ESTIMATING SPOTTED KNAPWEED INTAKE OF SHEEP USING NIRS TECHNOLOGY

by

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ABSTRACT

Targeted grazing is proving to be effective in controling spotted knapweed infestations. Maximizing the potential of targeted grazing requires a method to determine the botanical composition of individual diets of grazing animals over time Fecal near infrared reflectance spectroscopy (NIRS) has been used to estimate the botanical composition of sheep consuming, leafy spurge, mountain big sage brush, and juniper, but has not been used to estimate dietary composition of sheep consuming spotted knapweed. Fecal NIRS spectra collected from three feeding trials were used to develop modified partial least squares regression equations to predict percent spotted knapweed in sheep diets. Independent validation of individualy developed equations resulted in R^2 values of .22 - .72. An equation developed by combining data from all three trials resulted in acceptable levels of precision (R^2= .96) and was used to analyze data collected from range fecal sampling trials conducted in 2006. Two fecal sampling trials were conducted in 2006 to determine NIR's ability to detect differences in dietary composition of sheep grazing spotted knapweed infested range over time. Approximately 90 fecal samples were collected on July 13 and again on August 15 from a band of 900 ewes grazing spotted knapweed infested range to determine changes in diet over time. Fecal samples from five randomly selected ewes in the same band were collected weekly (June 22 – August 17) to detect variation in indiviudal intake over time On July 13, 55% of ewes from had 0-5% spotted knapweed in their diets while 44% had 5-20% spotted knapweed in their diets. On August 15, 1% of ewes, had <10% spotted knapweed in their diets and 44% had 20-25% spotted knapweed in their diets. With the exception of July 6, individual intake of spotted knapweed was similar (P > .05) from June 22, through July 20, but was greater (P > .05)from July 27 through August 17 than from June 22 through July 20. These results suggest that the appropriate time to apply grazing for spotted knapweed control is later in the growing season when sheep are including more of the target plant in their diet.

CHAPTER 1

INTRODUCTION

Spotted knapweed (*Centaurea stoebe* L. ssp micranthos (Gugler) Hayek), was introduced from Euarsia in the late 1800s, and now infests every county in Washington, Idaho, Wyoming and Montana (MWSCC 2008). It is an aggressive invasive species that has replaced native perrenials on foothill ranges and pastures in the Northern Rocky Mountains (Tyser and Key 1988). Monotypic infestations of spotted knapweed can reduce species richness, water infiltration, increase soil erosion and reduce livestock and wildlife forage (Lacey et al. 1989, Watson and Renney 1974). Management of spotted knapweed using chemical and mechanical methods, fire and biological agents has been successful to some degree but is often constrained by cost, social and environmental concerns (Griffith and Lacey 1991).

Domestic livestock grazing as a weed management tool has been used to control noxious weeds. Grazing offers a potential cost effective alternative weed management tool for landowners and an economic return for livestock producers (Lacey 1987). By selecting some plants and avoiding others, small ruminants can change the competitive interactions between plants and consequently affect the structure of the ecosystem (Walker and Hodgkinson 1999). Sheep are well suited for weed control because of their morphology and dietary preferences. They are roughage eaters (Hofmann 1993) but typically consume a mixed diet of forbs, grasses and shrubs. Sheep preference for some

types of forbs over grasses or shrubs (Hanley 1982) make them ideal for controlling broad-leaved weeds.

Prescribed or targeted grazing of spotted knapweed using sheep is proving to be a successful management tool. However, an effective method to determine the botanical composition of individual diets under typical grazing conditions throughout the grazing period is necessary to maximize the potential of sheep in this role (Walker and Hodgkinson 1999).

Fecal near infrared reflectance spectroscopy has been used to predict the chemical (Lyons and Stuth 1992) and botanical (Walker et al. 1998, Walker et al. 2002, and Walker et al. 2007) composition of diets. It has also proven to be a more rapid and cost effective tool for determining diets than the common method of microhistological analysis (Walker et al. 1998). Fecal NIRS has been used to estimate the percentage of leafy spurge (Walker 1998), mountain big sage brush (Walker et al 2002), and juniper (Walker et al. 2007) in the diets of sheep and goats. Fecal NIRS can be used to estimate differences in botanical composition between treatment groups or of individual animals within a group (Walker et al.1998, 2002, 2007). Although it is rapid and cost effective relative to other methods of diet determination (Holechek et al. 1982), it has not been used to determine the botanical composition of diets from sheep grazing spotted knapweed infested rangeland. Therefore the objectives of this study were to develop and validate a fecal NIRS equation for estimating the percent knapweed in the diets of sheep and to determine if that equation could be used to estimate percent spotted knapweed in the diet of sheep grazing an infested landscape.

CHAPTER 2

LITERATURE REVIEW

History and Distribution of Spotted Knapweed

Spotted knapweed (*Centaurea stoebe* L.) is an invasive perennial forb introduced from Eurasia in the late 1800's as contaminates in alfalfa (Medicago sativa L.) and in discarded ship ballast. (Watson and Renney 1974). Spotted knapweed now infests every state except Alaska, Texas, Oklahoma, and Mississippi (USDA 2009 a) and is found in every county in Washington, Idaho Wyoming and Montana (Sheley et al. 1998). In Montana it is estimated to infest 1.5 million hectares (MWSSC 2008) and is spreading at a rate of 10%-27% per year (Lacey 1983).

Impacts of Spotted Knapweed

As densities of spotted knapweed increase, species richness and the frequency of many other species decline (Tyser and Key 1988). Lacey et al. (1989) observed 56% greater runoff and 192% more sediment yield in spotted knapweed-dominated plots than in bunchgrass-dominated plots. Spotted knapweed infestations have been shown to decrease available livestock and wildlife forage by reducing the yield of more desirable forage species (Watson and Renney 1974, Sheley et al. 1998, Rice et al. 1997). However studies show that spotted knapweed is grazed by sheep (Olson et al. 1997, Olson and Wallander 2001, Hale 2002), elk (*Cervus elaphus*), mule deer (*Odocoileus hemionus*),

white-tailed deer (*Odocoileus virginianus*) (Wright and Kelsey 1997). Cattle have also been reported to consume spotted knapweed early in the growing season (Henderson 2008). In addition to environmental degradation, Hirsch and Leitch calculated in 1996 that knapweed infestations cost Montana's economy more than $42 million in direct and indirect costs annually and could cost the industry $155 million per year if allowed to spread to their potential ecological range (Hirsch and Leitch 1996).

Biology of Spotted Knapweed

Spotted knapweed belongs to the Asteracea family and can be identified by its divided alternate leaves, black tipped bracts and purple flowers (USDA 2009). It has several characteristics that give it a competitive advantage over other introduced and native species and increase the challenges associated with its management. Spotted knapweed seizes available resources by germinating early, growing rapidly, and allocating resources to above ground biomass (Sheley et al. 1998). It is capable of reproducing vegetatively and is also a prolific seed producer, producing over 400 seeds per plant. (Watson and Renney 1974). Seed production by spotted knapweed plants in northern Idaho averaged up to 29,600 seeds m^{-2}, which is1,000 times the seed required to maintain an infestation (Shirman 1981).

