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Risk of Nitrate Toxicity when Grazing Annual Forages

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RISK OF NITRATE TOXICITY WHEN GRAZING ANNUAL FORAGES

by

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Annual forages provide a valuable grazing resource for producers; however, annuals are prone to accumulating nitrate and toxicity can be a potential challenge. There are multiple publications regarding nitrate toxicity, but few, if any, address grazing high nitrate forages. There is variability on what amount of nitrate is considered toxic to cattle, and information is not available on the frequency producers experience toxicity when feeding annual forages. To understand the incidence of nitrate toxicity in the North Central Region of the U.S., a survey was distributed through the "UNL BeefWatch" newsletter to producers. Though producers appeared concerned about nitrates in annual forages, only 38% have experienced an issue. Management decisions to test annual forages for nitrates did not change if a producer had previously experienced toxicity. Producers tended to experience nitrate toxicity more often when grazing (31%) compared to feeding hay (21%). This data agreed with a dataset of samples submitted to Ward Laboratories, in which 48% of fresh brassica samples, 23% of fresh annual grasses, and 5% of dry annual grasses analyzed would have been considered at risk for causing toxicity. However, the increased incidence of toxicity in pasture is smaller than expected based on the large proportion of fresh forages sampled and submitted to the commercial laboratory and considered toxic. Some mitigation factors may explain differences in toxicity risk for animals grazing compared to animals fed annual forage hay. Understanding these factors and the cost of not utilizing the forage is important for management decisions. Although these forages pose a

risk of toxicity, they provide a high quality feed source. An additional study was done to understand how the nutritive value of late-summer planted brassicas and small grains change through early winter. Even after the forage froze and was brown in January, these forages remained a highly digestible feed source, indicating producers can increase yields by delaying grazing in the fall, and still utilize the forage in the winter.

Keywords: nitrate toxicity, annual forages, brassicas, grazing, forage quality

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CHAPTER I. REVIEW OF LITERATURE

INTRODUCTION

In Nebraska, double-cropped annual forages, often referred to as cover crops, or forage cover crops are utilized to provide agronomic and conservation benefits, as well as a feed source for livestock. In Nebraska and much of the Midwest, these annual forages are planted most often in mid- to late-summer through early fall. They can be planted following wheat (Triticum), seed corn, corn silage (Zea *mays*), and soybean (Glycine *max*) harvest. A recent survey indicated wheat, seed corn, and corn silage acres accounted for 41% of the cover crops planted in Nebraska (Drewnoski et al., 2015). Small grains and brassicas are the most common forages utilized as cover crops in Nebraska (Drewnoski et al., 2015). Establishment costs discourage some producers from utilizing cover crops, but the potential to use cover crops as a forage source by grazing, ensiling, or haying, gives producers an option to offset the establishment cost and provide a potential net return.

Small grains and brassicas have been shown to be highly digestible (80-90% DM in vitro true digestibility) with moderate crude protein (15 to 20% CP when planted in mid- to late-summer and harvested in early fall (Coblentz and Walgenbach, 2010; Villalobos and Brummer, 2015; Villalobos and Brummer, 2017). Digestibility is greater in fall grown oats compared to late-summer grown oats due to lower neutral-detergent fiber (NDF) concentration and a greater proportion of water soluble carbohydrates (Contreras-Govea and Albrecht, 2006; Coblentz and Walgenbach, 2010). Studies in Wisconsin, Colorado, Nebraska, and Maine have all observed a relatively consistent quality maintained in late summer planted oats and brassicas when harvested throughout the fall (Coblentz and Walgenbach, 2010; Villalobos and Brummer, 2015; Wiedenhoeft and Barton, 1994).

Testing forages for nutritive value can be beneficial to producers to determine stocking rate, develop a grazing plan, and establish rental agreements. However, when tested, these annual forages frequently test high in nitrates. Consequently, producers must decide if the animals grazing would be at risk of nitrate toxicity, and if there is a risk, weigh the cost of not utilizing the forage against this risk (Kemp, 1982). Although multiple reviews have addressed nitrate toxicity (Wright and Davison, 1964; Leng, 2008; Russell, 2002; Hibberd, 1993; Crowley, 1985; Jones, 1988; Kemp, 1982; Lee and Beauchemin, 2014; Mohini et al., 2017; Brunning-fann and Kaneene, 1993; Klasing et al., 2005), few have adequately described nitrate toxicity in grazing situations. Kemp et al. (1982) compiled over 40 feeding studies into a review, and distinguished between fresh, dry, or ensiled forages and the risk of toxicity, but this summary still does not account for all of the factors that occur in grazing situations.

This review describes the potential for nitrate accumulation in annual forages, how nitrate toxicity occurs, the previous research that developed the guidelines used for nitrate toxicity, and why production systems grazing annual forage systems are unique in their potential for nitrate toxicity. This review also identifies gaps in research knowledge of nitrate toxicity and why this "old topic" should be readdressed to fit individual production scenarios. With the information provided, management decisions can be more confidently made by consultants and producers in order to utilize the high quality feed that annual forages can provide.

NITRATE ACCUMULATION IN FORAGES

Soil nitrate is the most common source of nitrogen used for plant growth that non-legume plants use for protein assimilation and other nitrogenous compounds necessary for growth. This occurs through reducing the absorbed nitrate and converting it into ammonia before assimilation (Bolan and Kemp, 2003). However, if plant uptake of nitrate is greater than utilization, nitrate

accumulation can occur. Accumulated nitrates can be toxic in high concentrations to ruminant animals consuming the forage (Wright and Davison, 1964).

Several plant and environmental factors can cause plants to accumulate nitrate. These include plant species, stage of maturity, nitrogen application, short-term drought, prolonged cloud cover, or cold temperatures (Wright and Davison, 1964). Crawford et al. (1961) planted several common annual and perennial forage species fertilized with ammonium nitrate in multiple experiments and concluded that the annual species accumulated greater concentrations of nitrate than perennial species. Other studies have noted species differences affecting nitrate accumulation, although nitrate concentrations varied appreciably for each species as well. For example, Kretschmer (1958), noted oats (Avena *sativa*) accumulated greater nitrate than multiple clover species (Trifolium), alfalfa (Medicago *sativa*), or tall fescue (Festuca *arundinacea*). Other studies and reviews have listed species prone to nitrate accumulation, and those that have caused nitrate toxicity. Many annuals commonly used as forage are included on these lists such as millet (Pennisetum *glaucum*), oats (Avena *sativa*), rapeseed (Brassica *napus*), rye (Secale *cereal*), sorghum (Sorghum *bicolor*), and sudangrass (Sorghum *sudanense*) (Provin and Pitt, 2003).

Fertilization rate and growth stage were two other nitrate accumulation factors observed by Crawford et al. (1961). When oats were fertilized from 0 to 224 kg N/ha, harvests at the vegetative, boot, and dough stage illustrated the most nitrate accumulated during the vegetative stage (Table 1). These data demonstrate that immature forages have higher concentrations of nitrate than mature forages, and that increasing fertilizer rate increases nitrate concentrations.

Other data in the study by Crawford et al. (1961) indicated that N fertilization rate affected nitrate accumulation more than the timing of N fertilization. This was demonstrated by fertilizing oats with 224 kg N/ha 19, 32, 43, or 59 days after seeding or shortly before harvest.

