# Evaluation of Nitrogen-Delivery Methods for Stocker Cattle Grazing Annual Ryegrass

by

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## Abstract

A 3-yr study was conducted to evaluate the efficacy of replacing N fertilizer with either interseeded annual legumes or supplementation with high-protein by-product feeds for stocker cattle grazing annual ryegrass. Each yr, 90 steers (initial BW, 225 ± 36 kg) were assigned to the following treatments, with or without monensin fed in a free-choice mineral supplement: 1) fertilization of annual ryegrass (*Lolium multiflorum* Lam.) with 112 kg N/ha in split application (NFERT), 2) fertilization of annual ryegrass with 56 kg N/ha at planting and interseeded crimson clover (*Trifolium incarnatum* L.; CC), 3) fertilization of annual ryegrass with 56 kg N/ha at planting and interseeded arrowleaf clover (*Trifolium vesiculosum* Savi; AC), 4) fertilization of annual ryegrass with 56 kg N/ha and supplementation of dried distillers grains plus solubles at 0.65% BW daily (DDGS), and 5) fertilization of annual ryegrass with 56 kg N/ha and supplementation with whole cottonseed at 0.65% BW daily (WCS). Grazing was initiated on December 14, 2015 (Yr 1), February 15, 2017 (Yr 2), and December 21, 2017 (Yr 3). Steers were weighed unshrunk every 28 d, and forage mass (FM) was measured concurrently using the destructive harvest/disk meter double-sampling method. Thirty 0.81-ha paddocks were stocked initially with three 'tester' steers, and stocking density was adjusted using 'put-and-take' steers based on changes in FM and steer BW to maintain a forage allowance (FA) of 1 kg DM/kg steer BW. Grazing was discontinued on May 11, 2016 in Yr 1, May 10, 2017 in Yr 2, and February 19, 2018 in Yr 3 following 140, 84, and 56 d

of grazing, respectively. Data for all steers (i.e., tester and put-and-take steers) were used to determine stocking density, forage allowance, and grazing-d/ha. Total gain/ha was calculated for each pasture as the ADG of tester steers and grazing-d/ha of both tester and put-and-take steers (Beck et al., 2011). Dependent variables evaluated included ADG, total gain/ha, stocking density, and FA, and FM. Forage nutritive and chemical compositional data included the dependent variables IVTD and concentrations of CP and DIP. Data were analyzed using PROC MIXED of SAS 9.4 (SAS Inst. Inc., Cary, NC.) for a 5 × 2 factorial design consisting of 5 N-delivery methods and 2 levels (+/-) of monensin. Dependent variables for pasture botanical composition included pasture clover percentage and pasture clover DM mass. Clover mass was calculated as total forage DM mass multiplied by clover percentage per pasture. Data were analyzed using PROC MIXED of SAS 9.4 (SAS Inst. Inc., Cary, N.C.) for a 2 × 2 factorial design consisting of two clover species and two levels (+/-) of monensin. Main effects were N-delivery method, ionophore, year, and their 2-way interactions. Because there were no significant 3-way interactions detected for any of the dependent variables evaluated, their sum of squares and associated df were apportioned to the model error term (residual) for significance testing. The PDIFF option of LSMEANS was used to separate means when protected by F-test at $\alpha = 0.10$. Forage mass (FM) was affected by yr ($P < 0.0001$) and N-delivery method ($P = 0.004$) with FM being greatest in Yr 2, intermediate in Yr 1, and least in Yr 3 as a result of differences in temperature and precipitation experienced among the 3-yr of the study. Forage mass was greatest for NFERT, DDGS, and WCS,

intermediate for CC, and least for AC. Average daily gain ($P = 0.001$), total gain/ha ($P < 0.0001$), stocking density ($P < 0.0001$), and grazing-days ($P < 0.0001$) were greater for NFERT, DDGS, and WCS than CC and AC. Average daily gain ($P < 0.0001$), total gain/ha $P < 0.0001$, stocking density ($P < 0.0001$), and grazing-days ($P < 0.0001$) were affected by yr. Forage IVTD ($P < 0.0001$), CP ($P < 0.0001$), and DIP ($P < 0.0001$) were affected by yr, and DIP was also affected by N-delivery method ($P = 0.0006$) such that NFERT, AC, DDGS, and WCS were greater than CC. Clover presence ($P = 0.001$; $P < 0.0001$) and clover DM mass ($P = 0.003$; $P < 0.0001$) were affected by year and clover species, respectively. Monensin inclusion affected clover presence ($P = 0.01$) and clover mass ($P = 0.02$). Results are interpreted to mean that supplementation with a high-protein by-product feed for cattle grazing annual ryegrass maintained or improved cattle performance characteristics, stocking densities, and grazing-days compared with fertilized annual ryegrass or annual ryegrass interseeded with annual clovers, and that yr more so than N-delivery method affected forage nutritive value.

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## Cool-Season Annual Forages Utilized Throughout the Southeastern US

### *Annual Ryegrass*

History

Annual ryegrass (*Lolium multiflorum* Lam.), also known as Italian ryegrass, is a cool-season annual bunchgrass with dark, shiny leaves with smooth edges that may grow to a height of 0.91 meters (Ball et al. 2015). Annual ryegrass is indigenous to southern Europe where it was grown in meadows (Holt, 1976) and under irrigation for cut forage (Beddows, 1953) as early as the thirteenth century. In temperate areas where annual ryegrass and perennial ryegrass (*Lolium perenne* L.) are adapted, they are the primary grasses used for forage and silage in dairy and animal production, including the temperate areas of Europe, the British Isles, New Zealand, Australia, the Americas, and Japan (Jung et al., 1996).

In the United Kingdom, Continental Europe, and Ireland, annual ryegrass and perennial ryegrass form the basis for most pasture systems and silage production. Whereas there is some interseeding of clovers and polyculture forage systems, the majority of pastures are monocultures that are top-dressed with N fertilizer (Van Wijk and Reheul, 1991). In New Zealand, ryegrass is the predominant forage utilized in pasture mixtures, with perennial ryegrass and white clover (*Trifolium repens*) composing the base for permanent pasture systems for sheep, dairy production, and cattle (Hunt and Easton, 1989). In the southeastern United States, annual ryegrass is managed as a winter annual where it is planted in the fall and grazed throughout the winter and must be replanted each year due to stand die-off. The lack of stand persistence may be attributed to high summer temperatures, high humidity, drought, disease, or nematodes (Jung et al., 1996). Wheeler and Hill (1957) reported that annual ryegrass seed production in the United States averaged 15 million kg between 1939 and 1945, and that commercial production of annual ryegrass seed was less than 25 years old at that time. This observation led Evers et al. (1997) to speculate that annual ryegrass production began to increase in the US during the 1930s.

There have been several cultivars of annual ryegrass developed by universities in the Southeast, including ‘Gulf’ and ‘Marshall’ for winter grazing (Jung et al., 1996). Marshall annual ryegrass was the result of 29 years of natural selection from ‘Common’ annual ryegrass that had been grown as a reseeding stand under grazing conditions and was developed by the Mississippi Agricultural and Forestry Experiment Station in 1980 at the North Mississippi Branch Station in Holly Springs. Marshall is a late-maturing, diploid annual ryegrass that typically matures two weeks later than Gulf annual ryegrass. This late maturation allows Marshall to produce forage longer than other diploid varieties of annual ryegrass during the spring (Arnold et al., 1981).

Management, Growth Characteristics, and Productivity

Annual ryegrass is a cool-season annual bunchgrass that lacks rhizomes and will initiate flowering when daylength reaches 11 hours (Hall, 1992). Annual ryegrass has a more upright growth habit and wider, laxer leaves that are typically rolled in the sheath compared with perennial ryegrass (Evan, 1964). Annual ryegrass has shiny dark green leaves with smooth edges and clasping auricles. When seed heads emerge, annual ryegrass can reach heights of almost a meter and can produce 4,483 to 13,450 kg/ha (Lacefield et al., 2003). A 2-year study evaluated cultivar effect on forage yield of annual ryegrass when 280 kg N/ha were applied. Across both years, forage yield ranged from 6,000 kg/ha to 13,000 kg/ha among all cultivars evaluated (Redfearn et al., 2002). These forage yield ranges were corroborated in a 3-year study in which Italian and Westerwolds annual ryegrass were fertilized with 225 kg N/ ha, and forage yields averaged 9,755 kg/ ha for Italian annual ryegrass and 11,774 kg/ ha for Westerwolds annual ryegrass (Kunelius and Narasimhalu, 1983). A sward of ryegrass, perennial or annual, is composed of a population of competing tillers. Establishment of new tillers is affected by management and environment (Hunt and Field, 1979), with tiller density increasing under frequent cutting or grazing (Hunt and Mortimer, 1982).

Annual ryegrass can be planted in either a prepared seedbed at a depth of 0.6 to 1.3 cm or overseeded into a dormant warm-season perennial pasture (Evers et al., 1997). Typically, a seeding rate of 600 seedlings/$m^2$ is necessary for a satisfactory stand of annual ryegrass (Evers and Nelson, 1994). In the Lower South, annual ryegrass can be planted from mid-September to early October on a prepared seedbed (Kee et al., 1995), and mid-October is recommended for overseeding annual ryegrass into a warm-season perennial pasture (Alison and Ashley, 1993). Sod should be cut or grazed to a height of 10 cm or shorter for an open-type sod such as ‘Coastal’ bermudagrass for sodseeded annual ryegrass (Evers and Nelson, 1994), and for dense sods such as bahiagrass and common bermudagrass, disking may be required to ensure adequate light reaches the soil surface (Evers et al., 1997). Utley et al., (1976) evaluated annual ryegrass planted in a prepared seedbed or interseeded into perennial sods including Coastal bermudagrass, Pensacola bahiagrass, or Coastcross-1 bermudagrass. Forage production from January to April was greater (5.09 MT/ha) for annual ryegrass planted in a prepared seedbed than sod-seeded annual ryegrass (2.67 MT/ha).

In the Gulf Coast, annual ryegrass production can persist from November through May, whereas in more northern regions production typically lasts from February to May (Ball et al., 2015). Most annual ryegrass production encompasses an area from central Texas through southeastern Oklahoma to the mid-Atlantic Coast. By 1992, a survey of Extension forage specialists reported an estimated 1.1 million ha of annual ryegrass grown in the US (Evers et al., 1997). This estimate has been confirmed by Ball et al. (2007), who reported more than 1 million ha of annual ryegrass is grown in the Southeast.

Nelson et al. (1992) reported that annual ryegrass seed germinates when day/night temperatures range between 15/2 and 35/22$^{o}$ C. Weihing (1963) reported a cessation of annual ryegrass growth when the average daily temperature declined below 6$^{o}$ C in the Gulf Coast region. As daily temperature rose, there was an increase in growth rate with a peak at 18$^{o}$ C. Jung et al. (1996) noted that annual ryegrass does not go into dormancy in the southern US, and thus winterhardiness is dependent upon survival of tillers during periods of rapid temperature change. As noted by Hillard et al. (1992), annual ryegrass can be grown in areas with as little as 500 mm of rainfall during the growing season but is well adapted to areas with high rainfall. Annual ryegrass can grow well on soils ranging from deep sandy conditions to poorly drained clay soils, and grows best with a soil pH greater than 5.7.

Annual ryegrass responds positively to N fertilization under favorable climatic conditions (Evers et al., 1997). As foliar N concentration increases, rate of photosynthesis increases, thus leading to an increase in growth (Hull and Mooney, 1990). When N fertilizer was applied to annual ryegrass at rates ranging from 0 to 250 kg N/ha, a linear response in kg DM/ha was reported for fertilization rates up to 150 kg N/ha in Argentina, where 0 kg N/ ha represented 37 and 34% of DM production in the 250 kg N/ ha treatment during the 2 years of the trial. Nitrogen-use efficiency of annual ryegrass declined with increasing N fertilization rate from 44.20 to 52.21 kg DM/kg N at 50 kg N/ ha to 15.02 to 17.61 kg DM/kg N at 250 kg N/ha (Marino et al., 2004).

Nutrient Characteristics and Animal Performance

Annual ryegrass is grown in much of the Southern Region of the United States due to its uniform growth distribution, sward stability under relatively wet grazing conditions, and high nutritive value (Lippke and Ellis, 1997). Vegetative growth from annual ryegrass has DM digestibility values of > 70%, and in some cases can exceed 80% during the first few weeks of the grazing season. Protein values can range from 20 to 30% (Lippke, 1986). However, digestibility declines as the plant matures due to decreased levels of soluble plant components, total sugars and proteins, and increased fiber (Parks et al., 1964). This generalization was confirmed by Lippke and Forbes (1994), who evaluated hand-plucked and total-sward samples of ryegrass-rye in south Texas for evaluation of CP, NDF, and ADF. The authors noted no consistent change in CP, NDF, or ADF concentrations through the first 3 months of the project, but once rapid spring growth initiated, CP declined as NDF and ADF increased, resulting in reduced forage quality (Lippke and Forbes, 1994). In a comparison of annual ryegrass varieties, Redfearn et al. (2002) reported CP values ranging from 255 g CP/kg DM in December to 129 g CP/kg DM in May, NDF values ranging from 371 g NDF/kg DM in December to 605 g NDF/kg DM in May, and in vitro true digestibility (IVTD) values ranging from 849 g IVTD/kg DM in December to 685 g IVTD/ kg DM in May. These data agree with reports from Parks et al. (1964) and Lippke and Forbes (1994).

A 4-year study (Bagley et al., 1988) evaluated the effect of various cool-season annual forage mixtures on grazing steer performance. Treatments consisted of: 1) rye-ryegrass-arrowleaf clover, 2) rye-ryegrass-ladino clover, 3) ryegrass-arrowleaf clover, and 4) ryegrass plus N fertilizer. Eight 2.02-ha pastures were used during each year in which 128 steer calves were allotted to the 8 pastures. Across all treatments, ADG averaged above 0.91 kg/d from November through April and declined to approximately 0.68 kg/d in May. Stocking rates did not differ among treatments, and ranged from 2.84 to 3.14 steers/ha. However, grazing-days were greater for treatments that contained rye than ryegrass-clover and N-fertilized ryegrass pastures (157 vs 149 d), and ryegrass-clover pastures had grazing-days comparable to N-fertilized ryegrass pastures (149 vs 148 d; Bagley et al., 1988).

Islam et al. (2011) compared growth performance of steers grazing either rye-annual ryegrass pastures or ‘Texoma’ tall fescue in a 4-year study. Eight 0.81-ha paddocks were stocked with two steers per paddock with put-and-take animals in order to control forage growth. Over the 5-yr study, grazing-days were greater in the annual system (448 d) than the perennial system (385). The authors noted that during years with limited rainfall, the annual systems provided more grazing-days than the perennial systems, and during years with adequate rainfall, both systems had increased grazing-days. Average daily gains were similar for the annual system and perennial system, and ranged from 0.62 kg/d to 1.33 kg/d. However, total gains varied among treatment and year, and were dependent upon grazing-days of the system during a particular year (Islam et al., 2011).

*Clovers*

There are about 250 species of true clovers belonging to the genus *Trifolium*. One-third of true clover species are perennials, and the remainder are annuals. Clovers grow in temperate regions of the world and require cool, moist climates, or will grow when cool climatic conditions prevail. There are three primary centers of clover diversity including the Eurasian center with 150 to 160 species, the American center with 60 to 65 species, and the African center (south of the Sahara) with 25 to 30 species (Taylor, 1985). The origin of the genus is not known; however, two locations have been hypothesized. The first is western North America from where the genus spread via the land bridge of the Iberian straits to Asia, and then to Africa and Europe (Zohary, 1972); and the second is the Mediterranean area due to the diversity in chromosome number and form found in this area (Taylor et al., 1980).

During the Iron Age when cattle were domesticated and forests were cleared, clovers and grasses increased in production (Glob, 1970). Red clover (*Trifolium pretense* L.) was domesticated in Central Europe in the 18$^{th}$ century due to the progress made in cattle production and availability of adapted genotypes (Kupzow, 1980). Clovers were popular due to their contribution to honey production, which was a major source of concentrated sugar (Evans, 1957). Clovers were also utilized by American Indians for vegetables, and some species were thought to possess medicinal characteristics such as cleaning the blood, soothing nerves, promoting sleep, and restoring fertility (Turner and Kuhnlien, 1982). Clover was probably spread to the USA and other areas by hayloft seed and seed mixed with hay and bedding (Carrier and Bort, 1916).

In order for biological N fixation to occur, nodulation of clover roots by *Rhizobacterium* must occur. Before the discovery of the relationship between clovers and *Rhizobacterium*, it was common practice to transfer soil from a pasture that contained a specific clover to a new field in which the clover was being established (Taylor, 1985). Early studies showed that leguminous plants had preferences for certain bacteria, and that nodulation would only occur when these bacteria were present. Certain bacteria were able to nodulate multiple leguminous species, and these were called "cross-inoculation" groups (Fred et al., 1932). Clover rhizobia are aerobic, gram-negative, nonsporulating rods that are motile due to peritrichous flagellation, and they grow well when cultured at 28 to 30$^{o}$C, but some strains can grow at 35$^{o}$C (El Essawi and Abdel Ghaffar, 1967). Briefly, nodulation occurs when *Rhizobacteria* attach to the root surface and infect the root hair. The infection thread grows to the base of the hair, where an organized mass of root tissue is formed and forms the nodule (Dart, 1974).

Crimson Clover

Crimson clover (*Trifolium incarnatum* L.) is a winter annual that was introduced to the United States in 1818 (Kephart, 1920). Crimson clover is an important annual legume for winter grazing in the South and is characterized by rapid growth in the fall and spring, allowing it to be integrated easily into cropping sequences. Some other characteristics that support the use of crimson clover in the South are: 1) the ability to grow under a wide range of climatic and soil conditions, 2) efficient N fixation, and 3) effective association with other crops (Hollowell and Knight, 1962).

Crimson clover possesses a central taproot and many fibrous roots, and soil moisture and tilth influence root development. Under favorable growth conditions, seedlings will grow rapidly and form a dense crown (Knight, 1969). Low germination temperature can accelerate plant development, shorten the vegetative phase, cause earlier flowering and maturity, and accelerate the formation of generative organs. Short daylength prolongs the vegetative stage and the extent of branching (von Gliemeroth, 1943). Whereas the leaves and stems of crimson clover resemble those of red clover, they differ due to their rounded tips and the absence of leaf marks (Knight, 1969). Stand density will alter the number of stems present, with thin stands producing a larger rosette and more stems (Knight and Hollowell, 1959).

Crimson clover was initially used as a winter cover and green-manure crop. As a green manure, it enables corn to produce at a level comparable to that from 75 to 100 kg N fertilizer/ha (Cope, 1955). Across a 2-year study in Mississippi, crimson clover fixed an average of 155 kg N/ha (Brink, 1990). Use of green manures declined after 1960; however, crimson clover remained important in many crop rotations and as a self-reseeder in peach, pecan, and other orchards (Bregger, 1951; Donnelly and Cope, 1961; Hollowell and Knight, 1962). Crimson clover is well suited for sandy and clay soils and has the ability to tolerate medium soil acidity (Hollowell and Knight, 1962). It does well on well-drained, fertile soils, but is not suited to low or wet soils subject to overflow or soils that are poorly drained (Rogers, 1947).

Planting crimson clover in a prepared seedbed in the fall will allow for the earliest growth (Donnelly and Cope, 1961). Seeding rates range from 12 to 33 kg/ha, depending on intended grazing intensity (Knight, 1967). In the Southeast, crimson clover should be established in late August or early October (Ball et al., 2015). Crimson clover can be sod-seeded into grass sods (Coats, 1957). For reseeding to be successful, close grazing or mowing of the grass in late summer is required, and heavy grass residue should be removed (Hoveland et al., 1971). Interseeding crimson clover into adapted winter-annual grasses can increase total forage yields and extend the grazing season. Including grasses also may reduce the incidence of bloat (Donnelly and Cope, 1961).

Knight (1970) evaluated crimson clover production when overseeded into a bermudagrass sod. Crimson clover was seeded at a rate of 33.6 kg/ha and received 224 kg N/ha compared with bermudagrass plots that either did not receive N fertilizer or received 224 kg N/ha. Forage yield samples were collected from the middle of subplots with a sickle-bar mower in 0.91-m strips when the clover height had reached 15 cm, and the bermudagrass was harvested at 4-week intervals. Across the 4 years of the trial, crimson clover and bermudagrass produced an average of 13 tons DM/ha compared with 9 and 2.5 tons DM/ha for the fertilized and nonfertilized bermudagrass, respectively, and crimson clover accounted for 22 % of the total forage yield (Knight, 1970).

Knight and Hollowell (1962) evaluated forage production under different defoliation intensities for ‘Dixie’ and ‘Chief’ crimson clover. Crimson clover was planted at a rate of 13.6 kg seed/ha in 1.5 × 3.6-m plots and fertilized with 227 kg 0-9-17. Forage was clipped to a 5 cm stubble height with a sickle-bar mower. Average forage yield across the 5-year trial was 4,694 kg/ha for Dixie and 4,637kg/ha for Chief (Knight and Hollowell, 1962). A 3-year study by Pederson and Ball (1991) evaluated seasonal and total forage yield of seven clover varieties at four locations in the southern half of Alabama: Fairhope, Monroeville, Marion Junction, and Tallassee. The clovers evaluated were arrowleaf clover, ball clover, berseem clover, crimson clover, red clover, subterranean clover, and white clover. Forage was harvested to a 7.62-cm stubble height at approximately 30-day intervals from a 0.76 × 6.1-m section in the center of each plot. Over the three years and four locations, production averaged 3,892 kg/ha for arrowleaf clover, 3,097 for ball clover, 4,793 kg/ha for berseem clover, 3,999 kg/ha for crimson clover, 4,367 kg/ha for red clover, 2,085 kg/ha for subterranean clover, and 3,097 kg/ha for white clover (Pedersen and Ball, 1991).