Spotted knapweed seeds are capable of germinating under a broad range of environmental conditions (Watson and Renney 1974), but may also remain dormant in the soil seed bank for many years. After 8 years of dormancy, 25% of buried seeds remain viable (Davis et al. 1993). Animals may also contribute to seed dispersal by

transporting seeds externally, or by ingesting them. Flower heads can also be transported to new locations when they attach to vehicle undercarriages, as well as shoes and clothing (Sheley et al. 1999).

Spotted knapweed also synthesizes cnicin, a sesquiterpene lactone, that is bitter tasting to livestock (Kelsey and Locken 1987). Cnicin has anti-microbial properties that can reduce rumen microbial activity and inhibit digestion when spotted knapweed leaves and flower buds exceeds 70% of the diet (Olson and Kelsey 1997). Plants two years or older, with one or more stems have the lowest levels of cnicin. Concentrations are generally higher in leaves of spotted knapweed compared to flower buds which are higher in concentrations than stems. Concentrations of cnicins decrease in stems and increase in leaves from early to mid growing season and regrowth may have lower concentrations than mature growth throughout the plant (Olson and Kelsey 1997). When collected in August total plant concentrations of cnicin were greatest in plants less than one year old having only rosette leaves and no stems (Locken and Kelsey 1987).

Prescribed Grazing

Prescribed or targeted grazing with domestic livestock as part of a weed management strategy may provide a cost-effective alternative for landowners and an economic return for livestock producers (Lacey 1987). Using sheep to control spotted knapweed may provide an alternative when other methods such as mechanical and chemical are constrained due to economic, social, and environmental concerns (Olson and Wallander 1994).

Targeted Grazing

Targeted grazing is the application of a specific kind of livestock at a determined season, duration, and intensity to accomplish defined vegetation or landscape goals(Launchbaugh and Walker 2006). Targeted grazing differs from traditional management in that it refocuses outputs of grazing from livestock production to vegetation and landscape enhancement (Launchbaugh and Walker 2006). Several examples of successful targeted grazing schemes include the use of goats for kudzu management, sheep used to create fire breaks around communities and both sheep and goats used to control noxious and invasive weeds (Launchbaugh and Walker 2006).

The goal of targeted grazing in controlling most noxious and invasive weeds is to reduce seed production and maximize damage to the target plant while minimizing the stress placed on desirable species by applying the proper animal at the proper timing, and intensity (Launchbaugh and Walker 2006, Willson et al. 2006). A sheep's morphology and preference for forbs over grasses and shrubs make them ideal for this purpose (Hanley 1982). Sheep have a smaller body size, relative to cattle, and narrow muzzles and cleft upper lips that enable them to be more discriminating than cattle when selecting plants and plant parts (Arnold and Dudzinski 1978). They are also able to consume plants that maybe poisonous to cattle (Sharrow and Mosher 1982, Ralphs et al. 1991) and plants that contain secondary compounds such as cnicin found in spotted knapweed (Olson and Kelsey 1997). Targeted grazing prescriptions using sheep have been well established for such noxious and invasive weeds as leafy spurge (*Euphorbia esua* L.),

tansy ragwort (*Senecio jacobaea* L.) and downy brome (*Bromus tectorum* L.) (Willson et al. 2006).

Spotted Knapweed Control

As with other noxious and invasive weeds, managing large infestations of spotted knapweed using sheep as targeted grazers requires two goals. The first is to reduce the ability of plants to produce seed. The second is to place sufficient stress on the target plant to reduce its competitive ability while minimizing the impact on desirable forages (Launchbaugh and Walker 2006; Willson et al. 2006). Doing so requires applying the proper animal at the proper timing and intensity (Launchbaugh and Walker 2006).

Defoliation of spotted knapweed reduces its vigor and seed production, increasing resources available for desirable forages (Sheley et al 2003). In a study by Lacey et al. (1994), monthly defoliation of spotted knapweed plants in a green house did not reduce total biomass of clipped plants, but did result in a reduction of carbohydrate concentrations by 50% in roots, stems and crowns. Total foliage was not affected, but root and crown growth decreased 0 and 70% respectively when spotted knapweed was subjected to seven levels of defoliation and three levels of competition with blue bunch wheatgrass (*Pseudoroegneria spicta*) (Kennett et al. 1992).

Although it contains defensive mechanisms to deter herbivory, spotted knapweed is a nutritious livestock and wildlife forage, particularly early in the growing season (Kelsey and Mihalovich 1987, Olson and Wallander 2001, Hale 2002) and several grazing studies in Montana and Idaho have shown that sheep readily graze spotted

knapweed even when other high quality forage is available (Olson and Walander 2001, Hale 2002, Henderson 2008, Thrift 2008).

In a south western Montana study, areas repeatedly grazed by yearling ewes for three growing seasons had lower densities of seedlings, rosettes, and mature spotted knapweed plants than ungrazed areas (Olson et al 1997). In addition, the proportion of young plants in the population was less in grazed than ungrazed areas. Continuous grazing can also reduce the number of viable seeds produced by spotted knapweed. In the same study by Olson et al. (1997) viable seed in grazed areas was reduced by 54% while the number of viable seeds in the soil of the ungrazed control sites increased 88% during the same period.

While repeated grazing can have a negative impact on spotted knapweed plants and populations, it can also negatively impact native populations and other desirable forages, reducing their competitive ability as well. After three years of repeated grazing, root and shoot biomass of grazed Idaho fescue (*Festuca idahoensis*) plants were 38 and 27% less than ungrazed plants while spotted knapweed root and shoot biomass were unaffected (Olson et al. 1997). Frequency of invasive Kentucky blue grass (*Poa pratensis*) increased 35% after three years of repeated grazing and bare ground increased from 2.2 to 5.6% in grazed areas (Olson et al.1997).

Timing of a defoliation event may be as important as frequency and or severity in meeting the goals of targeted grazing. After 3 years of repeated mowing treatments, a single mowing of plants in the flowering or seed productions stage can reduce adult plant density by 85% (Rinella et al. 2001). Mowing during the flowering or bud stage can

reduce seed germination by 70% and the number of seed producing plants by 91% (Watson and Renney 1974).

Several studies have been conducted to establish the appropriate time to apply grazing to spotted knapweed. Benzel (2008) conducted a two year clipping study that simulated grazing of spotted knapweed at three growth stages (rossette, bolting, late-bud/ early flowering, and full flowering). Treatments reduced the number of viable seeds produced at all stages and combinations of stages. Removing 35 to 40% of above ground biomass at the rosette stage reduced the number of viable seeds per plant by 77 - 88% compared to the control. The effects of clipping in August were the same as clipping in June + July, June + August, July +August and June + July + August which was a reduction in 95 - 100% of viable seeds produced (Benzel et al 2009). The author concluded that grazing spotted knapweed in the bolting stage would require an additional defoliation in the late bud/early flowering stage. However, if it was grazed during the late bud/early flowering stage before seed set, defoliation would only have to occur once and sheep would not have to be quarantined to avoid spreading seed (Benzel et al 2009).

A two year study in Idaho demonstrated that sheep preferred spotted knapweed in the rosette stage to any other growth stage, but their utilization of spotted knapweed was greater than grasses and forbs in July during the bolting stage than in June during the rosette stage (Hale 2002). This led Hale to recommend grazing spotted knapweed with sheep should be applied in mid-summer when plants were in the bolting stage.