The oats were then all harvested on day 77 after seeding. The resulting nitrate concentrations were 0.91, 0.88, 1.38, and 0.94% (2,093, 2,024, 3,174, 2,162 mg NO_3-N/kg DM) respectively. Although Crawford et al. (1961) was unable to measure leaching fertilizer in the soil, which could have influenced the result, the data shows increasing fertilizer rates will increase nitrate in the forage.

When the 0 to 224 kg N/ha fertilized oats were harvested, stems, leaves, and heads were separated to determine the nitrate partitioning among the various plant parts, the stems accumulated the most nitrate followed by the leaves with little nitrate in the heads. The difference in plant parts increased as more fertilizer was applied. At the boot stage, stems ranged from 0.2 to 1.4% NO_3DM (460 to 3,220 mg NO_3-N/kg of DM) leaves from 0.2 to 0.5% NO_3DM (460 to 1,150 mg NO_3-N/kg of DM) and heads remained fairly constant at approximately 0.1% DM NO_3 (230 mg NO_3-N/kg of DM). Additionally, the nitrate differences in the stems and leaves narrowed during the dough stage as leaves accumulated more nitrate than observed in the boot stage (Crawford et al., 1961).

Maynard et al. (1976) analyzed vegetables for nitrate concentration and observed similar plant part effects of nitrate accumulation as Crawford et al. (1961). The fruit or flower had lower nitrate accumulation than the leaf, followed by the stem. The highest concentrations of nitrate were found in the lower 1/3 of the stem. Maynard et al. (1976) noted roots also can accumulate substantial nitrate in concentrations that fall between the upper and lower stem concentrations, however plant species has an effect on root nitrate accumulation. Radish and beet roots accumulate high nitrates, while sweet potatoes and carrots do not (Maynard et al., 1976).

The last factor evaluated in the Crawford et al., (1961) study was the effect of light intensity on nitrate accumulation. Plants shaded to an estimated 60% light intensity had much

higher accumulation than plants under full light (Crawford et al., 1961). Even within a 24 hr period growing conditions such as daylight and temperature can have significant impacts on nitrate concentrations in the plant. A study in New York demonstrated considerable diurnal nitrate accumulation, beet plants fluctuated from approximately 0.7 to 0.2% NO_3-N DM (7,000 to 2,000 mg NO_3-N/kg DM) from sunrise to sunset respectively (Minotti and Stankey, 1973).

A study by George et al. (1971) illustrated the effect of light intensity on photosynthesis. Photosynthesis, and thus light intensity, drives protein and organic compound synthesis, in which the plant utilizes nitrate (Bolan and Kemp, 2003). Phipps (1975) noted a negative correlation in nitrate accumulation and water soluble carbohydrate accumulation. This relationship was expected as decreasing light intensity increases nitrate concentrations by slowing the photochemical reduction of nitrate in the plant. An additional explanation for the nitrate build up is that the water soluble carbohydrates provide the energy for the enzymes that reduce nitrate, and so lower water soluble carbohydrates would decrease nitrate utilization by the plant (Phipps, 1975).

In 1958, a study by Kretschmer in the Everglades measured nitrate concentrations from early December through late January in oats and recorded rainfall and temperature. Kretschmer (1958) observed a decrease in nitrate concentrations following the first observed frost damage, and then the nitrate concentrations steadily increased through the remaining observations. Daily low temperatures (-1.1 to 18.3° C range) correlated with increasing nitrate content more than daily high temperatures (Kretschmer, 1958). Additionally, a smaller difference between the daily high and low for the day resulted in higher nitrate content.

Drought, hail, frost, and disease have been noted to increase plant accumulation of nitrate (Bolan and Kemp, 2003). A multiple regression analysis of climate information by Dickson and

Macpherson (1976) indicated that the amount of sunshine hours for 10 days prior to harvest was the most correlated climate variable that affected nitrate accumulation. To give a simplified explanation, situations where photosynthesis or growth rate is suppressed for a period of time will cause nitrate accumulation in plants (Bolan and Kemp, 2003).

Susceptibility of cover crops to accumulate nitrate

In Nebraska farming systems, cover crops utilized in the spring and fall have multiple factors that can make their use as a forage resource susceptible to accumulating high concentrations of nitrate. As previously discussed, annual forages are more prone to accumulate nitrate than perennial forages (Crawford et al., 1961). Brassicas and small grains, the most common species utilized as cover crops in Nebraska, are known to accumulate high concentrations of nitrates (McCartney et al., 2009). It is common for cattle to graze late-summer planted forages starting in late fall through the early winter and for cattle to graze fall planted winter hardy forages in the early spring. These grazing systems cause grazing to coincide with cool temperatures and shorter day lengths which can lead to increased nitrate concentrations (Bolan and Kemp, 2003). Additionally, these cover crops tend to have relatively short growing windows resulting in forages often being grazed when quite immature. Unfortunately, the vegetative, early growth stage corresponded with the highest nitrate concentrations (Crawford et al., 1961; Bolan and Kemp, 2003).

As mentioned previously, one cropping system often uses cover crops is hybrid seed corn production. Nebraska is a top producer of seed corn, and harvested 223,000 acres of seed corn in 2013 (Stovall, 2016). With the early harvest in this system, cover crops work into the system for multiple agronomic benefits, and to scavenge excess nutrients. Seed corn is harvested earlier than corn for grain, and often leaves more nitrogen in the soil following harvest than other

cropping systems. Cropping systems that leave excess fertilizer in the soil benefit from cover crops capturing and recycling those nutrients back into the soil for the next crop to use, but there is a risk of accumulating high nitrate concentrations in the cover crop plants which can be potentially toxic to ruminants if the cover crop is used as a forage source.

NITRATE TOXICITY IN RUMINANTS

Dietary nitrate is consumed and enters the rumen where the microbial population converts nitrate to nitrite, and then ammonia. Ammonia is then utilized by bacteria to grow resulting in protein in the form of the bacteria themselves, that the animal can utilize (Undersander et al., 1999). The excess ammonia produced travels through the portal blood, enters the urea cycle in the liver, and is either recycled back to the rumen or travels to the kidneys and excreted in urine (MacKown and Weik, 2004). However, if nitrate is consumed at high concentrations in the diet, animal health can be affected because the bacteria that convert the intermediate, nitrite to ammonia can be overwhelmed resulting in nitrite being absorbed into the bloodstream.

To understand what rumen populations are responsible for nitrate reduction, Lin et al. (2011) did an in vitro study in which they compared the rate of nitrate reduction in whole rumen fluid, the bacterial fraction, the protozoa fraction, and the fungi fraction. The study also had a non-supplemented control and a urea supplement to compare to the nitrate supplement. All were sourced from the same animal un-adapted to a high nitrate diet. Lin et al. (2011) found the whole rumen fluid, protozoa, and bacteria were responsible for nitrate reduction, but the bacteria took approximately 12 hours to become as effective a reducing nitrate as the protozoa or whole rumen fluid. Additionally, nitrate decreased methane production, increased acetate production, and decreased propionate and butyrate (Lin et al., 2011). A study by Lewis (1951b) concluded that

the optimal pH for nitrate reduction is 6.5 and 5.6 for nitrite reduction in vitro. The reported pHs in the study by Lin et al. ranged from 6.5 to 6.82 (Lin et al., 2011). The pH evidence suggests a higher energy diet may favor nitrite reduction and prevent nitrite accumulation in the rumen.

