is responsible for resistance against mechanical stress resulting from grazing livestock (Hay et al., 1989). As a perennial species, its vegetative reproduction is supported by self regeneration from season to season but crucially, it exhibits low competitive ability against grasses (Rochon et al., 2004). Thus, long-term investigations of grass clover mixtures have shown that the survival of white clover depends mainly on the competitiveness of associated grasses (Rochon et al., 2004).

Davies (2001) in a detailed discussion on competition between grass and legumes in established pastures made several important conclusions that are relevant to this study. He noted that the extent to which grass-clover relationship is influenced by temperature and N is strongly dependent on the stage of development of the canopy. Canopy development effects, Davies (2001) suggested, comprised of three stages with variable duration: 1) active increase in light capture, 2) light capture and, 3) maturation. The species with the highest rate of leaf area expansion will increase its share of the light intercepted at the expense of its competitor. This tends to be a general problem in binary mixtures of ryegrass-clover swards thus clover suppression is inevitable. Davies (2001) made another conclusion that avoidance of desiccation may also, at least partly, account for the low and relatively protected position which clover comes to occupy in mixed sward in winter. In the absence of defoliation, differing height responses of grass and clover to winter temperatures can result in clover suffering severe competition for light (Davies, 2001). Weller and Cooper (2001) reported the mean clover composition in two grazing seasons to be 272.3 g kg⁻¹ DM and 307.0 g kg⁻¹ DM. This is an indication of the dominance of ryegrass when grown in mixture with clover.

Since more grass leaf than clover leaf is present in the upper layers of the sward in the spring, it is not surprising to find that spring defoliation can be beneficial in terms of clover composition (Davies and Evans, 1990). Rochon et al. (2004) suggested that the timing of the first harvest cut or grazing is also crucial for determining the competitiveness of white clover and sustaining it. Schulte and Neuteboom (2002) observed that in grazed swards, white clover colonizes areas of damaged swards caused by heavy grazing and trampling of animals. In this situation, weeds can overgrow the white clover resulting in patchiness and a failure to achieve the ideal balance of grass and clover (Schulte and Neuteboom, 2002). Carrere et al. (2001) studying how the vertical and horizontal structure of a perennial ryegrass and white clover sward influences grazing reported that in mixed patches of a strip sward, clover was also more defoliated than ryegrass (30.0 vs. 18.0%). These authors concluded that for continuously stocked ryegrass-clover mixtures, differential defoliation of species varies according to vertical distribution of leaves but is little affected by horizontal structure of canopy. When grazed by sheep, which have a high capacity of selective grazing, the degree of mixing between ryegrass and clover has little effect on the pattern of species defoliation (Carrere et al., 2001). The reasons suggested for this trend was that sheep were able discriminate not only among patches with or without white clover but also for clover within small patches where the two species are present (Carrere et al., 2001).

Davies (2001) suggested three ways in which grazing animals may affect the relationship between grass and clover; 1) grazing intensity (such that the remaining herbage includes more of one species than others), 2) deposition of dung and urine resulting in: (a) uneven pattern of N distribution in the soils and (b) subsequent avoidance

of recently contaminated areas, and by (3) actively selecting clover rich areas. Stocking rates influences clover-grass sward because it determines grazing intensity. At a high stocking density, clover composition of pastures generally decline due to intense selection of clover by grazing animals (Curll et al., 1985). These authors reported that increasing the stocking from 25 to 55 yearling sheep ha⁻¹ reduced herbage accumulation by 40%, whether or not N fertilizer was applied. The increased stocking rate increased the density of ryegrass tillers, but reduced the density of clover stolons and the clover composition of the sward (Curll et al., 1985). Uneven deposition of dung (feces) and urine by grazing animals can influence the cover of grass-white clover swards dynamics in mixed pastures (Schwinning and Parsons, 1996; Gillet et al., 2009). In a study of white clover under grazing conditions, Laidlaw and Vertès (1993) reported that the return of urine reduces stolon population density and N₂ fixation of white clover by indirectly stimulating grass growth. These authors suggested an additional effect of feces return on grazed grass-clover pastures is the rejection of herbage around dung pats resulting in changes in grass and clover morphology. In general, both sheep and cattle show active selection for clover in mixed swards even in swards where clover proportion is low, thus disadvantageous for clover existence in mixed pastures (Davies, 2001).

Rochon et al. (2004) suggested that it is generally true that measures, which promote the growth and competitive ability of grasses, particularly the application of N fertilizers, reduce white clover in the sward. Curll et al. (1985) showed that application of 200 kg N ha⁻¹ annually increased herbage accumulation by 20% but substantially reduced the clover content. Rochon et al. (2004) posited that if white clover is desired in

the sward, then the amount of N- fertilizer has to be reduced with consequences for grazing management.

Spatial System of White Clover-Ryegrass Pastures

The use of large-scale spatial patterns, whether in the height, density, or species composition of vegetation, are one of the most demonstrable and widely recognized features of heterogeneity in large herbivore grazing systems (Parsons and Dumont, 2003). Baumont et al. (2002) suggested that vegetation characteristics such as herbage mass, sward structure vertical and horizontal availability of preferred plant species and spatial distribution influence behavior and intake of ruminants. Thus, to understand how their existence relates to grazing process, and what the implication of patterns are for plants, animals, and land users, requires adding spatial concepts, and dynamics to our knowledge of interactions between plants and animals (Parsons and Dumont, 2003).

Pasture utilization by grazing animals remains a complex biological process that is not well understood, even with the ongoing grazing behavioral research (Burns and Sollenberger, 2003). Rook et al. (2002) suggested that an understanding of herbivores in response to differences in sward state and relative availability of the component plant species, and the feedback effects of these strategies on subsequent sward state, is an essential prerequisite to development of sustainable grazing systems. The efficient use of pastures by grazing livestock in multi-species sward requires an understanding of preference and selection by animals (Rutter et al., 2004). In understanding grazing behavioral patterns of cattle subject to adjacent monocultures of a legume-grass base system, the term preference and selection must be distinguished. Parsons et al. (1994)

defines preference as what the animals select when given the minimum physical constraints, while selection is preference modified by environmental circumstances.

This modification is becoming increasingly important in many temperate grassland based livestock systems with increasing incorporation of legumes in grass swards (Rutter et al., 2002). To know what the animals want to eat is usually achieved by grazing the two herbage species as spatially separate but adjacent monocultures with animals given free choice to either grass or clover whenever they want (Rutter et al., 2004). In a study of dietary preference of dairy cows grazing ryegrass and white clover, Rutter et al. (2004) reported that clover formed 63.2% of the total herbage intake of dairy cows grazing in paddocks that contained a spatially separated system of 25% clover and 75% grass in adjacent monocultures. In paddocks that contained 75% clover and 25% grass in spatially separated adjacent monocultures, clover formed 84.5% of the total herbage intake of dairy cattle. The mean clover intake of dairy cows in their study was 73.8% between the two clover groups offered. These authors suggested that this is what cows would select if offered 50% clover and 50% grass (by ground area). The general trend in their study also showed a decline in the preference of clover during the course of the day (Rutter et al., 2004). Another observation in that study was that intake rates were higher for cows grazing clover ($41.3 \text{ g min}^{-1} \text{ DM}$) in adjacent monocultures paddock of equal size of pure grass and pure clover than cows grazing grass ($27.5 \text{ g min}^{-1} \text{ DM}$) and were higher in the evening than in the morning (Rutter et al., 2004). These authors concluded that cows showed active selection for clover and that these results indicated that preference for clover was partial and not absolute. Studying ingestive behavior of heifers grazing monocultures of ryegrass and white clover, Rutter et al. (2002) reported

that heifers grazing grass spent longer time eating (536 min d⁻¹ versus 436 min d⁻¹) compared to those of clover. In that study the authors also reported that instantaneous dry matter intake rates min⁻¹ were identical on grass and clover swards (both 12.9 g DM min⁻¹), which gave rise to greater daily intakes of grass compared with clover (6.93 kg DM d⁻¹ versus 5.61 kg DM d⁻¹). Clover, however, had a higher dry organic matter digestibility (DOMD) than ryegrass (599 vs. 772 g kg⁻¹) and so animals on the two different swards had similar intakes of digestible organic matter (4.17 kg d⁻¹ versus 4.27 kg d⁻¹) (Rutter et al., 2002). Similar ADG (0.97 kg d⁻¹ versus 0.99 kg d⁻¹) was reported for heifers grazing grass or clover pastures, respectively (Rutter et al., 2002). These authors suggested that the heifers were regulating their intake by adapting to a foraging strategy aimed at minimizing grazing time.

Since grazing animals generally consume forages selectively, prediction of their nutrient intake and of the location and intensity of the impact on the heterogeneous vegetations need an understanding of the animals' foraging decisions (Prache et al., 2006). When sward height is similar for both species at the beginning of grazing, sheep are assumed to spend most time feeding from species allowing the highest intake rate (Prache et al., 2006). Champion et al. (2004) suggested that the existence of this partial preference is likely to lead to selective grazing by sheep when they are grazing mixtures of grass and clover.

Parsons et al. (1994) studied diet preference of sheep on ryegrass and white clover on swards that contained adjacent monocultures of grass and clover and observed their intake behavior. Their study used experimental paddocks that contained 20, 50, and 80%

white clover by ground area to distinguished partial preference from indifference and reported the following results:

- 1) The proportion of time grazing on clover portions was different for the proportion of clover (20, 50 and 80%) in the experimental paddock. The mean percentage time grazing the three proportions of clover during their test periods were 49.5%, 77.6%, and 72.7% respectively. They suggested that this was evidence that animals did not graze at random (indifference), but grazed preferentially.
- 2) In relation to diet selection of sheep, clover content in the diet was higher in the morning periods than in the afternoon periods of grazing, followed by a return to high clover diet content each morning. Intake of grass increased during the course of the day, greater in the afternoon compared to the morning periods. This general pattern was observed for both physiology (dry or lactating ewes) and background combinations (grass, clover, and grass/clover treatments) throughout the study periods.