Over a two year period on central Montana rangeland, sheep grazing a light infestation (13% spotted knapweed by weight) ate fewer graminoids in June than July

(17% vs. 55% of their diet, respectively), whereas sheep in the moderate infestation (36% spotted knapweed) ate fewer graminoids in July (45% in June vs. 20% in July). In the moderate infestation, relative utilization of spotted knapweed was greater in July than June (50% vs. 35%, respectively), but averaged 46% in the light infestation (Thrift et al. 2008). Although sheep did not preferentially consume spotted knapweed, an average utilization of 45%, led the authors to conclude that managing spotted knapweed with grazing could be accomplished by applying sheep to infested rangelands in either June or July. However, decreasing the utilization of desirable gramminoids required grazing light infestations in June and moderate infestations in July.

In a two year study of an Idaho fescue, blue bunch dominated plant community, sequential cattle and sheep grazing in either June or July to control spotted knapweed resulted in greater than 60% utilization of spotted knapweed while maintaining less than 50% utilization of desirable gramminoids (Henderson 2008). Although spotted knapweed utilization by cattle declined between June and July (43% vs 39 %), cattle plus sheep utilization remained unchanged in both years at 61%, (Henderson 2008).

Methods of Diet Determination

Timing of defoliation is critical to meeting targeted grazing goals and substantial research has been done to establish the appropriate point in the grazing season for applying sheep to spotted knapweed infestations to meet those goals. However, most research has reported utilization using indirect range sampling techniques rather than estimating intake of the animal itself (Hale 2002, Henderson 2008, Olson and Wallander

2001, Thrift 2008). Maximizing the potential for utilizing livestock as a rangeland improvement tool requires a cost effective method to determine botanical composition of individual animal diets under typical grazing conditions (Walker et al. 1998, Walker and Hodgkinson 1999).

Procedures used for estimating the botanical composition of the range herbivore's diet include direct observation, utilization techniques, cannulation sampling, and fecal analysis. Important attributes of an effective method for determining grazing animal diets include: free animal movement and completely natural selection of all available plants and plant parts regardless of pasture size regardless of terrain; must be useful for wild and domesticated animals; not require slaughter of test animals; and require minimum animal care (Holechek et al. 1982).

Direct Observation

Simplicity, minor equipment requirements, and ease of use are major advantages of direct observation (Holechek et al. 1982). Difficulty in species identification and quantification of how much of a plant was consumed are important problems associated with this procedure. Although results from direct observation of tame animals have been proven to be consistent with data from esophageally fistulated animals, (Free et al. 1970, Sanders et al. 1980), it is not practical for use on large brush infested pastures with rough terrain (Sanders et al. 1980). Factors influencing the accuracy and precision of the direct observation procedure also include the training of the observer, complexity of the plant

community present, and/or phenological development of individual plants (Holechek and Gross 1982).

Utilization Techniques

Utilization is one of the oldest approaches used to evaluate the grazing animal's diet. The advantages of this approach include speed and the fact it provides information on where and to what degree a range is being used (Holechek et al. 1982). A serious problem with any utilization technique is that large scale losses of plant parts from weathering, trampling and animals other than those of interest can greatly confound results. Further, when forage is actively growing, regrowth after defoliation can make accurate estimates of utilization difficult to obtain (Cook and Stoddart 1953).

Cannulation and Stomach Analysis Methods

Cannulation methods are accurate but are costly and require considerable time. Stomach analysis involves animal sacrifice (Mcinnis et al 1982), and may be biased toward less digestible materials in the diet (Vavra and Holechek 1980).

Fecal Sampling

Fecal sampling offers several advantages which lend itself as an effective tool for diet determination. These include: not interfering with grazing habits of domestic or wild animals, unlimited sampling, and very little sampling equipment (Mcinnis et al. 1983). The primary limitation of fecal analysis include: differential plant digestion resulting in accuracy problems (Mcinnis et al. 1983). and some species may become unidentifiable in

the feces (Slater and Jones 1977). Identification is further complicated by aging of fecal material before sample collection.

Methods of Fecal Analysis

Microhistological Analysis

Microhistological analysis is the most widely used method for quantifying botanical composition of masticated forage or fecal material. The technique of microhistological analysis to estimate diet composition was pioneered by Baumgartner and Martin (1939) and further advanced by others such as Free et al. (1970) Mohammad et al. (1995) and Alipayo et al. (1992) who determined it to be adequate in analyzing esophageal, rumen and fecal samples to determine diets of cattle, sheep and goats. Studies have shown microhistological analysis can give an accurate representation of percent diet botanical composition if observers had compounded diets to check their accuracy (Holecheck and Gross 1982). However, it has analysis has been limited by differential plant digestibility, observer error (Vavra et al. 1978; Bartolome et al. 1995), expense, which can be as high as $430/diet (LLSP 2008) and slow analysis time (Davitt 2009 personel correspondence). These limitations often result in less than ideal or ineffective sampling strategies due to the inability to accommodate the amount of analytical work required (Foley et al. 1998).

Fecal NIRS

Fecal analysis using near infrared reflectance spectroscopy (NIRS) has been used to estimated the chemical (Lyons and Stuth 1992) and botanical (Walker et al, 1998, 2002, 2007) composition of diets. NIRS is the process of exposing samples to light energy. Absorption of light energy by C-H, N-H and O-H bonds in a sample allow an estimation of the content. Analysis of these bonds is essential as they are the primary constituents of the organic molecules in forages (Stuth et al. 2003). The absorption, measured as log(1/Reflectance), of light waves create spectral features that are combined with reliable compositional analysis of the sample material in a predictive statistical model (Foley et al. 1998). Establishing the relationship between log(1/Reflectance) values and known reference values is called calibration of an NIRS method, and using the relationship to determine the amount of a component in a new sample is called NIRS estimation or prediction (Williams 2001). Validation refers to the ability of the calibration equation to predict the reference value of samples not part of the data set used in the development of the calibration equation (Walker et al. 2002).

The relationship between spectral features and reference data is expressed in the form of a regression equation. Reference values are used as the independent X variable and mathematical combinations of log(1/R) values at various wavelengths as the dependent Y variables. R^2 (coefficient of determination), slope (between reference or actual and predicted values) and standard error of prediction (SEP; error caused by both lack of precision and lack of accuracy) are the statistics most commonly used to evaluate the effectiveness of the developed equation (Williams 2001).

Fecal NIRS equations for predicting the diets of grazing animals are developed and validated by comparing fecal samples of animals with samples of known composition. A calibration model's reference population must be large enough to encompass expected variations in the analyzed samples (Van Kempen 1996). Therefore these equations must include a broad population of plant species representing the diverse conditions that grazing livestock may encounter (Stuth et al. 2003). Variations include not only plant species, tissue type, and physiological age, but drying process, operating temperatures and particle size (Batten 1998) as well.

Several studies estimating the percent composition of chemically defended and noxious weeds in ruminant diets using NIRS analysis of fecal material have been conducted. When comparing NIRS to the traditional technique of microhistological analysis, Walker et. al (1998) found that it could be used in feed trials to estimate percent leafy spurge in the diets of 20 goats and 19 sheep with values of coefficient of simple correlation (r^2) as high as 0.96 and a slope of 0.96 for goats and r^2 of 0.96 and slope of 1.06 for sheep. Microhistological regression equations resulted in a $r^2 = .22$ and a slope = 0.90 for goats, $r^2 = 0.32$ and slope = 1.02 for sheep. With two feed trials using goats and sheep fed varying levels of leafy spurge from different sources, the study concluded that NIRS of fecal material could be used to screen large numbers of animals for phenotypic differences in diet selection and for making treatment comparisons.