A 2-year study (Mooso et al., 1990) was conducted to evaluate stocker cattle performance from annual ryegrass and clover mixtures seeded into a bermudagrass sod. Bermudagrass was harvested for hay, and two mixture treatments were planted in early October: 1) 'Marshall' annual ryegrass-'Osceola' white clover, or 2) ryegrass-white clover-'Dixie' crimson clover at with seeding rates of 28, 3.4, and 13.5 kg/ha for ryegrass, white clover, and crimson clover, respectively. Two seeding methods were used: 1) conventional seed drill and 2) broadcasting and disking the sod. Fall-weaned calves (initial BW, 281 kg) were grouped by sex and allotted to each mixture × planting method combination at 2.47 to 3.0 hd/ha when ryegrass was 25 cm tall. Cattle were weighed every 28 d, and stocking densities were adjusted to maintain a sward height of 15 to 25 cm. Forage production was similar for both sodseeding methods across both years, averaging 7,611 kg DM/ha. Overall, sodseeding method did not affect grazing-days, and in April the mixture containing crimson clover supported more grazing-days (47 d) than the ryegrass-white clover mixture (36 d). Average daily gain was not impacted by sodseeding method (0.99 vs 0.97 kg/d for drill and broadcast, respectively) or forage mixture (0.98 vs 0.97 kg/d for ryegrass-white clover and ryegrass-white clover-crimson clover, respectively). Similarly, total beef produced (kg/ha) was not affected by sodseeding method (520 vs 511 kg/ha for drill and broadcast, respectively) or by forage mixture (494 vs 532 kg/ha for ryegrass-white clover and ryegrass-white clover-crimson clover, respectively).

Hoveland et al. (1991) conducted a 3-yr grazing study to evaluate cattle performance from: 1) tall fescue fertilized with 135 kg N/ha, 2) tall fescue and interseeded ladino white clover and red clover, 3) tall fescue and interseeded birdsfood trefoil, or 4) annual ryegrass and interseeded crimson clover with 135 kg N/ha. Eight 2.02-h paddocks were planted with the 4 treatments with 2 replicates per treatment. Steers (initial BW, 227 kg) were placed on trial in early March with an initial stocking density of 2.47 steer/ha, and available forage was maintained using put-and-take animals. Steer weights were recorded every 28 d, and forage mass was recorded every 28 d using a 0.37-$m^2$ quadrat with 10 samples per pasture. Stocking densities were greater for winter-annual pastures (average 5.29 hd/ha) than the other three treatments (4.20, 3.03, 3.31 for fescue + N, fescue + trefoil, and fescue + clover, respectively). Average daily gains (kg/d) across the 3 years of the grazing trial were 0.88 for fescue + N, 1.03 for fescue + trefoil, 1.05 for fescue + clover, and 1.17 for the annual treatment. The authors conjectured that the overall high ADG may have been due to the drought conditions experienced during the trial, which may have reduced stem development and increased forage concentration of soluble carbohydrates. Total gain across the 3 years was greater for the winter-annual treatment (575 kg/ha) than the other treatments (429, 353, and 399 kg/ha for fescue + N, fescue + trefoil, and fescue + clover, respectively). The authors stated that the improved gain per acre for the winter-annual treatment was a result of high stocking rates and high ADG (Hoveland et al., 1991).

Arrowleaf Clover

Arrowleaf clover (*Trifolium vesiculosum* Savi) is a winter-annual clover that was first released in the United States in 1963 (Miller and Wells, 1985). Arrowleaf clover is native to Europe and Asia, ranging across the east and west Mediterranean region, Greece, the Balkan Peninsula, Crimea, western Caucasus, and southern Russia. In the United States, arrowleaf clover has been grown widely in the Southeast, ranging from Texas and Oklahoma to Georgia and South Carolina, and north into Arkansas and Tennessee (Duke, 1981). Arrowleaf clover can be cut for hay or silage, grazed, or used as green manure or for seed production (Miller and Wells, 1985).

Arrowleaf clover can grow in a variety of soils including clay, silty loam, and sandy soils, but root knot nematodes may be an issue in sandy soils. Arrowleaf clover has good drought tolerance but cannot grow well in poorly drained soils. Warm temperate climates are necessary for arrowleaf clove to thrive (Duke, 1981). Arrowleaf clover should be planted between September and November (Ball et al., 2015); however, location dictates optimum planting date. In south Georgia, seeding usually occurs from October 15 to 31, whereas in central Alabama and Mississippi, seeding should occur from September 15 to 30. The difference in planting date is due to arrowleaf clover's high-temperature dormancy (Miller and Wells, 1985). It has been reported that germination will occur when temperatures are constant at 4.5 $^{o}$C for 16 hours and 21 $^{o}$ C for 8 hours (Hoveland and Elkins, 1965). Evers (1980) reported that germination was reduced by day/night temperatures of 30/20 and 35/25$^{o}$ C, and that improved germination occurred when temperatures were 15/5, 20/10, or 25/10$^{o}$ C.

Arrowleaf clover responds well when soil pH is about 6.0, but it can grow when soil pH ranges from 5.0 to 7.5 (Hoveland et al., 1969). Arrowleaf clover can be seeded as a monoculture, with companion grass species, or overseeded into a sod of bermudagrass or bahiagrass. Seeding into a firm prepared seedbed is most likely to produce a successful clover stand, and can be accomplished with broadcasting or drilling seed to a 6 to 12 mm depth followed by a cultipacker. When overseeded into a sod, arrowleaf seed may be broadcast if excess grass is removed or drilled using a drill that creates a furrow in the sod. Disking the sod can provide a better seedbed and reduce competition from the grass (Miller and Wells, 1985).

Arrowleaf clover production was evaluated when overseeded into a bermudagrass sod (Knight, 1970). Arrowleaf clover was seeded at a rate of 16.8 kg/ha and received 224 kg N/ha and compared with bermudagrass plots that either did not receive N fertilizer or received 224 kg N /ha. Forage yield samples were collected from the middle of subplots with a sickle-bar mower in 0.91-m strips when the clover growth reached 15 cm height, and the bermudagrass was harvested at 4-week intervals. Across the 4 years of the trial, arrowleaf clover and bermudagrass produced an average of just over 12 tons DM/ha compared with 9 and 2.5 tons DM/ha for the fertilized and nonfertilized bermudagrass, respectively, and arrowleaf clover accounted for 17 % of the total forage yield (Knight, 1970).

A 2-year study (Evers, 1985) evaluated arrowleaf clover and subterranean clover forage mass and N contributions when overseeded into bermudagrass and bahiagrass sods. Arrowleaf clover was broadcast-seeded at 4 kg/ha, and subterranean clover was seeded at 15 kg/ha in October of each year. Plots containing clover were fertilized with 112 kg N/ha in split applications on June 1 and August 1, and plots that did not contain clover were fertilized with either 0, 84, 168, 252, or 336 kg N/ha in split applications on 1 April, 1 June, and 1 August. Plots with clover were harvested in December and March of the first year, but not until April of the second year due to defoliation by wild geese. All plots were harvested from May through October and were harvested with a flail mower that cut an 80-cm wide strip through the center of the plot to a height of 3 cm. Nitrogen fertilizer equivalency was determined via linear regression comparing clover-grass forage yields to forage yields at varying N rates. Forage yields were not different between grass-clover mixtures and bermudagrass fertilized with 168 kg N/ha or bahiagrass fertilized with 252 kg N/ha. Grass-clover N accumulation was equal to or exceeded fertilization rates of grass at 252 kg/N ha and, when mixtures were compared with unfertilized grass, N accumulation ranged from 117 to 157 kg N/ha. Coastal bermudagrass would have required 127 or 160 kg N/ha for yields to be comparable to that of overseeded arrowleaf and subterranean clover, respectively, and Pensacola bahiagrass would have required 211 or 254 kg N/ha for yields to be comparable to that of overseeded arrowleaf and subterranean clover, respectively (Evers, 1985).

A 3-year experiment was conducted in Headland, AL that evaluated cow-calf performance from winter annuals that were sod-seeded into bermudagrass compared with fertilization of the bermudagrass sod (Hoveland et al., 1978). Treatments were: 1) not overseeded and fertilized with 112 kg N/ha, 2) overseeded with 'Gulf' ryegrass at 22 kg/ha, 3) 'Wrens Abruzzi' rye, 'Yuchi' arrowleaf clover, and 'Autauga' crimson clover seeded at 67, 9, and 11 kg/ha respectively, and 4) overseeded with arrowleaf and crimson clovers. Rye-clover plots (0.7 ha) were planted in mid-October with a grain drill, and clover-only and ryegrass treatments were broadcast-seeded in November. Non-overseeded bermudagrass was fertilized in April and July at 56 kg N/ha at each application, rye-clover plots were fertilized with 56 kg N/ha in November and January, and ryegrass plots received 56 kg N/ha in January, April, and July. One cow-calf pair was assigned to each plot, with additional pairs being added during peak forage growth periods. Calves were weaned in early October and cows remained on pasture until there was insufficient forage available or at time of seeding. Pastures that contained rye and clovers provided the earliest grazing, with initiation dates between January 2 to 14 across the 3 years, and provided 9 months of grazing. Crimson clover was available in limited amounts in late February and continued through late April. Arrowleaf clover had limited availability in early March, but much of its production occurred in April, May, and June. Pastures that contained clover were initially stocked between March 4 to 17, and crimson clover was prevalent until late April, and arrowleaf clover was available through June. Pastures overseeded with ryegrass were stocked from February 7 to March 4, and ryegrass was available until mid-May. Non-overseeded bermudagrass pastures were stocked from March 27 to April 9. Grazing was increased by about 3 months when rye and clover were overseeded compared with bermudagrass alone, with ryegrass accounting for nearly 2 months and crimson-arrowleaf clover accounting for 1 month. Calf gain/ha was increased by 91% when rye and clovers were overseeded (628 kg/ha), 44% with overseeded ryegrass (473 kg/ha), and 40% for overseeded clovers (460 kg/ha) compared with non-overseeded grass (328 kg/ha). Calf daily gain was increased by 22% when rye and clovers were overseeded (0.87 kg/d), 12% for overseeded ryegrass (0.80 kg/d), and 24% with overseeded clovers (0.89 kg/d) compared with non-overseeded grass (0.71 kg/d). Cow gain/ha was greatest with overseeded clovers (325 kg/ha), intermediate for rye-clover (269 kg/ha) and ryegrass (217 kg/ha), and least for non-overseeded grass (183 kg/ha). Cow ADG followed the same pattern and was greatest with overseeded clovers (0.62 kg/d), intermediate for rye-clover (0.41 kg/d) and ryegrass (0.37 kg/d), and least for non-overseeded grass (0.22 kg/d; Hoveland et al., 1978).

A 4-year study (Ocumpaugh, 1990) was conducted in Beeville, TX that compared yearling heifer performance from overseeded arrowleaf clover or subterranean clover in bermudagrass with N-fertilized bermudagrass. The N-fertilized bermudagrass treatment received 56 kg N/ha in February and August, and arrowleaf clover and subterranean clover were planted at 11.2 kg/ha using a no-till drill. Variable stocking densities were utilized when rainfall was adequate to obtain uniform grazing due to pastures ranging in size from 2.02 to 3.64 ha. Stocking densities were fixed in 2 of 4 years due to limited rainfall. Heifer ADG across the 4 years was 0.90 kg/d for the arrowleaf clover treatment, 0.76 kg/d for subterranean clover, and 0.73 kg/d for bermudagrass fertilized with N. Total gain (kg/ha) across the 4 years was 239 for arrowleaf clover, 198 for subterranean clover, and 174 for N-fertilized bermudagrass (Ocumpaugh, 1990).

## Stocker Cattle Production in the Southeastern United States

### *Overview and Economic Impact*

In the South, beef cattle production consists primarily of cow-calf, commercial and purebred operations (Ball et al., 2015). Hoveland (1986) noted that beef production in the Southeast was inefficient. To illustrate, they stated that with a 70% calf crop, weaning weights of 160 kg/calf, and 1.6 ha per cow-calf pair, the average cattleman was only producing 70 kg/ha of calf annually. However, by simply utilizing recommended management practices, productivity could be doubled or tripled. The greatest increase in productivity on the same land area could be achieved with beef stocker production, wherein weaned calves are stocked on high-quality, cool-season annual or perennial pastures. Stocker production is a high-risk enterprise compared with cow-calf production due to purchase and sale of calves, health care, production and management of high-quality forages, and appropriate supplementation strategies that require a high level of skill and experience (Hoveland, 1986). Traditionally, small-grain (e.g., rye and oats) pastures have been used in the South, and calf ADG of 1 kg are typical (Utley et al., 1975). Hoveland and Anthony (1977) noted that rye-ryegrass-arrowleaf clover pastures in Alabama supported 0.9 kg ADG and a grazing season of 229 d in central Alabama.

The stocker sector has been profitable in the southern United States for many years (Prevatt et al., 2011b). Sustained profitability is predicated upon the ability of stocker producers to purchase calves in the fall of the year when prices are typically less due to large supply of calves from cow-calf operators who do not want to feed and care for the calves during the winter (Rankins Jr. and Prevatt, 2013). The system that has historically provided the best opportunity for profitability is acquisition of lightweight calves in the fall and increasing BW by 100 to 200 kg for sale in the spring (Prevatt et al., 2011a). When calves were managed to add 130 kg or more of BW and sold as a uniform load, the increase in price for the lot ranged from \$0.11 to \$0.22/kg (Prevatt et al., 2011a), and the value of added gain ranged from \$0.16 to \$0.55/kg BW gain (Rankins, Jr. and Prevatt, 2013). These increases were corroborated by a report that showed from 2000 to 2011 the value of BW gain for stocker calves increased by 134% (Beck et al., 2013). In an evaluation of 37 grazing experiments that included many types of forage systems, Ball and Prevatt (2009) noted that 8 of the top 10 systems included legumes, the most economical costs of BW gain were tall fescue with clover (\$0.66/kg BW gain), and a combination of rye and ryegrass resulted in \$1.32/kg BW gain. However, none of the trials evaluated benefits of ionophores, and few utilized growth-promoting implants, both of which have been shown to increase ADG.

*Stocking Rate*

Petersen et al. (1965) noted that production per animal and per ha were dependent upon the stocking rate of the pasture. If the stocking rate was too low, then forage would not be fully utilized and production per ha would be low; but if the stocking rate was too high, then production per animal would be reduced due to insufficient forage. Furthermore, under- or overstocking may result in injury and unwanted changes in sward composition. The authors reported that there was a linear increase in gain per ha as stocking rate increased, and that gain per animal is inversely related to stocking rate once the rate at which forage is consumed surpasses the rate of growth of forage available for grazing. Ball et al. (2015) have noted that grazing animals can positively or negatively impact a pasture and the forage species present. These changes are brought about through grazing and trampling of plants by animals and the excretion of dung and urine, and the interrelationship of animals and plants in a pasture are complex. Ball et al. (2015) and Petersen et al. (1965) concur that stocking rate greatly influences animal productivity, and that optimum stocking rate of a pasture is influenced by: 1) forage production, 2) accessibility of forage to animals, 3) nutritive value of the forage, 4) species composition of pasture, and 5) seasonal variations in forage production. Allen et al. (2011) provided objectives for considering stocking methods: 1) to allocate nutrition among classes of livestock, 2) improve forage utilization, 3) reduce negative impacts to soil and plants, 4) extend the grazing season, and 5) accomplish an experimental objective.

Allen et al. (2011) observed that, of 20 examples of stocking methods evaluated, all fell into one of two categories, either continuous or rotational stocking. Ball et al. (2015) described continuous stocking wherein animals are maintained on a single pasture while grazing, which allows for selective grazing by animals unless the pasture is overstocked. Selective or preferential grazing can lead to overgrazing of certain plants and undergrazing of other plants, and if stocking rates are not adjusted to match amounts of available forage, the pasture may be over- or understocked and its utilization efficiency adversely impacted. The authors further describe rotational stocking as subdividing pasture into multiple paddocks and grazing each paddock for a set period while the nongrazed paddocks are allowed to rest and regrowth can occur. Rotational grazing allows for increased carrying capacity, potentially 20 to 30% higher, compared with continuous stocking (Ball et al., 2015).

Blaser et al. (1956) conducted a 6-year evaluation of different forage mixtures on steer performance in northern Virginia. Forage mixtures were planted in 0.81-ha pastures with 3 replications each and consisted of orchardgrass fertilized with 242 kg N/ha, tall fescue fertilized with 242 kg N/ha, orchardgrass + ladino clover, tall fescue + ladino clover, bluegrass + white clover, and orchardgrass + lespedeza + redtop. Pastures were subdivided to allow for rotational grazing, and rotations lasted between 4 and 12 days, depending on stage of forage growth. Management strategies for forage growth were: 1) orchardgrass and tall fescue pastures fertilized or interseeded with clover were grazed when forage heights reached 15.24 to 30.48 cm and was discontinued when forage heights were 5.08 cm; 2) lespedeza-orchardgrass-white clover was grazed when heights reached 12.7 to 25.4 cm and was discontinued when heights were 2.5 cm; and 3) bluegrass-white clover was grazed when heights reached 10.16 to 17.78 cm and was discontinued when heights were 1.91 cm. Grazing was initiated in the spring (early April) when adequate forage was available and was terminated in the fall (late October to early November) when forage was too short to graze. There were between six and eight 28-d weigh periods, with the first six periods of the season used for ADG evaluation. Put-and-take management was utilized with six tester animals, yearlings or 2-yr-old cattle, allotted to each of 18 paddocks, and grazer animals utilized when necessary. Steer ADG was greatest for orchardgrass-ladino clover (0.54 kg/d), orchardgrass-lespedeza-white clover (0.54 kg/d), and bluegrass-white clover (0.51 kg/d), intermediate for fertilized orchardgrass (0.49 kg/d) and tall fescue-ladino clover (0.45 kg/d), and least for fertilized tall fescue (0.40 kg/d). Steer-grazing-days/ha were greatest for fertilized tall fescue (1,015 d), intermediate for tall fescue-ladino clover (773 d), fertilized orchardgrass (766 d), bluegrass-white clover (692 d), and orchardgrass-ladino clover (637 d), and least for orchardgrass-lespedeza-white clover (504 d). Total gain was least for orchardgrass-lespedeza-white clover (300 kg/ha), and for the other five treatments ranged from 400 to 373 kg/ha.

Hafley (1996) evaluated yearling steer performance in Louisiana from 'Surrey' and 'Marshall' annual ryegrasses under continuous and rotational grazing management in a 2-year study. Ryegrass was sod-seeded in late October into a warm-season perennial grass sod in one 7.3- and three 8.1-ha pastures that were divided into 6 paddocks of equal size (1.2 or 1.34 ha). In the first year, 93 yearling steers were allotted to paddocks at stocking rates of 3 steers/ha for continuous stocking and 6 steers/ha for rotational stocking, and the trial lasted 71 days. Due to limited number of animals, only 4 of 6 paddocks were used in each pasture, and the rotational group was not replicated. Steers in the rotational stocking group grazed paddocks for 3.5 days, and paddocks were rested for 14 days. In the second year, 62 steers were allotted to pasture with 3 steers/ha for continuous stocking and 5.4 steers/ha for the rotational stocking to ensure replication of the rotational stocking treatment, and the trial lasted 84 days. Steers in the rotationally stocked group grazed paddocks for 12.6 days and paddocks were rested for 25 days. Steers were weighed every 28 days. Steer ADG were greater for the continuously grazed paddocks (1.18 kg) than rotationally grazed paddocks (0.92 kg). However, total gain was greater for the rotationally grazed (403 kg/ha) than continuously grazed paddocks (301 kg/ha).

Vendramini et al. (2006) conducted a 2-year trial evaluating the effect of level of supplementation on early-weaned calves grazing a mixture of annual ryegrass and rye in

Florida. Annual ryegrass (‘Jumbo; 20 kg/ha) and a commercial-blend rye (Grazemaster; 80 kg/ha) were overseeded on a glyphosate-treated bahiagrass sod. Three weeks after planting, pastures received 40 kg N/ha, 17 kg P/ha, and 66 kg K/ha, and additional applications of 40 kg N/ha in late December, early February, and early March of each year. Two early-weaned calves, one steer and one heifer, were assigned to each of 9 0.2-ha pastures, and put-and-take animals were used to reduce herbage allowance differences among pastures. Calves were weighed every 28 days. Pastures were subdivided for rotational grazing, and each sub-section was grazed for 7 days and allowed to rest for 21 days. Three levels of supplementation were provided daily: 10, 15, or 20 g/kg of calf BW. Supplement contained 146 g CP/kg and 700 g TDN/kg and consisted mainly of wheat middlings, soybean hulls, soybean meal, and cottonseed meal. Herbage allowance did not differ among treatments (0.62 kg DM/kg BW), but was greater in the second year (0.8 kg DM/kg BW) than the first year (0.4 kg DM/kg BW). Calf ADG and gain/ha increased linearly with increasing supplement level: 10 g/kg BW (0.74 kg/d; 950 kg/ha), 15 g/kg BW (0.81 kg/d; 1080 kg/ha), and 20 g/kg BW (0.89 kg/d; 1320 kg/ha). Stocking rate also increased as supplement level increased: 10 g/kg BW (5.5 AU/ha, 1 AU = 500 kg $BW^{0.75}$), 15 g/kg BW (5.9 AU/ha), and 20 g/kg BW (6.5 AU/ha). An economic analysis indicated that, as supplement level increased, concentrate cost and income increased: 10 g/kg BW ($600/ha; $2,100/ha), 15 g/kg BW ($970/ha; $2,370/ha), and 20 g/kg BW ($1,430/ha; $2,900/ha). However, there was no difference in gross return among treatments: 10 g/kg BW ($1,500/ha), 15 g/kg BW ($1,400/ha), and 20 g/kg BW ($1,470/ha).

*Growth Promoting Technologies*

Growth-promoting technologies (ionophores and anabolic implants) have the potential to improve animal performance during the stockering and feedlot phases of the beef production cycle (Stackhouse-Lawson et al., 2013). Growth-promoting technologies improve animal performance via different mechanisms, including altering digestive processes and the partitioning of nutrients post-absorption (NRC, 2016). Stackhouse-Lawson et al. (2013) noted an additive effect on ADG, final BW, and HCW when multiple growth-promoting technologies were administered to feedlot cattle.