Stilmant et al. (2005) reported that there was a preference for white clover in mixed sward with perennial ryegrass varieties sward, this was particularly evident where white clover composition was more variable between plots. Rook et al. (2002) reported that sheep displayed a partial preference for a diet containing 60% clover. In their study, however, the partial preference for clover led to a rapid depletion of this species relative to grass. They suggested that at the time of grazing, the clover had not fully adapted to grazing and had a low amount of lamina present, although they did not record measurement for this parameter (Rook et al., 2002). Total clover herbage mass decreased from 3051 to 1895 kg DM ha⁻¹ between the start and end of the study. Daily herbage

intake by sheep on white clover was initially maintained around 1.1 kg DM head⁻¹ d⁻¹ while for grass, intake was initially maintained at around 0.7 kg DM head⁻¹ d⁻¹, giving a sum dry matter intake per day of 1.8 kg head⁻¹ (Rook et al., 2002). Grazing time on white clover increased, although herbage mass declined over the period of the study. Despite a reduction in white clover herbage mass the animals were actually attempting to maintain their dietary preference, despite having to graze longer on account of reduced intake rate of clover as sward height and the inability to maintain total intake decreased (Rook et al., 2002). In a study of selection and ingestive behavior of fallow deer and sheep grazing on adjacent monocultures of white clover and tall fescue (*Festuca arundinacea* cv. Manade; F), Piasentier et al. (2007) reported that deer grazed a higher proportion of clover than sheep (53% vs. 37%) and on average, both ruminant species spent more time grazing on clover than on fescue monocultures (257 min d⁻¹ versus 164 min d⁻¹).

Dumont et al. (2002) stated that understanding the distribution of grazing activity and its management is valuable to ensure the sustainability and productivity of heterogeneous grasslands. These authors posited that controlled behavioral studies can provide insight into the cognitive abilities of herbivores and suggest new approaches to improve their grazing distribution. Rutter (2006) in a review of diet preference for grass and legumes in free-ranging domestic sheep and cattle reported that both cattle and sheep eat a mixed diet and showed partial preference of approximately 70% for clover. There was a diurnal pattern to preference, with stronger preference for clover in the morning, with the proportion of grass in the diet increasing towards the evening (Rutter, 2006).

Champion et al. (2004) indicated that in utilizing spatially separated grass-legume systems; sheep and dairy cattle achieved higher intake from grass and clover when these

are offered as separate monoculture compared with animals grazing a traditional mixed sward. The intake benefits of lower selection cost have the potential to be exploited on farm to increase intake and production (Champion, et al., 2004).

Two important components of ruminant nutrition are carbon (C) and N which are important for energy and protein synthesis. White clover has a higher proportion of N in relation to C than grass (Whitehead, 1995). Rutter (2006) suggested that balancing the C and N concentration of the diet is important, as eating a diet too rich in N will have implication for the animal's energy budget because extra energy will need to be expended by the animals to process excess N ingested, digested, and absorbed. Rutter (2006) posited that it is unlikely that a single plant species will have the perfect balance of nutrients to meet an animal's nutritional needs, and so the animals will need to select a variety of plant species in order to provide an optimum balance of nutrients. Thus, there must have existed strong evolutionary pressure for ruminants to adopt a diet selection strategy that optimizes their intake of nutrients, especially C and N, as the energetic costs associated with getting it wrong would have placed them at a competitive disadvantage with more efficient foragers (Rutter, 2006).

CHAPTER III

MATERIALS AND METHODS

Study Site

The study was conducted at the Brown Loam Branch Experiment Station at Raymond, MS during the winter-spring grazing season of 2007-08 and 2008-09. Soils at the experimental pastures are predominantly Loring silt loam (fine-silty, mixed, thermic Typic Fragiudalfs).

Treatments

There were four forage system treatments established with the cool season forage species annual ryegrass (*Lolium multiflorum* Lam. cv. Marshall) and the legume white clover (*Trifolium repens* L. cv Durana) as; (1) monoculture grass (MG), (2) monoculture legume (ML), (3) a binary mixture of grass and legume (MIX), and (4) spatially separated adjacent monoculture of grass (SSG) and legume (SSL) within the same paddock (SS). Two levels of stocking rate (SR; 3 or 6 steers ha⁻¹) were imposed on each of the four forage systems to give a 4 × 2 factorial arrangement of treatments. There were two replications of each treatment combination in a completely randomized design experiment.

Animal and Pasture Management

Paddock size was 0.34 or 0.67 ha. In the first year of the study, pastures were established in the early fall of 2007 and grazing commenced 14 Dec. 2007 and ended 4 June 2008 when forage allowance on pastures did not continue to support acceptable animal performance. In the second year of the study, pastures were established in early fall of 2008 but due to poor forage growth, grazing did not commence until 30 Jan. 2009 and ended 17 June 2009. At establishment all pastures were fertilized with P and K based on pre-plant soil tests. In addition, 60 kg N ha⁻¹ was applied in split applications 3 wk after the emergence of ryegrass, at the initiation of grazing, and again in March of each season to monoculture grass stands only (MG and SSG) for a total of 180 kg N ha⁻¹ applied annually. No fertilizer N was applied to monoculture or mixed legume stands. In this study, a total of 32 Angus crossbred yearling beef steers in 2008 (initial body weight [BW] of 236 kg) and Angus crossbred heifers in 2009 (initial BW of 245 kg) were used. Two steers or heifers, grouped by body weight and temperament, were randomly assigned to each of the 16 pastures. Animals had access to a continuous supply of fresh in each paddock unit through a self-regulated trough and tap water supply system.

Measurements

Pastures were measured every 14 d to monitor herbage mass using a double sampling technique (Burns et al., 1989). The sward height was measured using a falling plate disk meter with 20 contacts per experimental unit except in the spatially separated monoculture where there were 20 contacts each for the grass and clover component. Thus, estimates of herbage mass were taken in each paddock, and in the case of the adjacent monocultures, on each forage component. In each paddock, the first disk meter

contact site was selected by walking a randomly selected number of steps into the pasture from the gate. Thereafter, a fixed number of steps, estimated to cover five diagonal transects (a “zigzag” pattern) in each paddock, was used to determine the rest of the contact sites so as to spatially cover the entire paddock. After taking disk measurements in each paddock, herbage from three 0.25-m² quadrats were harvested at 2.5 cm above the soil surface to represent the lowest, mean and tallest disk meter readings recorded in the paddock in order to calibrate the indirect estimates (disk reading) with direct estimates (harvested samples). The harvested herbage was dried in a forced-air oven at 60°C for 72 hours in order to determine dry matter (DM) concentration. A regression equation was developed with direct measurements (DM weight of clipped samples) and indirect estimates (disk readings). Herbage mass on pasture was estimated using the mean of the 20 disk readings per pasture. Herbage mass per period was calculated as the average of herbage mass estimates taken at Days 0, 14, 28 within each 28-d period.

Herbage accumulation, a measure of pasture growth rate, was estimated every 28 d using two 1-m² circular enclosure cages for each paddock and two each on the SSG and SSL component of the SS paddock. Cages were placed at the beginning of grazing at random sites estimated (by disk measurement) to represent the mean herbage mass of the pasture. At 28-d intervals, coinciding with animal weighing days, cages were moved to be placed at new sites representing the current average herbage mass, and disk measurements were taken from the previously enclosed area. Herbage accumulation was calculated as the change in herbage mass estimates in the caged area from when the cages were placed to when they were moved every 28 d.

Forage allowance was calculated for each pasture as average herbage mass divided by the average total animal weight on that pasture during that 28-d period (Sollenberger et al., 2005). Average herbage mass in forage allowance calculations was the sum of herbage mass at Day 0 and herbage mass at Day 28 plus herbage accumulation for that period, divided by two.

Botanical composition of MIX pastures was estimated monthly using a double sampling technique of visual estimates in a 0.25 m² quadrat calibrated with actual DM weight of the botanical components. In each MIX paddock, 30 visual estimates were taken by dropping a circular 0.25-m² quadrat the percentage clover in the mixture, after which three 0.25- m² quadrat samples representing the high, mean, and low visual estimates were harvested and hand separated to determine actual ratio of each forage component. These samples were oven dried as described above to obtain dry weight of each component, and the ratio calculated. These data were used to quantify a regression relationship between the indirect visual estimates and direct estimates. The average of the visual estimates across each paddock was inserted in the regression equation to obtain the average proportion of clover in the mixture.

Herbage within each cage was randomly hand-plucked to represent the portion of canopy that was grazed for each paddock. Samples were oven dried (55 to 60°C), ground to pass a 2-mm stainless steel screen using a Wiley Mill (Model 4; Thomas Scientific, Swedesboro, NJ), and stored in airtight sterile plastic bags at room temperature until analyzed. A micro-Kjeldahl technique was used to determine N concentration and crude protein (CP) concentration was calculated by multiplying N by 6.25. The wet chemistry method (modified ANKOM system) according to procedures of Goering and Van Soest

(1970) was used to determine acid detergent fiber (ADF) and neutral detergent fiber (NDF). A modified (ANKOM system) version of Tilley and Terry (1963) was used to determine in vitro dry matter digestibility (IVDMD) and in vitro digestibility of the NDF fraction (IVDNDF).

At the initiation of grazing and every 28 d thereafter, all animals were weighed. Weights were taken at 0800 h following a 16-h feed and water fast. Average daily gain (ADG) was calculated each 28-d period throughout the grazing season. Liveweight gain (LWG) per unit area was calculated for the entire grazing season as $ADG \times \text{number of animal grazing days on each experimental unit}$.

Statistical Analysis

Data were analyzed by fitting models using PROC MIXED in SAS. For season long averages, year was considered a fixed effect and repeated measure, but monthly period was not written as an experimental variable in the model. Analyzed this way, the annual response is calculated as the average monthly responses. To evaluate the pattern of monthly responses during the season, data were analyzed separately by year because grazing did not begin at the same time in both years, resulting in a different number of 28-d periods for each year (six in Year 1 and five in Year 2). Further, what would be considered Period 1 each year corresponds to different weather conditions and different stage of pasture maturity, so “Period 1” comparisons between years would not be valid. Similarly, comparisons of “February” between years would not be valid because February represents the third month of grazing in Year 1 but the first month of grazing in Year 2. Monthly period (for ADG, herbage mass, herbage accumulation, and forage allowance) or samplings dates (for forage nutritive value parameters) were considered as

repeated measures. Responses involving animals (i.e., ADG and forage allowance) were analyzed for the two stocking rates and the four forage systems, MG, ML, MIX, and SS (i.e., a 4×2 factorial). For forage responses, however, the monoculture components of the SS system, SSG and SSL, were treated as if they were separate treatments, thus analyzed as five forage system “components” (SS is split into SSG and SSL) at two levels of SR (i.e., a 5×2 factorial).

In 2009, there were occurrences during the last 28-d period, including moving animals back and forth to insert estrus synchronization treatment and for artificial insemination that may have affected normal experimental responses. Based on this, it was decided to not include animal performance or average herbage mass responses for the last period in any statistical analysis because data may not be valid reflection of treatment effects. Herbage accumulation and forage nutritive value data were still included, however, because those data were not affected by these occurrences and they may be useful information about annual ryegrass and white clover late end-of-season characteristics.