When the ruminant consumes high levels of nitrate, the conversion of the nitrite to ammonia is limited because this reduction typically occurs at a slower rate than the reduction of nitrate to nitrite (Lewis, 1951a). Lewis (1951a) initially recognized the rate limiting step in a study measuring ruminal nitrate, nitrite, and ammonia after sheep were administered nitrate through a stomach tube (Lewis et al., 1951a). This rate limiting step results in the buildup of the intermediate nitrite. Both nitrate and nitrite are water soluble and easily enter the bloodstream through the rumen (Wang et al., 1961). One mechanism used to absorb nitrate is the Cl^-/HCO_3^- exchange, and nitrate has been noted to inhibit Cl^- absorption in the rumen (Würmli et al., 1987).

If nitrite enters the blood, it will convert ferrous hemoglobin to ferric methemoglobin, which is unable to carry oxygen (Burrows et al., 1987). Consequently the signs of nitrate toxicity result from a lack of oxygen and asphyxiation. Some signs include a staggering gait, rapid breathing, collapse, abortion, and death (Bolan and Kemp, 2003). In cattle, clinical signs begin to appear when 40% (Hibberd et al., 1993) to 60% (Burrows et al., 1987) of the total hemoglobin is converted to methemoglobin, and death occurs when 70-90% of hemoglobin has been converted (Hibberd et al., 1993, Burrows et al., 1987). However, there is some discrepancy on when signs and death occur as other publications report adverse effects occurring after 20% of hemoglobin has been converted to methemoglobin and death when 60% has been converted (Al-Qudah et al., 2009).

Treatment

Blood naturally contains limited amounts of NADH methemoglobin reductase, an enzyme capable of converting methemoglobin back to hemoglobin. However, this process is slow (Mansouri and Lurie, 1993). There are not good treatment options for livestock experiencing methemoglobinemia. Methylene blue is one treatment option, and this product speeds up the conversion of methemoglobin back to hemoglobin by transferring an electron from the reductase to the methemoglobin (USP Conv., 2008). This treatment was noted to be effective in cattle as early as 1940. Bradley et al., (1940) used an intravenous injection of a 4% methylene blue solution at a rate of 2 g per 500 lb of BW to treat cattle with signs of nitrate toxicity. However, there are no commercial veterinary methylene blue products in the U.S. or Canada, which requires veterinarians to use human products. These have had some success, but treating multiple cattle experiencing nitrate toxicity simultaneously can make acquiring enough methylene blue difficult (USP Conv., 2008). Treating livestock with methylene blue is an off label use, as it is not specifically approved for veterinary use, even though it is considered an acceptable treatment for emergency methemoglobinemia (2008 USP Conv., 2008). As an off label use, the treatment must be administered by a vet, and the dose, as well as number of doses is determined by the vet. The limited research on the long term effects of this treatment in livestock leads to carcinogenic and withdrawal time concerns (USP Conv., 2008).

Adaptation

The most recent research focus on nitrate, involves utilized nitrate supplements as method to reduce methane production in ruminants. Nitrate metabolism is a preferred hydrogen sink to methane production, and the potential to utilize nitrate as a non-protein nitrogen source that can reduce methane production is thoroughly discussed in a review by Leng (2008). Because

of this potential, Lee et al. (2015a) supplemented encapsulated nitrate to beef heifers to determine if cattle can safely be fed nitrate in a feedlot setting without experiencing toxicity. The dietary nitrate content was increased from 0.11 to 4.8% NO_3 (253 to 11,040 mg NO_3-N/ kg DM) in a diet containing mostly barley silage (50%) and dry rolled corn (40%). A 14-day adaptation period was used, and the resulting methemoglobin concentrations did not increase with increasing nitrate in the diet. However, it is important to note that cattle sorted some encapsulated nitrate pellets out in the high diet inclusions, and had a slower rate of intake than lower inclusion heifers (Lee et al., 2015a). The maximum methemoglobin concentration reached by any heifers in the study reached 17.3% (Lee et al., 2015a). A study by Alaboudi and Jones (1985) acclimated 55 kg sheep to a 2.5 g KNO_3/kg BW (23,000 mg NO_3-N/kg DM assuming 2.5% BW DMI) diet. When using the rumen fluid from adapted or un-adapted sheep, they saw the adapted sheep rumen fluid reduced nitrate at a rate three times the rate of the un-adapted rumen fluid and reduced nitrite at a rate five times faster. Rumen fluid collected from sheep on a 5000 mg NO_3-N/kg DM diet was more effective at reducing nitrate than rumen fluid from sheep on a low nitrate diet (Sinclair and Jones, 1964).

Not all studies successfully adapted animals to increasing levels of nitrate in the diet. By increasing the nitrate dose given daily, Cheng et al. (1985) noted an increased capability of the rumen fluid from treated animals to reduce nitrate and nitrite at a faster rate than controlled animals. These holestein cows were given 0.1 g NO_3/kg BW (920 mg NO_3-N/kg DM) on day one, and increased by 0.1 g NO3/ kg BW per day. All four treatment cows died after reaching 0.4 g NO_3/kg BW (3,680 mg NO_3-N/kg DM) on day 4, but this dataset still showed the rumen's capacity to increase nitrate and nitrite reduction rates and the potential for a more gradual adaptation (Cheng et al., 1985). Increasing the dietary nitrate daily likely caused the adaptation

period to fail as one day was not long enough for adaptation to be successful (Cheng et al., 1985).

In a study by Kemp et al., (1977) potassium nitrate was dosed for 18 days to cattle. The peak nitrate concentration in the rumen fluid occurred the day of the initial dose, however, the nitrite concentrations in the rumen fluid and methemoglobin concentrations in the blood remained above 25% through day 18. Unfortunately, samples were not taken past day 18 to observe how long the cattle took to adapt to the dietary nitrate in order to have normal methemoglobin levels.

Establishing guidelines

Nitrate toxicity was first recorded by Mayo in 1895 (Wright and Davison, 1964). Since then multiple cases of nitrate toxicity in ruminants receiving a high amount of nitrate in their diet have been reported. The initial work quantifying the risk of nitrate toxicity and what levels of nitrate can be safely fed was conducted by Bradley et al. in 1940. The study started following about 15 cases in which cattle fed oat hay or straw aborted or died. To determine the toxic agent, Bradley et al. made an oat hay extract from feed samples. One extract contained the equivalent of 128 g KNO_3 and was given to a 116 kg calf (6167 mg NO_3-N/kg DM assuming DMI of 2.5% BW) and the other was identical except 70% of the nitrate was removed by crystallization. Then the extracts were administered via a stomach tube to test animals, confirming nitrate was the cause when the nitrate treated calf had identical symptoms to the case study calves, while the low nitrate calf remained normal. Following this conclusion, the objective of the next study was to establish the minimum lethal dose for nitrate (Bradley et al., 1940).