<u>Ionophores</u>

Ionophores are a class of carboxylic polyether antibiotics produced by *Streptomyces* species (Westly, 1978). There are three types of ionophores approved for use in the United States, including: monensin produced by *S. cinnamonensis* (Haney and Hoehn, 1968), lasalocid produced by *S. lasaliensis* (Bergen et al., 1951), and laidlomycin produced by *S. eurodicus* (Kitame, 1974). Shumard and Callender (1968) reported that ionophores were first incorporated in poultry feed to alleviate coccidiosis. The Food and Drug Administration approved the use of ionophores for ruminant feeds in the mid-1970s (Russell and Strobel, 1989).

Ionophores are metal/proton antiporters that can complex with either monovalent or divalent cations (Russell and Houlihan, 2003). Monensin and laidlomycin bind with either $Na^+$ or $K^+$, whereas lasalocid preferentially binds $K^+$ but will also bind $Na^+$, $Ca^{++}$, or $Mg^{++}$ (Russell, 2002). Lasalocid is not an obligatory cation/$H^+$ antiporter, unlike monensin and laidlomycin, and can exchange $Ca^{++}$ for 2 $K^+$ (Haynes et al., 1980). Ionophores operate by dissipating ion gradients within the membrane of bacterial cells

(Russell, 2002). Ionophores are composed of a backbone that contains strategically spaced oxygen atoms (Pressman, 1976). The backbone is capable of conformational changes that place the oxygens in a structure resembling a ring or cavity into which the cation or proton fits. The neutral oxygens ligand with the cation through ion-dipole interactions. This conformation places the polar ionophore-cation complex in the interior of the structure with hydrocarbon groups oriented to the outside (Pressman, 1976). When binding with a cation occurs, a lipophilic, cyclic cation-ionophore complex is formed that can diffuse through the interior of the biomolecular membrane structure (Bergen and Bates, 1984). For efficient transport to occur, certain kinetic criteria must be met: 1) complexation-decomplexation reactions must be rapid in high-dielectric regions, 2) transport activation energy cannot rise excessively when the ion solvation shell is exchanged for the ionophore oxygen system, and 3) high stability can be achieved when the cation-ionophore complex enters the low-dielectric region of the membrane interior (Pressman, 1976).

Pressman (1976) notes that carboxylic ionophores are composed of oxygenated heterocyclic rings formed through head-to-tail hydrogen bonding along with a single terminal carboxyl group. All the carboxylic ionophores complex with cations only in the deprotonated anionic form. They can carry protons in the protonated carboxylic form and carry ions as electrically neutral zwitterions. The ideal cycle of cation-for-proton exchange by carboxylic ionophores begins when a protonated ionophore within a membrane diffuses to one surface and the proton is released. This traps the ionophore at the polar surface due to the increased polarity of the charged anionic form. The charged ionophore anion discovers a complexible cation and engulfs the cation and displaces the attached solvent. This leads to the creation of a zwitterionic complex in which the charged portions are located internally, allowing the zwitterion complex to break away from the polar surface and traverse the nonpolar interior (Pressman, 1976). Once the zwitterion complex reaches the other polar surface, the electrostatic forces that had stabilized it are no longer greater than the unfavorable $\Delta G$ (Gibbs free energy change) of cyclization, and the cation is released. Once this occurs, the ionophore reverts to the anionic form and, after being protonated, will traverse the membrane again and repeat the cycle (Bergen and Bates, 1984). Ion flux is determined thermodynamically by the respective chemical gradients of the cation and proton (Bergen and Bates, 1984). Additionally, equilibrium is pH-sensitive and independent of membrane potential. Carboxylic ionophores are regarded as exchange diffusion carriers (Pressman, 1976).

Ionophores are generally more toxic to Gram-positive bacteria than Gram-negative bacteria (Russell, 2002), which leads to a shift in the microbial population within the rumen. Gram-positive bacteria are more sensitive to ionophores due to the lack of an outer cell membrane. Gram-positive bacteria possess a thick peptidoglycan layer, about 30 nm wide, that lays above the cell membrane phospholipid bilayer. Lipoteichoic acid chains extend from the peptidoglycan surface and form a glycocalyx that provides protection to the cell but is not as effective a barrier as the outer membrane of Gram-negative cells (Russell, 2002). Like Gram-positive bacteria, Gram-negative bacteria possess a peptidoglycan layer that lays above the phospholipid bilayer of the cell membrane. However, the Gram-negative peptidoglycan layer is much thinner than that of the Gram-positive cell. Gram-negative cells possess an outer membrane that is composed of a phospholipid layer adjacent to the peptidoglycan layer as well as a lipopolysaccharide layer that interacts with the environment. Porins are embedded in the outer membrane and allow the passage of low-molecular-weight compounds, whereas high-molecular-weight compounds are excluded (Russell, 2002). These physiological differences between Gram-positive and Gram-negative bacteria are a major factor as to why ionophores affect Gram-positive bacteria to a greater extent than Gram-negative bacteria.

Mitchell (1961) proposed that bacteria utilize a membrane-bound ATPase or electron transfer system to transfer protons across the cell membrane. The process was referred to as chemiosmotic coupling that was caused by the driving force of a given chemical reaction channeling a chemical component or group along a pathway specified by the physical organization of the system (Mitchell, 1961). Bergen and Bates (1984) reported that there is evidence that these gradients exist in mitochondria and bacteria and can be generated from the transfer of electrons down an electron transport chain or by proton extrusion coupled with the hydrolysis of a phosphoryl bond in ATP (Bergen and Bates, 1984). An electrochemical gradient of hydrogen ions allows for the conservation of energy at the membrane level, and ATPase activity is found in association with many bacterial membranes (Harold, 1972). An $F_o$-$F_1$ ATPase is found in all cells. The system serves to extrude protons in anaerobic bacteria that rely on substrate concentration phosphorylation for ATP production. The $F_1$ portion hydrolyzes ATP to ADP + Pi, while the $F_o$ portion functions as a channel for protons to reenter the cell. Proton extrusion is linked to ATP cleavage, and ATP synthesis is coupled to the proton motive force during oxidative phosphorylation through the influx of protons through the $F_o$ channel (Wilson and Smith, 1978). Proton motive force is the electrical potential differential across the membrane and the chemical gradient derived from the difference in the pH values of the aqueous solution on either side of the membrane (Bergen and Bates, 1984).

Ionophores improve the efficiency of growing and finishing cattle through increased energy metabolism efficiency, improved nitrogen metabolism, and retardation of digestive disorders, mainly chronic lactic acidosis and bloat (Bergen and Bates, 1984). In a review of 136 comparisons of growth-promoting technologies, Bretschneider et al. (2008) reported that feeding monensin or lasalocid increased ADG by 0.075 and 0.078 kg/d. The authors further note a quadratic effect of monensin and lasalocid dose on ADG, with the greatest gains observed at 100 mg/100 kg BW for monensin and lasalocid.

Horn et al. (1981) evaluated the impact of feeding monensin on performance of stocker cattle grazing wheat pasture in Oklahoma. Heifers were assigned to 1 of 3 treatments: 1) no supplement, 2) 0.91 $kg \cdot head^{-1} \cdot day^{-1}$ of a pelleted supplement, or 3) 0.91 $kg \cdot head^{-1} \cdot day^{-1}$ of the same pelleted supplement that contained 100 mg monensin. Heifers grazed wheat pastures for 112 days in year 1 (November to March) and 113 days in year 2 (December to March). In year 1, ADG for heifers that received monensin (0.73 kg) was improved by 0.09 and 0.18 kg compared with heifers that received supplement with no monensin (0.64 kg) and unsupplemented heifers (0.55 kg), respectively. In year 2, ADG of heifers that were fed monensin (0.63 kg) was 0.07 kg greater than heifers that received supplement without monensin (0.56 kg) and unsupplemented heifers (0.55 kg).

Beck et al. (2014) reported on the impact of stacking growth-promoting technologies on performance of cattle grazing wheat, and the economic impact of the technologies. Twenty-four ha of wheat pasture were subdivided into 15 1.6-ha paddocks that were each stocked with 4 steers for fall grazing in each of 2 years, and in the spring these paddocks were further divided into 30 0.8-ha paddocks that were each stocked with 4 steers/ha). Steers were assigned to one of 3 supplementation regimens: 1) free-choice non-medicated mineral, 2) monensin-fortified (1.78 g monensin/kg) mineral, and 3) monensin-fortified (0.33 g monensin/kg) pressed protein block; and either implanted with 40 mg trenbolone acetate/8 mg estradiol/29 mg tylosin tartrate (Component TE-G) or nonimplanted. Fall grazing was initiated in early November each year and terminated in mid-February, and spring grazing was initiated in late February with a different set of steers and terminated in early May. The authors reported a lack of interaction between supplement treatment and implant, and because effects were additive in nature, they presented supplement and implant data separately. Inclusion of monensin increased ADG by 0.07 (mineral; 1.22 kg) and 0.09 (protein block; 1.24 kg) kg compared with the non-medicated free-choice mineral (1.15 kg). Similarly, total gain was increased for the medicated mineral (93 kg BW) and protein block (93 kg BW) compared with the non-medicated free-choice mineral (86 kg BW). Implanting improved ADG by 0.14 kg, and total gain by 10 kg over non-implanted steers. The combination of monensin supplementation and implantation decreased cost of gain by 26%.

Growth-Promoting Implants

Growth-promoting implants in current use are typically composed of estrogens, androgens, or synthetic forms of these hormones (Preston, 1999). They are implanted in the middle third of the ear of cattle and sheep, and the hormone is contained within a matrix of either lactose, cholesterol, or large polymer of polyethylene glycol (Brandt, 1997). Early uses of hormones to enhance animal production were iodinated proteins fed to dairy cattle to improve milk production and estrogen implants to increase fat deposition in growing chickens (NRC, 1966). The first use of hormonal implants in beef cattle and sheep was in 1954, when DES (diethylstilbestrol) was used for lean meat promotion and enhanced growth (Raun, 1997).

The NRC (2016) states that the mode of action on the enhancement of rate of protein accretion is not fully understood. However, a number of theories include: increased synthesis and secretion of endogenous GH, increased androgen receptors in cells, and decreased muscle protein turnover (Preston, 1999). The earliest explanation was that implants cause increased synthesis and release of endogenous GH based upon observations of increased concentrations of circulating GH and insulin (Preston, 1975), increased number of acidophilic cells in the anterior pituitary (Clegg and Cole, 1954), and increased size of the anterior pituitary (Preston, 1975). Sheep treated with TBA (trenbolone acetate) were reported to have androgen receptors in the cytosol of skeletal muscle (Sinnett-Smith et al., 1987). Thomson et al. (1996) reported that steers implanted with hormones had enhanced protein synthesis without reduced protein degradation, which indicated that growth factors in the serum indirectly affected muscle protein metabolism. Frey et al. (1995) noted that IGF-1 and IGF binding protein-3 were increased in the serum of implanted steers, and that satellite cells from implanted steers had increased responsiveness to growth factors (IGF-1 and fibroblast growth factor).

Paisley et al. (1997) evaluated effect of implant type on performance of stocker steers grazing dormant tallgrass prairie in Oklahoma. Three hundred steers were assigned to 1 of 4 treatments: 1) no implant, 2) implanted with Synovex-C, 3) implanted with Synovex-S, or 4) implanted with Revalor-G. Steers were placed on native tallgrass prairie pastures in October and provided 1.4 kg/d of a 25% CP supplement, fed 3 times/week, that provided 100 mg monensin·steer$^{-1}$·day$^{-1}$. Steers were weighed in January (midpoint) and March (final). Steer ADG (0.32, 0.32, and 0.35 kg) and total gain (52, 53, and 57 kg/steer) were greater for steers implanted with Synovex-C, Synovex-S, and Revalor-G, respectively, than non-implanted steers (0.28 kg; 46 kg/steer). Among the implants, Revalor-G had improved ADG and total gain compared with Synovex-C, but not Synovex-S.

Coffey et al. (2001) reported on the impact of implanting steers grazing tall fescue with low or high levels of endophyte infection in Arkansas. Steers were assigned to one of two implantation regimens, no implant or implanted with Revalor-G, and placed on pasture in April with either low- or high-endophyte infection levels. Stocking rates were 4.5 to 4.9 steers/ha due to variations in pasture size, and grazing lasted for 64 days. Steer ADG was greater for steers grazing low-endophyte tall fescue (0.78 kg) than steers that grazed high-endophyte fescue (0.54 kg), and had greater BW gain (50 vs 34 kg). Implantation with Revalor-G also improved ADG (0.70 vs 0.61 kg) and BW gain (45 vs 39 kg) compared with nonimplanted steers.

McMurphy et al. (2011) conducted a 2-year experiment on the effect of implant type and protein supplement source on stocker cattle grazing Old World bluestem (OWB) and native tallgrass range pastures in Oklahoma. Twelve OWB pastures were stocked at a rate of 1.56 steers/ha, and 3 tallgrass pastures were stocked at 0.45 steers/ha. In May and June of yr 1 and yr 2, respectively, 196 steers were evenly distributed among the OWB and tallgrass pastures and assigned to one of three implantation treatments: 1) no implant, 2) Ralgro, and 3) Component TE-G, such that all implant treatments were represented uniformly across pasture type, and pastures were grazed for 126 days. In late July, pastures were assigned to 1 of 3 supplementation regimens: 1) no supplement, 2) cottonseed meal-based supplement, and 3) DDGS-based supplement; supplements were designed to provide 125 mg monensin·steer$^{-1}$·day$^{-1}$ and were fed 3 times/week at a rate of 0.95 kg/steer. The authors reported no implant × supplement interaction and concluded that the gain improvements from implants and supplements were additive in nature. Supplementation improved steer ADG over unsupplemented steers, and DDGS supplementation had greater ADG than CSM supplementation (0.98, 0.93, 0.81 kg for DDGS, CSM, no supplement, respectively). Similarly, total BW gain was improved for supplemented steers over unsupplemented steers, and for steers supplemented with DDGS compared with those supplemented with CSM (78, 74, 64 kg BW for DDGS, CSM, and no supplement, respectively). Economic impact was evaluated for the different supplementation and implantation strategies. Value of gain was $1.36/kg of BW gain, cost of gain for feed was $0.76 for CSM and $0.48 for DDGS, and cost of gain for implants were $0.22 for Ralgro and $0.12 for Component TE-G.

## Supplementation of Grazing Cattle

Kunkle et al. (2000) noted that cattle supplementation is implemented for a variety of reasons, including greater economic returns, correcting a nutrient deficiency, conservation of forage, improved forage utilization, improved animal performance, and(or) cattle behavior management. A challenge faced by nutritionists and cattle producers is to supply nutrients in a cost-effective manner that meets the needs of the animals and producer’s management goals. Nutrient requirements for beef cattle vary based upon body weight, level of production, genetic makeup, and environmental conditions. Supplementation of grazing cattle is challenged further due to selective consumption of available plants and consumption of different plant parts that differ in nutrient composition. A supplementation strategy that is typically employed is provision of protein, vitamins, and minerals to rectify any forage deficiencies and, if cost-effective, provide supplemental energy (Kunkle et al., 2000). Poppi and McLennan (1995) note that the supply and delivery of amino acids (AA) and energy-yielding substrates to body tissues are the primary determinants of live weight gain within the genetic limits of the animal. Dietary protein content determines AA supply to the animal, and the extent of AA supply to the tissue is dependent upon the passage of AA, from undegraded plant protein and microbial crude protein (MCP), though the intestinal wall. For protein accretion, a supply of AA along with non-protein energy-yielding substrates are necessary (Poppi and McLennan, 1995).

*Energy Supplementation*

Forage-fed cattle typically have increased performance when energy supplementation is balanced with other nutrients, and cattle consuming lower-quality diets usually show greater improvement compared with higher-quality diets (Kunkle et al., 2000). Grazing cattle can be fed up to 0.5% BW of a concentrate without greatly reducing forage intake (Horn and McCollum, 1987). However, Bowman and Sanson (1996) reported that there was minimal effect on forage intake when cattle were fed grain-based supplements below .25% BW, but intake was negatively affected when supplement was fed above .25% BW. Moore et al. (1999) reported that associative effects of supplementation had been observed for grazing cattle, in agreement with NRC (2016) that associative effects impact intake and digestibility of forage, and that the effect may be greater or less than that of forage alone due to interactions between forage and concentrate.

Chase and Hibberd (1987) evaluated utilization of low-quality hay by cows supplemented with corn in Oklahoma. Twelve cows were block by BW into three groups, and a fourth group consisted of 4 ruminally cannulated heifers. The groups were placed in four 4 × 4 Latin squares with 14-day experimental periods, and treatments consisted of a cottonseed meal-based supplement (control) and 3 levels of corn (1, 2, and 3 kg/d), where corn replaced cottonseed meal to achieve a uniform 256 g/d of supplemental protein intake. The authors noted the hay contained 4.2% CP, 52.6% ADF, and 11.6% lignin. As the level of corn increased (0, 1, 2, 3 kg/d) a cubic effect was reported for hay OM digestibility (36.5, 35.1, 23.6, 18.9%; respectively). Hay intake decreased linearly (8,762, 8,177, 6,402, and 5,065 g/d) as corn level increased, but total tract digestible DM had a cubic response to increasing corn levels (3,531, 3,910, 3,308, 3,393 g/d). Substitution rates for corn were 0.83 g hay/g supplement at 1 kg corn, 2.68 g hay/g supplement at 2 kg corn, and 1.97 g hay/g supplement at 3 kg corn. Ruminal ammonia-N (mg/dl) declined as corn levels increased (2.20, 1.12, 0.88, 0.61; respectively), whereas total ruminal VFA concentration (mM) was not affected by corn level. The authors noted that ruminal ammonia appeared to limit forage utilization, and that ruminal degradable protein may need to be included.

Horn et al. (1995) conducted a 3-yr experiment to determine the impact on performance of high-starch and high-fiber energy supplements on stocker calves grazing wheat pasture in Oklahoma. Stocker cattle were assigned to either a control (mineral supplement only), high-starch supplement (corn-based), or high-fiber (soyhulls- and wheat middlings-based) supplement, the latter of which were fed at about 0.75% BW daily for 6 d/wk. In 2 of the 3 years, cattle were sent to a feedlot and fed a common finishing diet. Across the 3 years, supplementation improved ADG by 0.15 kg/d (0.92, 1.06, 1.08 for control, starch, and fiber, respectively). In the first year that cattle were sent to the feedlot, control calves were lighter (309 kg) than starch- (329 kg) and fiber- (328 kg) supplemented cattle, but control calves had greater ADG (1.72 kg/d) than starch- (1.62 kg/d) and fiber- (1.64 kg/d) supplemented cattle. Final BW was also greater for control calves (540 kg) than starch- (524 kg) and fiber- (525 kg) supplemented cattle. In the second year that calves were sent to the feedlot, control calves were lighter (381 kg) than starch- (391 kg) and fiber- (392 kg) supplemented cattle, but final weights were not different (563, 554, 564 kg for control, starch, and fiber, respectively). Average daily gains were also not different among treatments (1.52, 1.49, 1.54 kg/d for control, starch, and fiber, respectively).

Weissend (2015) conducted a 2-year experiment to compare type and amount of energy supplement on performance of stocker cattle grazing annual ryegrass in Alabama. Stocker cattle were assigned to 1 of 3 supplement types (cracked corn, soybean hulls, and citrus pulp) and 3 supplementation levels (0.25, 0.5, and 0.75% BW), or a negative control (no supplement). Cattle were stocked at 4.94 hd/ha. There was no supplement type × level interaction for either ADG or total gain/ha. Average daily gain was greatest for corn (1.17 kg/d) and soybean hulls (1.11 kg/d), intermediate for citrus pulp (1.08 kg/d), and least for the unsupplemented control (0.91 kg/d). Total gain/ha in the first year was greater for corn (630 kg/ha), citrus pulp (579 kg/ha), and soybean hulls (597 kg/ha) than the control (460 kg/ha), and in the second-year corn was the greatest (608 kg/ha), soybean hulls were intermediate and not different from corn (576 kg/ha), citrus pulp was not different from soybean hulls (554 kg/ha), and the control was not different from citrus pulp (506 kg/ha). Average daily gain by supplement level was greater for 0.25% BW (1.11 kg/d), 0.5% BW (1.11 kg/d), and 0.75% BW (1.14 kg/d) than the unsupplemented control (0.91 kg/d). Similarly, in the first-year total gain/ha was greater for 0.25% BW (593 kg/ha), 0.5% BW (584 kg/ha), and 0.75 % BW (628 kg/ha) than control (460 kg/ha), but in the second-year 0.25% BW (579 kg/ha) and 0.5% BW (588 kg/ha) were greatest, 0.75% BW (572 kg/ha) was intermediate, and control (506 kg/ha) was least.

*Protein Supplementation*

The NRC (2016) utilizes the metabolizable protein (MP) system, which separates protein requirements into microbial and host animal needs, and partitions protein into two classifications: ruminally degradable protein (RDP) and ruminally undegradable protein (RUP). The RDP supplies peptides, AA, and nonprotein nitrogen (NPN) for microbial metabolism and microbial crude protein (MCP) synthesis. Klopfenstein (1996) notes that RUP is supplied by forages or supplemental feeds. There are three sources of MP supply to the duodenum: MCP, RUP, and endogenous protein, but the endogenous protein does not contribute to the net supply of MP (NRC, 2016). Klopfenstein (1996) states that microbial protein requirements for growth should be met before a response to escape protein is detected. Microbial protein should be adequate to meet the needs of cattle at or near maintenance; however, growing cattle or lactating cows may require RUP in addition to MCP to meet MP requirements (Klopfenstein, 1996).