Regression analysis indicated that initial BW did not affect ADG, so covariate analysis was not used. Responses in this study were considered different at $P < 0.05$ unless otherwise indicated. Means separation was conducted using the PDIFF option ($P < 0.05$) in SAS.

CHAPTER IV

RESULTS

Weather

During the 2007–2008 growing season, several periods of low accumulated precipitation occurred in the months of November, December, January, March, and June compared to the 2008–2009 season and the 30-yr normal (Table 5). In the 2008–2009 growing season, precipitation was low in October, February, and May compared to 2007–2008 and the 30-yr normal average. During the 2007–2008 growing season, average precipitation was lower than the 2008–2009 and the 30-yr normal. Also during the 2008–2009 growing season, December and January mean air temperatures were lower compared to the 2007–2008 season. Average monthly air temperatures for the entire growing season were similar to long-term average (Table 5). Quantity and distribution of precipitation and air temperature have a major effect on forage production (Mouriño et al., 2003). In this study, the second year (2009) of grazing commenced in February due to restricting forage growth after seedling emergence, possibly because of cold temperatures and cloudy days in late fall to early winter.

Table 5. Monthly accumulated rainfall and mean air temperature at Brown Loam Experiment Station, Raymond, MS during September to June of 2007 to 2008 and 2008 to 2009.

Month	Accumulated rainfall			Average air temperature		
	2007-2008	2008-2009	30-yr avg.	2007-2008	2008-2009	30-yr avg.
	mm			°C		
Sep.	109.7	260.1	78.5	23.0	23.8	23.9
Oct.	65.5	39.6	87.9	18.0	17.8	17.9
Nov.	39.6	159.3	130.8	12.4	12.4	12.6
Dec.	36.8	227.3	136.9	12.3	8.9	8.6
Jan.	92.7	134.4	158.5	8.9	8.3	7.1
Feb.	175.0	95.5	121.9	9.8	9.7	9.4
Mar.	71.1	235.0	161.5	14.7	14.1	13.7
Apr.	98.8	94.0	150.9	17.4	16.9	17.4
May	103.4	16.8	122.2	21.0	22.3	21.9
June	49.8	210.1	118.6	26.8	26.6	25.6
Season						
Total	842.4	1472.1	1267.7			
Average	84.3	147.2	126.8	13.8	13.6	13.3

Herbage Mass

Analyzed across the two seasons, there was a system \times SR interaction effect ($P < 0.0001$) on average annual herbage mass. At the high SR, SSL had lesser average annual herbage mass than SSG (Table 6). At low SR, however, MG had the greatest average annual herbage mass, the legume components ML and SSL had similar average annual herbage mass and were the least, and MIX and SSG had similar average annual herbage mass, which was intermediate between MG and the monoculture legume components (Table 6). Within forage systems components, MG, MIX, and SSG had greater average annual herbage mass at low SR than at high SR, but SR did not affect average annual herbage mass of the legume plots, ML and SSL (Table 6).

Table 6. Average annual herbage mass of components of forage systems grazed using continuous stocking at two levels of stocking rate during the winter-spring season of 2008 and 2009.

System [†]	Stocking rate		P-value [‡]
	High	Low	
	——kg DM ha ⁻¹ ——		
MG	1960 ^{AB§}	3170 ^A	< 0.0001
ML	1910 ^{AB}	1980 ^C	0.7334
MIX	1920 ^{AB}	2800 ^B	< 0.0001
SSG	2250 ^A	2620 ^B	0.0491
SSL	1870 ^B	1990 ^C	0.5076

[†] MG = monoculture grass, ML = monoculture legume, MIX = a binary mixture of grass and legume, SSG = the grass component of a spatially separated grass and legume system in the same paddock, and SSL, the legume component of the spatially separated system.

[‡] P value to compare stocking rate means within forage system.

[§] Within columns, means followed by the same superscripts are not different ($P > 0.05$).

During 2008, there was a period \times system \times SR interaction effect ($P = 0.0006$) on average monthly herbage mass. In December at high SR, the two monoculture grass plots MG and SSG had greater herbage mass than ML, MIX and SSL (Table 7). At low SR, the two monoculture legume components, ML and SSL, had the least herbage mass. Stocking rate effect on herbage mass during this period was different only for MIX (Table 7). In January at high SR, herbage mass on MG was greater than SSL, while MIX and SSG were intermediate but not different than the other four forage systems. At low SR, herbage mass of MG, MIX and SSG were similar but greater than that of SSL. Within system components, herbage mass of MG, MIX and SSG were greater at low SR than at high SR, but SR did affect herbage mass of SSL. Herbage mass estimates on ML

pastures during this period were treated as missing data because weed infestation on these plots might have affected the accuracy of the disk measurements. For the rest of the duration of that study year (the next four 28-d periods), herbage mass was not different among systems components at high SR, and at low SR, MG and MIX consistently had greater herbage mass than ML, SSG and SSL. Also for the rest of the duration of the study, herbage mass of MG, and MIX was greater at low SR than at high SR and there was a trend ($P < 0.10$) for a similar response on SSG (Table 7). There was no SR effect on herbage mass of the monoculture legume components, ML and SSL.

Table 7. Average herbage mass of forage systems components grazed using continuous stocking at two levels of stocking rate for six 28-d periods during the winter-spring season of 2008.

Period	System [†]	Stocking rate		P-value [‡]
		High	Low	
		—kg DM ha ⁻¹ —		
Dec	MG	2850 ^{A§}	2710 ^B	0.374
	ML	2060 ^{BC}	1780 ^C	0.129
	MIX	2320 ^B	3080 ^A	< 0.0001
	SSG	2780 ^A	2940 ^{AB}	0.283
	SSL	1800 ^C	1770 ^C	0.814
Jan	MG	1990 ^A	2360 ^A	0.017
	ML	- [¶]	-	-
	MIX	1900 ^{AB}	2350 ^A	0.004
	SSG	1780 ^{AB}	2080 ^A	0.049
	SSL	1630 ^B	1540 ^B	0.589
Feb	MG	1660 ^A	2340 ^A	< 0.0001
	ML	1550 ^A	1580 ^B	0.833
	MIX	1700 ^A	2250 ^A	0.001
	SSG	1560 ^A	1850 ^B	0.065
	SSL	1510 ^A	1510 ^B	0.983
Mar	MG	1590 ^A	2890 ^A	< 0.0001
	ML	1840 ^A	1960 ^B	0.435
	MIX	1680 ^A	2680 ^A	< 0.0001
	SSG	1690 ^A	1960 ^B	0.079
	SSL	1670 ^A	1760 ^B	0.547
Apr	MG	1660 ^A	2340 ^A	< 0.0001
	ML	1550 ^A	1580 ^B	0.833
	MIX	1700 ^A	2250 ^A	0.001
	SSG	1560 ^A	1850 ^B	0.065
	SSL	1510 ^A	1510 ^B	0.985
May	MG	1590 ^A	2890 ^A	< 0.0001
	ML	1840 ^A	1960 ^B	0.435
	MIX	1680 ^A	2680 ^A	< 0.0001
	SSG	1690 ^A	1960 ^B	0.079
	SSL	1670 ^A	1760 ^B	0.547

[†] MG = monoculture grass, ML = monoculture legume, MIX = a binary mixture of grass and legume, SSG = the grass component of a spatially separated grass and legume system in the same paddock, and SSL, the legume component of the spatially separated system.

‡ *P* value to compare stocking rate means within forage system.

§ Within columns, means within periods followed by the same superscripts are not different ($P > 0.05$).

¶ Indicates missing data because measurements could not be taken.

In 2009, there was also a period \times system \times SR interaction effect ($P < 0.0001$) on average monthly herbage mass. Throughout that study year, at high SR monthly herbage mass of SSG was always ranked at the top and at low SR, MG was always ranked at the top, but their comparisons to the other forage systems components fluctuated (Table 8). At high SR, MG, ML, MIX and SSL had similar herbage mass but were lesser than SSG during the first two 28-d periods, February and March (Table 8). In the third 28-d period (April) herbage mass of SSG was greater than only MG and MIX, and in the fourth 28-d period (May), there were no differences among forage systems components (Table 8). At low SR, MG and SSG had greater herbage mass than ML during February. Also, MG had greater herbage mass than SSL. During March and April at low SR, MG clearly had greater herbage mass than all the others, and in May, MG, MIX, and SSG had similar herbage mass (Table 8). Further, the components with monoculture legume had the least herbage mass, different than all other systems components in April and May. In February and March, however, ML had the least but SSL was not different from MIX and SSG in February or from MIX March. Within systems, SR affected only MG herbage mass during February. In March, SR affected herbage mass in MG and MIX, but in the subsequent periods, SR affected all forage system components that included grass. Through the study year, SR had no effect on herbage mass of paddocks with monoculture legume components.

Table 8. Average herbage mass of forage systems grazed using continuous stocking at two levels of stocking rate for four 28-d periods during the winter-spring season of 2009.

Period	System [†]	Stocking rate		P-value [‡]
		High	Low	
——kg DM ha ⁻¹ ——				
Feb	MG	2030 ^{B§}	2660 ^A	0.013
	ML	1920 ^B	1940 ^C	0.919
	MIX	2100 ^B	2340 ^{ABC}	0.350
	SSG	2600 ^A	2540 ^{AB}	0.795
	SSL	1990 ^B	2110 ^{BC}	0.624
Mar	MG	1870 ^B	3750 ^A	< 0.0001
	ML	1930 ^B	2100 ^D	0.501
	MIX	1950 ^B	2730 ^{BC}	0.003
	SSG	2710 ^A	2860 ^B	0.556
	SSL	2180 ^B	2300 ^{CD}	0.624
Apr	MG	1670 ^C	5040 ^A	< 0.0001
	ML	2090 ^{ABC}	2340 ^C	0.315
	MIX	1720 ^{BC}	3430 ^B	< 0.0001
	SSG	2580 ^A	3380 ^B	0.002
	SSL	2200 ^{AB}	2500 ^C	0.229
May	MG	2000 ^A	3760 ^A	< 0.0001
	ML	2110 ^A	2310 ^B	0.404
	MIX	1950 ^A	3410 ^A	< 0.0001
	SSG	2420 ^A	3290 ^A	0.001
	SSL	2060 ^A	2340 ^B	0.263

[†] MG = monoculture grass, ML = monoculture legume, MIX = a binary mixture of grass and legume, SSG = the grass component of a spatially separated grass and legume system in the same paddock, and SSL, the legume component of the spatially separated system.

[‡] P value to compare stocking rate means within forage system.

[§] Within columns, means within periods followed by the same superscripts are not different ($P > 0.05$).