To establish the minimum lethal dose, nine calves were given various levels of potassium nitrate by a stomach tube. One calf was given 75 KNO_3/cwt, three 50 g KNO_3/cwt, one 30 g

KNO_3/cwt, and four 25 g KNO_3/cwt. Assuming DMI of 2.5% of BW these doses would equate to a feed concentration of 9231, 6179, 3702, and 3087 mg NO_3-N per kg of DM. All animals died except for two given the 25 g KNO_3/cwt, resulting in the LD_{50} (lethal dose for 50% of population) to be estimated at 25 g KNO_3/cwt (3087 mg NO_3-N/kg DM assuming 2.5% BW DMI), although the authors acknowledged that more data was needed to establish a more accurate recommendation. Given the nitrate levels tested and the results, Bradley et al. decided to suggest that the safe level of nitrate should be set at 1.5% KNO_3 (2100 mg NO_3-N/kg DM).

In 1966, Crawford et al. set out to confirm, or update the lethal dose recommendations proposed by Bradley (1940) as well as observe how toxicity affected growth and milk production. Crawford recognized that drenching nitrate in trials to determine toxicity is not directly comparable to production scenarios when the nitrate is consumed in feed.

Crawford et al. (1966) used three different studies utilizing yearling, 2 year, and mature female dairy cattle. The nitrate sources ranged from heavily fertilized oats hayed in the boot stage containing up to 2.3% NO_3 (5290 mg NO_3-N/kg DM), stomach tube/ drenched sodium nitrate, and hay top-dressed with calcium nitrate, potassium nitrate, or sodium nitrate salts dissolved in water before treating hay. The study noted some consumption effects, as heifers did not eat the top-dressed hay as well as the control hay. They also measured the maximum methemoglobin reached in each treatment and noted the peak occurred 3 hours after drenching, 4.5 hours after feeding if the cattle were fed two times per day, and 8 hours after feeding if the cattle were only fed once a day.

Crawford et al. (1966) suggested that the LD_{50} for nitrate toxicity to be 15g/100lb BW (3040 mg NO_3-N/kg DM assuming 2.5% BW DMI) if the nitrate is administered directly through a drench or stomach tube, and to be 45 g/100 lb. BW (9119 mg NO_3-N/kg DM assuming 2.5%

BW DMI) if the nitrate was fed through top-dressed hay (Crawford et al., 1966). Thus when animals received nitrate via a drench the LD_{50} estimated by Crawford et al. (1966) was similar to what Bradley et al. (1940) suggested, but the LD_{50} for animals fed nitrate through top-dressed hay was three times higher.

There have been multiple studies on nitrate toxicity and case studies on nitrate toxicity since Bradley et al. (1940). Table 2 summarized some of these studies with the dose of nitrate given, how the dose was given, and the resulting health effects observed. Most of the previous studies shown on the table administer nitrate directly through a cannula or by drenching the test animal. There are multiple other studies, and some administer nitrate through the diet such as the previous study discussed by Crawford et al. (1966), and as well as case studies in which nitrate toxicity was the diagnosed cause of death (Al-Qudah et al., 2009; Bradley et al., 1940; Hasley, 1998) that were not included in the table.

The study by Simon et al. (1959) dosed dairy heifers with three levels of nitrate through a cannula with the goal of quantifying the dose of nitrate necessary to cause abortion. The initial dose given was close to the LD_{50} of 25 g KNO_3/cwt (or 3087 mg NO_3-N/kg DM assuming 2.5% BW DMI) that was suggested by Bradley et al. (1940). They gave 140 g KNO_3 to a 700 lb heifer which would have equated to 20 g KNO_3/cwt (or 2887 mg NO_3-N/kg of DM assuming 2.5% BW DMI) and resulted in all three treatment animals dying. Simon et al. (1959) then gave a 100 g KNO_3 dose (14 g KNO_3/cwt; equivalent of 2253 mg NO_3-N/kg of DM assuming 2.5% BW DMI) and all three treatment animals aborted, but survived. The lowest nitrate dose was 70 g KNO_3 (10 g KNO_3/cwt; equivalent to 1599 mg NO_3-N/kg of DM assuming 2.5% BW DMI) which resulted in no adverse health effects (Simon et al., 1959).

Davison et al. (1964) also wanted to quantify the reproductive effects of nitrate in dairy heifers. Davison et al. (1964) observed abortion at 440 mg $NaNO_3$/kg BW (2181 mg NO_3-N/kg of DM assuming 2.5% BW DMI) and both abortion and death when dosed 660 mg $NaNO_3$/kg BW $NaNO_3$ (3301 mg NO_3-N/kg of DM assuming 2.5% BW DMI) (Davison et al., 1964).

At a glance, the animals Davison et al. (1964) were affected at a higher level of nitrate than the animals observed by Simon et al. (1959) since a much smaller proportion of animals experienced toxicity but some key experiment differences make the trials difficult to directly compare. For one, Davison et al. top-dressed the nitrate dose onto feed in a solution, and the study by Crawford (1966) demonstrated the difference between a dose placed directly into the rumen, and a dose ingested through feed with a slower rate of intake. Additionally, the heifers in the study by Davison et al. (1964) received an additional energy supplement through corn, resulting in a presumably higher quality diet than the heifers in the study by Simon et al. (1959), although Simon et al. does not report a diet. The diet quality difference was shown to be important in later studies, specifically the study by Burrows et al. (1987) in which the highest energy diet resulted in the least methemoglobin formation (Burrows et al., 1987).

Other study treatments summarized in Table 2 include studies by Setchel and Williams (1962) Wang et al. (1961), Lewis et al. (1951), and Hymas and Mesler (1960). The study by Setchel and Williams (1962) contained both chronic and acute toxicity experiments, but the table only reports the results from the acute experiment. Only one ewe, and two weathers were utilized. All animals were given 20 g $NaNO_3$ with a stomach tube, but because of weight differences, this was equivalent to approximately 3123 mg/kg NO_3-N (assume 2.5% BW DMI) for the ewe, which did not have signs of toxicity, and 3747 mg/kg NO_3-N (assume 2.5% BW DMI) for the wethers that both died from toxicity. The study by Wang et al. (1961) was done to

better understand the time course of nitrate reduction in ruminants, and so a lower dose was given. This dose of 100 g KNO_3 (1647 mg/kg NO_3-N DM assuming 2.5% BW DMI) was low enough that no adverse health signs were noted, but an appreciable peak amount of methemoglobin was measured at about 15% of hemoglobin (Wang et al., 1961). The main takeaway from the study by Wang et al. was that nitrate and nitrite are readily absorbed through the rumen wall into the blood stream, and that the time max methemoglobin formed corresponds to the time maximum nitrite production in the rumen occurred (Wang et al., 1961), this has been confirmed by others including Kemp et al. (1977). In the study by Wang et al., (1961) this occurred approximately 3 hours after dosing, however the peak time has been noted to change if fed nitrate through hay rather than drenched, or if an animal is fed once or twice a day (Kemp et al., 1977; Crawford et al., 1966)

The study by Lewis used multiple nitrate dose concentrations to better understand the reduction of nitrate in the rumen, and to observe the subsequent methemoglobin produced (Lewis et al., 1951a). Through this study, Lewis et al. (1951a) identified the conversion of nitrite to ammonia as the rate limiting step, and found that a dose of 25 g $NaNO_3$ (2561 mg NO_3-N /kg DM) would result in 60% of hemoglobin being converted to methemoglobin when placed in the rumen of three-year old wethers fed 1.6 kg of hay per day. They also noted that 50-60% methemoglobin resulted in the first observed signs of nitrate toxicity (Lewis et al., 1951a). In the literature, there are studies in which ruminants tolerated a much higher dose of nitrate than what the authors expected. For example, in a study by Hymas and Mesler (1960) a Guernsey bull was initially dosed with the equivalent of 4094 and 5888 mg NO_3-N /kg DM through a stomach tube and survived both treatments. Then, a dose of 6780 mg NO_3-N /kg was given before successfully causing the animal to die from nitrate toxicity. Death was observed for a Guernsey steer given a

dose equivalent to 8538 mg NO_3-N /kg that subsequently caused death in the same experiment. All of the doses given in the study would have been expected to cause death based on previous research, but the bull tolerated two high doses without reported clinical signs of toxicity.