In a study estimating percent Mountain Big Sage brush (*Artemisia tridentata* Nutt. ssp. vaseyana (Rydb) Beetle.) in sheep diets, high precision but questionable accuracy of developed NIRS equations led Walker et al. (2002) to the conclusion that variation in

plant species to be estimated and the forages used as back ground feed in diets should include as much variation as possible. The same study also led the authors to the conclusion that the decreased cost and ability to rapidly process samples compared to microhistological analysis would allow NIRS to be more readily used for studies involving large scale fecal sampling.

Whitworth (2002) developed NIRS equations to estimate percent composition of Redberry and Ashe (*Juniperus ashei* Buchh. and *J. pinchotii* Sudw.) Juniper types could not be differentiated in this trial, but overall juniper intake was estimated. Even though low precision (R^2=0.624) was reported for predicting juniper in a diet, internal NIRS calibrations produced better predictions than microhistological analysis.

CHAPTER 3

MATERIALS AND METHODS

NIRS Calibration

Following fecal NIRS protocol established by Walker et al. (2002) three feeding trials were conducted over the summers of 2005 (Trial I) and 2006 (Trials II and III) at Ft. Ellis Research Farm, Bozeman, Montana. Their purpose was to provide fecal samples for developing and validating NIRS equations to estimate percent spotted knapweed in sheep diets. Background feeds and spotted knapweed for all three trials were collected from various locations to be representative of typical forages found in western Montana and for the purpose of creating as much diversity in the diets as possible. Back ground forages and spotted knapweed were ground in a hammer mill through a 2.54 cm screen to facilitate mixing. Subsamples were taken and dry matter was calculated for all background forages and spotted knapweeds prior to mixing diets. Molasses was added at 3% of the total diets during mixing to help adhere feed particles together and reduce sorting.

All animal procedures were approved by the Montana State University Institutional Animal Care and Use Committee (Protocol # AA027).

Trial I

Twelve 68± 4.5 kg mature ewes were randomly assigned one of twelve diets consisting of four types of spotted knapweed (pre-bud and flowering from low and

upland areas) and three base or background feeds consisting of smooth brome (*Bromus inermis* Leyss.)/alfalfa (*Medicago sativa* L), tall fescue (*Festuca arundinacea* Schreb.) and crested wheatgrass (*Agropyron cristatum* (L.) Gaertn). Total diets were fed at 2.5% DM of body weight. Spotted knapweed was fed at 0% of the diet days 1-7, 5% fed days 8-14 and 50% on days 15-21.

Trial II

Because adult ewes were unavailable, nine 63± 4.5 kg yearling rams were used for the study in 2006. This was not consider an issue due to data from Texas (Walker 2006, personnel communication) that indicates NIRS predictions do not differ between males, wethers or female sheep. The yearling rams were randomly assigned individual diets containing three levels (0, 15 and 30% DM) of spotted knapweed (pre-bud and flowering mixed) and 3 background diets of smooth brome/alfalfa, orchard grass (*Dactylis glomerata* L.) / alfalfa, and mixed grass/alfalfa. To determine the effects of green forages on NIRS ability to estimate composition, Trial II also consisted of three 63 ± 4.5 kg yearling rams were individually fed a diet of crested wheatgrass/smoothbrome and spotted knapweed fed cut fresh each day prior to feeding at approximately the same time of day and location. Dry matter for both the background forages and the spotted knapweed was determined prior to the first day of the trial and diets were mixed to approximate 0, 15 and 30% spotted knapweed when mixed with the background feed. Each day, prior to feeding, samples of the fresh background feed and kanpweed were

sampled and dry matter determined. The resulting average composition of the fresh diets was 0, 19 and 38% spotted knapweed. The total diets were fed at 2.7% body weight.

Trial III

To determine if NIRS spectral output is a function of the percent or the total amount of spotted knapweed in the diet, the same 12 yearling rams used in Trial II were fed diets that contained similar amounts of spotted knapweed (.27 kg DM). Rams were assigned to one of 3 diets with 4 animals per diet. Background forage was fed at 1.54, 1.18 and .82 kg DM for diets fed at 2.5, 2.0 and 1.5% body weight and contained 15, 19 and 25% spotted knapweed respectively. Background forage used in the trial was a combination of the three forages used in Trial II plus an additional grass hay mixed in equal amounts to create one feed. Diets were fed for 7 days.

Fecal Sampling

Fecal samples were collected on Days 5, 6 and 7 of each feeding period in each trial. Approximately 12-14 "pellets" were collected per sheep per day for a total of a 40 pellets. Samples were dried in a forced air oven at 55^{o} C for 24 hours. Dried subsamples were then ground in a cyclone mill to pass through a 1-millimeter screen. Samples were packed in labeled 125 ml glass containers and sent to Texas A&M Agricultural Experiment Station in San Angelo, Texas for NIRS determination.

Determining the Intake of Spotted Knapweed for Ewes on Range

Two fecal sampling trials were conducted at the Mannix ranch in west central, Montana over the 2006 grazing season (June –August) to characterize the variation in spotted knapweed intake of grazing ewes on open range. The first trial was conducted to determine spotted knapweed intake variability within a band of ewes at two times during the growing season and the second to determine weekly variation within individual sheep.

Study Site Description

The trials took place 5 km east of Helmville, Montana at about 1400 m elevation. The ecological site is a Silty, 380 to 480-mm precipitation zone in the foothills and mountains area (Thrift 2008), and is classified as a rough fescue (*Festuca campestris* Rydb.)/bluebunch wheatgrass (*Pseudoroegneria spicata* (Pursh) A. Löve ssp. s*picata*) habitat type (Mueggler and Stewart 1980). Soils are very deep, well-drained, and include Shawmut cobbly loam, Danvers clay loam, and Roy gravelly loam on an alluvial fan (USDA 2009 b). The 33-year average annual precipitation is 314 mm, with 56% occurring as rain between May and September (WRCC 2009). Dominant grass species included Idaho fescue, bluebunch wheatgrass, green needlegrass (*Nassella viridula* (Trin.) Barkworth), and Sandberg bluegrass (*Poa secunda* J. Presl) (Thrift et al. 2008). Dominant forbs in the study area were spotted knapweed, common dandelion (*Taraxacumofficinale* G.H. Weber ex Wiggers), western yarrow (*Achillea millefolium* L.), yellow salsify (*Tragopogon dubius* Scop.), lupine (*Lupinus* spp. L.), wild onion

(*Allium* spp. 16L.) and Mountain big sagebrush (*Artemisia tridentata* Nutt. ssp. *vaseyana*(Rydb.) Beetle)(Thrift 2008).

Pasture Characterization

Nineteen pastures were surveyed to characterize the vegetation present and better explain changes in knapweed intake over the summer. Transects were run on pastures 1 through 12 between June 21and June 23. Transects were run on pastures 13 through 19 between July 11 and July 13. The dates were chosen so that vegetative composition could be determined immediately prior to sheep grazing those particular pastures. Vegetation data were compiled using a double sample technique (BLM 1996) that consisted of a 300 meter transect started at a random recorded point and set on a degree heading that would allow the transect to cover an area representative of the vegetation and topography of each pasture. Transects consisted of 30 plots set 10 paces apart, with the first plot ten paces from the recorded starting point. A ¼ m^2 frame was used for determining percent composition of forage types (forbs, gramminoids and spotted knapweed) by weight in grams. Composition in all 30 plots was estimated visually. This required visually estimating then clipping and weighing one plot in the morning and one in the afternoon to adjust estimations. Four plots were chosen randomly from the thirty. The four plots were clipped and vegetation was sorted and weighed by forage type. Only plots containing spotted knapweed were clipped. Clipped weights were compared with estimated weights to create a correction factor and percent composition of each forage for each pasture was calculated using the corrected weight (BLM 1996).