Hafley et al. (1993) evaluated the effect of feeding heifers supplements with various amounts of degradable protein while grazing warm-season grass in Nebraska. Six mixed-stand pastures (4.05 ha each) consisting of big bluestem, switchgrass, and some indiangrass were each subdivided into 3 paddocks which the cattle rotated through, and cattle grazed 2 of the three paddocks (15 animals/paddock) at any one time at a stocking rate of 5.6 heifers/ha. Heifers were assigned to five treatments: 1) negative control (no supplement), 2) energy control, 3) energy control plus escape protein (0.18 $kg \cdot animal^{-1} \cdot day^{-1}$ of CP), 4) energy control plus rumen-degradable protein (0.18 $kg \cdot animal^{-1} \cdot day^{-1}$ of CP equivalents), and 5) combination treatment (0.36 $kg \cdot animal^{-1} \cdot day^{-1}$ of CP equivalents). Escape protein consisted of non-enzymatically browned soybean meal and feather meal, degradable protein consisted of corn steep liquor and urea, and the combination treatment was an additive mixture of the escape and degradable protein sources. All treatments were fed individually at a rate of 775 g DM/d. Grazing lasted 75 days (June to August), and cattle were rotated every two weeks between paddocks within pasture. Average daily gain was greatest for the combined protein treatment (1.08 kg/d) compared with the negative control (0.95 kg/d), energy control (0.94 kg/d), and escape protein (0.97 kg/d), but the rumen-degradable protein treatment was intermediate and did not differ from the other treatments (1.03 kg/d). The authors concluded from these data that cattle gains are enhanced by supplementation with rumen-degradable protein when grazing warm-season grass.

Anderson et al. (1988) conducted an experiment comparing level of escape protein supplement for steers grazing smooth bromegrass in Nebraska from April to July (trial 1) and August to November (trial 2). Steers were assigned to the following treatments: 1) no supplement, 2) 0.11 kg·animal$^{-1}$·d$^{1}$ of CP, 3) 0.23 kg·animal$^{-1}$·d$^{-1}$ of CP, or 4) 0.34 kg·animal$^{-1}$·d$^{-1}$ of CP as a supplement consisting of corn starch, bloodmeal, corn gluten feed, salt, and molasses; in the second trial, soyhulls were added for improved palatability. All steers received 582 g supplement DM/d with protein replacing corn starch in equal amounts that were fed individually daily using Calan gates. Steers grazed smooth bromegrass pasture at a stocking density of 4.1 steers/ha. Steer ADG was improved with protein supplementation (0.94, 1.06, and 1.01 kg/d; 0.111 0.23, 0.34 kg supplement/d, respectively) over the control (0.91 kg/d) in the first trial, and in the second trial all three protein supplement levels improved performance equally. The authors note that these data support the theory that even though actively growing cool-season grasses have high crude protein content, MP may still be deficient in ruminants.

Vendramini and Arthington (2008) compared supplementation strategies for early-weaned calves in Florida. In one experiment, calves grazed annual ryegrass from January to April (112 d) and then grazed stargrass from April to August (112 d). Supplement was fed at 1% BW as either soybean hulls or 80:20 soybean hulls and cottonseed meal, and supplement rate was adjusted every 28 d. In the second trial, calves were allotted to either annual ryegrass or bahiagrass and grazed from January to April. Steers grazing annual ryegrass received 1% BW of the soybean hulls and cottonseed meal used in the first experiment, and steers grazing bahiagrass received 2% BW of the same supplement. In the first experiment, there was no difference in ADG between soybean hulls (0.80 kg/d) and hulls plus cottonseed meal (0.76 kg/d) when calves grazed annual ryegrass. However, when calves grazed stargrass, ADG was greater for calves fed soybean hulls and cottonseed meal (0.67 kg/d) than those fed soybean hulls (0.58 kg/d).

In the second experiment, calves that grazed annual ryegrass and received 1% BW supplement had greater ADG (0.97 kg/d) than calves that grazed bahiagrass and received 2% BW supplement (0.76 kg/d).

Proteinaceous Corn By-Products

There are many protein by-product feeds that are derived from corn, but with the increased use of corn for industrial uses, especially ethanol production (Davis, 2001), distillers grains have become a readily available feed that is high in protein. Distillers grains are produced during dry-milling of corn grain for alcohol production. Stock et al. (1999) provide a detailed account of the dry-milling process. Briefly, grain is ground, and the starch is fermented by yeast to produce alcohol and the mash is processed to remove the alcohol. After the alcohol is removed, the remaining slurry is called whole stillage. The whole stillage is strained and pressed to remove the course particles, which may be sold as wet distillers grains (WDG) or dry distillers grains (DDG). The remaining liquid fraction is termed thin stillage and contains fine grain particles and yeast, and may contain up to 40% of the total residual DM. The thin stillage is evaporated to produce a syrup-like product called condensed distillers solubles (CDS) that contains 20-35% DM. The CDS can be added to WDG or DDG to produce wet distillers grains plus solubles (WDGS) or dried distillers grains plus solubles (DDGS). The NRC (2016) reports that DDGS contains on a DM basis: 10.73 % fat, 33.66 % NDF, 16.17 % ADF, and 30.79 % CP.

Stock et al. (1999) note that starch represents two-thirds of corn grain DM. After fermentation, one-third of the original DM remains and, because the fermentative process only removes starch, all other nutrients are more concentrated. For example, Stock et al.

(1999) state that corn grain contains about 9% CP, and this increases to 27% CP (DM basis) in whole stillage. Similarly, fat concentration is greater in distillers grains because the oil remains after fermentation (Stock et al., 1999). The NRC (2016) reports that distillers grains plus solubles (DGS) contain approximately 31.5% CP, 10.5% fat, 6% starch, and 43.5% NDF (DM basis). Furthermore, DGS contains roughly 40% RDP and 60% RUP (NRC, 2016). Nutrient characteristics of DGS has been reported to be impacted by the facility where the grains were produced (Buckner et al., 2011) and by the amount of CDS added to DGS (Corrigan et al., 2009).

Griffin et al. (2012) examined the effect of level of DDGS supplementation on performance of steers grazing subirrigated Sandhills meadow in the summer in Nebraska. Steers were assigned to treatments consisting of: 1) unsupplemented, 2) low-level supplementation (0.6% BW), or 3) high-level supplementation (1.2% BW), and supplement amounts were not adjusted for BW gain during the study. Steers grazed subirrigated meadow for 91 d (May through August), and were individually fed supplement 6 d/wk. At termination of grazing, steers were shipped to a feedlot for 154 d, penned by treatment, and fed a common finishing diet. Average daily gain increased linearly with increasing supplement amount (0.89, 1.03, 1.19 kg/d), and final BW increased linearly with increasing level of supplement (361, 376, 387 kg for control, 0.6% BW, and 1.2% BW, respectively) at the end of the grazing period. At termination of the feedlot phase, final BW had increased linearly with increasing supplementation level (646, 664, 691 kg for control, 0.6 % BW, and 1.2% BW, respectively). However, ADG during finishing was not influenced by previous supplementation regimen (1.85, 1.87, 1.97 kg/d for control, 0.6 % BW, and 1.2% BW, respectively).

Jenkins et al. (2009) evaluated the impact of level of DDG supplementation on steer performance when grazing native pasture, and subsequent performance when grazing wheat pasture in Texas. Native pasture consisted primarily of buffalograss, blue grama, sideoats grama, and western wheatgrass. Steers were assigned to treatments consisting of 1) unsupplemented (0.0% BW), 2) 0.25% BW, 3) 0.5% BW and 4) 0.75% BW, and supplement level was based upon initial BW but not adjusted for BW gain during the 56-d grazing period (October to December). The weekly amount of supplement was prorated and fed 3 d/wk. After termination of grazing native pasture, steers were transferred to wheat pasture and grazed for 76 d. Steer ADG increased linearly as supplement level increased when grazing native pasture (0.27, 0.48, 0.64, 0.78 kg/d for 0.0, 0.25, 0.5, 0.75% BW, respectively). During the wheat pasture phase, unsupplemented steers had 0.11 to 0.18 kg greater ADG than supplemented steers (0.90, 0.73, 0.79, 0.74 kg/d for 0.0, 0.25, 0.5, 0.75% BW, respectively). Final BW was the same for unsupplemented steers (284 kg) and steers supplemented at 0.25% BW (284 kg), but less than steers supplemented at 0.5% BW (297 kg) and steers supplemented at 0.75% BW (298 kg). Supplement efficiency (kg BW added per kg of supplement consumed relative to unsupplemented control) for the entire system, native pasture and wheat grazing, compared with the unsupplemented control was 0.017 for 0.25% BW supplement treatment, 0.218 for the 0.5% BW supplement treatment, and 0.171 for the 0.75% BW supplement treatment. Incremental system supplement efficiency was 0.418 when going from 0.25 to 0.5% BW and 0.077 when going from 0.5 to 0.75% BW.

Greenquist et al. (2009) compared performance of steers fed DDGS while grazing smooth bromegrass with those that grazed fertilized smooth bromegrass in Nebraska.

Steers were allotted to treatments consisting of 1) smooth bromegrass fertilized with 90 kg N/ha and stocked initially at 9.2 animal unit month (AUM)/ha, 2) unfertilized smooth bromegrass initially stocked at 6.4 AUM/ha, and 3) unfertilized smooth bromegrass stocked at the same rate as the fertilized treatment with 2.3 kg of DDGS supplemented daily. Steers grazed the bromegrass pastures for 160 d (April through September). Final BW was greater for the supplemented steers (477 kg) than the control (440 kg) and the steers that grazed fertilized bromegrass (437 kg). Similarly, ADG was greater for DDGS-supplemented steers (0.92 kg/d) than control (0.68 kg/d) and steers grazing fertilized bromegrass (0.67 kg/d). Total gain (kg/ha) was greatest for DDGS-supplemented steers (404), intermediate for steers grazing fertilized bromegrass (302), and least for control steers (197), which this was due to the reduced stocking rate of the control treatment.

In a continuation of the study reported by Greenquist et al. (2009), Watson et al. (2012) conducted an economic evaluation on the impact of N fertilization and DDGS supplementation for stocker cattle grazing smooth bromegrass in Nebraska. Variables that were included for total cost analysis were: initial steer price plus interest, yardage, health and processing fees, death loss, cash rent plus interest, and fertilizer and DDGS costs for those treatments. Total cost ($/steer) was greatest for DDGS-supplemented steers (971.69), and did not differ between control (953.97) and steers that grazed fertilized bromegrass (951.14). However, total revenue ($/steer) and net return ($/steer) were greater for DDGS supplementation (989.24; 17.55) than control (947.77; -6.20) and steers grazing fertilized bromegrass (942.43; -8.71). Cost of gain ($/kg BW gained) and breakeven ($/kg final BW) were less for DDGS-supplemented steers (1.05; 2.04) than control (1.24; 2.19) and steers grazing fertilized bromegrass (1.25; 2.19).

Proteinaceous Cotton By-Products

Cottonseed production in the United States was estimated to be 71.5 million tons in 1994, 60 to 65% of which was processed for oilseed meal and the rest was fed directly to ruminants as whole cottonseed (Eng, 1995). By-product feeds from cotton processing include: gin trash, gin motes, whole cottonseed, de-linted cottonseed, cottonseed hulls, cotton linters, and cottonseed meal (Rogers et al., 2002). Davis and Harland (1946) reported whole cottonseed containing high levels of energy, protein, P, and degradable fiber. The NRC (2016) reports whole cottonseed contains on a DM basis: 19.45 % fat, 47.82 % NDF, 42.85 % ADF, and 22.87 % CP.

Gadberry et al. (2005) evaluated replacing corn and cottonseed meal with de-oiled rice bran and whole cottonseed on digestion of a hay-based diet. To evaluate digestion characteristics, a 3 × 3 Latin square was utilized to compare corn plus cottonseed meal (71:29, w/w DM; CCSM), de-oiled rice bran and whole cottonseed (62:38, w/w DM; DRCS), or extrusion-processed de-oiled rice bran and whole cottonseed (62:38, w/w DM; EXT). Steers had free-choice access to grass hay (10.9% CP, 42.3% ADF, 71.8% NDF) and were supplemented at the rate of 10 g/kg BW daily. Adaptation periods lasted 14 d and, beginning on d 1 of 5-d fecal collection periods, steers received pulse doses of Yb-labeled hay and Dy-labeled supplement. Hay (4 g) and supplement (5 g) were incubated in situ for 6, 12, 24, 36, 48, and 72 h, and fecal samples were collected at 4, 8, 12, 16, 20, 30, 36, 48, 54, 60, 72, 84, and 96 h beginning on d 1 of collection. Total tract DM digestibility of hay tended to be greater for DRCS (41.7%) and EXT (36.9%) than CCSM (26.7%); however, total tract DM digestibility of supplement was greater for CCSM (95.8%) than DRCS (90.5%) and EXT (88.1%). Overall, total tract digestibility of the total diet did not differ among treatments CCSM (68.9%), DRCS (70.5%), and EXT (69.1%). Treatment did not affect ruminal degradability of supplement (48.9, 50.4, and 52.6% for CCSM, DRCS, and EXT, respectively) or hay (31.1, 30.9, and 30.6% for CCSM, DRCS, and EXT, respectively). Further, the authors compared steer performance in a 63-d feedlot trial when fed grass hay and either corn grain and cottonseed meal (CCSM), de-oiled rice bran and whole cottonseed (DRCS), or extruded de-oiled rice bran and whole cottonseed (EXT). Supplements were fed at 10 g/kg BW daily and hay was provided free-choice. Hay DMI/steer was 0.2 kg/d greater for CCSM (5.3) than DRCS and EXT (5.1 each). Steer ADG (1.3, 1.2 and 1.2 kg/d) and final BW (291, 291and 291 kg) was not affected by supplement type (CCSM, DRCS, and EXT, respectively).

Horner et al. (1988) evaluated ruminal fermentation changes as level of whole cottonseed in a TMR increased. Diets comprised either 0, 5, 15, or 30% whole cottonseed (WCS) such that approximately 80% of the WCS replaced concentrate and 20% replaced corn silage. Digestion flasks received 5 g substrate 3 times daily and were emptied every 24 h for sampling. Experimental periods lasted for 7 d with 4-d adaptation periods and 3-d ruminal fluid collection periods, and were repeated three times. At each collection period, MCP, VFA, and ammonia were determined, and pH was recorded at 0, 2, 4, and 6 h postfeeding. As WCS amount increased, pH increased and was greatest at the 30% inclusion level across all measurement times (6.07, 6.10, 6.14, and 6.20 for 0, 5, 15 and 30% WCS, respectively). An increase in ammonia concentration of 61.3% was reported when WCS increased from 0 to 30% of the diet, and 5 and 15% WCS were not different from 0%. Microbial protein (mg/dl was depressed with inclusion of WCS at all levels. Total VFA concentration was decreased 7% when WCS increased from 0 to 30%. Molar proportion (mol/100 mol) of acetate increased (56.3, 61.3, 66.4 and 66.8) and propionate decreased (28.9, 23.4, 21.6 and 21.1) as WCS increased (0, 5, 15 and 30%, respectively).

Poore et al. (2006) examined the effect of supplementing whole cottonseed to heifers grazing stockpiled tall fescue in North Carolina over 2 years. Heifers strip-grazed stockpiled tall fescue from December to February and November to February in years 1 and 2, respectively. Heifers were weighed every 28 d for BCS determination and adjustment of forage allocations. Whole cottonseed was supplied at 0.33% BW daily. Heifers supplemented with WCS had improved ADG (0.51 vs. 0.35 kg/d) and BCS change (0.42 vs 0.05) over heifers that did not receive WCS. Stocking rate did not differ between heifers that received WCS and heifers that were not supplemented (7.4 vs 6.9 heifers/ha).

Chase et al. (1994) investigated the impact of gossypol-containing diets on the growth and reproductive development of Brahman bulls in Texas. Weanling bulls were assigned to one of six groups and assigned to diets consisting of bermudagrass hay and dietary CP in the form of: 1) SBM as 18.9% of diet, as fed, 2) CSM as 19.8 % of diet, as fed, or 3) WCS as 41.1 % of diet, as fed to provide 0, 6, or 60 mg gossypol/kg BW daily, respectively. Bulls were fed for 196 d and weighed at 28-d intervals. Body weight gain was less for bulls fed WCS (113 kg) than SBM (148 kg) and CSM (151 kg). Bull ADG was 0.76, 0.77, and 0.58 kg/d for SBM, CSM, and WCS, respectively.

Cranston et al. (2006) conducted two studies to determine the impact of replacing cotton by-products with whole cottonseed or pelleted cottonseed on finishing beef cattle in Texas. In the first experiment, 120 steers were assigned to pens (5 steers/pen) that had been assigned to treatments consisting of whole cottonseed (WCS) replacing cottonseed meal in a steam-flaked corn-based diet (CON), or equivalent amounts of cottonseed meal and cottonseed oil as found in whole cottonseed were fed (EQU). In the second experiment, 150 steers were assigned to pens (5 steers/pen) that had been assigned to the same treatments as in experiment 1 or to a pelleted cottonseed (PCS) treatment that was used in place of WCS. Diets were mixed daily and delivered at amounts so that less than 0.5 kg were left in bunks. In experiment 1, final BW (567, 574 and 560 kg) and ADG (1.57, 1.61 and 1.53 kg) did not differ among treatments (CON, WCS, and EQU, respectively). Steers fed the CON diet had improved DMI (kg/d) and G:F (8.11 and 0.193, respectively) compared with WCS (8.70 and 0.179, respectively) and EQU (8.29 and 0.182, respectively). In experiment 2, final BW (590, 589, and 595 kg) and ADG (1.47, 1.46, and 1.49 kg/d) did not differ among treatments (CON, WCS, and PCS, respectively). Unlike experiment 1, DMI (kg/d) and G:F were improved for WCS (8.00 and 0.182, respectively) and PCS (8.07 and 0.185, respectively) compared with CON (8.46 and 0.174, respectively).

## Nutrient Cycling

### *Nitrogen, Phosphorus, and Potassium Cycles*

Nitrogen, P, and K are economically important mineral nutrients for agricultural production. How these minerals move through the environment is important to understand to reduce the risk of overapplication and contamination of soil and water resources. Nitrogen and P are more labile than K, but all three can move through agricultural ecosystems.

Nitrogen Cycle

McNeill and Unkovich (2007) discuss the N cycle in depth. The major sources of N inputs include biological or industrial fixation of atmospheric dinitrogen to $NH_3$, dry deposition via decomposition of particulate matter, wet deposition from rain and lightning strikes, and organic sources from plants, animals, and microorganisms. The principal forms of N returned from the atmosphere include $NH_4^+$, $NH_3$, and $NO_3^-$. Organic N deposition also occurs in form of amine aerosols, organic nitrates, and particulate N from dust, pollen, and bacteria, and may account for up to 30% of N deposited in some ecosystems (Neff et al., 2002).

For organic forms of N to become plant-available, decomposition of plant matter, animal waste, root exudate, and rhizodeposits must occur. After decomposition, mineralization of organic N occurs within the biologically active surface soil (0-5 cm) via aerobic and anaerobic heterotrophic microorganisms and produces $NH_4^+$ and $NH_3$ (McNeill and Unkovich, 2007). A continuous cycle of mineral N incorporation into soil microbial organic material, and the concomitant release of immobilized N to the soluble mineral N pool is referred to as mineralization-immobilization turnover (Jansson and Persson, 1982). Ammonium may be oxidized to $NO_3^-$ from $NO_2^-$ via nitrification by autotrophic or heterotrophic soil microbes (Wood, 1990). Nitrification is influenced by pH, moisture, temperature, and aeration of the soil (McNeill and Unkovich, 2007). Soil N may be in the forms of $NH_4^+$ (Chaillou and Lamaze, 1997), $NO_3^-$ or $NO_2^-$ (Darwinkel, 1975), or simple organic forms (Lipson and Monson, 1998) for plant N acquisition. Ammonium is taken up by plant roots where it is converted to glutamine in order to avoid $NH_4^+$ toxicity (Brugiere et al., 1997), which is utilized for amino acid or protein production (McNeill and Unkovich, 2007). Nitrate reductase is required for the assimilation of $NO_3^-$ and converts nitrate to $NO_2^-$. Nitrite reductase then converts $NO_2^-$ to $NH_4^+$, which is then converted to glutamine and utilized by the plant (McNeill and Unkovich, 2007).

Nitrogen losses occur via ammonia volatilization, gaseous N emissions as $NO_x$ and $N_2$, nitrate leaching, and organic and inorganic N transport via wind and water erosion. Ammonia volatilization may occur at the soil surface when free ammonia is present and increases with increasing pH and temperature (McNeill and Unkovich, 2007). Nitrogen losses from ammonia volatilization of fertilizer can exceed 50% of applied N in rain-fed agricultural systems, and up to 80% in flooded systems (Freney, 1997). Denitrification is the reduction of $NO_2^-$ and $NO_3^-$ to NO and $N_2O$, which may be further reduced to $N_2$ (Knowles, 1982). Heterotrophic bacteria (Payne, 1981) and fungi (Shoun et al., 1981) are the primary microbes responsible for denitrification due to their use of N oxides as terminal electron acceptors and organic C as electron donors when oxygen availability is limited (McNeill and Unkovich, 2007). Due to the negative charge of nitrate anions and soil particles, $NO_3^-$ is not retained and leaches below the plant root zone and into groundwater. Nitrate leaching is impacted by the quantity of water going through the soil and the concentration of $NO_3^-$. Leaching may be increased due to fire, harvest, fallowing, cultivation, and grazing which disturb the ecosystem. Erosion removes N via wind and water when the soil has been disturbed such as by ploughing, clear-felling, overgrazing, irrigation, mining, and construction (McNeill and Unkovich, 2007). Galloway et al. (1995) estimated that globally 20 million metric tons of dissolved

N and 20 million metric tons of particulate N are being removed by rivers to coastal waterways annually.

Phosphorus Cycle

In the top 20 cm of soil, P concentrations range from 200 to 2,000 ppm, of which 20 to 80% is present as organic P. The primary avenues of P input are weathering of parent material, fertilization, and plant and animal residues. There is very little atmospheric deposition of P (Bünemann and Condron, 2007), but phosphorus can be delivered through the air as dust particles, insect remains, pollen, spores, and leaf fragments (Duce et al., 1991). The most common primary P mineral is apatite (Frossard et al., 1995), which is transformed into various secondary compounds in highly weathered soil (Walker and Syers, 1976). Phosphorus is generally found in three forms within soils: organic phosphorus, calcium-bound inorganic phosphorus, and iron-aluminum-bound inorganic phosphorus (Brady and Weil, 2002).