Herbage Accumulation

There was a main effect of system for average annual herbage accumulation across the two years ($P < 0.0001$). Herbage accumulation was not different between the two monoculture grass plots, MG and SSG, or the two monoculture legume plots, ML and SSL (Fig. 1). The monoculture grass components MG and SSG had greater average annual herbage accumulation than that of the monoculture legume components ML and SSL. Also, MIX had greater herbage accumulation than plots of ML and SSL but less than MG. There was a main effect of SR ($P = 0.038$) on average annual herbage accumulation across the two years, with lower herbage accumulation at high SR (21.3 kg DM ha⁻¹ d⁻¹) than at low SR (26.9 kg DM ha⁻¹ d⁻¹).

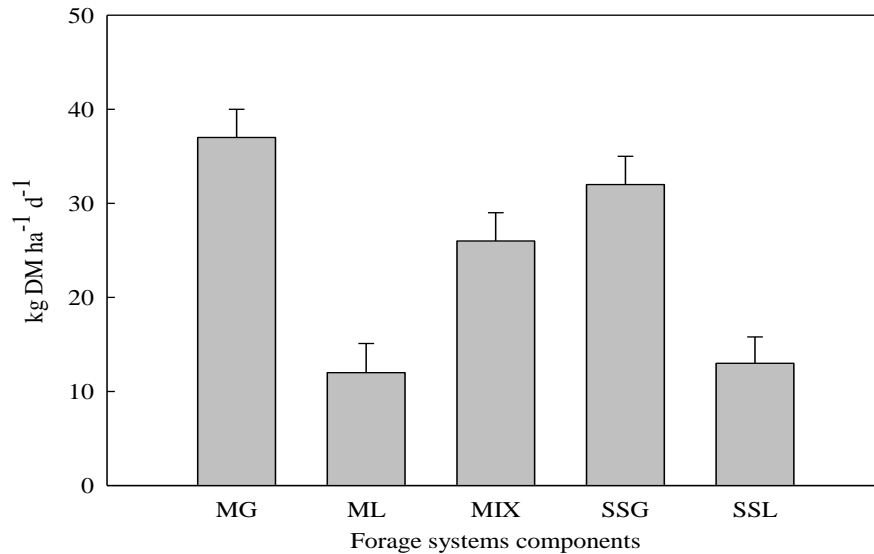


Figure 1. Average herbage accumulation of forage systems components grazed using continuous stocking at two levels of stocking rate during the winter-spring seasons of 2008 and 2009.

In 2008, there was a period \times forage system interaction effect ($P < 0.0001$) on monthly herbage accumulation (Table 9). In December, the two monoculture grass plots MG and SSG had greater herbage accumulation than plots of ML and SSL. In January, forage systems components were not different in herbage accumulation (Table 9). In February, MG had greater herbage accumulation than SSL and plots of ML, MIX, and SSG were intermediate but not different than either. In March, plots of MG and SSG had greater herbage accumulation than ML, MIX, and SSL. Herbage accumulation during April for plots of MG and SSG were greater than ML, MIX, and SSL. Also, MIX had greater herbage accumulation than ML. Herbage accumulation during May was similar among forage system components (Table 9).

Table 9. Average herbage accumulation of forage systems grazed using continuous stocking for six 28-d periods during the winter-spring season of 2008.

System [†]	Period					
	Dec	Jan	Feb	Mar	Apr	May
	kg DM ha ⁻¹ d ⁻¹					
MG	64.0 ^{Aa‡}	10.0 ^{Ac}	20.0 ^{Ac}	51.0 ^{Aa}	47.0 ^{Ab}	-
ML	7.0 ^{Bb}	-	7.0 ^{ABb}	35.0 ^{Ba}	9.0 ^{Cb}	11.0 ^{Ab}
MIX	- [§]	7.0 ^{Ab}	15.0 ^{ABab}	28.0 ^{Ba}	24.0 ^{Ba}	12.0 ^{Aab}
SSG	69.0 ^{Aa}	12.0 ^{Ab}	10.0 ^{ABb}	61.0 ^{Aa}	55.0 ^{Aa}	13.0 ^{Ab}
SSL	13.0 ^{Bab}	3.0 ^{Ab}	3.0 ^{Bb}	30.0 ^{Ba}	17.0 ^{BCab}	11.0 ^{Ab}

[†] MG = monoculture grass, ML = monoculture legume, MIX = a binary mixture of grass and legume, SSG = the grass component of a spatially separated grass and legume system in the same paddock, and SSL, the legume component of the spatially separated system.

[‡] Within columns, means followed by the same uppercase letter superscripts, and within rows, means followed by the same lowercase letter superscripts are not different ($P > 0.05$).

[§] Indicates missing data because measurements could not be taken.

During 2009, there was a period \times system \times SR interaction effect ($P < 0.0001$) on monthly herbage accumulation (Table 10). In February at high SR, SSG had greater herbage accumulation than all other forage systems components, but at low SR, herbage accumulation was not different among forage system components. Stocking rate effect on herbage accumulation during this period was only different for SSG (Table 10). In March at high SR, herbage accumulation was greatest on MG forage system component. Herbage accumulation on SSG was greater than on plots of ML, MIX, and SSL also MIX had greater herbage accumulation than the two forage system components of monoculture legumes ML and SSL. The identical trend was observed in herbage accumulation at low SR during this period (Table 10). Within forage system components, herbage accumulation on plots of MG and MIX was greater at low SR than at high SR and there was a trend ($P < 0.10$) for a similar response on SSG (Table 10). Stocking rate had no effect on herbage accumulation for the monoculture legume components ML and SSL. In April at high SR, SSL had greater herbage accumulation than MG and MIX but no other forage system components were different during this period. At low SR, herbage accumulation on MG and MIX was greater than on ML, SSG, and SSL. Also SSG had greater herbage accumulation than ML and SR effect on herbage accumulation during this period was higher at low SR than at high SR for MG, MIX, and SSG. Stocking rate had no effect on herbage accumulation for plots of ML and SSL during April (Table 10). During the last two 28-d periods of this study (May and June), herbage accumulation was not different among forage system components within SR level. Within system components, herbage accumulation was greater at high SR only

in MG and MIX but there was no SR effect in any of the other forage system components. In June, there was no SR effect on herbage accumulation within forage system components (Table 10).

Table 10. Average herbage accumulation of forage systems grazed using continuous stocking at two levels of stocking rate for five 28-d periods during the winter-spring season of 2009.

Period	System [†]	Stocking rate		P-value [‡]
		High	Low	
		— kg DM ha ⁻¹ d ⁻¹ —		
Feb	MG	20.0 ^{B§}	26.0 ^A	0.519
	ML	8.0 ^B	10.0 ^A	0.756
	MIX	17.0 ^B	12.0 ^A	0.505
	SSG	39.0 ^A	23.0 ^A	0.043
	SSL	12.0 ^B	11.0 ^A	0.887
Mar	MG	83.0 ^A	105.0 ^A	0.022
	ML	15.0 ^D	9.0 ^D	0.558
	MIX	35.0 ^C	54.0 ^C	0.028
	SSG	61.0 ^B	77.0 ^B	0.074
	SSL	11.0 ^D	7.0 ^D	0.629
Apr	MG	8.0 ^B	117.0 ^A	< 0.0001
	ML	17.0 ^{AB}	19.0 ^C	0.796
	MIX	5.0 ^B	100.0 ^A	< 0.0001
	SSG	20.0 ^{AB}	42.0 ^B	0.020
	SSL	25.0 ^A	24.0 ^{BC}	0.919
May	MG	18.0 ^A	0.7 ^A	0.029
	ML	15.0 ^A	7.0 ^A	0.345
	MIX	22.0 ^A	0.3 ^A	0.029
	SSG	18.0 ^A	2.0 ^A	0.113
	SSL	13.0 ^A	10.0 ^A	0.775
Jun	MG	9.0 ^A	0.0 ^A	0.260
	ML	8.0 ^A	0.5 ^A	0.461
	MIX	6.0 ^A	3.0 ^A	0.731
	SSG	0.4 ^A	3.0 ^A	0.760
	SSL	3.0 ^A	4.0 ^A	0.848

[†] MG = monoculture grass, ML = monoculture legume, MIX = a binary mixture of grass and legume, SSG = the grass component of a spatially separated grass and legume system in the same paddock, and SSL, the legume component of the spatially separated system.

‡ *P* value to compare stocking rate means within forage system.

§ Within columns, means followed by the same superscripts are not different ($P > 0.05$).

Forage Allowance

There was a system \times SR interaction effect ($P = 0.0015$) and a year \times SR interaction effect ($P = 0.01$) on average annual forage allowance. At high SR, forage allowance was not different among forage systems but at low SR, MG and MIX had greater forage allowance than ML and SS (Table 11). Within forage systems and years, forage allowance was greater at low SR than at high SR (Table 11).

Table 11. Average forage allowance of steers and heifers on four forage systems grazed using continuous stocking at two levels of stocking rate during the winter-spring season of 2008 and 2009.

System [†]	Stocking rate		<i>P</i> -value [‡]
	High	Low	
	—kg DM kg ⁻¹ LW—		
MG	1.2 ^{A§}	3.4 ^A	< 0.0001
ML	1.1 ^A	2.2 ^B	< 0.0001
MIX	1.2 ^A	3.1 ^A	< 0.0001
SS	1.2 ^A	2.5 ^B	< 0.0001
Year			
2008	1.1 ^A	2.5 ^B	< 0.0001
2009	1.2 ^A	3.2 ^A	< 0.0001

[†] MG = monoculture grass, ML = monoculture legume, MIX = a binary mixture of grass and legume, SS = spatially separated grass and legume system in the same paddock.

‡ *P* value to compare stocking rate means within forage system or year.

§ Within columns, means followed by the same superscripts are not different ($P > 0.05$).

During 2008, there was a system \times SR interaction effect ($P < 0.0001$) on monthly forage allowance (Fig. 2). At high SR, forage allowance was not different among forage

systems but at low SR, SS had lower forage allowance than MG and MIX. Within each forage system, there was greater forage allowance at low SR than at high SR (Fig. 2). Data from ML forage system was not used to make means comparisons because of missing data.