Sampling Trial 1, Determining Flock Intake

Two fecal collections were made to determine variability in spotted knapweed intake of a flock of approximately 900 ewes with their lambs grazing under normal range conditions. The first collection took place July 13 and the second, August 15. The evening prior to sampling, sheep were moved to fresh bed ground. The next morning, as ewes and lambs were leaving the bed ground fresh fecal samples were collected from approximately 10% of the ewes in the flock.

Sampling Trial 2, Determining Individual Intake

To determine variation in individual intake of spotted knapweed, five ewes were randomly selected from the flock of 900 and ear tagged. Fecal samples were collected from the ear tagged ewes on; June 22 and 29, July 6,13,20,and 27, August 3, 10, and 17. For each collection, individual ewes were located in the flock through identifying their ear tags and then followed until they defecated. To help avoid degradation, samples were immediately collected in sealable plastic bags and placed in a cooler with ice.

Zero Knapweed Sampling

To create a baseline diet for enhancing the ability of NIRS to estimate dietary composition, fecal samples were included that contained no spotted knapweed. This was accomplished by placing 5 ewes in an 18 x 18 m^2 enclosure representative of the area grazed by the flock but containing no spotted knapweed and allowed to graze for 4 days (July 10 – 13). Salt and water was provided and the enclosure was moved when

necessary to ensure adequate feed was made available. Fecal samples were collected on day 5 (July 14).

Fecal Samples

For all sampling trials, at least 21 grams of fecal material per ewe were collected. Samples were first air dried in paper bags for at least 24 hours. Samples were dried in a forced air oven at 55^{o} C for 24 hours, and sub samples were ground in a cyclone mill to pass a 1 mm screen. Ground subsamples were packed in labeled 125 ml. glass containers and sent to Texas A&M Agricultural Experiment Station in San Angelo Texas for NIRS analysis.

NIRS Analysis

Fecal Analysis

At Texas, spectral data were obtained by first conditioning collected fecal samples for 24 hours in an environment with constant temperature and humidity (21^{o} C, 65%). Samples were then packed in quarter-cup sample cells with a near-infrared, transparent, quartz cover glass. Cells were scanned 32 times using a scanning reflectance monochrometer (Model 6500, NIRSystems Inc., Silver Springs, MD). Reflected energy (log [1/R], where R = reflectance) was measured, averaged over the 32 scans and recorded at 2-nanometer intervals from 1,000 to 2,500 nm.

Reflected energy data were transformed using multiplicative scatter correction (Geladi et al. 1985) and a 2,8,8,1 math treatment using WINISI II software (ISI, 1999) in which the first number is the order of the derivative, the second number is the gap

(number of points over which the derivative is calculated), and the third and fourth numbers are the smooth (number of points in a moving average and the number of nanometers over which the second smoothing is applied, respectively). Data pretreatment has the effects of removing nonlinearity caused by light scatter, correcting for baseline drift and enhancing absorption peaks (Williams 2001).

Equation Analysis

Modified partial least squares regression (Martens and Naes, 1987) equations were developed using NIRS spectra from fecal samples (Feeding Trials I, II and III) as independent variables and percent knapweed in diets as the dependent reference data. NIRS data from feeding Trials I, II and III, were divided into two data sets for calibration and validation analysis. Trial I used mature ewes, and spotted knapweed and base forages collected during the 2005 growing season. Data from that trial were considered one data set. Trials II and III took place during the summer of 2006 using yearling rams with different spotted knapweed and base forages than those used in 2005. Data from those two trials were combined and considered the second data set. Final calibrations were developed using the data combined from Trials I, II ,and III. Validation of developed equations was conducted to determine their ability to estimate percent knapweed in the fecal samples. Internal validation refers to using samples from the same set as those used to develop the equation. External or independent validation refers to using samples not from the same sample set as the developed equation. Internal and independent validation statistics from the data sets were compared separately. Statistics used to evaluate developed equations included coefficient of determination (R^2), standard

error of prediction (SEP; error caused by lack of precision and lack of accuracy), and the slope of the line between actual and predicted values (an indicator of accuracy) (Naes et al. 2002) The final calibration equation (Trials I, II, and III combined) was used in Sampling Trials 1 and 2 fecal analysis.

Percent spotted knapweed in the diet was analyzed separately for each sampling trial using the GLM procedure of SAS (SAS 2004) with date of fecal collection in the model. Means were separated using Least Significant Differences when the $P < 0.05$.

CHAPTER 4

RESULTS AND DISCUSSION

Developing and Validating NIRS Estimation Equations

Precision and accuracy of internal and independent fecal NIRS calibrations validations for predicting percent spotted knapweed in diets of sheep are presented in Table 1. Internal validation statistics are similar to those reported for leafy spurge (Walker et al. 1998), mountain big sage brush (Walker et al.2002) and juniper (Walker 2007). The independent validation statistics for this trial were lower than those for mountain big sage brush but higher than those reported for juniper (Walker et al.2002, Whitworth 2002). The calibration equation developed using Trial I results estimated values obtained in Trials II and III poorly (R^2=.22). However the calibration equation developed from Trails II and III data estimated values obtained in Trial I with R^2=.72. This compares to R^2= .56 for predicting juniper consumption by goats (Walker 2007).

Table 1. Internal and independent validation of calibration equations developed using fecal samples from Trials I, II, and III.

	Internal Validation		Independent Validation		
Calibration Data Set	Trial I	Trail II & III	Trial I, II & III	Trial I Estimating Trial II & III	Trial II &III Estimating Trail I
N	36	24	60	36	24
R^2	0.99	0.93	0.96	0.22	0.72
Slope	0.99	0.93	0.96	0.84	0.55
SEP	2.06	2.49	3.33	15.21	13.33

The reason for the differences in R^2 values may come from the need to include as much diversity in the base diets as possible in order to create a more robust and reliable predictive equation (Walker et al. 2002). The chemical analysis of base forages utilized in trial I and trial II and III are presented in tables 2 and 3 respectively. Base forages used in trial I were all low quality and were similar in nutritive value. These values suggest that the base forages used in this trial were not as diverse as desirable. Trial I also differed from Trials II and III in that mature ewes were used rather than yearling rams. Combining all three trials included the diversity from each trial and increased predictive power of the calibration equation (Table 4). Internal fecal NIRS validation of the calibration equations calculated from the combined trials (I, II, II) provided acceptable precision (R^2= .96 and SEP = 3.35) and accuracy (slope = .96). The combined feeding trial calibration equation predicted intake reasonably well in each of the individual feeding trials (R^2 = .76 to .99). The predictive ability of the equation is adequate to to identify animals or periods of high consumption versus low consumption by distinguishing general differences in consumption of spotted knapweed by sheep (Williams 2001).

The R^2 value for the three fresh forage samples (.93) were similar to the R^2 from the other trials. This supports the conclusion reached by Walker et al. (2002) that NIRS equations developed using dry samples can be used to predict general trends in dry matter consumption of a specific fresh forage.