Plant and animal residues provide organic P to the soil, which must be decomposed and mineralized to become available to plants (Brady and Weil, 2002). Heterotrophic bacteria and fungi may break down organically bound phosphate using enzymes such as phosphatase, phytase, and nucleotidase (Ehrlich, 1981). Microbial breakdown of organic phosphates and release to ground water constitutes the primary source of within-sediment phosphorus (Krajewski et al., 1994). Phosphorus is generally take up by plants in the form of phosphate ions ($HPO_4^{-2}$ and $H_2PO_4^{-}$), and the species of phosphate ion is dictated by soil pH; $HPO_4^{-2}$ is present in alkaline soils and $H_2PO_4^{-}$ present in acidic soils (Brady and Weil, 2002).

Brady and Weil (2002) note that soluble phosphorus, while plant-available, is rapidly removed from solution and immobilized. The fate of P is determined by soil pH, with low pH leading to formation of iron-, aluminum-, or magnesium-hydroxyphosphate precipitates, and high pH leading to formation of calcium-phosphate compounds. As these phosphate-containing compounds age, they become more insoluble due to additional bonding to soil cations and reduction in surface area. Additionally, P immobilization can occur through binding by soil, particularly those with high clay content, mainly 1:1 clays (Brady and Weil, 2002). Immobilized P may be returned to the soluble phase through the action of protozoa and fungi (Coleman et al., 1978) and earthworms (Mackay et al., 1982). The presence of a P sink such as a plant root reduces the inorganic P concentration in the soil, leading to increased mineralization and reducing the time inorganic P is in the soil and minimizing inorganic fixation, thus increasing P turnover (Blair and Bowland, 1978).

Phosphorus losses occur from runoff, leaching, and removal of crops. Phosphorus losses, as surface runoff and subsurface flow, in Ohio averaged 0.55 kg P/ha when orchardgrass/Kentucky bluestem pastures were grazed during the summer, and 4.1 kg P/ha when pastures were grazed during the summer and hay was fed during the winter on those pastures (Owens et al., 2003). The authors (Owens et al., 2003) note that the increased P loss from the hay- fed portion occurred because the hay provided the majority of P.  In Indiana, total P losses of 12% were reported over a 25-yr period from continuous cultivation (Berber, 1979).

Potassium Cycle

Potassium is taken up by plants in amounts that is second only to N. Soil type has a large impact on K, with sandy soils having more potential for leaching (Kayser and Isselstein, 2005) than soils containing clay, where K is adsorbed in the soil solution (Rowell, 2014). Potassium inputs to the soil include weathering of K-containing minerals (micas and feldspar), animal and plant residues, and fertilizer application (Brady and Weil, 2002). Potassium in the cation form may be converted to chemically fixed forms via precipitation and adsorption and can be released to the cation form by solubilization and desorption (Haynes and Williams, 1993). The fate of K derived from fertilizer or urine is determined by the potential of plants to take up and soils to adsorb K (Kayser and Isselstein, 2005). Under grazing conditions, K distribution and that of other nutrients is localized around water sources, feeders, and shade, and the amount accumulated is impacted by soil characteristics, climate, and animal behavior (Owens et al., 2003).

There is little volatile loss of K (Kayser and Isselstein, 2005). Potassium losses from leaching are generally small and ranged from 5 to 31 kg K/ha in mown grasslands in England (Alfaro et al., 2004), and 2 kg K/ha (Early et al., 1998) to 48 kg K/ha (Di and Cameron, 2004) in grazed pastures in New Zealand. Potassium concentrations in topsoil can impact Ca and Mg dynamics (Kayser and Isselstein, 2005), and can cause increased leaching of Ca and Mg (Sakadevan et al., 1993). The largest loss of K is through the removal of forage from pasture systems (Kayser and Isselstein, 2005).

*Nitrogen Fixation by Legumes*

The use of pasture legumes to maintain high total forage production without using fertilizer N and to improve soil N status has long been acknowledged (Ledgard and Steele, 1992). Brady and Weil (2002) noted that, apart from plant photosynthesis, biological N-fixation may be the most important biochemical process for life on the planet. The conversion of inert atmospheric dinitrogen gas to N-containing organic compounds is performed by certain microorganisms, including bacteria, actinomycetes, and cyanobacteria. As a result of the N cycle, these N-containing compounds become available to other organisms after N-fixation occurs (Brady and Weil, 2002).

Nitrogen fixation associated with legumes results from a symbiotic relationship between the legume and *Rhizobium* bacteria that are specific for each legume species. For N fixation to occur, colonization of legume roots by the bacteria must occur. Ledgard and Steele (1992) described the steps necessary for nodule formation. First, the appropriate *Rhizobium* strains must be present in the soil. Secondly, the *Rhizobium* must proliferate around host plant roots, and then induce deformation of root-hairs and enter the deformed root-hair. Once in the root-hair, an infection thread is produced and enters the root cortical cells, which stimulates cell division and nodule formation. Lastly, the infection thread releases bacteria that multiply and differentiate into bacteroids. The nodules with successful bacteriod development are where N-fixation occurs (Ledgard and Weil, 1992).

Bergersen (1971) discussed the biochemistry of N-fixation within legume nodules. Briefly, the reduction of $N_2$ to $NH_3$ by nitrogenase requires large amounts of ATP to break the triple bond between the N atoms. Oxygen and $H_2$ are necessary for oxidative phosphorylation and supplying ATP. Nitrogenase, however, is deactivated by

$O_2$. Leghemoglobin is produced to protect nitrogenase while allowing oxygen to be present for respiration to occur. Nitrogenous compounds are actively taken up in the endodermal and pericycle cells of nodular vascular tissue and exported (Bergensen, 1971). The N fixed by bacteroids has three possible destinations: being utilized by the host plant, becoming available to non-fixing companion plants, or immobilization by heterotrophic microorganisms and incorporation into soil OM (Brady and Weil, 2002).

It has been estimated that biological N-fixation input in terrestrial ecosystems accounts for 40 million metric tons of N per year (Galloway et al., 1995). In Argentina, it was reported that white clover fixed an average of 38 kg N/ha when grown with tall fescue, and lotus fixed an average of 31 kg N/ha when grown with tall fescue. Additionally, N-fixation was determined across forage types based upon different grazing intensities: high (800 to 1,200 kg DM/ha residual forage) or low (1,800 to 2,200 kg DM/ha residual forage), and was 39 kg N/ha for high and 30 kg N/ha for low (Refi and Excuder, 1998). In Iowa, total seasonal N fixation from red clover interseeded with orchardgrass was reported to range from 66 to 195 kg N/ha (Farnham and George, 1994). In Texas, it was reported that maximum N fixation across the 2 years of the study were 296 kg N/ha for arrowleaf clover and 189 kg N/ha for crimson clover. In addition, the authors note that the percentage of plant N derived from N fixation was greater than 75% for all clovers evaluated (Evers and Parsons, 2011).

Factors that impact N transfer include: biomass ratio between donor and companion plants, root turnover rate, C allocation (Rasmussen et al., 2007), rate of nodule turnover and the amount of rhizodeposited N (Phillips et al., 2006; De Graff et al., 2007), and the presence of mycorrhizal fungi (Moyer-Henry et al., 2006). In Sweden, N transfer from red clover to perennial ryegrass was 7 kg N/ha and 68 kg N/ha in harvested and intact stands, respectively, from which harvested samples had been clipped to 0.06-m stubble height twice when clover was at late vegetative stage and then at flowering (Dahlin and Stenberg, 2010). In Minnesota, N transfer from alfalfa, birdsfoot trefoil, red clover, and white clover to reed canarygrass was evaluated. Nitrogen fixation (kg N/ha), over the 4 years of the study, averaged 175 for alfalfa, 77 for birdsfoot trefoil, 63 for red clover, and 9 for white clover. There was little N transfer from legume to grass during the establishment year, but it averaged 28 kg N/ha in yr 2, 10 kg N/ha in yr3, and 6 kg N/ha in yr 4 (Heichel and Henjum, 1991).

*Impact of Grazing Cattle on Nutrient Cycling*

Fertilization leads to increased cycling of nutrients within the soil-plant-animal continuum, and acts as a catalyst for recycling processes (Dubeux et al., 2006). Grazing by herbivores increases nutrient cycling via degradation and return of nutrients to the soil (Bardgett and Wardle, 2003). Increasing stocking rates allow for enhanced consumption of herbage by livestock, which increases nutrient returns to the soil as excreta (Thomas, 1992). However, nutrient recovery from excreta by plants is variable due to the random distribution of excreta within pastures (White et al., 2001). Excreta may cover between 30 and 40% of a pasture, but these areas may account for up to 70% of the forage production due to high nutrient concentrations (Haynes and Williams, 1993). Areas where animals lounge, where waterers and shade are, tend to have elevated concentrations of soil nutrients due to high excreta deposition (Mathews et al., 1996).

Cattle return about three-fourths of N, four-fifths of P, and nine-tenths of K of ingested nutrients as excreta (Brady and Weil, 2002). Dubeux et al. (2006) reported that soil P (21 vs 10 mg/kg), K (103 vs 52 mg/kg), and Mg (198 vs 122 mg/kg) concentrations were greater within 8 m from a water source than more than 16 m of the water source when cattle grazed bahiagrass in Florida. The authors postulated that these differences were due to greater excretal deposition near the waterers. Additionally, herbage mass accumulation was greater within 8 m (40 kg DM/ha·d) of the waterer compared with greater than 16 m (20 kg DM/ha·d; Dubeux et al., 2006). Similarly, in Hawaii when cattle grazed kikuyugrass, N, P, and K accumulated within 15 m of shade at one site, and P, K, and Mg accumulated within 15 m of water sources at another site (Mathews et al., 1999).

## II. Effects of Nitrogen-delivery method and monensin on stocker cattle production from annual ryegrass

### Introduction

Input costs and high land prices are major challenges facing the beef cattle industry. To be economically viable, beef production systems must effectively exploit the capacity of the ruminant animal to consume and efficiently convert forage to liveweight gain. To realize this production strategy to its full potential, an abundant supply of high-quality forage must be continuously available. In the Southeast, this has traditionally been achieved by grazing small-grain and other cool-season annual grasses (Utley et al., 1975). Annual ryegrass (*Lolium multiflorum* Lam.) is a cool-season annual bunchgrass (Hall, 1992) that can produce between 6,000 and 13,000 kg/ha of forage DM when fertilized (Redfearn et al., 2002), and there are more than 1 million ha of ryegrass grown annually in the Southeast (Ball et al., 2007).

Nitrogen (N) fertilizer represents the single greatest input cost of forage production for grazing by stocker cattle, and costs rose steadily from the mid-1990s through the early-2010s, followed by a small decline in the late 2010s. Lower-cost N fertilizer alternatives would be economically advantageous to cattle producers, including interseeded legumes or supplementation with high-protein by-product feeds. Crimson clover (*Trifolium incarnatum* L.) and arrowleaf clover (*Trifolium vesiculosum* Savi) are annual legumes that are well adapted to the Southeast (Ball et al., 2015). Crimson clover has been reported to fix an average of 155 kg N/ha (Brink, 1990), and arrowleaf clover averaged 137 kg N/ha (Evers, 1985). Pederson and Ball (1991) reported mean forage DM mass (kg/ha) of 3,999 for crimson clover and 3,892 for arrowleaf clover. Knight (1970) reported an increase of 3.6 MT DM/ha for bermudagrass (*Cynodon dactylon*) interseeded with crimson clover, and 2.7 MT DM/ha for bermudagrass interseeded with arrowleaf clover.

Distillers dried grains plus solubles (DDGS) are a by-product of alcohol production from dry-milling of corn. The NRC (2016) reports that DDGS contains on a DM basis: 10.73% fat, 33.66% NDF, 16.17% ADF, and 30.79% CP consisting of 40% RDP and 60% RUP fractions. Supplementation of stocker cattle with DDGS (2.3 $kg \cdot hd^{-1} \cdot d^{-1}$) increased ADG by 0.25 kg/d and total gain by 101 kg/ha over fertilized smooth bromegrass (Greenquist et al., 2009). Whole cottonseed (WCS) is a by-product of the cotton ginning process. The NRC (2016) reports that whole cottonseed contains on a DM basis: 19.45% fat, 47.82% NDF, 42.85% ADF, and 22.87% CP consisting of 70% RDP and 30% RUP (NRC, 2000). Poore et al. (2006) noted an increase in ADG of 0.16 kg/d for heifers supplemented at 0.33% BW with WCS compared with unsupplemented heifers. The efficacy of feeding an ionophore to grazing cattle has been long established. Horn et al. (1981) reported an increase in ADG of 0.08 kg when monensin was included in a supplement to cattle on wheat pasture, and this was corroborated by Bretschneider et al. (2008) who reported an increase of 0.075 kg/d when monensin was fed.

The objective of this study was to evaluate the efficacy and economic feasibility of replacing N fertilizer with either interseeded legumes or supplementation with high-protein by-products, with or without monensin, for stocker cattle production from annual ryegrass.

## Materials and Methods

All experimental procedures were implemented according to a protocol approved by the Auburn University Animal Care and Use Committee (PRN 2014-2438).

### *Treatment Structure*

Treatments were randomly assigned to thirty 0.81-ha pastures in Yr 1 of a 3-yr study with the restriction that the same treatment could not be applied to adjacent pastures. Treatments in Yr 2 and 3 were maintained on the same pastures to which they had been assigned in Yr 1. Treatment structure was a completely randomized 5 × 2 factorial with five N-delivery methods, with or without monensin (n = 3 pastures/treatment) provided in a custom-formulated compressed mineral block (Ridley Block Operations Mankota, MN). Nitrogen-delivery methods included: annual ryegrass fertilized with 112 kg N/ha in a split-application (NFERT), annual ryegrass interseeded with crimson clover and fertilized with 56 kg N/ha at time of establishment (CC), annual ryegrass interseeded with arrowleaf clover and fertilized with 56 kg N/ha at time of establishment (AC), annual ryegrass fertilized with 56 kg N/ha and cattle supplemented with distillers dried grains plus solubles at the rate of 0.65% BW daily (DDGS), and annual ryegrass fertilized at 56 kg N/ha and cattle supplemented with whole cottonseed at the rate of 0.65% BW daily (WCS). Interseeding and supplementation rates were selected to deliver an additional 56 kg N/ha that the NFERT treatment received in the second of the split-application.

*Pasture Establishment*

A 3-yr winter grazing trial was conducted at the E.V. Smith Research Center located 48 km from Auburn University in Milstead, AL (32.443$^{o}$ N lat., 85.897$^{o}$ W long.). Soil characteristics at initiation of the study were: 6.1 soil pH, 37 kg P/ha, 202 kg K/ha, 686 kg Mg/ha, and 2,118 kg Ca/ha. Thirty 0.81-ha paddocks that consisted of a fine sandy loam were used. These paddocks had previously been planted to annual ryegrass for the last five yrs, and prior to that with warm-season grasses including: bermudagrass (*Cynodon dactylon*), bahiagrass (*Paspalum notatum*), and dallisgrass (*Paspalum dilatatum*) for summer grazing.

Each year in early October prior to planting, pastures were fertilized with 329 kg of 17-17-17 fertilizer to provide 56 kg N/ha. Pastures were planted on Oct. 16, 2015, Dec. 23, 2016, and Oct. 25, 2017 in Yr 1, 2, and 3, respectively; planting was delayed in Yr 2 compared with Yr 1 and 3 because of exceptionally dry soil conditions resulting from below-average precipitation and ongoing drought conditions earlier in the fall. Each year, pastures assigned to NFERT, DDGS, and WCS were seeded at a rate of 34 kg/ha of 'Marshall' annual ryegrass, and the interseeded clover pastures were seeded at a rate of 17 kg/ha of 'Marshall' annual ryegrass and 34 kg/ha of 'Dixie' crimson clover or 9 kg/ha of 'Blackhawk' arrowleaf clover (Wax Seed Company, Amory, MS) to a depth of 0.6 cm into a prepared seedbed. Pastures assigned to the NFERT N-delivery method received an additional 56 kg N/ha as liquid N (28% N solution of ammonium nitrate and ammonium thiosulfate that provided 5% S) on Feb. 23, 2016, March 20, 2017, and Jan. 24, 2018.

*Animal and Pasture Management*

Pastures were initially stocked with 90 crossbred steers (3 steers/pasture) of no more than ⅛ *Bos indicus* influence with an initial weight of 225 ± 10, 256 ± 15, and 239 ± 15 kg in Yr 1, 2, and 3, respectively. Cattle were procured through open-bid contract with a stocker producer in Reform, AL and were delivered in late December of each yr. Upon delivery, calves were quarantined for 30 d on dormant mixed-grass paddocks and fed corn silage and grass hay at a maintenance level of intake in Yr 1 and 3. Because of delayed planting and turnout due to drought in the late fall and early winter of Yr 2, calves were placed on dormant mixed-grass paddocks and fed a 50:50 blend of corn gluten feed and soybean meal for a targeted gain of 0.25 kg/d. Prior to study initiation, calves were stratified by BW, randomly assigned to pastures and ear-tagged for identification. Calves were implanted with Ralgro® in Yr 1 and 2 and Synovex-S® in Yr 3. Throughout the study, calves had access to clean water and a complete pressed-mineral block with a targeted intake of 57 to113 $g \cdot hd^{-1} \cdot d^{-1}$ that contained: Ca 4.70-5.70%, P 4.0%, NaCl 16.90-19.90%, Mg 0.20%, K 1.50%, Co 10 ppm, Cu 1,000 ppm, I 140 ppm, Mn 3,950 ppm, Se 13.3 ppm, Zn 4,000 ppm, Vit. A 45,400 IU/kg, Vit. D-3 11,350 IU/kg, and Vit. E 11.35 IU/kg. Half of the blocks were nonmedicated, and the other half contained monensin at 1,620 g/ton to provide 50 to 200 $mg \cdot hd^{-1} \cdot d^{-1}$. Supplement was provided once daily at approximately 0800 hr. Calves were weighed every 28 d following feed restriction for 24 h in order to derive shrunk weights. Weights were used to adjust supplement rates for the succeeding 28-d period, and to adjust stocking densities in order to maintain a uniform forage allowance across all treatments of 1 kg forage DM/kg steer BW using put-and-take steers. Cattle were weighed and turned out onto pastures for grazing on December 14, 2015 (Yr 1), February 15, 2017 (Yr 2), and December 21, 2017 (Yr 3). Mean initial forage mass (kg/ha) was 1,068, 539, and 619, and mean initial forage allowance was 1.04, 1.03, and 0.94 in Yr 1, 2, and 3, respectively. Grazing was terminated on May 11, 2016 (140 d), May 10, 2017 (84 d), and February 19, 2018 (56 d) when forage quantity and quality could no longer maintain an ADG of 0.68 kg/d.

Forage mass was determined using the double-sampling method described by Frame (1981). Twenty-five forage heights were recorded from each 0.81-ha pasture using a 0.25-$m^2$ disk meter. Seventy-two calibration samples were taken from the 18 pastures assigned to the NFERT, CC, and AC treatments by recording forage heights and clipping forage to a stubble height of approximately 5 cm. Samples were placed in individual plastic bags and placed in a cooler for transport to the Auburn University Ruminant Nutrition Laboratory where they were transferred to individual paper bags and dried at $60^o$ C to a constant weight. After drying, sample weights were plotted against their respective height values, and the resultant prediction equations ($r^2$ ranged from 0.10 to 0.88, mean $r^2 = 0.64$) were used to determine the approximate forage DM mass for each pasture and to adjust stocking densities. The overall prediction equation was: $y = 88.755x + 106.25$.

*Economic Evaluation*

An economic evaluation of N-delivery methods was conducted to compare the N-fertilized pasture system to the interseeded-clover and protein byproduct-supplemented pastures on an input cost/ha and cost of gain basis. Costs included variable input costs of N fertilizer, labor, seed, supplements, and machinery. The hourly cost of equipment ($25.00) used during the experiment was determined previously by Prevatt et al. (2008) and multiplied by the hr of actual use time as recorded for each system. Diesel costs used were determined from the average retail cost of diesel during the 3 yr of the experiment. Labor costs were the number of hours of labor per system multiplied by $9.00/hr. The price of 17-17-17, DDGS, and WCS were $415, $110, and $205/ton, respectively. Fertilizer and supplement costs were determined from the Alabama Weekly Feedstuff/Production Cost Report. Formulas and prices used to determine input costs are provided in the Appendix.

*Statistical Analysis*

Data were analyzed using PROC MIXED of SAS 9.4 (SAS Inst. Inc., Cary, NC.) for a 5 × 2 factorial design consisting of 5 N-delivery methods and 2 levels (+/-) of monensin. Data for all steers (i.e., testers and put-and-take steers) were used to determine stocking density, forage allowance, and grazing d/ha. Total gain/ha was calculated for each pasture as the ADG of tester steers and grazing d/ha of both tester and put-and-take steers (Beck et al., 2011). Dependent variables evaluated included ADG, total gain/ha, stocking density, grazing d/ha, forage allowance, and forage mass. Main effects were N-delivery method, ionophore, year, and their 2-way interactions. Because there were no significant 3-way interactions detected for any of the dependent variables evaluated, their sum of squares and associated df were apportioned to the model error term (residual) for significance testing. The PDIFF option of LSMEANS was used to separate means when protected by F-test at $\alpha = 0.10$, trends were declared at $\geq 0.10$ to $\leq 0.15$.