Few studies observe nitrate levels on fresh, grazed forages. One of the few studies was done by Dickson and Macpherson (1976), when they grazed perennial ryegrass pasture under three different fertilization treatments with lactating ewes for two years. The fertilizer treatment pastures were split into 6 or 7 paddocks to be rotationally grazed, and the nitrate content of the pastures ranged from 0.03% to 0.67% NO_3-N DM (300 to 6700 mg NO_3-N/kg DM). In both years, there were no health issues and the maximum methemoglobin reached on the most heavily fertilized feed was 0.2 g per 100 ml blood (12.5-25% methemoglobin assuming 8 and 16 g/dL hemoglobin in sheep). The 6700 mg NO_3-N /kg would have been considered a very toxic level in the diet for ruminants based on traditional recommendations. The fact that the ewes were grazing, and a gradual adaptation likely occurred as the pastures increased in nitrate concentrations over time, allowed the sheep to graze with no adverse consequences. However, some reviews and studies indicate fewer cases of nitrate toxicity in sheep and that sheep are less susceptible than cattle (Sinclair and Jones, 1964) which could also have influenced the results by Dickson and Macpherson (1976). Another study by Phipps (1975) fertilized herbage that reached 0.76% NO_3-N (7600 mg NO_3-N/kg DM) and expected to see increased methemoglobin and signs of nitrate toxicity in dairy cattle grazing the herbage, but no adverse health consequences occurred, including no difference in blood methemoglobin (Phipps, 1975).

Multiple guidelines have been put together for producers to utilize when making management decisions. However, these guidelines are not consistent and might not apply to every scenario. Table 3 provides a few examples of state extension program recommendations

for feeding nitrate containing feed to non-gestating cattle. Most of these extension resources differentiate between the toxic concentration of nitrate in feed and the toxic concentration in water. Some also provide more conservative recommendations for gestating cows compared to other cattle types (yearlings, bulls, open cows, etc.), and in the "caution feeding" category, further specify the need to dilute the intake of high nitrate feeds by mixing with low nitrate feeds. The state extension recommendations are most similar when looking at the safe level of nitrate in the diet, but some programs are more conservative than others when evaluating the toxic level of nitrate.

Chronic and acute toxicity

In the literature, there are two symptom categories for nitrate toxicity, chronic and acute. According to a review by Wright and Davison (1964) acute toxicity occurs when an animal dies or collapses shortly after consuming the toxic agent, and chronic toxicity is any lesions formed or poor production that occurs for an extended period of time before the animal fully recovers or dies (Wright and Davison, 1964). Multiple publications follow this example to define chronic and acute symptoms, but these are also misleading. By the simple definition, acute toxicity refers to adverse effects resulting from a single or multiple doses of the toxic substance given in a short period of time, while chronic toxicity refers to toxicity caused by long term exposure to a toxic agent. In the case of nitrate toxicity, cattle are always exposed to nitrate through plant material in the diet. The compound nitrate itself is not toxic, rather high doses of nitrate become toxic if the ruminal microorganisms are not able to break down the intermediate, nitrite, fast enough to prevent nitrite from building up. Nitrite is the toxic compound (Hibberd et al., 1993). Bruning-fann and Kaneene (1993) wrote a thorough review on nitrate toxicity, addressing chronic and acute signs, and the studies done on each.

There are multiple claims of chronic symptoms cattle and other species experience when exposed to non-lethal concentrations of nitrate for an extended period of time. Some chronic effects attributed to nitrate toxicity include depressed gain and milk yield, abortion, vitamin A deficiency, and thyroid issues (Hibberd et al., 1993; Wright and Davison, 1964). The data leading to the belief that nitrate interferes with thyroid function appears to originate from a study by Wyngaarden et al. (1952), in which nitrate blocked iodine uptake in rat thyroids (Wright and Davison, 1964). However, Bloomfield et al. (1962) did a study on sheep and did not observe any thyroid effects similar to what was noted in rats. A study by McIlwain and Schipper (1963) dosed calves with nitrate in their water source and measured serum carotene and vitamin A, and observed a slight decrease in vitamin A and increase in carotene as the nitrate dose increased. The same study also had a nitrate treatment in which they also supplemented a small dose of E. coli organisms in the drinking source and observed slightly higher methemoglobin levels in the E. coli dosed calves. However, the increase was slight and the authors concluded further research was needed, before drawing conclusions about the vitamin A production result, or the E. coli effect on nitrate toxicity susceptibility.

Jainudeen et al. (1964) used Holstein heifers to observe any effects of nitrate toxicosis on the thyroid or endocrine response by feeding 0, 440, or 660 mg NO_3/kg BW (0, 2181, and 3301 mg NO_3-N /kg DM respectively). The nitrate given through top-dressed hay, and the heifers were grouped into treatments that started began three estrous cycles before breeding, 40 days pregnant, or 150 days pregnant. All continued treatment until 30 days post calving following which all were necropsied. The only response was a slightly heavier pituitary gland, and the researchers concluded that in ruminants, it is unlikely nitrate affects vitamin A or iodine balance, or the thyroid (Jainudeen et al., 1964).

One concern of chronic nitrate toxicity is decreased gain and performance. Nitrate is reduced to nitrite, and nitrite is known to be antimicrobial and is used in the meat-processing industry for that purpose (Marais et al., 1988). Marias et al. (1988) performed an in vitro study with rumen fluid from sheep, and compared bacterial populations as well as the difference in digestion when potassium nitrate was added to the rumen fluid. The feed (Kikuya grass) was incubated from 0 to 72 hours in order to understand the rate and extent of digestion. The nitrate addition reduced the rate of digestibility although the extent of digestibility did not change. When tungstate was added as an inhibitor of nitrate reductase, the digestibility rate was not decreased to the extent it was when no nitrate reductase inhibitor was added, indicating the reduced digestibility occurred due to the presence of nitrite. The nitrate treatment reduced total microbial populations compared to the control, specifically cellulolytic and xylanolytic populations. Marias et al. (1988) acknowledges challenges with in vitro studies, but concluded that due to the decreased bacterial populations, the performance of animals on high nitrate diets could be inhibited.