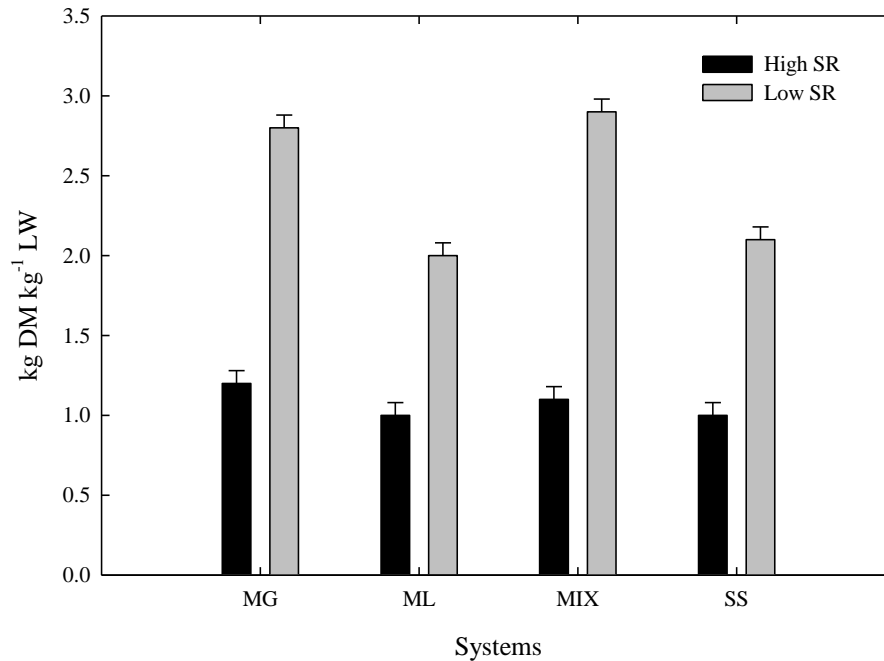


Figure 2. Average forage allowance of steers on forage systems grazed using continuous stocking at two levels of stocking rate during the winter-spring season of 2008.

During 2008, there was also a period \times SR interaction effect ($P < 0.0001$) on monthly forage allowance (Table 12). At high SR, December had the greatest forage allowance and at low SR, December had the greatest forage allowance and March had greater forage allowance than the periods of April and May. Within each period, there was greater forage allowance at low SR than at high SR (Table 12).

Table 12. Average forage allowance of steers at the six periods grazed using continuous stocking at two levels of stocking rate during the winter-spring season of 2008.

Period	Stocking rate		P-value [†]
	High	Low	
	—kg DM kg ⁻¹ LW—		
Dec	1.70 ^{A‡}	3.50 ^A	< 0.0001
Jan	- [§]	-	-
Feb	-	-	-
Mar	0.90 ^B	2.50 ^B	< 0.0001
Apr	0.80 ^B	1.90 ^C	< 0.0001
May	0.80 ^B	2.00 ^C	< 0.0001

[†] P value to compare stocking rate means within periods.

[‡] Within columns, means followed by the same superscripts are not different ($P > 0.05$).

[§] Indicates missing data because measurement could not be taken.

In 2009, there was a system \times SR interaction effect ($P < 0.0001$) (Table 13) and period \times SR interaction ($P = 0.0054$; Table 14) on monthly forage allowance. At high SR, forage allowance was not different between ML and SS and values for MG and MIX was not computed because herbage mass measurements could not be taken on those plots (Table 13). At low SR, forage allowance was different in the following order MG > MIX > SS > ML. Within system for ML and SS, forage allowance was greater at low SR than at high SR (Table 13).

Table 13. Average forage allowance of heifers on four forage systems grazed using continuous stocking at two levels of stocking rate during the winter-spring season of 2009.

System [†]	Stocking rate		P-value [‡]
	High	Low	
	—kg DM kg ⁻¹ LW—		
MG	- [§]	4.00 ^A	-
ML	1.20 ^{A¶}	2.40 ^D	< 0.0001
MIX	-	3.20 ^B	-
SS	1.30 ^A	2.90 ^C	< 0.0001

[†] MG = monoculture grass, ML = monoculture legume, MIX = a binary mixture of grass and legume, SS = spatially separated grass and legume system in the same paddock.

[‡] P value to compare stocking rate means within forage system.

[§] Indicates missing data because measurement could not be taken.

[¶] Within columns, means within stocking rates followed by the same superscripts are not different ($P > 0.05$).

At high SR, forage allowance during the period of February was greater than April and forage allowance during March was intermediate and not different than either (Table 14). At low SR, forage allowance during the period of April was greater than February and May, also during the period of March forage allowance was greater than the period of May (Table 14). Within periods, there was greater forage allowance at low SR than at high SR (Table 14).

Table 14. Average forage allowance of heifers at the four periods grazed using continuous stocking at two levels of stocking rate during the winter-spring season of 2009.

Period	Stocking rate		<i>P</i> -value [†]
	High	Low	
	—kg DM kg ⁻¹ LW—		
Feb	1.40 ^{A‡}	3.00 ^{BC}	< 0.0001
Mar	1.20 ^{AB}	3.20 ^{AB}	< 0.0001
Apr	1.00 ^B	3.50 ^A	< 0.0001
May	- [§]	2.80 ^C	-

[†] *P* value to compare stocking rate means within periods.

[‡] Within columns, means followed by the same superscripts are not different ($P > 0.05$).

[§] Indicates missing data because measurement could not be taken.

Forage Nutritive Value

Across years, there was a main effect of forage system components ($P < 0.05$) on average season-long forage chemical fractions (Table 15). Herbage in the monoculture legume components ML and SSL had greater CP and IVDNDF and lower NDF and ADF concentrations than herbage in MG, MIX and SSG (Table 15). Also, IVDMD in monoculture legume components ML, SSL, was greater than in the monoculture grass components MG and SSG, but IVDMD of MIX was intermediate but not different from either of those two groups.

Table 15. Average crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), in vitro dry matter digestibility (IVDMD), and in vitro digestibility of NDF (IVDNDF) concentrations of herbage in components of forage systems across grazing season and year.

System [†]	Nutritive value				
	CP	NDF	ADF	IVDMD	IVDNDF
g kg ⁻¹ DM					
MG	137.8 ^{B‡}	525.6 ^A	279.2 ^A	802.6 ^B	843.7 ^B
ML	274.0 ^A	365.3 ^B	227.0 ^B	858.3 ^A	917.0 ^A
MIX	140.2 ^B	493.6 ^A	266.0 ^A	816.1 ^{AB}	860.5 ^B
SSG	150.6 ^B	517.0 ^A	271.8 ^A	811.0 ^B	852.3 ^B
SSL	266.3 ^A	370.9 ^B	224.7 ^B	861.7 ^A	917.0 ^A

[†] MG = monoculture grass, ML = monoculture legume, MIX = a binary mixture of grass and legume, SSG = the grass component of a spatially separated grass and legume system in the same paddock, and SSL = the legume component of the spatially separated system.

[‡] Within column, means followed by the same superscripts are not different ($P > 0.05$).

In 2008 (Table 16) and 2009 (Table 17), there was a period × forage system interaction effect on forage chemical fractions ($P < 0.05$). Within sampling dates (note that the January 2008 sample was missed, however, the February 2008 sample is of herbage from the cage enclosures that were placed on 18 January, so represents the 28-d growth period) (Table 16), herbage in the monoculture legume components ML and SSL had higher or similar CP, lower NDF and lower or similar ADF concentrations than herbage in MG, MIX, and SSG. Throughout most of the grazing season, IVDMD among forage systems was generally not different but at the last sampling date (May), IVDMD was higher for herbage in the legume monoculture ML and SSL than for MG, MIX, and SSG. Among all forage systems, IVDNDF was not different during December, February,

and April (Table 16). In March, herbage in components of forage systems ML and SSL had greater IVDNDF than herbage in MG and MIX (Table 16). In May and June, IVDNDF for herbage in ML and SSL was greater than herbage in MG, MIX, and SSG (Table 16).

Across sampling dates in 2008, CP in monoculture legume plots ML and SSL remained constant throughout the duration of the study. High CP in February for ML seems to be an aberration. On the other hand, CP in forage system components that included grass remained constant from December to April but decreased in May and June. This decrease was more pronounced in the monoculture grass plots compared to MIX. Within all forage system components in 2008, NDF and ADF generally remained constant from December to March or April, but increased in May to June. Somewhat similarly in terms of trend in forage nutritive value characteristics, IVDMD and IVDNDF remained constant within all forage system components from December to April but decreased in May and June. Compared to monoculture legume components, the magnitude of decrease was larger in forage system components that included grass.

In 2009, there was also a period \times forage system interaction effect on forage chemical fractions ($P < 0.05$). Within sampling dates, similar to the pattern in 2008 (Table 17), the monoculture legume components ML and SSL had higher or similar CP, lower NDF and lower or similar ADF concentrations than MG, MIX, and SSG. At the first sampling date coinciding with the initiation of grazing, ML and SSL had lower IVDMD than herbage in MG and MIX (Table 17). Also, IVDMD of SSG was intermediate and not different from any other forage system components (Table 17). For the late February and April sampling, IVDMD of herbage among all forage system

components was generally similar within sampling date, but March, May, and June, IVDMD was generally greater in the monoculture legumes components than those that included grass, especially at the last two sampling times (Table 17). On the other hand, IVDNDF was not different among forage system components at the first two sampling times, but thereafter, monoculture legume components had increasingly greater IVDNDF compared to monoculture grass components, while the MIX treatment was generally intermediate between those two groups (Table 17).

Across sampling times during 2009 season, CP of system components that included grass peaked after the first 28 d of the grazing then steadily declined as the season progressed (Table 17). On monoculture legume plots, however, CP remained constant through the season then decreased in June. Also, both NDF and ADF tended to increase as the season progressed but the magnitude of increase was more marked for the components that included grass compared with legume monocultures (Table 17). On the other hand, IVDMD and IVDNDF responses were opposite in terms of numerical value but similar in terms of nutritive value, that is, the values decreased as the season progressed, with the magnitude of decreased being more marked in the system components with grass compared to legume monocultures.

Table 16. Mean crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), in vitro dry matter digestibility (IVDMD), and IVD of NDF (IVDNDF) concentrations of herbage in forage system components grazed at two levels of stocking rate using continuous stocking during the winter-spring season 2008.