## Results and Discussion

*Temperature and Precipitation*

Monthly mean and 30-yr average monthly temperatures from August to May of each yr at the research station are presented in Figure 1, and monthly and 30-yr average monthly precipitation totals from August to May of each yr at the research station are presented in Figure 2. In Yr 1, monthly mean temperatures approximated 30-yr averages. Precipitation was lower than average preceding and at time of planting in September and October, respectively, but greater than average rainfall in November and December allowed for acceptable forage production with a start date for grazing of Dec 14, 2015. Adequate rainfall throughout the remainder of the winter and spring allowed for forage production that supported a typical 140-d grazing season. As in Yr 1, mean monthly temperatures in Yr 2 were very similar to 30-yr averages. However, precipitation was extremely low in September, October and November, which delayed planting until Dec 23, 2016. During and after December, rainfall was adequate to support high forage mass production that enabled cattle to be turned out on Feb 15, 2017 for an 84-d grazing season. In Yr 3, mean temperatures were similar to 30-yr averages from October through December, followed by a much colder-than-average January and a very warm February.

Precipitation was very low in September but was near average in October and allowed for pastures to be planted on Oct 25, 2017. After planting, precipitation was well below average from November through April, which limited forage production but enabled cattle to be turned out on Dec 21, 2017. However, due to lack of rainfall, particularly in January, coupled with extreme fluctuations in temperature during the same period, forage production was limited, and the grazing season lasted 56 d.

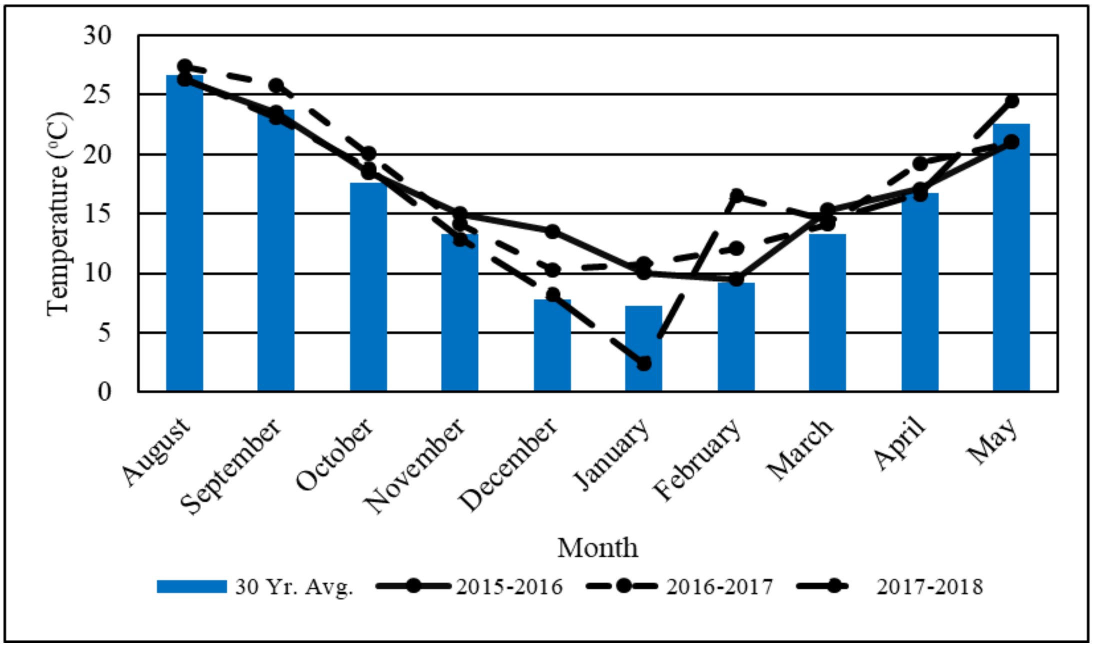

Figure 1. Monthly and 30-yr average temperatures From August to May by yr at E.V. Smith Research Center, Milstead, AL.

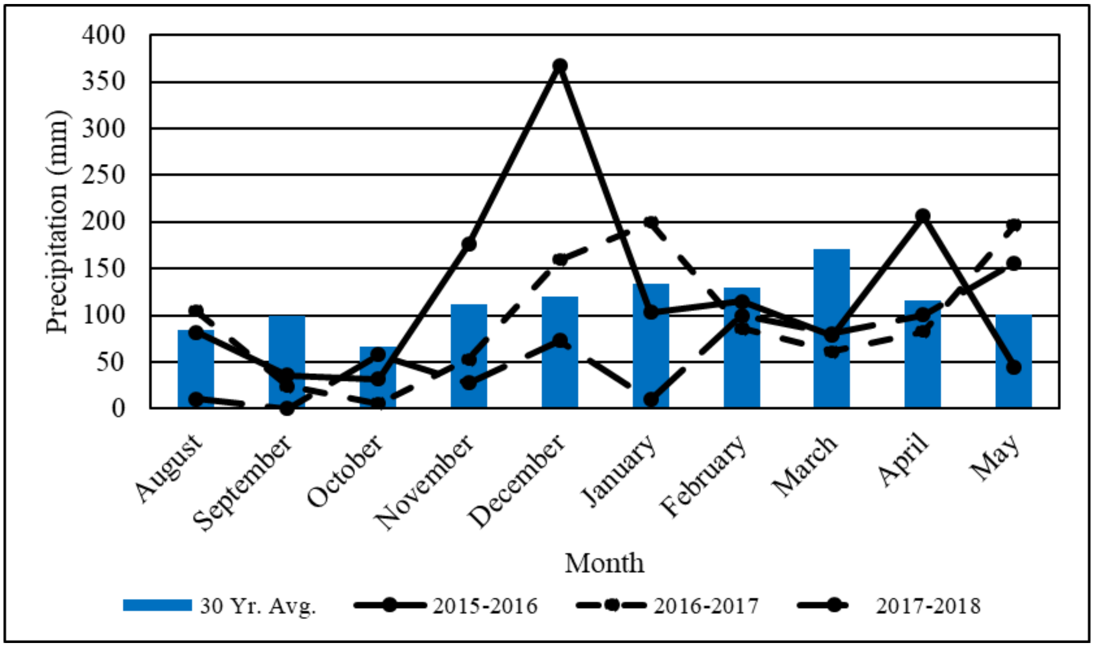

Figure 2. Monthly and 30-yr average monthly precipitation totals from August to May by yr at E.V. Smith Research Center, Milstead, AL.

*Forage Metrics*

Forage mass (kg DM/ha; Table 1) was not impacted by feeding monensin (*P* = 0.58), nor were any N-delivery method × monensin (*P* = 0.78), N-delivery method × yr (*P* = 0.99), or monensin × yr interactions (*P* = 0.65) detected. Forage mass was different among N-delivery methods (*P* = 0.004) and yr (*P* < 0.0001). Forage mass was greatest for NFERT, DDGS and WCS, intermediate for CC, and least for AC. Year 2 had greater forage mass than Yr 1, which had greater forage mass than Yr 3. Weather greatly impacted forage production and thus mass across the 3 yr of the study. A drought in September and October of Yr 2 delayed planting, but greater than average rainfall in December and January boosted forage production potential. Precipitation and temperature in Yr 1 generally followed the 30-yr average, with elevated rainfall in November and December. The difference in timing of excess rainfall between Yrs 1 and 2 may account for the greater forage mass observed in Yr 2, even though the grazing season was shortened due to drought the previous fall. Year 3 weather conditions were largely responsible for the decreased forage mass observed in that year. Very low January temperatures and high February temperatures along with below-average precipitation from November through March greatly limited the forage production capacity of the cool-season forages used in this study. Forage mass values of 2,061 kg DM/ha (Hafley, 1996) and 1,493 kg DM/ha (Mullenix et al., 2014) have been reported for continuously grazed annual ryegrass in multi-yr grazing trials. These values are greater than those reported herein, which may be due in part to use of more conservative stocking rates in those studies as well as marked differences in weather conditions.

Table 1. Mean forage mass (kg DM/ha) of annual ryegrass as affected by different N-delivery methods and year

| Item | |
|---|---|
| N-delivery method[1] | Forage mass |
| NFERT | $972^{a}$ |
| CC | $850^{b}$ |
| AC | $730^{c}$ |
| DDGS | $934^{ab}$ |
| WCS | $913^{ab}$ |
| | |
| <u>Year</u> | |
| 1 | $913^{e}$ |
| 2 | $1208^{d}$ |
| 3 | $519^{f}$ |

$^{a\text{-}c}$ within a column, means without a common superscript differ ($P < 0.10$; SEM = 47.4).
$^{d\text{-}f}$ within a column, means without a common superscript differ ($P < 0.10$; SEM = 36.4).
[1] NFERT= annual ryegrass fertilized with 112 kg N/ha in split application, CC = annual ryegrass fertilized with 56 kg N/ha and interseeded with crimson clover, AC = annual ryegrass fertilized with 56 kg N/ha and interseeded with arrowleaf clover, DDGS = annual ryegrass fertilized with 56 kg N/ha and cattle supplemented with dried distillers grains plus solubles at 0.65% BW/d, WCS = annual ryegrass fertilized with 56 kg N/ha and cattle supplemented with whole cottonseed at 0.65% BW/d.

Forage allowance (kg DM/kg steer BW; Table 2) did not differ among N-delivery methods (*P* = 0.64) or monensin status (*P* = 0.96), and there were no N-delivery method × monensin (*P* = 0.84), N-delivery method × yr (*P* = 0.28), or monensin × yr (*P* = 0.82) interactions. A trend (*P* = 0.11) was noted for FA by yr, with FA values tending to be slightly greater in Yr 1 (1.02) and 2 (1.04) than Yr 3 (0.94). These values may be compared with those derived by Rouquette et al. (2018) from an 11-yr grazing experiment with bermudagrass pastures that were overseeded with annual ryegrass or arrowleaf clover to extend the warm-season grazing period. In their study, the relationship between calf ADG and ryegrass DM mass was optimized at a FA of approximately 1.3 and 1.5 kg DM/kg BW for arrowleaf clover and ryegrass, respectively. The relationship between ADG and FA has been reported to be more or less linear up to a FA of 3 kg DM/kg BW (McCartor and Rouquette, 1977). However, a nonlinear regression model indicated a FA of 1.8 was necessary to maintain an ADG of 0.9 kg (Beck et al., 2013). More recent reports indicate greater ADG at lesser FA than those reported by Beck et al. (2013). Mullenix et al. (2014) reported steer ADG of 1.2 kg/d with FA of 1.36 for annual ryegrass and small-grain pastures. Similarly, Marchant et al. (2018) reported ADG of 1.44 kg/d at a FA of 0.89 for annual ryegrass and small-grain pastures. Weissend (2015) noted a mean ADG of 1.06 kg/d with a FA of 0.52 for annual ryegrass. Redfearn et al. (1985) reported that ADG was not negatively impacted until a FA of 0.21 had been realized for small-grain pastures.

Table 2. Mean forage allowance (kg DM/kg BW) of annual ryegrass as affected by N-delivery method, year, and monensin

| Item | |
|---|---|
| N-delivery method[1] | Mean |
| NFERT | 1.02 |
| CC | 0.93 |
| AC | 1.00 |
| DDGS | 1.04 |
| WCS | 1.01 |
| | |
| Monensin | |
| With | 1.00 |
| Without | 1.00 |

[1] NFERT= annual ryegrass fertilized with 112 kg N/ha in split application, CC = annual ryegrass fertilized with 56 kg N/ha and interseeded with crimson clover, AC = annual ryegrass fertilized with 56 kg N/ha and interseeded with arrowleaf clover, DDGS = annual ryegrass fertilized with 56 kg N/ha and cattle supplemented with dried distillers grains plus solubles at 0.65% BW/d, WCS = annual ryegrass fertilized with 56 kg N/ha and cattle supplemented with whole cottonseed at 0.65% BW/d.

*Cattle Performance*

Average daily gain (kg/d; Table 3) was not affected by monensin inclusion ($P$ = 0.45), and there were no N-delivery method × monensin ($P$ = 0.14) or monensin × yr ($P$ = 0.34) interactions. A N-delivery method × yr interaction was detected ($P$ = 0.08) in Yr 1 such that ADG was greatest for DDGS and did not differ from NFERT or WCS, and NFERT and WCS did not differ from CC or AC. In Yr 2, ADG differed between WCS and AC, with NFERT, CC, and DDGS being intermediate but not different from either WCS or AC. In Yr 3, ADG was greatest for NFERT and DDGS, AC and WCS had intermediate ADG that was not different from DDGS, and CC had the least ADG. Across all years, ADG was greater ($P$ = 0.001) for NFERT, DDGS, and WCS than CC and AC, and ADG was greater ($P$ < 0.0001) in Yr 2 and 3 than Yr 1.

Table 3. Average daily gain (kg/d) of steers grazing annual ryegrass as affected by N-delivery method, year, and monensin

| Item | Year | | | |
|---|---|---|---|---|
| N-delivery method[1] | 1 | 2 | 3 | Mean |
| NFERT | $1.19^{bcd}$ | $1.50^{acd}$ | $1.68^{ac}$ | $1.49^{h}$ |
| CC | $1.09^{bd}$ | $1.51^{acd}$ | $1.15^{be}$ | $1.25^{i}$ |
| AC | $1.12^{bd}$ | $1.37^{ad}$ | $1.38^{ad}$ | $1.29^{i}$ |
| DDGS | $1.37^{bc}$ | $1.55^{abcd}$ | $1.60^{acd}$ | $1.50^{h}$ |
| WCS | $1.20^{bcd}$ | $1.66^{ac}$ | $1.47^{ad}$ | $1.45^{h}$ |
| Mean | $1.19^{f}$ | $1.52^{g}$ | $1.46^{g}$ | |
| | | | | |
| <u>Monensin</u> | | | | |
| With | 1.41 | | | |
| Without | 1.37 | | | |

$^{a\text{-}b}$ within a row, means without a common superscript differ ($P$ < 0.10; SEM=0.09).
$^{c\text{-}e}$ within a column, means without a common superscript differ ($P$ < 0.10; SEM=0.09).
$^{f\text{-}g}$ within a row, means without a common superscript differ ($P$ < 0.10; SEM=0.04).
$^{h\text{-}i}$ within a column, means without a common superscript differ ($P$ < 0.10; SEM=0.05).
[1] NFERT= annual ryegrass fertilized with 112 kg N/ha in split application, CC = annual ryegrass fertilized with 56 kg N/ha and interseeded with crimson clover, AC = annual ryegrass fertilized with 56 kg N/ha and interseeded with arrowleaf clover, DDGS = annual ryegrass fertilized with 56 kg N/ha and cattle supplemented with dried distillers grains plus solubles at 0.65% BW/d, WCS = annual ryegrass fertilized with 56 kg N/ha and cattle supplemented with whole cottonseed at 0.65% BW/d.

Bagley et al. (1988) evaluated steer performance from annual ryegrass fertilized at 34 kg/ha, rye-annual ryegrass-arrowleaf clover, rye-ryegrass-ladino clover, and annual ryegrass-arrowleaf clover over 4 years. They reported ADG of 1.00 kg/d for steers grazing annual ryegrass-arrowleaf clover and 0.93 kg/d for N-fertilized annual ryegrass. Greater ADG in the current study may have been due in part to the use of monensin. Mooso et al. (1990) reported an ADG of 0.97 kg/d for stocker cattle grazing annual ryegrass-white clover-crimson clover pastures. Mullenix et al. (2014) and Marchant et al. (2018) reported ADG of 1.37 and 1.44 kg/d for cattle grazing monocultures of triticale, wheat and annual ryegrass, or mixtures of these forages, respectively, which were similar to values reported in the current study. Weissend (2015) reported a mean ADG of 1.12

kg/d for cattle fed energy supplements when grazing annual ryegrass, and an ADG of 0.91 kg/d for unsupplemented cattle. Griffin et al. (2012) reported a linear increase in ADG as level of DDGS supplementation was increased from 0 to 0.6 to 1.2% BW (0.89, 1.03, 1.19 kg/d, respectively) for cattle grazing subirrigated Sandhills meadow. Greenquist et al. (2009) reported similar daily gains (0.92 kg/d) for cattle grazing smooth bromegrass and receiving DDGS at 0.6% BW/daily. Poore et al. (2006) supplemented heifers grazing tall fescue with whole cottonseed and reported an ADG of 0.51 kg/d, which is less than from the current study and due most likely to differences between forage species.

Total gain (kg/ha; Table 4) was not affected by monensin inclusion ($P = 0.19$), and there were no N-delivery method × yr ($P = 0.25$) or monensin × yr ($P = 0.50$) interactions. A N-delivery method × monensin interaction ($P = 0.04$) was detected such that total gain was greatest for NFERT, DDGS and WCS, intermediate for CC, and least for AC when monensin was fed. However, total gain was greatest for DDGS and NFERT, NFERT and WCS did not differ, WCS and AC did not differ, and AC and CC did not differ when monensin was not fed. Across both levels of monensin inclusion, total gain was greatest ($P < 0.0001$) for NFERT, DDGS and WCS than CC and AC. Also, total gain was greater ($P < 0.0001$; Table 5) in Yr 1 and 2 than Yr 3. Reduced forage mass due to weather conditions in Yr 3 limited the grazing season to 56 d compared with 140 and 84 in Yr 1 and 2, respectively, and reduced forage mass impacted stocking densities, which resulted in lower total gain/ha in Yr 3.

Table 4. Total gain (kg/ha) of steers grazing annual ryegrass as affected by N-delivery method and monensin

| Item | N-delivery method[1] | | | | | |
|---|---|---|---|---|---|---|
| Monensin | NFERT | CC | AC | DDGS | WCS | Mean |
| With | 462[a] | 347[b] | 280[c] | 435[a] | 486[ae] | 402 |
| Without | 411[ab] | 302[d] | 340[cd] | 457[a] | 383[bcf] | 379 |
| Mean | 436[g] | 324[h] | 310[h] | 446[g] | 435[g] | |

[a-d] within a row, means without a common superscript differ (*P* < 0.10; SEM = 28.17).
[e-f] within a column, means without a common superscript differ (*P* < 0.10; SEM = 28.17).
[g-h] within a row, means without a common superscript differ (*P* < 0.10; SEM = 19.92).
[1] NFERT= annual ryegrass fertilized with 112 kg N/ha in split application, CC = annual ryegrass fertilized with 56 kg N/ha and interseeded with crimson clover, AC = annual ryegrass fertilized with 56 kg N/ha and interseeded with arrowleaf clover, DDGS = annual ryegrass fertilized with 56 kg N/ha and cattle supplemented with dried distillers grains plus solubles at 0.65% BW/d, WCS = annual ryegrass fertilized with 56 kg N/ha and cattle supplemented with whole cottonseed at 0.65% BW/d.

Table 5. Total gain (kg/ha) of steers grazing annual ryegrass as affected by year

| Year | Total gain |
|---|---|
| 1 | 480[a] |
| 2 | 472[a] |
| 3 | 219[b] |

[a-b] within a column, means without a common superscript differ (*P* < 0.10; SEM = 15.4).

Mooso et al. (1990) reported total gains of 494 and 532 kg/ha for steers grazing annual ryegrass-white clover and annual ryegrass-white clover-crimson clover pastures, respectively. These values are greater than those in the current study and reflect longer grazing seasons. Hoveland et al. (1991) reported total gains of 575 kg/ha for annual ryegrass-crimson clover and concluded that the high gains were due to high stocking rates and ADG. Hoveland et al. (1978) had previously reported total gains of 628 kg/ha from rye-arrowleaf clover-crimson clover, 473 kg/ha from ryegrass, and 460 kg/ha from arrowleaf clover-crimson clover overseeded into dormant bermudagrass. Marchant et al. (2018) reported a mean total gain of 541 kg/ha for steers grazing mixtures of wheat, triticale, and annual ryegrass. Greenquist et al. (2009) reported total gains of 404 kg/ha for DDGS-supplemented cattle grazing smooth bromegrass. Weissend (2015) reported total gains of 591 kg/ha for steers grazing annual ryegrass and fed energy supplements.

Stocking density (steers/ha; Table 6) was not affected by monensin ($P = 0.27$), nor was a monensin × year interaction ($P = 0.90$) detected. There was a N-delivery method × year interaction ($P = 0.08$) such that stocking density was greater for NFERT, DDGS and WCS than CC and AC in Yr 1. In Yr 2, stocking density was greater for NFERT than all other N-delivery methods. In Yr 3, WCS had the greatest stocking density and was not different than DDGS, DDGS was not different than NFERT, NFERT and CC were not different, and CC did not differ from AC. Across all years, stocking density was greater ($P < 0.0001$) for NFERT, DDGS and WCS than CC and AC. Furthermore, stocking density was greatest ($P < 0.0001$) in Yr 2, intermediate in Yr 1, and least in Yr 3. Stocking densities were adjusted based upon available forage DM. Forage mass in Yr 2 was plentiful due to favorable growing conditions of mild temperatures and greater than average rainfall in December and January when annual ryegrass and clovers, particularly crimson clover, were emerging and initiating vegetative growth. Weather conditions in Yr 1 were also favorable to cool-season forage production; however, greater than average rainfall occurred in November and December before forages had initiated vegetative growth. As such, forage productivity was less than in Yr 2. Poor growing conditions in Yr 3 limited forage production and reduced stocking density compared with Yr 1 and 2. A N-delivery method × monensin interaction ($P = 0.10$) was detected for stocking density. When monensin was fed, stocking density was greatest for NFERT and WCS, DDGS was intermediate and did not differ from CC, and CC and AC were not different. When monensin was not fed, stocking density was greatest for NFERT, DDGS and WCS, WCS and CC were not different, and CC and AC were not different. Whole cottonseed was the only N-delivery method for which monensin inclusion affected ($P$ = 0.10) stocking density, with stocking density being greater when monensin was fed than not fed.