Even though the antimicrobial effect of nitrate can be demonstrated, in vitro studies are unable to demonstrate the rapid turnover and adaptation of the microbes in the rumen. In fact, multiple studies used nitrate in the diet as a rumen degradable protein source to replace urea and found no differences in performance (Nolan et al., 2010; Huyen et al., 2010; Phuc et al., 2010; Sophea and Preston, 2010). Ewes grazing three pastures fertilized at different rates (100, 400, or 700 kg ha^{-1} N) gained more prior to weaning on the pasture most heavily fertilized. The pasture most heavily fertilized had a higher concentration of nitrates reaching 6,700 and 4,600 mg NO_3-N/kg DM in year one and two respectively. However, yield and intake data were not reported which could heavily influence the performance (Dickson and Macphearson, 1976).

In the study by Crawford et al. (1966) heifers on 1.24% nitrate (2852 mg NO_3-N/kg DM) oat hay gained significantly more than heifers on 0.08% nitrate (184 mg NO_3-N/kg DM) hay. The results from this study were not inferred as nitrate improving performance, but rather that if fed in hay instead of drenched directly into the rumen cattle can tolerate and grow normally on a higher nitrate load than the threshold suggested by Bradley et al., (1940) previously. Crawford et al. (1966) was unable to observe a significant difference in total milk production, similar to the total milk production results observed by Davison et al. 1964. Although nitrate does not appear to affect the volume of milk produced, studies have noted slight changes in milk composition. Davison et al. (1964) noted a slight increase in the nitrate content of milk as the nitrate dose increased.

The other potential cause of decreased performance appears to be reflected by the decreased rate of intake. Decreased intake in feedlot steers with an added 1% sodium nitrate in the diet was observed by Weichenthal et al. (1963), and when Hale et al. (1962) added 1% potassium nitrate to a finishing steer diet. However, Hale et al. (1962) did not observe a significant difference in average daily gain even though intake was reduced. When offered a preference, cattle in a study by Hymas and Mesler (1960) drank more water from a water source with less nitrate. In a study feeding encapsulated nitrate, the rate of intake decreased, and more sorting occurred as higher inclusions of nitrate were in the diet (Lee et al., 2015a).

When discussing nitrate toxicity symptoms, it is important to note the difference in acute, chronic, and sub-lethal signs. Abortion is probably the most concerning non-lethal sign of nitrate toxicity and has been considered either acute or chronic by researchers. Although some consider this a chronic sign of nitrate toxicity, most occur after nitrate concentrations reach near lethal levels (Crawford et al., 1966). In a case study paper by Ozmen et al. (2005) claims of chronic

toxicity causing abortion are made, however, Ozmen et al. (2005) defines a chronic nitrate toxicity as “a form of nitrate poisoning in which clinical signs of the disease are not observed.” (Ozmen et al., 2005). Technically, a chronic toxicity refers to the length of exposure rather than severity of the sign of toxicity, making the study by Ozmen et al. (2005) one of multiple examples in which nitrate is given blame for chronic effects rather than only acute toxicity effects.

When nitrate toxicity causes abortion, the lack of oxygen is the underlining cause. Nitrate limits blood oxygen through methemoglobin formation and/or a vasodilating effect (Malestein et al., 1980). Previous research using human blood demonstrated that equivalent doses of nitrite form methemoglobin in fetal blood faster than in maternal blood when tested in vitro (Malestein et al., 1980). A study by Malestein et al. (1980) was done to better understand abortion in ruminants exposed to nitrate by measuring maternal and fetal blood nitrite concentration and methemoglobin formation as well as the dam’s heart rate and respiration. Eight cows were given an intravenous or oral dose of potassium nitrite at partus, and 4 cows remained untreated and were used as controls. When comparing this study to others, it is important to note nitrite, not nitrate was administered. Nitrite treatment was given at partus in order to have access to the fetal blood. The animals were treated with 9-10 mg NO_2/ kg BW via intravenous, or 30 mg NO_2/ kg BW given orally. Nitrite rapidly increased in maternal blood after administration, but not in fetal blood. The maximum methemoglobin range reached in maternal blood of animals dosed with nitrite at partus reached 30-48% methemoglobin, while fetal blood only reached maximum levels of 3-11.2% methemoglobin. Although actual abortion could not be observed by waiting until partus to challenge animals with a nitrite load, the data demonstrated that the lack of oxygen transferred from maternal blood through the placenta, rather than methemoglobin formation in

fetal blood causes nitrate induced abortions (Malestein et al., 1980). Some indicate the last trimester of pregnancy is the most susceptible to abortion due to nitrate toxicity (Ozmen, 2005).

There is not a consistent dose of nitrate that will induce abortions. A study by Davison et al. (1964) used 45 dairy heifers challenged with a sodium nitrate load top-dressed onto feed at 440 mg/kg BW or 660 mg/kg BW (2181 and 3301 mg NO_3-N/kg DM). The 45 animals were split into 4 different treatments. The treatments in this study started feeding sodium nitrate three cycles before breeding, on the 40th day of pregnancy, or at 150 days of gestation. The 440 mg/kg BW treatment caused 1 abortion, and the 660 mg/kg NaNO3 caused 2 abortions. The 660 mg/kg treatment also caused 2 animals to die, and 1 animal collapsed twice. The animal that collapsed reached 93% methemoglobin, and was fed hay without nitrate on both evenings following her collapse before being put back on treatment the next morning. The collapses occurred following 162, and 181 days of being fed nitrate. The heifer that collapsed still gave birth to a live calf (Davison et al., 1964).

A few studies have observed nitrate's effect on other reproductive measures. In the previously discussed study by Davison et al. (1964), the heifers challenged with the 660 mg/kg sodium nitrate had lower conception rates than the animals treated with 440 mg/kg BW sodium nitrate and the untreated control animals. However, with less than 15 animals in each treatment, conception conclusions are hard to confidently establish. Laven et al. (2002) did a study with the objective to observe embryo growth and survivability in dairy heifers on heavily fertilized spring pasture. The study had fertilized pastures with nitrate concentrations ranging from 1932-3200 mg NO_3-N/kg DM and had a control forage with 1132 mg NO_3-N/kg DM. The high nitrate feed was grazed for 6 weeks following a 1 week adaptation period that included grazing the pasture during the day and fed a TMR in the evening. There were 48 heifers utilized that started the study 20-57

days pregnant. The study was a 2x2 factorial observing both forage nitrate concentration as well as two levels of concentrate supplementation. The results found higher milk and plasma urea and ammonia concentrations in animals on the fertilized pastures. However, embryo growth and survival was not affected when pregnancy was confirmed at the conclusion of the trial (Laven et al., 2002).

A study by Sinclair and Jones (1964) utilized 30 ewes, and dosed them with and equivalent of 1.5% KNO_3 (2100 mg NO_3-N /kg DM) in the diet by drenching dissolved potassium nitrate directly in the rumen through the entire breeding season and one treatment group continued the 1.5% KNO_3 diet (2100 mg NO_3-N /kg DM) through topdressed hay until lambing. Although the 1.5% KNO_3 (2100 mg NO_3-N /kg DM) was suggested to be the minimal lethal dose for nitrate in cattle diets by Bradley et al. (1940), there were no adverse chronic or acute breeding effects observed in this study (Sinclair and Jones, 1964). The authors recognize sheep may be less susceptible than cattle, but the methemoglobin concentrations were not different between the nitrate groups and control group. This was unexpected but could be attributed to the nitrate dose being too low to cause a difference, or if the blood samples were not taken at an appropriate time to demonstrate peak methemoglobin concentrations (Sinclair and Jones, 1964).