System [†]	Sampling date					
	19 Dec	14 Feb	10 Mar	10 Apr	6 May	3 Jun
	CP g kg ⁻¹					
MG	181.9 ^{Dab‡}	164.7 ^{Cb}	160.7 ^{Bb}	217.2 ^{Ba}	126.0 ^{Bb}	79.2 ^{Bc}
ML	249.6 ^{ABbc}	330.9 ^{Aa}	237.0 ^{Ac}	255.4 ^{ABbc}	279.6 ^{Ab}	259.3 ^{Abc}
MIX	189.1 ^{BCa}	179.6 ^{Cab}	157.0 ^{Bbc}	150.7 ^{Cb}	135.1 ^{Bc}	77.9 ^{Bd}
SSG	195.6 ^{Cab}	162.1 ^{Cb}	174.1 ^{Bb}	222.1 ^{Ba}	106.6 ^{Bc}	97.6 ^{Bc}
SSL	261.7 ^{Aa}	265.4 ^{Ba}	250.0 ^{Aa}	288.1 ^{Aa}	267.8 ^{Aa}	249.8 ^{Aa}
	NDF g kg ⁻¹					
MG	445.5 ^{Ad}	480.7 ^{ABd}	460.3 ^{Ad}	533.9 ^{Ac}	581.9 ^{Ab}	682.3 ^{Aa}
ML	325.2 ^{Bcd}	357.2 ^{Cbc}	298.1 ^{Cd}	405.2 ^{Ba}	386.7 ^{Bab}	422.3 ^{Ca}
MIX	448.9 ^{Ac}	448.8 ^{Bc}	436.3 ^{Ac}	530.5 ^{Ab}	545.5 ^{Ab}	680.5 ^{Aa}
SSG	462.3 ^{Ad}	517.5 ^{Ac}	418.4 ^{Ad}	542.9 ^{Abc}	575.8 ^{Ab}	628.4 ^{Ba}
SSL	337.5 ^{Bc}	349.9 ^{Cc}	356.9 ^{Bbc}	390.6 ^{Bab}	395.0 ^{Bab}	418.2 ^{Ca}
	ADF g kg ⁻¹					
MG	235.5 ^{Ad}	240.0 ^{ABd}	223.6 ^{Ad}	282.4 ^{Ac}	316.7 ^{Ab}	376.7 ^{Aa}
ML	215.8 ^{Abc}	208.8 ^{Bbc}	184.6 ^{Bc}	262.6 ^{Aa}	237.2 ^{Bab}	249.8 ^{Ca}
MIX	235.2 ^{Ac}	226.5 ^{Bc}	225.4 ^{Ac}	278.2 ^{Ab}	295.2 ^{Ab}	377.3 ^{Aa}
SSG	245.7 ^{Ac}	259.0 ^{Abc}	206.3 ^{ABd}	282.0 ^{Ab}	308.7 ^{Aab}	326.5 ^{Ba}
SSL	223.4 ^{Ab}	213.3 ^{Bb}	198.0 ^{ABb}	259.7 ^{Aa}	249.8 ^{Ba}	258.4 ^{Ca}
	IVDMD g kg ⁻¹					
MG	897.3 ^{ABa}	864.7 ^{ABa}	865.0 ^{Aa}	876.3 ^{Aa}	766.6 ^{Ab}	582.5 ^{Bc}
ML	837.8 ^{Bb}	906.9 ^{Aa}	914.8 ^{Aa}	865.6 ^{Aab}	806.5 ^{Ab}	829.0 ^{Ab}
MIX	899.7 ^{ABa}	893.4 ^{ABa}	869.3 ^{Aa}	858.4 ^{Aa}	785.4 ^{Ab}	574.3 ^{Bc}
SSG	906.9 ^{Aa}	842.4 ^{Bbc}	892.1 ^{Aab}	892.6 ^{Aab}	809.2 ^{Ac}	612.9 ^{Bd}
SSL	853.0 ^{ABab}	854.0 ^{ABab}	907.9 ^{Aa}	860.8 ^{Aab}	821.6 ^{Ab}	828.1 ^{Ab}
	IVDNDF g kg ⁻¹					
MG	937.4 ^{Aa}	908.5 ^{Aa}	910.0 ^{Ba}	907.0 ^{Aa}	805.4 ^{Bb}	639.5 ^{Bc}
ML	903.1 ^{Ac}	949.4 ^{Aab}	963.9 ^{Aa}	915.9 ^{Abc}	892.8 ^{Ac}	881.0 ^{Ac}
MIX	938.9 ^{Aa}	931.7 ^{Aab}	912.2 ^{Bab}	893.1 ^{Ab}	831.6 ^{Bc}	632.6 ^{Bd}
SSG	938.6 ^{Aa}	886.7 ^{Bb}	928.0 ^{ABa}	919.8 ^{Aab}	844.7 ^{Bc}	667.0 ^{Bd}
SSL	922.7 ^{Aab}	914.1 ^{ABbc}	956.6 ^{Aa}	910.7 ^{Abc}	892.8 ^{Abc}	876.0 ^{Ac}

† MG = monoculture grass, ML = monoculture legume, MIX = a binary mixture of grass and legume, SSG = the grass component of a spatially separated grass and legume system in the same paddock, and SSL = the legume component of spatially separated system.

‡ Within column, means followed by the same uppercase letter superscripts, and within rows, means followed by the same lowercase letter superscripts are not different ($P > 0.05$).

Table 17. Average crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), in vitro dry matter digestibility (IVDMD), and IVD of NDF (IVDNDF) concentrations of herbage in forage system components grazed at two levels of stocking rate using continuous stocking during the winter-spring season 2009.

System [†]	Sampling date					
	30 Jan	27 Feb	25 Mar	23 Apr	21 May	17 Jun
	CP g kg ⁻¹					
MG	127.8 ^{Bab‡}	170.7 ^{Ba}	140.3 ^{Bab}	131.7 ^{Bab}	82.2 ^{Bb}	72.1 ^{Bb}
ML	301.0 ^{Aa}	264.3 ^{Aa}	302.1 ^{Aa}	323.9 ^{Aa}	307.3 ^{Aa}	188.3 ^{Ab}
MIX	123.6 ^{Bb}	229.8 ^{ABa}	94.4 ^{Bb}	140.7 ^{Bb}	97.1 ^{Bb}	102.2 ^{Bb}
SSG	138.0 ^{Bbc}	271.7 ^{Aa}	150.0 ^{Bb}	129.0 ^{Bbc}	92.6 ^{Bbc}	78.6 ^{Bc}
SSL	282.0 ^{Aab}	218.7 ^{ABbc}	307.2 ^{Aa}	320.2 ^{Aa}	298.3 ^{Aa}	185.6 ^{Ac}
	NDF g kg ⁻¹					
MG	372.4 ^{ABc}	424.8 ^{Ac}	517.1 ^{Ab}	481.7 ^{Ab}	638.7 ^{Aa}	687.9 ^{Aa}
ML	333.1 ^{Bc}	398.3 ^{ABab}	330.6 ^{Cc}	335.8 ^{Bc}	350.2 ^{Bb}	440.6 ^{Ca}
MIX	370.2 ^{ABc}	363.6 ^{BCc}	431.6 ^{Bb}	460.0 ^{Ab}	609.0 ^{Aa}	598.1 ^{Ba}
SSG	415.8 ^{Ac^d}	377.2 ^{BC^d}	503.9 ^{Ab}	466.8 ^{Abc}	636.9 ^{Aa}	658.3 ^{Aa}
SSL	334.4 ^{Bb}	340.4 ^{Cb}	351.3 ^{Cb}	384.0 ^{Bab}	371.7 ^{Bab}	420.3 ^{Ca}
	ADF g kg ⁻¹					
MG	182.1 ^{Af}	212.8 ^{Ae}	286.8 ^{Ac}	251.9 ^{Ad}	353.9 ^{Ab}	390.0 ^{Aa}
ML	188.1 ^{Ac}	207.7 ^{Abc}	220.2 ^{Bb}	217.6 ^{Bb}	223.5 ^{Bb}	308.3 ^{Ba}
MIX	179.4 ^{Ac}	192.1 ^{ABc}	228.5 ^{Bb}	238.7 ^{ABb}	345.1 ^{Aa}	370.5 ^{Aa}
SSG	195.0 ^{Ad}	197.0 ^{AB^d}	283.8 ^{Ab}	243.1 ^{ABc}	349.4 ^{Aa}	364.8 ^{Aa}
SSL	186.7 ^{Ac^d}	171.7 ^{B^d}	212.7 ^{Bbc}	222.1 ^{Bb}	220.5 ^{Bb}	285.6 ^{Ba}
	IVDMD g kg ⁻¹					
MG	940.9 ^{Aa}	917.5 ^{Aa}	854.8 ^{Bb}	876.0 ^{Ab}	681.1 ^{Bc}	508.6 ^{C^d}
ML	901.1 ^{Bab}	910.9 ^{Aa}	881.4 ^{ABbc}	891.5 ^{Aabc}	865.9 ^{Ac}	687.5 ^{A^d}
MIX	940.3 ^{Aa}	915.6 ^{Aab}	907.7 ^{Abc}	879.7 ^{Ac}	699.1 ^{B^d}	570.8 ^{B^e}
SSG	919.0 ^{ABa}	902.2 ^{Aab}	873.6 ^{Bc}	882.7 ^{Abc}	675.3 ^{B^d}	523.5 ^{C^e}
SSL	906.4 ^{Ba}	918.9 ^{Aa}	906.1 ^{Aa}	902.9 ^{Aa}	866.4 ^{Ab}	714.4 ^{Ac}
	IVDNDF g kg ⁻¹					
MG	966.5 ^{Aa}	956.4 ^{Aa}	890.4 ^{Cb}	908.6 ^{Cb}	734.8 ^{Bc}	559.6 ^{C^d}
ML	940.4 ^{Aab}	955.1 ^{Aa}	936.1 ^{Aab}	944.0 ^{ABab}	922.2 ^{Ab}	790.0 ^{Ac}
MIX	964.7 ^{Aa}	954.6 ^{Aab}	935.2 ^{ABbc}	915.3 ^{BCc}	749.4 ^{B^d}	666.3 ^{B^e}
SSG	946.2 ^{Aa}	955.1 ^{Aa}	906.4 ^{BCb}	916.4 ^{BCb}	732.0 ^{Bc}	587.1 ^{C^d}
SSL	954.8 ^{Aa}	960.1 ^{Aa}	946.4 ^{Aab}	947.8 ^{Aa}	918.6 ^{Ab}	799.3 ^{Ac}

† MG = monoculture grass, ML = monoculture legume, MIX = a binary mixture of grass and legume, SSG = the grass component of a spatially separated grass and legume system in the same paddock, and SSL = the legume component of spatially separated system.

‡ Within column, means followed by the same uppercase letter superscripts, and within rows, means followed by the same lowercase letter superscripts are not different ($P > 0.05$).

Average Daily Gain

Analyzed across years, there was a main effect of system ($P = 0.04$) on ADG of steers and heifers (Fig. 3). Animals grazing SS had greater ADG than ML but neither was different from MG or MIX. Also, there was a main effect of SR on ADG ($P = 0.01$). At low SR, ADG (1.09 kg d^{-1}) was greater than at high SR (0.97 kg d^{-1}) (Fig. 3).