Table 6. Stocking density (steer/ha) of steers grazing annual ryegrass as affected by N-delivery method, monensin, and year

| Item | N-delivery method[1] | | | | | |
|---|---|---|---|---|---|---|
| Year | NFERT | CC | AC | DDGS | WCS | Mean |
| 1 | $3.28^{af}$ | $2.70^{bf}$ | $2.61^{bf}$ | $3.09^{af}$ | $3.09^{af}$ | $2.95^{k}$ |
| 2 | $4.49^{ae}$ | $4.32^{be}$ | $4.12^{be}$ | $4.32^{be}$ | $4.32^{be}$ | $4.42^{j}$ |
| 3 | $2.68^{bcg}$ | $2.26^{cdf}$ | $1.96^{dg}$ | $2.99^{abf}$ | $3.29^{af}$ | $2.63^{l}$ |
| Mean | $3.63^{h}$ | $3.09^{i}$ | $2.89^{i}$ | $3.49^{h}$ | $3.57^{h}$ | |
| | | | | | | |
| <u>Monensin</u> | | | | | | |
| With | $3.72^{m}$ | $3.18^{no}$ | $2.85^{o}$ | $3.37^{n}$ | $3.82^{mp}$ | 3.39 |
| Without | $3.54^{m}$ | $3.01^{no}$ | $2.93^{o}$ | $3.61^{m}$ | $3.32^{mnq}$ | 3.28 |
| Mean | $3.63^{h}$ | $3.09^{i}$ | $2.89^{i}$ | $3.49^{h}$ | $3.57^{h}$ | |

$^{a-d}$ within a row, means without a common superscript differ ($P$ < 0.10; SEM = 0.18).
$^{e-g}$ within a column, means without a common superscript differ ($P$ < 0.10; SEM = 0.18).
$^{h-i}$ within a row, means without a common superscript differ ($P$ < 0.10; SEM = 0.11).
$^{j-l}$ within a column, means without a common superscript differ ($P$ < 0.10; SEM = 0.08).
$^{m-o}$ within a row, means without a common superscript differ ($P$ < 0.10; SEM = 0.15).
$^{p-q}$ within a column, means without a common superscript differ ($P$ < 0.10; SEM = 0.15).
[1] NFERT= annual ryegrass fertilized with 112 kg N/ha in split application, CC = annual ryegrass fertilized with 56 kg N/ha and interseeded with crimson clover, AC = annual ryegrass fertilized with 56 kg N/ha and interseeded with arrowleaf clover, DDGS = annual ryegrass fertilized with 56 kg N/ha and cattle supplemented with dried distillers grains plus solubles at 0.65% BW/d, WCS = annual ryegrass fertilized with 56 kg N/ha and cattle supplemented with whole cottonseed at 0.65% BW/d.

Petersen et al. (1965) noted a positive linear relationship between gain/ha and stocking density, and that gain per animal was inversely related to stocking density once the rate at which forage was consumed surpassed the rate of growth of forage available for grazing. Furthermore, under- or overstocking may result in injury to and unwanted changes in sward composition. Bagley et al. (1988) reported stocking densities of 2.84 to

3.14 hd/ha for cattle grazing mixtures of rye, ryegrass, arrowleaf clover, and ladino clover. These densities are less than those in the current study and may reflect using visual appraisal of pasture to adjust stocking density as opposed to the double-sampling method used in this study. Hoveland et al. (1991) reported stocking densities of 5.29 hd/ha for mixed rye, annual ryegrass, and crimson clover pastures. Stocking densities of 3.5 and 2.94 steers/ha were reported by Mullenix (2014) and Marchant et al. (2018), respectively for cattle grazing monocultures or mixtures, respectively, of wheat, triticale, and ryegrass.

Grazing-days/ha (steer days/ha; Table 7) were not affected ($P = 0.29$) by feeding monensin, nor were there any N-delivery method × monensin ($P = 0.29$) or monensin × year ($P = 0.88$) interactions. A N-delivery method × year interaction ($P < 0.0001$) was detected for grazing-days/ha. In Yr 1, NFERT, DDGS and WCS had greater grazing-days than CC and AC. In Yr 2, NFERT was greater than AC, and CC, DDGS and WCS were intermediate and not different from NFERT or AC. In Yr 3, grazing-days/ha were greatest for WCS, NFERT and DDGS, DDGS and NFERT were not different from CC, and NFERT and CC were not different from AC. Across all years, grazing-days/ha were greater ($P < 0.0001$) for NFERT, DDGS and WCS than CC and AC, and across all N-delivery methods and both levels of monensin grazing-days/ha were greatest ($P < 0.0001$) for Yr 1, intermediate for Yr 2, and least for Yr 3. Grazing-days/ha were impacted by time of planting and forage productivity. Forage was planted at the appropriate time (October) in Yr 1 and 3, whereas drought delayed planting in Yr 2 until December. Cattle were put on pasture in December in Yr 1 and 3, and February in Yr 2. This delay reduced the available grazing days in Yr 2 because these cool-season forages typically are productive from December to May or June (Ball et al., 2015). Weather conditions also impacted forage DM production as described above, with forage mass production being greatest in Yr 2, intermediate in Yr 1, and least in Yr 3. These differences in forage mass impacted grazing-days/ha due to changes in stocking densities that were implemented to maintain a uniform forage allowance across treatments. Forage was able to be grazed until May in Yr 1 and 2 due to favorable temperatures and precipitation during the late winter and spring. However, in Yr 3 grazing was terminated in February due to limited forage mass caused by temperature extremes in January and February, and a lack of precipitation from November through March.

Table 7. Steer-grazing-days (d/ha) of steers grazing annual ryegrass as affected by N-delivery method and year

| | Year | | | |
|---|---|---|---|---|
| N-delivery method[1] | 1 | 2 | 3 | Mean |
| NFERT | $444^{ad}$ | $346^{bd}$ | $150^{cdef}$ | $313^{j}$ |
| CC | $340^{ae}$ | $305^{ade}$ | $127^{bef}$ | $257^{k}$ |
| AC | $329^{ae}$ | $294^{ae}$ | $110^{bf}$ | $244^{k}$ |
| DDGS | $432^{ad}$ | $305^{bde}$ | $167^{cde}$ | $302^{j}$ |
| WCS | $432^{ad}$ | $305^{bde}$ | $184^{cd}$ | $307^{j}$ |
| Mean | $395^{g}$ | $311^{h}$ | $148^{i}$ | |

$^{a-c}$ within a row, means without a common superscript differ ($P$ < 0.10; SEM=18.0).
$^{d-f}$ within a column, means without a common superscript differ ($P$ < 0.10; SEM=18.0).
$^{g-i}$ within a row, means without a common superscript differ ($P$ < 0.10; SEM=8.0).
$^{j-k}$ within a row, means without a common superscript differ ($P$ < 0.10; SEM=10.4).
[1] NFERT= annual ryegrass fertilized with 112 kg N/ha in split application, CC = annual ryegrass fertilized with 56 kg N/ha and interseeded with crimson clover, AC = annual ryegrass fertilized with 56 kg N/ha and interseeded with arrowleaf clover, DDGS = annual ryegrass fertilized with 56 kg N/ha and cattle supplemented with dried distillers grains plus solubles at 0.65% BW/d, WCS = annual ryegrass fertilized with 56 kg N/ha and cattle supplemented with whole cottonseed at 0.65% BW/d.

Islam et al. (2011) noted greater number of grazing-days/ha for rye-annual ryegrass pastures (448) than tall fescue pastures (385). Mean grazing-days/ha of 375 and

439 were reported by Marchant et al. (2018) and Mullenix et al. (2014) for cattle grazing mixtures or monocultures, respectively, of ryegrass, triticale, and wheat. Myer et al. (2008) reported 366 grazing-days/ha for annual ryegrass.

*Economic evaluation of N-delivery methods*

Economic evaluations of the interseeded-clover and supplemented N-delivery methods compared with the NFERT treatment were conducted (Table 8). Variables included cost of N fertilizer (17-17-17), seed, supplement, labor, planting, fuel, and machinery costs. Labor and fuel cost for feeding supplements were not included, as this was done in conjunction with daily checks on cattle. Inputs costs ($/ha) for CC, AC, DDGS, and WCS were 76, 60, 59, and 59%, respectively, of cost of NFERT. Cost of gain, prorated over all steers, ($/kg) for CC, AC, DDGS, and WCS were 102, 84, 57, and 60 %, respectively, of the cost of gain from NFERT. The discrepancy between the input costs and cost of gain, particularly for CC, was due to less gain/ha realized for CC than the supplemented treatments. AC also had less gain/ha than the supplemented treatments; however, because input costs were less for AC than CC, there was not as great of a difference between input costs and cost of gain. Decisions on allocation of financial resources is based upon enterprise-specific considerations. Sustained profitability is predicated upon the ability of stocker producers to purchase calves in the fall of the year when prices are typically lower due to large supply of calves from cow-calf operators who do not want to feed and care for the calves during the winter (Rankins Jr. and Prevatt, 2013). The system that has historically provided the best opportunity for profitability is acquisition of lightweight calves in the fall and increasing BW by 100 to

200 kg for sale in the spring (Prevatt et al., 2011). Based upon the three years of the current study, fertilization at half of the agronomic rate for annual ryegrass and provision of proteinaceous by-product feeds realized the least cost of gain.

Table 8. Estimated input costs ($/ha) and cost of gain ($/kg) associated with N-delivery methods for grazed annual ryegrass

| | N-delivery method[1] | | | | |
|---|---|---|---|---|---|
| Item | NFERT | CC | AC | DDGS | WCS |
| Fertilizer, $/ha | 316.32 | 157.92 | 157.92 | 157.92 | 157.92 |
| Seed, $/ha | 25.16 | 107.85 | 33.58 | 25.16 | 25.16 |
| Supplement, $/ha | 0.00 | 0.00 | 0.00 | 3.65 | 6.82 |
| Labor, $/ha | 9.34 | 7.41 | 7.41 | 7.41 | 7.41 |
| Fuel, $/ha | 94.48 | 62.98 | 62.98 | 62.98 | 62.98 |
| Machine cost, $/ha | 25.93 | 20.68 | 20.68 | 20.68 | 20.68 |
| Input costs, $/ha | 471.23 | 356.84 | 282.57 | 277.80 | 280.97 |
| Cost of gain, $/kg | 1.08 | 1.10 | 0.91 | 0.62 | 0.65 |

[1] NFERT= annual ryegrass fertilized with 112 kg N/ha in split application, CC = annual ryegrass fertilized with 56 kg N/ha and interseeded with crimson clover, AC = annual ryegrass fertilized with 56 kg N/ha and interseeded with arrowleaf clover, DDGS = annual ryegrass fertilized with 56 kg N/ha and cattle supplemented with dried distillers grains plus solubles at 0.65% BW/d, WCS = annual ryegrass fertilized with 56 kg N/ha and cattle supplemented with whole cottonseed at 0.65% BW/d.

## Summary and Conclusion

Weather conditions and N-delivery method greatly impacted forage production and length of grazing season in the current study. Forage allowance was successfully maintained at 1 kg forage DM/kg steer BW by periodically adjusting stocking density on the basis of available forage DM. Average daily gain, total gain/ha, stocking densities, and grazing-d/ha were greater for NFERT, DDGS and WCS than CC and AC. Monensin inclusion improved total gain/ha and stocking densities compared with no monensin. Cost of gain was least for treatments receiving supplement and greatest for the interseeded crimson clover treatment due primarily to lower gain/ha from the latter. Results are interpreted to mean that supplementation with a high-protein by-product feed for cattle grazing annual ryegrass can maintain or improve cattle performance characteristics, requires greater stocking densities, and results in more grazing-days/ha than fertilized annual ryegrass or annual ryegrass interseeded with annual clovers, and may be more economically viable based upon input costs; however cattle purchase and sale prices may affect net income, which was not evaluated in the current study.

## III. Effects of nitrogen-delivery method and monensin on productivity, nutritive value, and botanical composition of grazed annual ryegrass pasture

### Introduction

Nitrogen (N) fertilizer represents the single greatest input cost of forage production, with costs rising steadily from the mid-1990s through the early 2010s, followed by a small decline in the late 2010s. Alternatives to N fertilizer that can maintain or enhance forage yield and nutritive value would be advantageous for forage producers. Alternative N-delivery methods include, but are not limited to, interseeded legumes and provision of high-protein feeds to grazing cattle.

Annual ryegrass (*Lolium multiflorum* Lam.) is a cool-season annual bunchgrass (Hall, 1992) that can produce between 6,000 and 13,000 kg/ha of forage DM when fertilized (Redfearn et al., 2002), and there are more than 1 million ha of annual ryegrass grown annually in the Southeast (Ball et al., 2007). Crimson clover (*Trifolium incarnatum* L.) and arrowleaf clover (*Trifolium vesiculosum* Savi) are annual legumes that are well adapted to the Southeast (Ball et al., 2015). Crimson clover has been reported to fix an average of 155 kg N/ha (Brink, 1990), and arrowleaf clover averaged 137 kg N/ha (Evers, 1985). Pederson and Ball (1991) reported mean forage DM mass (kg/ha) of 3,999 for crimson clover and 3,892 for arrowleaf clover.

Dry matter yield at any stage of forage regrowth is reduced when N fertilization rates are reduced (Peyraud and Astigarraga, 1998). Cool-season grass/legume mixtures have been shown to have similar forage DM yields as cool-season grass monocultures, and greater forage DM yield than legume alone (Deak et al., 2007; Kunelius and Narasimhalu, 1983; Springer et al., 2007). A linear relationship between forage CP concentration and high N fertilizer application rates (800 kg/ha) has been reported for perennial ryegrass (Reid, 1966), and a marked reduction in CP concentration has been observed when application of N fertilizer is reduced (Blaser, 1964), resulting in as much as a 2-fold reduction (Nowakowski, 1962). Several beneficial aspects of clover inclusion in pastures have been reported, including increased forage CP concentration, greater forage DM digestibility, and improved forage mineral composition for cattle, resulting in increased intake and weight gain (Marten, 1985). Cattle return about three-fourths of N, four-fifths of P, and nine-tenths of K ingested in excreta (Brady and Weil, 2002). Forage CP concentrations were less for pastures where dried distillers grains plus solubles were fed than pastures that received N fertilizer (Greenquist et al., 2009; Watson et al., 2012).

The objective of this experiment was to evaluate the effects of N-delivery method and provision of monensin on forage nutritive value and clover composition of interseeded annual ryegrass pasture grazed by stocker cattle.

## Materials and Methods

All experimental procedures were implemented according to a protocol approved by the Auburn University Animal Care and Use Committee (PRN 2014-2438).

### *Treatment Structure*

Treatments were randomly assigned to thirty 0.81-ha pastures in Yr 1 of a 3-yr study, with the restriction that the same treatment could not be applied to adjacent pastures. Treatments in Yr 2 and 3 were maintained on the same pastures to which they had been assigned in Yr 1. Treatment structure was a completely randomized 5 × 2 factorial with five N-delivery methods, with or without monensin (n = 3 pastures/treatment) provided in a custom-formulated, compressed mineral block (Ridley Block Operations Mankota, MN). Nitrogen-delivery methods included: annual ryegrass fertilized with 112 kg N/ha in a split-application (NFERT), annual ryegrass interseeded with crimson clover and fertilized with 56 kg N/ha at time of establishment (CC), annual ryegrass interseeded with arrowleaf clover and fertilized with 56 kg N/ha at time of establishment (AC), annual ryegrass fertilized with 56 kg N/ha and cattle supplemented with distillers dried grains plus solubles at the rate of 0.65% BW daily (DDGS), and annual ryegrass fertilized at 56 kg N/ha and cattle supplemented with whole cottonseed at the rate of 0.65% BW daily (WCS). Interseeding and supplementation rates were selected to approximate the delivery of an additional 56 kg N/ha that the NFERT treatment received in the second of the split-application.

*Pasture Establishment*

A 3-yr winter grazing trial was conducted at the E.V. Smith Research Center located 48 km from Auburn University in Milstead, AL (32.443$^{o}$ N lat., 85.897$^{o}$ W long.). Thirty 0.81-ha paddocks that consisted of a fine sandy loam were used. These paddocks had previously been planted to annual ryegrass, and prior to that with warm-season grasses including bermudagrass (*Cynodon dactylon*), bahiagrass (*Paspalum notatum*), and dallisgrass (*Paspalum dilatatum*) for summer grazing.

Each year in early October prior to planting, pastures received 329 kg of 17-17-17 fertilizer to provide 56 kg N/ha. Pastures were planted on Oct. 16, 2015, Dec. 23, 2016, and Oct. 25, 2017 in Yr 1, 2, and 3, respectively; planting was delayed in Yr 2 compared with Yr 1 and 3 because of exceptionally dry soil conditions resulting from below-average precipitation earlier in the fall. Each year, pastures assigned to NFERT, DDGS, and WCS were seeded at a rate of 34 kg/ha of 'Marshall' annual ryegrass, and the interseeded pastures were seeded at a rate of 17 kg/ha of 'Marshall' annual ryegrass and either 34 kg/ha of 'Dixie' crimson clover or 9 kg/ha of 'Blackhawk' arrowleaf clover (Wax Seed Company, Amory, MS) to a depth of 0.6 cm into a prepared seedbed. Pastures assigned to the NFERT N-delivery method received an additional 56 kg N/ha as liquid N (28% N solution of ammonium nitrate and ammonium thiosulfate that provided 5% S) on Feb. 23, 2016, March 20, 2017, and Jan. 24, 2018.

*Pasture Management and Forage Analysis*

As reported previously (see Chapter II), pastures were initially stocked with 90 'tester' steers (3 steers/pasture), and 'put-and-take' steers were used to maintain a forage allowance target of 1 kg forage DM/kg steer BW. Cattle were weighed every 28 d in the morning following feed restriction for 24 hr in order to derive shrunk weights, and forage mass was determined concurrently using the double-sampling method reported by Frame (1981). Twenty-five forage heights were recorded from each 0.81-ha pasture using a 0.25-$m^2$ disk meter. Seventy-two calibration samples were taken from the 18 pastures assigned to the NFERT, CC and AC treatments by recording forage heights and clipping forage to a stubble height of approximately 5 cm. Samples for nutritive value analysis were collected at the same time from the top 20 cm of the sward canopy and consisted of 10 to 12 grab samples per pasture. Samples were placed in individual plastic bags and stored in a cooler for transport to the Auburn University Ruminant Nutrition Laboratory where they were transferred to individual paper bags and dried at $60^{o}$ C to a constant weight. After drying, sample weights were plotted against their respective height values, and the resultant prediction equations ($y = 88.755x + 106.25$; $r^2$ ranged from 0.10 to 0.88, mean $r^2 = 0.64$) were used to determine the approximate forage DM mass for each pasture and to adjust stocking densities. To determine clover percentage in pastures, samples were hand separated into clover and grass botanical components. After drying, clover and grass components were weighed individually and summed to determine total sample weight. Clover percentage was calculated as clover weight divided by total weight of the sample and multiplied by 100. Clover mass of the pasture was calculated as clover percent of pasture multiplied by the forage mass of pasture at each collection period.

Dried forage samples were ground to pass through a 1-mm screen in a Wiley mill (Thomas Scientific, Swedesboro, NJ). Dry matter concentration was conducted by drying samples at $100^{0}$ C for 12 hr according to AOAC procedures (1995). Forage concentration of N was determined by the Kjeldahl procedure according to AOAC procedures (1995). Samples were analyzed for IVTD by the Van Soest (1991) modification of the Tilley and Terry procedure (1963) using the Daisy II® incubator system (Ankom Technology, Macedon, NY). Ruminal fluid for batch-culture fermentation was collected from a fistulated Holstein dairy cow maintained at the Auburn University College of Veterinary Medicine dairy facility. The cow had free access to bermudagrass pasture and was limit-fed a supplement containing cracked corn, distillers dried grains, corn gluten feed, soyhull pellets, soybean meal, cottonseed meal, and cottonseed hulls. Fluid was placed in preheated insulated containers and transported immediately to the Auburn University Ruminant Nutrition Laboratory for processing. Forage degradable intake protein (DIP) was analyzed using a *Streptomyces griseus* protease procedure (Type XIV Bacterial; Sigma-Aldrich, Co. St. Louis, MO) as described by Mathis et al. (2001). Briefly, forage samples were weighed out to obtain 15 mg of N based on N concentration of the forage sample and placed in 125-mL Erlenmeyer flasks. Forty mL of a borate-phosphate buffer solution were added to each flask and incubated at 39$^o$ C for 1 hr in a shaking water bath. After incubation, 10 mL of protease solution was added to each flask and incubated for 48 hr at 39$^o$ C in a shaking water bath. After 48 hr, samples were filtered through Whatman #540 filter paper using a cone-shaped funnel and rinsed with 400 mL of distilled water to remove any incubation media. After rinsing, samples were dried in a 100$^o$ C oven overnight to obtain residual DM weight. Samples were then analyzed for N using the Kjeldahl assay with a Kjeltec Analyzer Unit Foss Tecator (Hoganas, Sweden). Percentage undegradable intake protein (UIP) was calculated by dividing the mg of residual N by the mg of initial N and multiplying by 100. Percentage degradable intake protein (DIP) was calculated by subtracting percentage UIP from 100 for each sample.

*Statistical Analysis*

Forage quality data were analyzed using PROC MIXED of SAS 9.4 (SAS Inst. Inc., Cary, NC.) for a 5 × 2 factorial design consisting of 5 N-delivery methods and 2 levels (+/-) of monensin. Dependent variables included forage percentage IVTD, concentrations of CP and DIP. Pasture botanical composition data were analyzed using PROC MIXED of SAS 9.4 (SAS Inst. Inc., Cary, N.C.) for a 2 × 2 factorial design consisting of 2 clover species and 2 levels (+/-) of monensin. Dependent variables included pasture clover percentage and pasture clover DM mass. Clover mass was calculated as total forage DM mass multiplied by clover percentage per pasture. Main effects were N-delivery method, ionophore, yr, and their 2-way interactions. Because there were no significant 3-way interactions detected for any of the dependent variables evaluated, their sum of squares and associated df were apportioned to the model error term (residual) for significance testing. Month was included as a repeated measure for all dependent variables. The PDIFF option of LSMEANS was used to separate means when protected by F-test at $\alpha = 0.10$, trends were declared at $\geq 0.10$ to $\leq 0.15$.