FACTORS THAT INFLUENCE TOXICITY WHEN GRAZING COVER CROPS

When grazing annual forages, there are multiple mitigation factors that may allow the animal to tolerate higher levels of nitrate than what traditional guidelines would indicate. Understanding these factors, as well as the key components that influence toxicity, a producer can better make management decisions for their operation. As the responses to nitrate in controlled research settings has varied, it is important to consider what factors influence the risk of toxicity, and

where the toxic "threshold" is for each production scenario. Lee et al. (2015a) summarized factors that determine toxicity into four categories; 1) nitrate levels in the diet, 2) nitrate consumption rate, 3) nitrate and nitrite reduction rates, and 4) ruminal passage rate (Lee et al., 2015a). A large portion of previous research simply focuses on the nitrate levels in the diet. Some research has observed rate of intake and methods of manipulating the other factors to reduce risk when a producer has feed high in nitrate.

1) Nitrate Levels in the Diet

Measuring nitrate in the available forage, fed or grazed, is a critical first step in preventing nitrate toxicity. Forages can be measured for nitrates with quick-assay methods as well as longer lab methods analyzing oven-dried or fresh samples (MacKown and Weik, 2004). The analysis method can influence the estimated nitrate levels in the forage (MacKown and Weik, 2004). Additionally, sampling method should be taken into consideration when interpreting nitrate analysis results. As stated previously, the leaves contain less nitrate than the stem (Wright and Davison, 1964). The nitrate recommendations are based on total nitrate consumed in the diet. For a grazing animal, an accurate diet sample estimate would be representative, but sampling accurately is difficult. A ground level sample will indicate higher levels of nitrate than what the animal is actually consuming, but can be uniformly done and repeated by producers.

When cattle graze annual forages, animal behavior strongly influences the diet through selectivity. Unlike when fed hay, the cattle are not forced to consume the entire plant. When grazing forages, cattle can be selective and typically eat the leaves initially before the stems (Chacon et al., 1978) consuming less nitrates than lab analysis would predict if samples were taken from ground level. By the time the animal consumes the stem and lower portions of the plant, depending on the stocking rate, the animal may have partially self-adapted to higher nitrate

concentrations, and the overall risk of toxicity is decreased. Grazing management plays a large role in the potential decreased risk of toxicity. If too many animals are allowed access and the forage is overstocked, they cannot be as selective, meaning the stem will be consumed earlier than if cattle were lightly stocked on the forage. The same concept applies to strip grazing management strategies. By strip grazing, cattle cannot be as selective in their diet.

2) Nitrate Consumption Rate

Lee et al. (2015a) attempted to study a slow release form of encapsulated nitrate to mimic a slower consumption rate as opposed to previous studies with readily absorbed supplemental nitrate drenched, given as a bolus, or placed into the rumen through a cannula. In this study, there were two experiments, the first had five heifers fed at 75% ad libitum intake and the second fed eight heifers at ad libitum. The diet was formulated so that the encapsulated nitrate was necessary to meet protein requirements. Nitrate was increased in both experiments from 1% to 5.8% of the diet DM (2,300 to 13,340 mg NO_3-N/kg DM) in five, four day step ups. On the restricted diet, one animal experienced nitrate toxicity and had 59% methemoglobin in the blood when fed 2.9% NO_3 (6670 mg NO_3-N/kg DM) in the diet, and another heifer went off feed when 2% (4600 mg NO_3-N/kg DM) of the diet was nitrate. Zero animals in the ad libitum fed trial were noted to experience any sign of toxicity. Consumption rate decreased linearly in the restricted diets from 0-3 hours after feeding as nitrate was increased. The unrestricted animals did not differ in their feed consumption rates, but there was a tendency for total intake to decrease with increasing nitrate ($P = 0.06$), and the feed was sorted more as the nitrate in the diet increased ($P < 0.05$). This study demonstrated the reduced risk for nitrate toxicity when animals were fed ad libitum (Lee et al., 2015a).

The results by Crawford et al. (1966) indicated nitrate consumed as hay (as opposed to a stomach tube) had an LD_{50} three times higher than what was originally found by Bradley and et al. (1940) when nitrate salts were drenched directly into the rumen. The resulting doses were 45 g/100 lb BW (9119 mg/kg NO_3-N DM assuming 2.5% BW DMI) vs. 15g/100lb BW (3040 mg/kg NO_3-N DM assuming 2.5% BW DMI) (Crawford et al., 1966). The higher tolerance of nitrate was attributed to a slower consumption rate of nitrate (Crawford et al., 1966). Later, Geurink et al. (1979) illustrated the slower consumption rate affecting toxicity, as well. The results in the study by Lee et al. (2015a) provide an additional explanation for the three times higher tolerance observed by Crawford and others (1966).

The consumption rate is a major consideration in nitrate toxicity while grazing annual forages. Grazing situations typically allow a slower consumption rate relative to a bunk feeding scenario for cattle unless a management strategy such as strip grazing is implemented. By strip grazing, an animal can rapidly consume large bites of forage after gaining access to a new area of the field.

3) Nitrate and Nitrite Reduction Rates

From the factors influencing toxicity discussed by Lee et al. (2015a), Geurink et al. (1979) provides evidence on how different nitrate sources can change how the nitrate compound is metabolized. Over a six-year period, Geurink et al. (1979) conducted approximately 40 experiments with the objective of determining more accurate nitrate toxicity thresholds. One experiment, in vitro, showed 80% of the nitrate was available after 20 minutes when a dry hay was submerged in distilled water, while freshly chopped turnips and grass only resulted in a maximum of 30% nitrate present after 20 minutes.

Grazed annual forages are more comparable to fresh chopped turnips and grass that released nitrate into the water at a slower rate than hay, indicating the nitrate will be available for reduction by microbes at a slower rate, further reducing the buildup of nitrite in the rumen and potential absorption of that nitrite into the bloodstream. Kemp (1982) used the compiled data from about 40 studies to create graphs illustrating the difference in the risk of nitrate toxicity when feeding a dried or pre-wilted forage, compared to a fresh forage. The figures he created are shown below (Figure 1). In the figures by Kemp, the reduced risk of toxicity for cattle consuming fresh forage is clearly illustrated. For example, if an animal is consuming 0.6 kg DM/ 100 kg BW, the level of nitrate in the feed that will result in approximately 20% methemoglobin is 2.0% NO_3 (4600 mg NO_3-N/ kg DM) for hay or pre-wilted silage, and 3.5% NO_3 (8050 mg NO_3-N/ kg DM) if fed fresh herbage.

Ruminal pH affects the rate both nitrate and nitrite are reduced by microbes in the rumen. According to Lewis et al. (1951b) the optimal pH to reduce nitrate is approximately 6.5, and the optimal pH to reduce nitrite is approximately 5.6. Later, pH observations by Tillman et al. (1965) agreed with the lower pH favoring nitrite reduction, and observed the increased nitrite absorption into the bloodstream when pH was higher.

Annual forages such as small grains and brassicas are highly digestible (Coblentz and Walgenbach, 2010; Villalobos and Brummer, 2015; Villalobos and Brummer, 2017). Brassicas in particular can be compared more closely to a concentrate than to a roughage as they are very digestible, and low in fiber (Villalobos and Brummer, 2015). A highly digestible diet would likely decrease ruminal pH, favoring nitrite reduction (Sapiro et al., 1949).