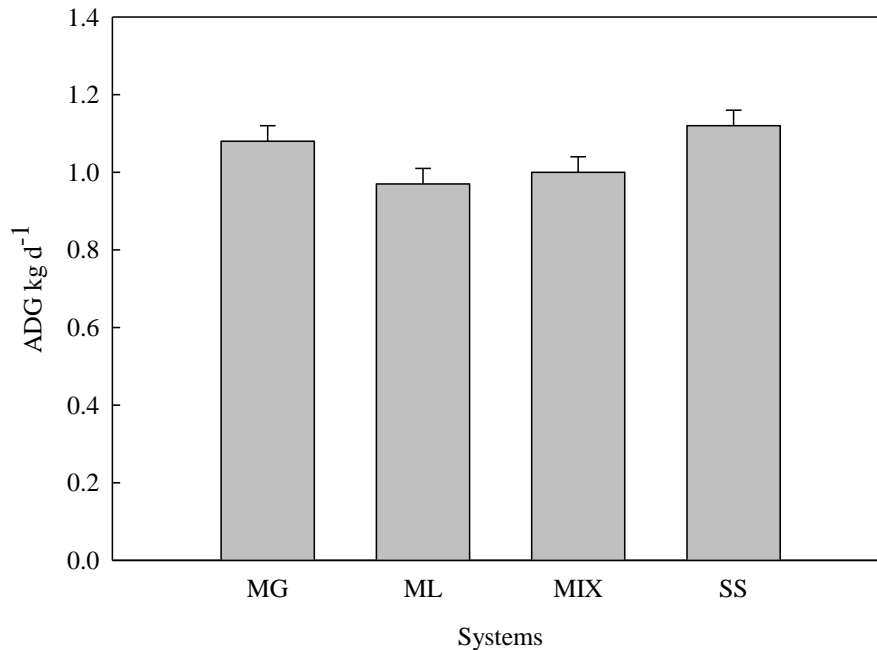


Figure 3. Average daily gain of steers and heifers grazed on four forage systems at two levels of stocking rate using continuous stocking during the winter-spring season of 2008 and 2009.

In 2008, there was a trend for a period \times system \times SR interaction effect ($P = 0.06$) on ADG, however, there was a period \times SR interaction effect ($P = 0.0405$). For the first 28 d of grazing, ADG was lower on ML than SS or MIX at the high SR, but ADG in MG was not different than any forage system. Animals had to be moved because forage was limited in the MG system, affecting gain data during the next two 28-d periods (January and February), so ADG means could not be calculated. There were no differences in ADG among the other forage systems. In March, ADG was similar among forage systems at high SR but at low SR, ML had lower ADG compared to MG and MIX, while SS was intermediate but not different from any other forage system. In April, MIX had lower ADG than MG and SS at the high SR, but ADG of ML was not different than any of the forage systems. At low SR, there was no difference in ADG among systems. In May, there was no difference in ADG among forage system at the high SR, but at low SR, ADG of SS and ML was greater than that of MG, and ADG of MIX was lower than ML but not different than SS or MG. There were no SR effects within forage systems during December, January, or April. In February and March, animals on MG and MIX had greater ADG at low SR than at high SR, but during the period of May, animals on high SR had greater ADG than animals on low SR (Table 18).

Table 18. Average daily gain of steers grazing four forage systems at two levels of stocking rate for six 28-d periods using continuous stocking during the winter-spring season of 2008.

Period	System [†]	Stocking rate		P-value [‡]
		High	Low	
		—————kg d ⁻¹ —————		
Dec	MG	0.37 ^{AB§}	0.65 ^A	0.2484
	ML	0.12 ^B	0.10 ^B	0.9539
	MIX	0.66 ^A	0.49 ^{AB}	0.4789
	SS	0.61 ^A	0.36 ^{AB}	0.2986
Jan	MG	0.77 ^A	0.78 ^A	0.9728
	ML	- [¶]	-	-
	MIX	0.67 ^A	0.86 ^A	0.3853
	SS	0.69 ^A	0.81 ^A	0.6092
Feb	MG	0.51 ^A	1.20 ^A	0.0043
	ML	-	-	-
	MIX	0.46 ^A	1.15 ^A	0.0043
	SS	0.79 ^A	0.82 ^A	0.8875
Mar	MG	0.60 ^A	1.20 ^A	0.0112
	ML	0.93 ^A	0.67 ^B	0.2641
	MIX	0.71 ^A	1.18 ^A	0.0499
	SS	0.78 ^A	1.10 ^{AB}	0.2123
Apr	MG	1.72 ^A	1.54 ^A	0.4536
	ML	1.49 ^{AB}	1.49 ^A	0.9728
	MIX	1.12 ^B	1.55 ^A	0.0725
	SS	1.65 ^A	1.65 ^A	1.0000
May	MG	0.99 ^A	0.51 ^C	0.0441
	ML	0.89 ^A	1.22 ^A	0.1741
	MIX	0.79 ^A	0.70 ^{BC}	0.7077
	SS	1.10 ^A	1.04 ^{AB}	0.9185

[†] MG = monoculture grass, ML = monoculture legume, MIX = a binary mixture of grass and legume, SS = spatially separated grass and legume system in the same paddock.

‡ P value to compare stocking rate means within forage system.

§ Within each period in the same column, means followed by the same superscripts are not different ($P > 0.05$).

¶ Indicates missing data because measurements could not be taken.

During 2009, there were main effect of system ($P = 0.012$), stocking rate ($P = 0.0038$) and period ($P < 0.0001$) on ADG (Fig. 4). Generally, animals on MG and SS had greater ADG than animals on ML, but ADG of animals on MIX was intermediate but not different from any other forage system and ADG at low SR (1.23 kg d^{-1}), was greater than ADG at high SR (1.16 kg d^{-1}). On average, ADG increased after the first 28 d of grazing, remained level during March and April periods, and then declined somewhat in May (Fig. 4).

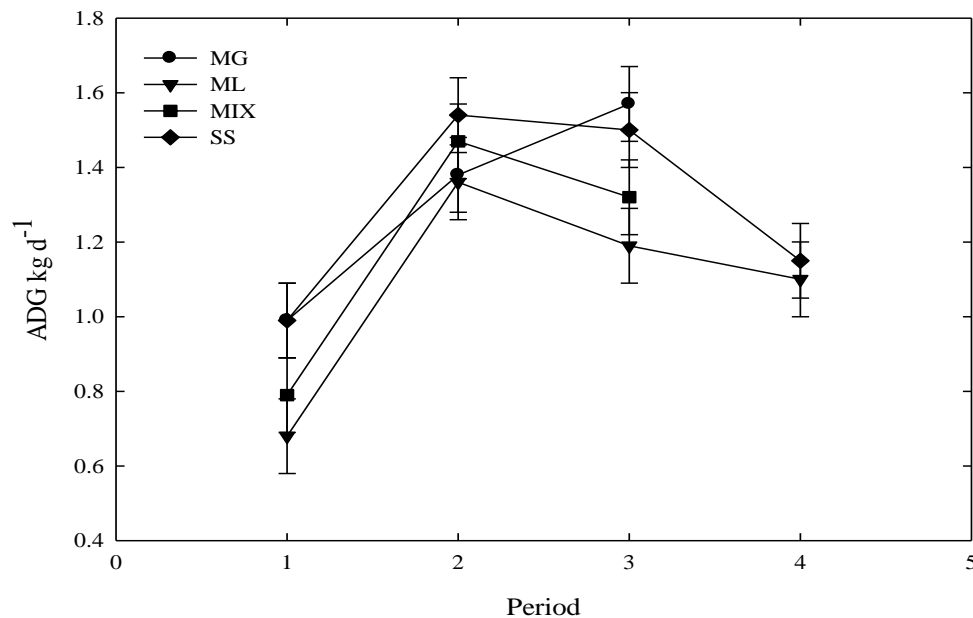


Figure 4. Average daily gain of heifers grazed on forage systems for four 28-d periods using continuous stocking during the winter-spring season of 2009. Period 1 = Feb, 2 = Mar, 3 = Apr, 4 = May

Across years, there was a system \times SR interaction effect ($P = 0.017$) on LWG (Table 19). Within each forage system, LWG was greater at high SR than at low SR (Table 19) but there was different separation of forage system means within SR level, which partially explains the interaction. At high SR, LWG on SS was greater than on MG, ML, and MIX. At low SR, LWG on SS, MG, and MIX was similar and all were greater LWG than ML (Table 19).

Table 19. Average liveweight gain of steers and heifers grazed using continuous stocking on forage systems at two levels of stocking rate during the winter-spring season across years.

System [†]	Stocking rate		P-value [‡]
	High	Low	
	———LW kg ha ⁻¹ ———		
MG	713 ^{B§}	467 ^A	< 0.0001
ML	620 ^B	340 ^B	< 0.0001
Mix	631 ^B	452 ^A	0.0020
SS	888 ^A	454 ^A	< 0.0001

[†] MG = monoculture grass, ML = monoculture legume, MIX = a binary mixture of grass and legume, SS = spatially separated grass and legume system in the same paddock.

[‡] P value to compare stocking rate means within forage system.

[§] Within columns, means followed by the same superscripts are not different ($P > 0.05$).

CHAPTER V

DISCUSSION

Annual ryegrass is one of the most widely used cool season annual in pastures in the southeastern USA (Evers and Nelson, 2000) and its compatibility with clovers in mixtures, particularly white clover, is reported to be poor due to its aggressiveness under favorable conditions (Annicchiarico and Berardo, 1993). However, growing this grass as an adjacent monoculture in the same paddock has been suggested as a viable alternative, which has prompted several studies of their interactions between system components (Kennedy et al., 2007).