## Results and Discussion

### *Temperature and Precipitation*

Monthly mean and 30-yr average monthly temperatures from August to May of each yr at the research station are presented in Figure 1, and monthly and 30-yr average monthly precipitation totals from August to May of each yr at the research station are presented in Figure 2 (See Chapter II). In Yr 1, monthly mean temperatures approximated 30-yr averages. Precipitation was lower than average preceding and at time of planting in September and October, respectively, but greater than average rainfall in November and December allowed for acceptable forage production with a start date for grazing of Dec 14, 2015. Adequate rainfall throughout the remainder of the winter and spring allowed for forage production that supported a typical 140-d grazing season. As in Yr 1, mean monthly temperatures in Yr 2 were very similar to 30-yr averages. However, precipitation was extremely low in September, October and November, which delayed planting until Dec 23, 2016. During and after December, rainfall was adequate to support high forage mass production that enabled cattle to be turned out on Feb 15, 2017 for an 84-d grazing season. In Yr 3, mean temperatures were similar to 30-yr averages from October through December, followed by a much colder-than-average January and a very warm February. Precipitation was very low in September but was near average in October and allowed for pastures to be planted on Oct 25, 2017. After planting, precipitation was well below average from November through April, which limited forage production but enabled cattle to be turned out on Dec 21, 2017. However, due to lack of rainfall, particularly in January, coupled with extreme fluctuations in temperature during the same period, forage production was limited, and the grazing season only lasted 56 d.

*Forage Nutritive Value*

In vitro true digestibility of forage DM (Table 9) averaged 88.8% and was not impacted by N-delivery method ($P = 0.95$) or monensin inclusion ($P = 0.47$), nor were N-delivery method × monensin ($P = 0.69$), N-delivery method × yr ($P = 1.00$), or monensin × yr interactions ($P = 0.96$) detected. Forage digestibility differed among yr ($P < 0.0001$) and was greatest in Yr 3, intermediate in Yr 2, and least in Yr 1. Digestibility may have differed as a result of extreme differences in weather conditions that caused grazing to be discontinued in February of Yr 3 when forage was still actively growing; whereas grazing in Yr 1 and 2 was terminated in May when the forage was maturing. Digestibility values reported by Marchant et al. (2018) for annual ryegrass, wheat, and triticale polycultures

(90%) and Weissend (2015) for annual ryegrass (93%) are similar to those observed in Yr 3 of the current study, and greater than in Yr 1 and 2. Hafley (1996) reported annual ryegrass IVDMD values ranging from 59 to 74%, and Redfearn et al. (2002) reported values of 62 to 89%. The lower digestibility values reported by Hafley (1996) and Redfearn et al. (2002) may reflect differences in cultivars and stage of ryegrass maturity from those in the current study. Kunelius and Narasimhalu (1983) reported IVDMD values for legume/Italian ryegrass mixtures of 73.4% for Persian clover, 72.0% for red clover, 70.5% for alfalfa, and 71.4% for birdsfoot trefoil. These values are lower than those reported in the current study, possibly because their study was conducted from July to October and their use of an apparent-digestibility procedure as opposed to the true-digestibility procedure used here. Even with differences noted among yrs forage quality remained high and would be unlikely to impact cattle performance.

Table 9. Forage IVTD (% DM) of grazed annual ryegrass as affected by year

| Year | IVTD |
|---|---|
| 1 | $86.00^{c}$ |
| 2 | $87.50^{b}$ |
| 3 | $92.78^{a}$ |

$^{a\text{-}c}$ within a column, means without a common superscript differ (*P* < 0.10; SEM = 0.59).

Forage CP concentration (Table 10) averaged 16.6% and was not impacted by N-delivery method (*P* = 0.23) or monensin inclusion (*P* = 0.28); nor were N-delivery method × monensin (*P* = 0.89), N-delivery method × yr (*P* = 0.68), or monensin × yr (*P* = 0.68) interactions detected. Forage CP concentrations differed among yr (*P* < 0.0001) and was greatest in Yr 3, intermediate in Yr 2, and least in Yr 1.

Table 10. Forage crude protein (% DM) of grazed annual ryegrass as affected by year

| Year | CP |
|---|---|
| 1 | $14.1^{c}$ |
| 2 | $17.2^{b}$ |
| 3 | $18.6^{a}$ |

$^{a-c}$ within a column, means without a common superscript differ ($P < 0.10$; SEM = 0.45).

Concomitantly with IVTD, precipitation and temperature impacts on forage CP concentration may partly explain these differences. Less forage production and an earlier grazing season when forages were in a vegetative state may account for the greater protein concentration in Yr 3. Similarly, the later planting date in Yr 2 would result in forage remaining in a vegetative state later in the season and thus have greater CP concentration than in Yr 1, which was a more typical grazing season wherein the forage was entering the reproductive stage in the latter phase of the season. Mean forage protein concentrations were greater than the 10.2% recommended by the NRC (2016) for growing cattle to gain 1 kg/d. These values approximate those reported by Redfearn et al. (2002) that ranged from 29% in December to 10% in May, and those reported by Hafley (1996) of 10 to 27%. Weissend (2015) reported mean forage CP concentration of 18.5%, with a range of 14 to 23% for annual ryegrass. Kunelius and Narasimhalu (1983) reported CP concentrations for legume/Italian ryegrass mixtures of 16.6% for Persian clover mixture, 17.3% for red clover mixture, 17.9% for alfalfa mixture, and 15.0% for birdsfoot trefoil. These CP concentrations are similar to those observed in the current study. There were no differences among N-delivery methods in the current study; however, Watson et al. (2012) reported CP concentrations of 17.9 and 15.0% for N-fertilized smooth bromegrass and smooth bromegrass where cattle were supplemented with DDGS at 0.6% BW daily, respectively.

Degradable intake protein (% of forage CP; Table 11) was not affected (P = 0.37) by monensin inclusion, nor were N-delivery method × monensin (*P* = 0.48), N-delivery method × yr (*P* = 0.61), or monensin × yr (*P* = 0.59) interactions detected. Degradable intake protein was greater (*P* = 0.0006) for NFERT, AC, DDGS, and WCS than CC (92.3%), and greatest (*P* < 0.0001) in Yr 1, intermediate in Yr 2, and least in Yr 3.

Table 11. Forage degradable intake protein (% CP) of grazed annual ryegrass as affected by N-delivery method and year

| Item | DIP |
|---|---|
| N delivery method[1] | |
| NFERT | 93.5[a] |
| CC | 92.3[b] |
| AC | 93.1[a] |
| DDGS | 93.4[a] |
| WCS | 93.5[a] |
| | |
| Year | |
| 1 | 94.5[c] |
| 2 | 93.6[d] |
| 3 | 91.3[e] |

[a-b] within a column, means without a common superscript differ (*P* < 0.10; SEM = 0.23).
[c-e] within a column, means without a common superscript differ (*P* < 0.10; SEM = 0.18).
[1] NFERT= annual ryegrass fertilized with 112 kg N/ha in split application, CC = annual ryegrass fertilized with 56 kg N/ha and interseeded with crimson clover, AC = annual ryegrass fertilized with 56 kg N/ha and interseeded with arrowleaf clover, DDGS = annual ryegrass fertilized with 56 kg N/ha and cattle supplemented with dried distillers grains plus solubles at 0.65% BW/d, WCS = annual ryegrass fertilized with 56 kg N/ha and cattle supplemented with whole cottonseed at 0.65% BW/d.

A plausible reason for lower forage DIP for the CC N-delivery method treatment is not readily apparent apart from the fact that crimson clover is an early-maturing species, which may have reduced sward CP digestibility compared with the other treatments that were wholly or mostly dominated by annual ryegrass. The NRC (2016) reported annual ryegrass contains 74% DIP (% CP), which was corroborated by Vendramini et al. (2008) who reported in situ DIP concentrations of 70, 72, and 77 % for mixed pastures of rye-annual ryegrass that were fertilized at the rates of 0, 48, or 80 kg N/ha. Haugen et al. (2006) reported in situ-estimated DIP concentrations of 99, 98, and 99% for kura clover, birdsfoot trefoil, and alfalfa, respectively, whereas Coblentz et al. (1999) reported in vitro DIP concentrations of 75% each for alfalfa and red clover after a 48-hr incubation period. The differences in DIP values between those reported by Coblentz et al. (1999) and from the current study may be due to differences in concentration of *Streptomyces griseus* protease in the buffer solutions used, 0.066 units/ml and 0.33 units/ml, respectively. However, even with these differences in DIP among treatments and growing seasons, DIP remained above 90% of forage CP, which meets requirements for microbial protein synthesis. The NRC (2016) reports a required DIP concentrations of 60.2 (% of CP) for a 350-kg animal gaining 1.0 kg/d. The DIP concentrations reported herein are greater than those reported in other studies. Amrane and Michalet-Doreau (1993) reported CP degradability values of 80.9 and 79.0% for ryegrass and alfalfa, respectively. Similarly, Van Vuuren et al. (1990) reported CP digestibility of 76.8% for annual ryegrass. These values are different than those in the current study due possibly to the use of *in situ* procedures, whereas the current study utilized a protease/buffer solution. However, Vendramini et al. (2006) also utilized a protease/buffer solution and reported a DIP value of 73% for mixed rye-annual ryegrass that had been interseeded into dormant bahiagrass pasture.

*Clover Characteristics*

A monensin × yr interaction (P = 0.74) was not detected for clover abundance (% of sward DM; Table 12). A clover species × monensin interaction (P = 0.003) was detected such that clover abundance for CC with monensin was greater than CC without monensin. Clover abundance was less for AC than CC at both monensin inclusion levels, and monensin inclusion had no effect on clover presence in AC. A clover species × yr interaction (P = 0.0003) was also detected such that clover abundance for CC was greatest in Yr 1 and did not differ between Yr 2 and 3, whereas clover abundance for AC did not differ among years.

Table 12. Clover abundance (%) in grazed annual ryegrass as affected by clover species, year, and monensin inclusion

| Item | Clover species[1] | | Mean |
|---|---|---|---|
| Year | CC | AC | |
| 1 | $19.4^{ac}$ | $1.0^{b}$ | $10.2^{g}$ |
| 2 | $10.8^{ad}$ | $0.3^{b}$ | $5.6^{h}$ |
| 3 | $8.5^{ad}$ | $0.7^{b}$ | $4.6^{h}$ |
| Mean | $12.9^{e}$ | $0.7^{f}$ | |
| | | | |
| Monensin | | | |
| With | $16.7^{ik}$ | $0.4^{j}$ | $8.6^{m}$ |
| Without | $9.1^{il}$ | $0.9^{j}$ | $5.0^{n}$ |
| Mean | $12.9^{e}$ | $0.7^{f}$ | |

$^{a-b}$ within a row, means without a common superscript differ ($P < 0.10$; SEM = 1.69).
$^{c-d}$ within a column, means without a common superscript differ ($P < 0.10$; SEM = 1.69).
$^{e-f}$ within a row, means without a common superscript differ ($P < 0.10$; SEM = 0.99).
$^{g-h}$ within a column, means without a common superscript differ ($P < 0.10$; SEM = 1.20).
$^{i-j}$ within a row, means without a common superscript differ ($P < 0.10$; SEM = 1.38).
$^{k-l}$ within a column, means without a common superscript differ ($P < 0.10$; SEM = 1.38).
$^{m-n}$ within a column, means without a common superscript differ ($P < 0.10$; SEM = 0.99).
[1]CC = annual ryegrass fertilized with 56 kg N/ha and interseeded with crimson clover, AC = annual ryegrass fertilized with 56 kg N/ha and interseeded with arrowleaf clover.

Weather conditions would be expected to impact clover production and concomitantly the percentage of clover within pasture. Conditions in Yr 1 enabled planting in October as recommended and grazing through May, encompassing the growing seasons of both crimson and arrowleaf clover. Planting was delayed until December in Yr 2 due to drought, but conditions were favorable thereafter for growth even though crimson clover was entering reproductive growth phase in the latter half of the grazing season. In Yr 3 pastures were seeded at the recommended time, but forage production was limited due to widely extreme weather fluctuations in January and February, just prior to arrowleaf clover entering vegetative production and during principally vegetative growth of crimson clover. The reason for the extremely low productivity of AC across the 3-yr study is not readily evident, but a possible cause may have been competition with the annual ryegrass. Annual ryegrass was in vegetative production during February and March when arrowleaf clover normally initiates growth, and thus may have outcompeted the arrowleaf clover for resources and inhibited its growth. Comparatively, crimson clover initiates growth around the same time as annual ryegrass and thus was able to compete more effectively with annual ryegrass. Clover abundance in pastures supplied with monensin may have been greater than in those without monensin because monensin has been shown to reduce DMI for high-forage diets (Fox et al., 1988; NRC, 2016). Thus, lower grazing pressure from reduced DMI may have allowed for increased clover abundance. Clover abundance in the current study was less than that reported by Kunelius and Narasinhalu (1983), who reported 60% legume contribution and 40% Italian ryegrass in a study evaluating DM yield and nutritive quality of legume ryegrass mixtures. Hoveland et al. (1976) reported that, in rye-annual ryegrass-arrowleaf clover systems, arrowleaf clover represented 5 to 25% of forage mass from December to February and increased to 30 to 50% of forage mass in March and April. Sanderson et al. (2005) reported white clover ranged from 20 to 50% in binary mixture with orchardgrass and grazed from April to August. Deak et al. (2007) reported clover presence in binary systems that were mob-grazed over 3 yr. Red clover grown with tall fescue or chicory ranged from 51 to 27%, and white clover grown with Kentucky bluegrass or perennial ryegrass ranged from 66 to 10%. However, the growth distributions for these clovers differed, with red clover having a high abundance at the beginning of the 3-yr study and decreasing as time progressed, whereas white clover had low abundance at the beginning and increased as the study progressed. Solomon et al. (2011) observed white clover abundance in a mixture with annual ryegrass of 23 and 13% at high (6 hd/ha) and low (3 hd/ha) stocking rates, respectively, under continuous grazing. Clover abundance in the current study was generally less than reported in these studies, which may reflect use of perennial or biennial clovers in the northern regions of the US or Canada where weather conditions are more conducive to clover production (Deak et al., 2007; Kunelius and Narasinhalu (1983); Sanderson et al., 2005; Solomon et al., 2011). Hoveland et al. (1976) utilized arrowleaf clover; however, that study was conducted in northern GA where conditions may have been more conducive to arrowleaf clover production.

No monensin × yr interaction (*P* = 0.24) was detected for clover mass (kg/ha; Table 13). A clover species × monensin interaction (*P* = 0.001) was detected where CC with monensin had greatest clover mass than CC without monensin, but AC with and without monensin did not differ. A clover species × yr interaction (*P* = 0.007) was also detected such that clover mass for CC was greatest in Yr 1, intermediate in Yr 2, and least in Yr 3, whereas clover mass did not differ for AC among yrs. Across all yrs, clover mass for CC was greater (*P* < 0.0001) than AC. Clover mass was greater (*P* = 0.003) in Yr 1 than Yr 2 and 3, and clover mass was greater (*P* = 0.02) when monensin was fed than when it was not fed.

Table 13. Clover mass (kg/ha) in grazed annual ryegrass as affected by clover species, year, and monensin inclusion

| Item | Clover species[1] | | Mean |
|---|---|---|---|
| Year | CC | AC | |
| 1 | $202.8^{ac}$ | $10.3^{b}$ | $106.5^{h}$ |
| 2 | $112.1^{ad}$ | $4.2^{b}$ | $58.1^{i}$ |
| 3 | $38.9^{e}$ | 3.3 | $21.1^{i}$ |
| Mean | $117.9^{f}$ | $5.9^{g}$ | |
| | | | |
| Monensin | | | |
| With | $174.5^{jl}$ | $0^{k}$ | $86.6^{n}$ |
| Without | $61.3^{jm}$ | $13.1^{k}$ | $37.2^{o}$ |
| Mean | $117.9^{f}$ | $5.9^{g}$ | |

$^{a-b}$ within a row, means without a common superscript differ (*P* < 0.10; SEM = 24.8).
$^{c-e}$ within a column, means without a common superscript differ (*P* < 0.10; SEM = 24.8).
$^{f-g}$ within a row, means without a common superscript differ (*P* < 0.10; SEM = 14.46).
$^{h-i}$ within a column, means without a common superscript differ (*P* < 0.10; SEM = 17.54).
$^{j-k}$ within a row, means without a common superscript differ (*P* < 0.10; SEM = 20.25).
$^{l-m}$ within a column, means without a common superscript differ (*P* < 0.10; SEM = 20.25).
$^{n-o}$ within a column, means without a common superscript differ (*P* < 0.10; SEM = 14.46).
[1]CC = annual ryegrass fertilized with 56 kg N/ha and interseeded with crimson clover, AC = annual ryegrass fertilized with 56 kg N/ha and interseeded with arrowleaf clover.

Clover mass was calculated as forage mass (see Chapter 2) multiplied by percentage clover abundance. Because forage mass was greatest in Yr 1, intermediate in Yr 2, and least in Yr 3, clover masses follow the same trend for CC, whereas the lack of measurable arrowleaf clover production resulted in no differences for AC among yrs.

Differences in clover mass were also due to variable weather conditions each year as discussed for clover abundance. Similarly, reduced forage intake when monensin was fed may possibly explain the greater clover mass.

## Summary and Conclusion

Forage nutritive value was impacted by N-delivery method, yr, and feeding of monensin. *In vitro* true digestibility and forage CP concentration were not affected by N-delivery method or monensin but were affected by variable temperature and precipitation among years, which impacted forage planting date and how long they could be grazed. Degradable intake protein of forage was affected by N-delivery method and yr. Due to the low abundance of arrowleaf clover, 4 of the N-delivery methods contained a majority of annual ryegrass, which matures later than crimson clover and may partly explain the lower DIP observed for CC. Clover abundance and mass were impacted by clover species, monensin inclusion, and yr. Arrowleaf clover had very low contribution to the sward and mass, whereas crimson clover was relatively abundant throughout the grazing seasons. Monensin may have reduced intake when fed to cattle and thus resulted in greater clover mass when provided. Different weather conditions among yrs impacted clover abundance and mass, with the greatest abundance and mass occurring during the first yr when temperatures and precipitation were optimal for cool-season annual-forage production from December to May.

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Appendix 1. Economic Analysis Calculations

Prices were averaged across the three years of the study.

<u>N Fertilization</u>

17-17-17: $415/ton

Cost for NFERT at 112 kg N/ha in split application:
Total amount of 17-17-17 used/paddock = [total amount of N used ÷ percent N in 17-17-17]
= 112 kg N ÷ 0.17 N/kg 17-17-17
= 659 kg/ha 17-17-17
Total cost of 17-17-17= [total amount of 17-17-17 used × cost/kg 17-17-17]
= 659 kg/ha 17-17-17 × $0.48/kg
= $316.32/ha

Cost for CC, AC, DDGS, and WCS at 56 kg N/ha:
Total amount of 17-17-17 used/paddock = [total amount of N used ÷ percent N in 17-17-17]
= 56 kg N ÷ 0.17 N/kg 17-17-17
= 329 kg/ha 17-17-17
Total cost of 17-17-17= [total amount of 17-17-17 used × cost/kg 17-17-17]
= 329 kg/ha 17-17-17 × $0.48/kg
= $157.92/ha

<u>Seed</u>

Annual ryegrass $42/50-lb bag
Crimson clover $36/10-lb bag
Arrowleaf clover $42/10-lb bag

NFERT, DDGS, and WCS annual ryegrass monocultures:
Total cost of ryegrass seed = [total amount of ryegrass seed used × cost/kg ryegrass seed]
= 13.6 kg/ha ryegrass seed × $1.85/kg
= $25.16/ha

CC annual ryegrass/crimson clover mixture:
Total cost of ryegrass seed = [total amount of ryegrass seed used × cost/kg ryegrass seed]

= 6.08 kg/ha ryegrass seed × $1.85/kg
= $11.25/ha

Total cost of crimson clover seed = [total amount of crimson clover seed used × cost/kg ryegrass seed]
= 13.6 kg/ha ryegrass seed × $7.93/kg
= $107.85/ha

AC annual ryegrass/arrowleaf clover mixture:
Total cost of ryegrass seed = [total amount of ryegrass seed used × cost/kg ryegrass seed]
= 13.6 kg/ha ryegrass seed × $1.85/kg
= 25.16$/ha

Total cost of arrowleaf clover seed = [total amount of arrowleaf clover seed used × cost/kg ryegrass seed]
= 3.63 kg/ha ryegrass seed × $9.25/kg
=$ 33.58/ha

Supplement

DDGS $110/ton
WCS $205/ton
Total cost of DDGS = [total amount of DDGS used × cost/kg DDGS]
= 30.38 kg/ha DDGS × $0.12/kg
= $3.65/ha

Total cost of WCS = [total amount of WCS used × cost/kg WCS]
= 29.63 kg/ha WCS × $0.23/kg
= $6.82/ha

Labor

Establishment of pastures = [hr to establish pasture × labor rate]
= 0.62 hr/ha × $9.00/hr
= $5.56/ha

Fertilization:
Split application for NFERT = [ hr to fertilize × labor rate]
= 0.42 hr/ha × $9.00/hr
= $3.78/ha

Single application for CC, AC, DDGS, WCS = [ hr to fertilize × labor rate]
= 0.21 hr/ha × $9.00
= $1.85/ha

Fuel

Disking and seeding = [ gal diesel/ha × $/gal diesel]
= 12.35 gal/ha × $2.55/gal
= $31.49

Fertilization:

Split application for NFERT = [ gal diesel/ha × $/gal diesel]
= 24.70 gal/ha × $2.55/gal
= $62.99/ha

Single application for CC, AC, DDGS, WCS = [ gal diesel/ha × $/gal diesel]
= 12.35 gal/ha × $2.55/gal
= $31.49

Fixed cost of machinery

A rate of $25.00/hr (Prevatt et al., 2008) is used to calculate machinery costs for establishment and fertilization or pastures. Machinery costs were not included for feeding as feed was delivered during daily inspection of cattle.

Establishment cost = [hr to establish/ha × $25/hr]
= 0.61 hr/ha × $25/hr
= $15.43/ha

Fertilization:

Split application for NFERT = [hr to fertilize/ha × $25/hr]
= 0.42 hr/ha × $25.00/hr
= $10.50/ha

Single application for CC, AC, DDGS, WCS = [hr to fertilize/ha × $25/hr]
= 0.21 hr/ha × $25.00/hr
= $5.25/ha