Table 2. Percent dry matter (DM), crude protein (CP), neutral detergent fiber (NDF) and acid detergent fiber (ADF) of background forages and spotted knapweed used in Feeding Trial I.

	% DM	CP	NDF	ADF
		---------- % DM bases ----------		
Smooth brome/alfalfa hay	92.2	10.6	56.3	39.2
Tall fescue hay	92.7	8.3	57.5	35.9
Crested wheatgrass hay	92.6	11.2	64.6	39.4
Knapweed				
bud stage/dry site	92.8	13.6	44.2	34.2
flowering stage/dry site	93.3	13.2	47.8	37.5
bud stage/wet site	93.1	9.9	50.8	39.8
flowering stage/wet site	93.7	7.9	55.7	44.9

Table 3. Dry matter (DM), crude protein (CP), neutral detergent fiber (NDF) and acid detergent fiber (ADF) of background forages and spotted knapweed used in Feeding Trial II and III.

	% DM	CP	NDF	ADF
		-------- % DM Bases ---------		
Smooth brome/ alfalfa hay	93.3	19.7	59.9	40.5
Orchard grass/ alfalfa hay	93.8	12.0	60.0	40.3
Mixed grass/alfalfa hay	93.7	7.7	53.8	41.1
Knapweed	92.9	7.4	50.6	40.5
Fresh knapweed	45.8	8.5	54.7	44.7
Fresh smooth brome/crested wheatgrass	70.0	8.4	67.2	38.7
Grass Hay	94.6	7.7	59.9	40.5

Table 4. Number of samples (N), coefficient of determination (R^2), slope and standard error of prediction (SEP) for internal validation of equation developed using data from Trials I, II, and III combined.

	All	Trial I	Trial I and II	Trial II	Fresh Forage	Trial III
N	60	36	24	12	3	12
R^2	0.96	0.97	0.85	0.87	0.93	0.76
Slope	0.96	0.98	0.78	0.76	0.77	0.98
SEP	3.35	3.11	3.68	4.66	6.92	2.33

The relationship between the actual and predicted percent spotted knapweed in the diets of ewes in our feeding trials are shown in Figure 1. The solid line represents a perfect 1:1 relationship. The graph demonstrates the ability of fecal NIRS to identify general or large differences in spotted knapweed intake.

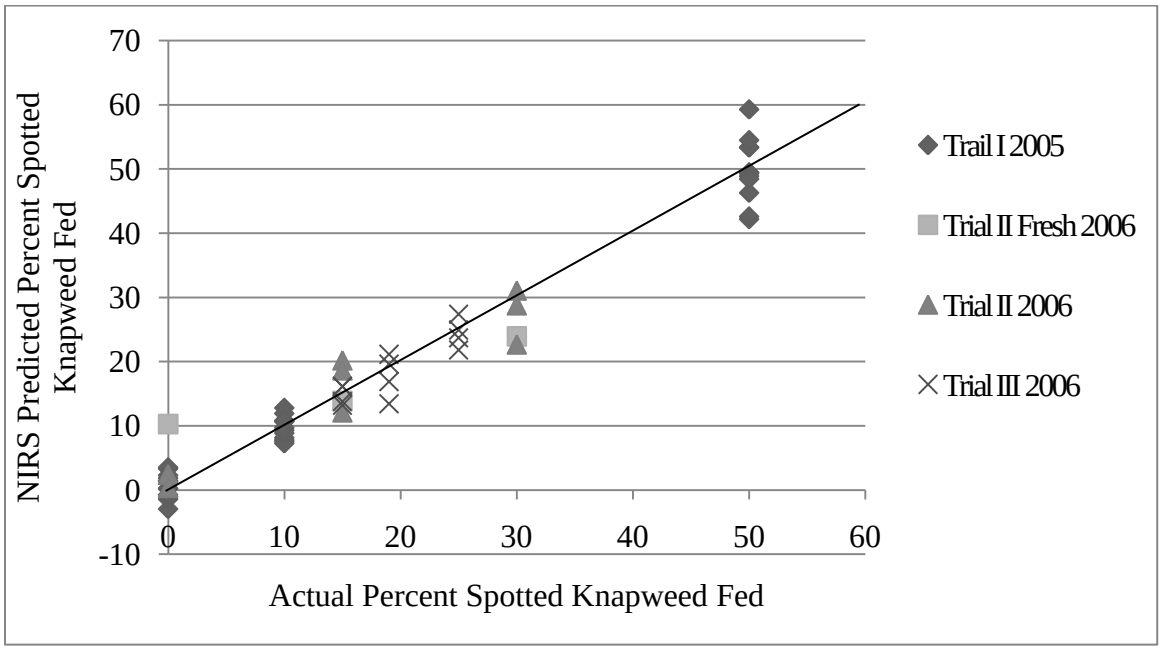


Figure 1. Internal calibration predictions of percent spotted knapweed in the diets of ewes by analyzing samples from Trials I, Trial II fresh forages, Trial II, and Trial III using the combined equation.

Trial III

Individual results from Trial III are presented in Table 5. In this trial, all sheep were fed .27 kg DM spotted knapweed at rates of 15, 20, and 25% in their diets. These results validate that the developed equation is estimating the percent spotted knapweed in the diet rather than the total amount consumed and are in agreement with previous work by Walker et al. (2002).

Table 5. Number of samples (N), standard error (SE), and means for knapweed consumption and predicted consumption from Trial III.

Percent knapweed in diet	N	Predicted Percent knapweed	SE
15	4	14.8[ac]	
20	4	17.8[ab]	1.27
25	4	24.5[bc]	

[a] Means differ P = 0.1346
[b] Means differ P = 0.0047
[c] Means differ P = 0.0004

Determining the Intake of Spotted Knapweed for Ewes on Range

Estimates of forage availability and dates grazed are presented in Table 6. All pastures used in this trial had an estimated knapweed concentration of less than 9%. Considerable variation existed between individual sites within each particular pasture. Grazing protocol involved short duration grazing to be concentrated on sites with the heaviest concentrations of spotted knapweed. The relative availability of spotted knapweed was calculated as the average of the amount available to the animal for a 3 day period prior to sampling.

Table 6. Biomass and availabilty in percent of total for spotted knapweed, grasses and other forbs in pastures grazed June 16 - August 17. [a]

		Biomass				Availability		
		---------- kg/ha ----------				---------- % ----------		
Pastures	Dates Grazed	Spotted Knapweed	Grass	Forbs	Total	Spotted Knapweed	Grass	Forbs
1	June 25 to 27; July 31 to August 1	13	679	418	1110	1.2	61.2	37.6
2	June 23 to 24; July 31	0	638	229	867	0	73.6	26.4
3	June 20 to 22; July 29 to 30	2	938	351	1291	0.1	72.7	27.2
4	August 2 to 4	5	374	342	721	0.7	51.8	47.4
5	June 16 to 19; July 27 to 28	0	374	165	539	0	69.4	30.6
6	August 7	0	351	295	645	0	54.3	45.7
7	July 1; August 7	12	628	329	968	1.2	64.9	33.9
8	June 28 to 30; August 5 to 6	1	1084	285	1370	0.1	79.1	20.8
9	August 8 to 10; August 18	70	526	280	875	7.9	60.1	31.9
10	July 2 to 7; August 11 to 13	78	654	407	1049	7.4	53.8	38.8
11	July 8 to 9; August 17	1	1382	391	1774	0.1	77.9	22.1
12	August 26	135	1226	150	1511	8.9	81.1	10.0
13	July 26	33	823	232	1088	3	75.5	21.3
14	July 10 to 12; August 15 to 16	0	586	223	809	0	72.5	27.5
15	July 24 to 25	11	459	338	808	1.4	56.8	41.8
16	July 19 to 20	0	320	174	494	0	64.8	35.2
17	July 16 to 18	2	874	188	1063	0.2	82.2	17.1
18	July 13 to 15	0	420	221	642	0.1	65.6	34.4
19	July 21 to 23	34	618	319	971	3.5	63.6	32.8