The first objective of this study was to quantify pasture productivity among forage systems under continuous stocking as influenced by two levels of stocking rate. The results showed that across years, average herbage mass was similar among forage systems components at high SR, but at low SR herbage mass was greater on MG compared to MIX and SSG. Also, at low SR all plots that contained grass (MG, MIX, and SSG) had greater herbage mass than monoculture legume plots (ML and SSL). Analyzed separately by year and including the 28-d period effect in the model (Tables 7 and 8), results showed that when there were differences in herbage mass, MG and SSG were typically greater than MIX. One possible explanation for this response may be because no fertilizer was applied on MIX pastures throughout the study. This difference

was not consistent throughout the study, however, as MIX had similar and sometimes even greater herbage mass than MG and SSG during some of the 28-d periods. Elgersma et al. (2000) reported that mixtures of perennial ryegrass and white clover receiving no N fertilizer had similar DM yield per annum as ryegrass receiving N fertilizer applied at 140 kg N ha⁻¹ yr⁻¹, similar to the general pattern observed in this study. In contrast, Filho et al. (2005) reported that, herbage mass in mixtures of perennial ryegrass-white clover was on average 37% less per ha than on pure ryegrass pastures in their grazing study. Williams et al. (2003a) reported that no differences in herbage mass were seen between white clover, perennial ryegrass and the mixture of the two under grazing, which was contrasting and similar to results obtained in this study. The differences that occurred in herbage mass between plots that contained grass compared to those that contained legume alone could be attributed to the differences in growth rate of these forage species. Annichiarico and Berardo (1993) suggested that there was greater aggressiveness of ryegrass compared to white clover. Gooding and Frame (1997) and Yu et al. (2008) indicated that greater tiller density of annual ryegrass compared to white clover stolon density may be partially responsible for the aggressive growth of grass. In this study, stocking rate affected herbage mass on forage systems components that included grass, but not monoculture legume. One possible reason for this response could be the explanation of Chapman et al. (2007) that chemical satiety in the case of pure clover meal could be a function of the rate of release of ammonia from the soluble or rapidly degradable protein fraction and subsequent uptake in the blood, thus leading to limited intake on clover. Hopkins (2000) suggested that herbage mass of pasture are influenced by several factors, namely forage species, fertilizer management, grazing intensity and

climatic factors e.g. temperature, rainfall among others, all of which may have had a role in the fluctuating response of herbage mass in this study.

Herbage accumulation is a key agronomic factor for pasture productivity and is influenced by animal grazing intensity (stocking rate) and fertilizer application. The average herbage accumulation (Fig. 1 and Tables 9 and 10) across the two years was greater on monoculture ryegrass components (MG and SSG) $35.0 \text{ kg DM ha}^{-1} \text{ d}^{-1}$ compared to monoculture legume (ML and SSL) $12.0 \text{ kg DM ha}^{-1} \text{ d}^{-1}$ and MIX being intermediate ($26.0 \text{ kg DM ha}^{-1} \text{ d}^{-1}$). Callow et al. (2001) reported in their study herbage accumulation rates for Italian ryegrass (*Lolium multiflorum* Lam.) ranged from 128 to $145 \text{ kg DM ha}^{-1} \text{ d}^{-1}$ which was higher than herbage accumulation rates recorded in this study. Sheldrick et al. (1993) reported herbage accumulation rates for white clover ranged from 10 to $80 \text{ kg DM ha}^{-1} \text{ d}^{-1}$ and in this study, white clover herbage accumulation was within this range. Améndola et al. (1997) in a study of grass-legume mixtures under grazing reported that herbage accumulation for white clover-ryegrass pastures was $60 \text{ kg DM ha}^{-1} \text{ d}^{-1}$ which was generally greater on average to herbage accumulation in this study on all forage systems components.

The proportion of clover in MIX pastures was evaluated only during 2009 winter-spring season and there was a main effect of stocking rate ($P = 0.01$). The percent clover at high SR (22.55 ± 2.93) was greater than low SR (13.39 ± 2.93) throughout the 2009 grazing season. The reason for this difference was due perhaps to greater grazing intensity on high SR resulting in a more open canopy thus allowing greater clover growth in combination with ryegrass. Yu et al. (2008) reported that the proportion of clover in the biomass of ryegrass-clover mixed pastures showed a grazing intensity gradation. In

their study, clover proportion never exceeded 20% in a range of four grazing intensities, with the lowest clover proportion in the low grazing intensity paddock and the highest clover proportion in the very high grazing intensity paddock. Clover proportion reported in other studies was 16.5% in mixture with Italian ryegrass (Annicchiarico and Berardo, 1993) and under rotational grazing mean proportion of clover in the mixture was 30% (Schils et al., 1999). Rook et al. (2002) reported mean proportion of clover herbage mass in mixtures with perennial ryegrass fell from 44% at the start of their study to 31% at completion this was still higher than the proportion of clover in mixtures in this study, which may indicate greater compatibility with perennial than annual ryegrass.

Sollenberger et al. (2005) suggested that stocking rates can affect animal performance greatly depending on forage species, forage mass and other sward canopy characteristics, hence there is additional merit for incorporating forage allowance since it takes in to account both stocking rate and sward characteristics. In this study, forage allowance was similar at high SR but at low SR, MG and MIX had greater forage allowance than ML and SS. Also, stocking rate had a definite and consistent effect on forage allowance throughout the study, greater at low SR than at high SR across systems, years and periods (Tables 11, 12, 13, 14 and Fig. 2). Stewart et al. (2007) reported that herbage allowance decreased as management intensity increased due to decreasing herbage mass and increasing stocking rate, similar to the trend observed in this study. Sollenberger and Moore (1997) suggested that once forage allowance is maintained above 1.0 kg DM kg⁻¹ BW then animal performance should not be affected. In this study regardless of stocking rate, forage allowance was always above this recommended level on the four forage systems.

Chemical composition of herbage in this study (Table 15) showed a greater forage nutritive value for white clover in pure stand (ML and SSL) compared to plots that contained annual ryegrass (MG, SSG and MIX) across the two seasons. When data were analyzed separately by year (Tables 16 and 17) forage nutritive value variables for monoculture legume plots (ML and SSL) were mostly greater but sometimes similar to plots that contained ryegrass (MG, SSG and MIX) during some of the 28-d periods. The lack of difference in forage nutritive value variables between MIX and plots of monoculture grass (MG and SSG) may be partially accounted for by the low proportion of clover in the mixture. Frame and Newbould (1986) suggested that for forage quality improvement, white clover should not be below 30% in the mixture. This proportion was not met in the current study. Also, forage nutritive value on plots of legume (ML and SSL) was consistent throughout the study but on plots that included grass (MG, SSG, and MIX) forage nutritive value declined over the duration of the study (Tables 16 and 17). Piasentier et al. (2007) reported that the chemical composition of white clover remained substantially stable during their experiment, which is in agreement to the pattern of response observed in this study. The values of forage quality characteristics reported in this study for white clover and annual ryegrass in pure and mixed stands were similar to and sometimes greater than those reported by other researchers (Griffiths et al., 1999; Redfearn et al., 2002; Rutter et al., 2002).

Griffiths et al. (1999) reported that the quantity of pasture available is one of the most important factors affecting animal performance. In the current study, forage system effect on ADG was different (Figs. 3 and 4) only between SS and ML. There was no clear indication in this study that herbage mass or forage allowance had any effect on

ADG. Rutter et al. (2002) reported growth rates of heifers over 20 wk grazing duration were similar for grass and clover (0.97 and 0.99 ± 0.107 kg d⁻¹, respectively) in contrast to the results obtained in the current study where MG (1.08 kg d⁻¹) had greater ADG than ML (0.97 kg d⁻¹). Yarrow and Penning (2001) reported ADG of heifers grazing low, medium and high white clover mixtures with ryegrass to be 0.72 , 0.74 and 0.90 kg d⁻¹, which were lower than those obtained on MIX plots in this study. Zaragoza-Ramirez et al. (2008) reported ADG ranging from 1.0 to 1.2 kg d⁻¹ for stocking rates of 8 to 2 steers ha⁻¹ grazing annual ryegrass.

Pasture productivity in terms of liveweight gain per unit area is a function of herbage allowance, forage quality and stocking rate (Mouriño et al., 2003). In this study, both forage system and stocking rate affected LWG. Liveweight gain (Table 19) on SS paddocks (888 kg ha⁻¹) at high SR rate exceeded those on MG, ML and MIX by a range of 175 to 268 kg ha⁻¹ and at low SR animals grazing paddocks of ML produced the lowest LWG ha⁻¹. The difference in LWG on SS system at high SR could be attributed to the differences in forage quality variables; animals on this system were exposed to a diet that allowed for selection of both legume and grass compared to those on ML, and had access to consistently higher forage quality compared to those on MG and MIX (Tables 15, 16, and 17). Within systems in this study, there was greater LWG at high SR compared to low SR. This trend was due primarily to difference in area grazed and since forage allowance was always above the recommended level regardless of stocking rate. Bouton et al. (2005) reported LWG of animals grazing white clover and tall fescue (*Festuca arundinacea* Schreb.) ranged between 166 to 245 kg ha⁻¹ during a spring grazing study. Curll et al. (1985) reported that sheep grazing clover-ryegrass pastures

had greater LWG per unit area on low SR than high SR. This observation by them was in contrast to the trend observed in this study. Bouton et al. (2005) reported that when proportion of white clover in a mixture with tall fescue was between 20 to 40%, improvement in animal gains was found in their study. This was in agreement to results reported by Yarrow and Penning (2001) showing increase in animal gains when clover was 20% in the sward. In the current study, clover proportion in MIX was marginally over 20% at high SR and less at low SR. This perhaps could partially explain, in addition to similar forage quality, why there was no difference in LWG between monoculture grass plots (MG and SSG) versus MIX.

CHAPTER VI

SUMMARY AND CONCLUSIONS

This study assessed pasture productivity and performance of grazing animals on a system of spatial separation of grass and legume (50:50 ratio) within the same paddock compared to monoculture of grass or legume (MG and ML) and a binary of grass-legume mixture (MIX). Across years, herbage mass was influenced by forage system components and stocking rate. At high SR herbage mass was similar among forage system components but at low SR, MG had the greatest herbage mass. Stocking rate did not affect herbage mass within the monoculture legume forage systems (ML and SSL).

Average herbage accumulation across years was similar among forage system components of MG and SSG and these had greater herbage accumulation than ML and SSL. Also, MIX had greater herbage accumulation than ML and SSL.

Forage allowance was not different among forage systems at high SR but at low SR MG and MIX had greater forage allowance than ML and SS. Within each forage system, stocking rate affected forage allowance greater at low SR compared to high SR.

Forage nutritive value variables in this study were consistently greater for herbage in ML and SSL compared to MG, MIX, and SSG, where forage quality was lower and declined as the season advanced.

Across years, ADG was greater on SS compared to ML forage system but neither was different than MG and MIX. Across the two winter-spring seasons at high SR, SS had the greatest LWG ha⁻¹, but at low SR, SS, MG, and MIX had similar LWG ha⁻¹ and all had greater LWG than ML.

The results from this study suggest that there is potential for grazing animals utilizing a system of spatially separated monoculture grasses and legumes within the same paddock. Future work should be geared towards the manipulation of stocking rate using a put-and-take technique (variable stocking) to determine pasture carrying capacity under this system. Also the exploration of other forage species for both the grass and legume component in this same system arrangement, for cool season as well as warm season forages should be pursued.

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