[a]Pastures 1-12 estimated June 21-23, Pastures 13-19 estimated July 11-13

Sampling Trial 1, Determining Flock Intake

Estimated intake of spotted knapweed of a random 10% of the ewes in the flock on July 13 and August 15 are presented in Table 7. Ewes were generally consuming a higher proportion of their diets in spotted knapweed in mid-August than in mid-July (P< 01). The estimated intake values for spotted knapweed in this sampling trial (5.04 and 20.70% for mid -July and mid- August) were similar to those observed in the weekly sampling trial during the same time period (5.34 verses 25.84 and 21.98 on July 13 versus August 10 and August 17). Figure 2 represents the percent of ewes consuming varying concentrations of spotted knapweed by sampling period. This figure shows a higher perecentage of the flock with > 15 % spotted knapweed in their diets in mid-August than in mid-July (19% vs. 5% respectively).

Table 7. Number of samples (N), estimated percent spotted knapweed in the diets and standard error (SE) from Sampling Trial 1.

Day of sampling	N	% Knapweed	SE
July 13	89	5.0	0.57
August 15	88	20.7[b]	0.58

[ab] Column means with different superscripts differ P < .01

The increased consumption of spotted knapweed in August versus July may be attributable to an increase in preference by sheep for the weed, or the sheep were becoming adapted to it. Because the band used in this study grazed the same pastures the previous grazing season, increased consumption may be attributed to an increased preference as other forages became less palatable.

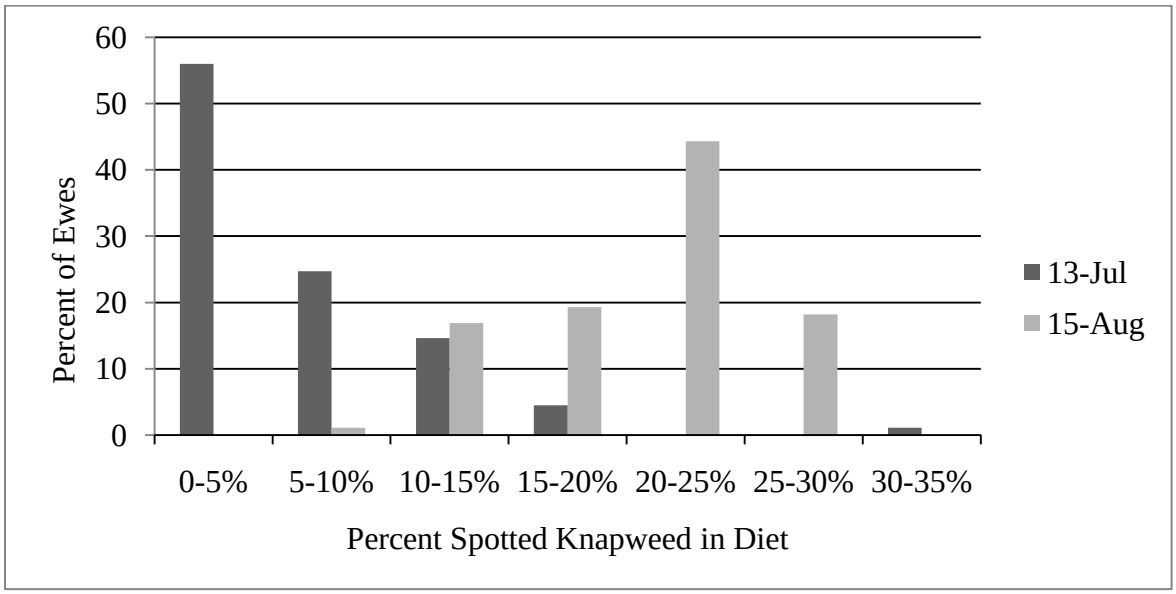


Figure 2. Percent of ewes in the flock listed by percent of spotted knapweed in their diets on 13 July and 15 August.

Sampling Trial 2, Determining Individual Intake

Spotted knapweed average intake by ewes, by week are reported in Table 8. Average intake was higher (P<.01) in July (July 6, 13, 27) than in June (June 22, 29). With the exception of the last sampling date in July compared with the first sampling day in August (July 27 compared with August 3), intake in August (August 3, 10, 17) was higher (P<.01) than in July (July 6, 13, 20). This trend can be seen in Figures 3 and 4. Figure 3 depicts average estimated spotted knapweed in the diets by week. Fgure 4 depicts the intake of each of the 5 sheep by week.

Table 8. Results from Sampling Trial 2. Number of samples analyzed from grazing animals in the corresponding pastures (N), percent knapweed available in the pastures grazed for nine weeks, estimated percent of the animals diets that were knapweed and standard error (SE) of the estimations.

		Percent knapweed	Percent knapweed	
Week	N	Available in Pasture[g]	Intake	SE
1(22-June)	5	0.09	2.7[ab]	
2(29-June)	5	0.65	0.7[a]	
3(6-July)	5	7.4	9.5[c]	
4(13-July)	5	0.03	5.3[abcd]	2.25
5(20-July)	5	0.1	5.0[abc]	
6(27-July)	5	3.01	16.5[e]	
7(3-August)	5	0.65	17.2[ef]	
8(10-August)	5	5.49	26.0	
9(17-August)	5	7.91	22.0[f]	

[abcdef] Column values with different superscripts differ P < .01.

[g] Represents an average of the composition of pastures sheep were grazing the previous three days.

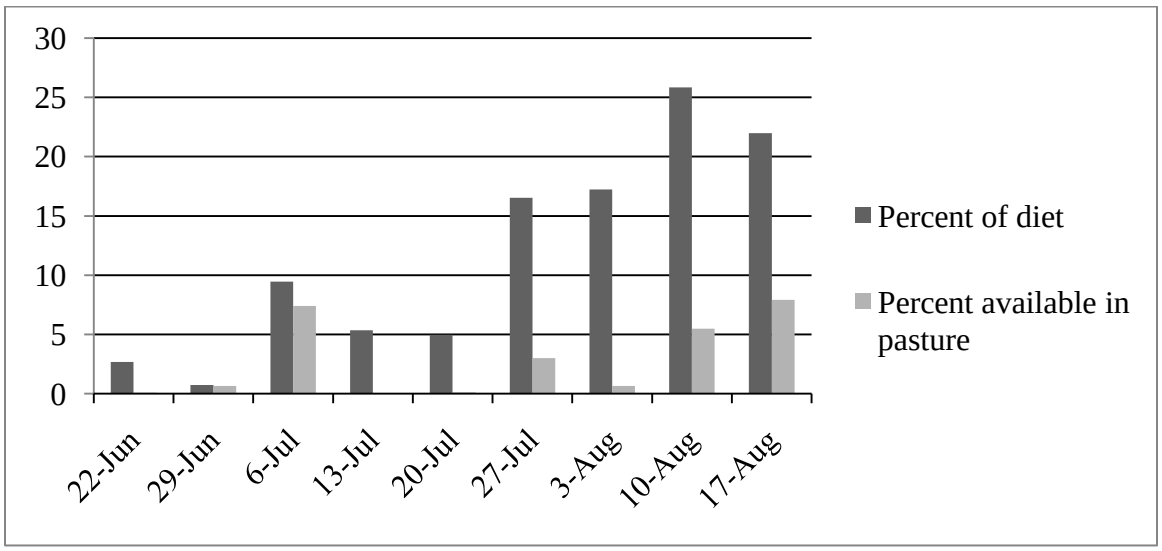


Figure 3. Average percent spotted knapweed in the diets of individual animals compared to the amount available in the pastures from June 22 through August 17.

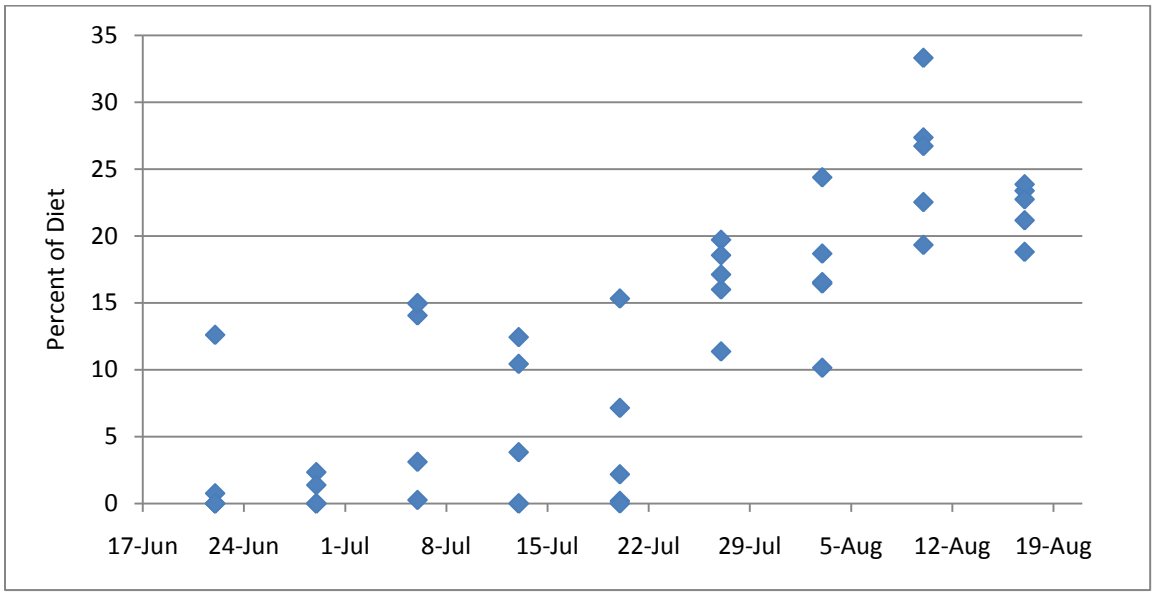


Figure 4. Individual intake of spotted knapweed by percent in the diets of five ewes grazing infested pastures from June 22 through August 17.

CHAPTER 5

CONCLUSIONS AND IMPLICATIONS

An equation can be developed to estimate percent spotted knapweed in the diets of sheep. However, lower precision and accuracy resulting from independent validations suggests caution should be used when applying fecal NIRS outside the parameters used to develop the predictive equation. Feeding trials used to produce fecal samples should use as much variety in background forages as possible and the total diets should be representative of those for which the developed equation will be used. By combining data from all three feeding trials we were able to develop an equation that could predict percent knapweed in the diets with an R^2 of .96 and a slope of .96 with internal validation. The equations predictive ability was adequate to identify general differences in consumption of spotted knapweed by sheep grazing infested rangeland and was used in two range sampling trials to determine differences in spotted knapweed intake by sheep over a grazing season.

Results from both sampling Trials I and II show that sheep included more spotted knapweed in their diets later in the growing season when plants are in full flower stage. These results are similar to Thrift et al. (2008) who reported that the diets of sheep grazing moderate infestations (36% spotted knapweed by weight), contained more spotted knapweed on average in July than in June (75% vs. 53%). Relative utilization of spotted knapweed was also greater in moderately infested pastures in July than June (50% vs. 35%) (Thrift et al. 2008). These estimates are higher than ours, but they

estimated botanical composition of sheep diets from clipped plots, whereas ours came from samples collected directly from the grazing animal. The areas infested by spotted knapweed on the sites used by Thrift also had greater concentrations of knapweed than our sites (36% vs. < 9%). It is also important to note that our estimates should be considered interval scale data, rather than exact percentages of spotted knapweed in the diet. Hale (2002) reported that sheep consumed spotted knapweed at all stages of growth, but preferred rosettes over bolting and flowering/seed set stage in feeding trials. This is in contrast to our study showing that sheep preferred spotted knapweed later in the growing season at the flowering stage. In this case, the difference may be that Hale used dried rather than fresh knapweed and background forages in his preference trial.

In this study, sheep were either selecting spotted knapweed later in the grazing season or, due to exposure to the plant, had become adapted to it. Because the flock used in this study had grazed the same pastures the previous summer, the increased consumption can be attributed to an increased preference as the season progressed. Cool season grasses typical of those found on our study site lose palatability and nutritional value more rapidly than spotted knapweed as the season progresses (Launchbaugh and Walker 2006, Olson and Wallander 2001). Cnicin levels in spotted knapweed are also higher in June than in August with maximum concentrations in July (Kelsey and Mihalovich 1987). Olson and Kelsy (1997) reported that cnicin concentrations were highest in leaves, intermediate in flowers and lowest in stems of spotted knapweed in mid-June and mid-July. The time of lowest concentrations of cnicn in the predominant plant parts consumed corresponds to the time of increased consumption.

The objective of grazing plans for noxious weeds and invasives should be to prevent seed production and reduce biomass (Wilson et al. 2006). Hale concluded that high stocking rates during the bolting stage would be the optimal grazing prescription for spotted knapweed while providing the best quality forage for grazing animals. Thrift et al. (2008) recommended grazing light infestations in June and moderate infestations in July to increase utilization of spotted knapweed while reducing the impact on desirable forages and suggested that late July would be the optimal time for grazing with sheep. Plants in the early flowering to seed formation are most susceptible to grazing pressure (Rinnella 1994). Our results support the suggestion that the optimal time for grazing would be later in the growing season, which corresponds to our data showing an increased percent spotted knapweed in the diets of sheep from late July to early August.

This study was the first to use fecal NIRS to estimate the percent spotted knapweed in the diets of individual animals under typical grazing conditions. Although the estimations of percent spotted knapweed may not represent precise percentages in the diet, we are confident that ewes grazing on foot hills range infested with spotted knapweed included more of the target plant in their diets later than earlier in the growing season. Applying sheep grazing to spotted knapweed infestations at the appropriate time is required to create the greatest impact on the target plant while minimizing impact on desirable forages in a targeted grazing prescription. Based on our data the appropriate time to for this would be late in the growing season during flowering, prior to viable seed production.

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