In the study by Tillman et al. (1965) the effects of molybdenum as a required cofactor for nitrate reduction and iron and copper as required cofactors for nitrite reduction. The study

observed the ruminal and blood concentrations of nitrate and nitrite in sheep adequate or deficient in these metals. Molybdenum status in the diet affected how fast nitrate was reduced and the molybdenum deficient diet resulted in less nitrate reduction, but nitrite reduction with copper and iron supplementation was not affected (Tillman et al., 1965).

A study by Sapiro et al. (1949) was one of the initial studies that observed energy supplementation's influence on nitrate toxicity (Sapiro et al., 1949). Trials in vitro utilizing rumen fluid from sheep on a poor quality diet retained nitrite in the rumen fluid for a longer period of time than the rumen fluid from a high quality diet. These results indicated either a favorable environment for nitrate reduction, or a less favorable environment for nitrite reduction. An additional in vitro trial by Sapiro et al. (1949) noticed an accelerated rate of nitrite disappearance when glucose was added to the rumen fluid from either a poor or high quality diet (Sapiro et al., 1949).

Following the in vitro observations, Sapiro et al. (1949) used sheep on a poor or high quality diet, and tested for methemoglobin in the blood following a potassium nitrate dose. Glucose was also administered to some test animals. Potassium nitrate and glucose were both dissolved in water and administered with a drench (Sapiro et al., 1949). When given no additional glucose, and 20 g of potassium nitrate/ 100 lb. BW on a poor quality diet resulted in more methemoglobin production than a 50 g potassium nitrate/ 100 lb. BW in a high quality diet in sheep (Sapiro et al., 1949). For both diets, additional glucose appeared to protect the animals from methemoglobin production, and there was no significant difference in the glucose effect on a high vs. low quality diet (Sapiro et al., 1949).

To quantify the effectiveness of corn supplementation on nitrate toxicity, Burrows et al. (1987) used three-year old cows on a prairie hay diet supplemented with different amounts of dry

rolled corn and challenged them with a nitrate load. There were two-week intervals between each animal's nitrate challenge with the intent to prevent the rumen bacteria from adapting to a nitrate load prior to treatment. In the study, cattle were fed 0, 1.6, or 3.2 kg of corn supplementation for 10 days, before administering a nitrate challenge. The challenge was done by dissolving sodium nitrate in water and administering it directly through a rumen cannula. The nitrate load administered was 0.3 g NaNO3/ kg BW (1967 mg NO_3-N/kg DM assuming 2.5% BW DMI). In total, there were 8 observations of animals supplemented 1.6 and 3.2 kg of corn each, and 12 observations of animals receiving no supplementation. In the observations, ruminal nitrite and blood methemoglobin were the only variables significantly affected by corn supplementation. Both ruminal nitrate, and methemoglobin linearly decreased ($P < 0.05$) with increasing corn supplementation for the overall mean, mean of the maximum, and the relative area under the curve. The mean of the maximum methemoglobin values also had a quadratic decrease ($P = 0.03$) with increasing corn supplementation. Eight of 12 head receiving 0 kg of corn and 2 of 8 receiving 1.6 kg showed signs of nitrate toxicity and had methemoglobin reach approximately 50% of hemoglobin before methylene blue was administered and the animals recovered. The results from this study demonstrated corn supplementation can mitigate the risk of nitrate toxicity in cattle and reduces methemoglobin formation in the blood. The 3.2 kg of corn supplemented provided the most mitigation, but there was not a treatment tested that contained more than 3.2 kg of corn (Burrows et al., 1987).

The two discussed studies demonstrate the benefit of high quality diets and additional energy supplementation provide for ruminants consuming high concentrations of nitrate. Multiple reviews give credit to the additional energy increasing microbial fermentation, accelerating the reduction of nitrate and nitrite (Hibberd et al., 1994; Wright and Davison, 1964; Brunning-Fann

and Kaneene, 1993). Ruminal pH was previously discussed and may also play a role in the effect energy supplementation has on nitrate toxicity as a lower ruminal pH provides a rumen environment that favors nitrite reduction, preventing methemoglobinemia.

In a review by Leng (2008), there is a thorough discussion on possible interactions between sulfur reducing bacteria and nitrate reducing bacteria. Takahashi et al. (1998) demonstrated sulfur's impact on nitrate reduction in the rumen through L-cysteine supplementation to sheep treated with a toxic dose of nitrate (0.45% NO3-N, 4500 mg NO_3-N/kg DM) through a stomach tube (Takahashi et al., 1998). Cysteine prevented the buildup of nitrite in the rumen, preventing methemoglobin formation in the blood (Takahashi et al., 1998). The level of cysteine supplemented was 60% of the "maximum allowance of dietary sulfur" for dairy cattle as established by the 1988 NRC standards (Takahashi et al., 1998). There have been similar interactions observed between nitrate and sulfur reducing bacteria in other ecosystems, demonstrating the capability of sulfur to compete with nitrate for hydrogen (Leng, 2008).

Brassicas have been observed to contain considerably high levels of sulfur. In New Zealand, a study observed kale to have 0.85%, rape 0.61%, swedes 0.56%, and turnips 0.69% sulfur on a dry matter basis (Sun et al., 2012). In fact, there are case studies in which cattle consuming diets largely composed by brassicas were diagnosed with polioencephalomalacia (PEM), a disorder caused by high sulfur diets (McKenzie et al., 2009; Gould, 2000). Although the high sulfur content in brassicas provides a risk of PEM, the high sulfur also provides a hydrogen sink to compete with nitrate in the rumen and consequently mitigate the risk of nitrate toxicity in brassica containing diets.

CONCLUSION- MANAGEMENT STRATEGIES AND CONSIDERATIONS

Although there is a significant amount of information published on nitrate toxicity in ruminant animals, there are multiple factors that make this toxicity more complex than it is often portrayed. Traditional recommendations differ in what is considered toxic as well as if there is a different level of nitrate considered toxic for gestating cattle compared to others. Kansas State provides guidelines and specifies that an animal with a compromised immune system is a higher risk animal than others. For each institution's recommendations, there are management strategies suggested to help producers reduce the risk of nitrate toxicity. There are multiple commonalities when viewing each set of recommendations.

When utilizing high nitrate forages, it is critical to not turn cattle out onto the forage when hungry. By doing this, the animals will not gorge themselves immediately on the forage and the slower rate of intake will help prevent nitrate toxicity and any bloat associated with rapid consumption of high quality annual forages. Additionally, it is always recommended to have a general idea of the nitrate content of the water source. Water can easily cause nitrate toxicity and a clean, fresh water source is essential for any successful livestock operation. Water concentrations of nitrate above 227 mg NO_3-N/L is considered high enough to cause acute symptoms and death (Adams et al., 1992) which is much lower than any suggested thresholds for dietary nitrate in the feed. In the case study by Al-Qudah et al. (2008) over half of the animals showed acute signs of nitrate toxicity (dead or aborted) within four hours of turnout. Although the nitrate level in the forage reached 1480 mg NO_3/kg (340 mg NO_3-N/kg), significant losses were incurred because the nitrate concentration of the drinking water was 1700 mg NO_3/kg (391 mg NO_3-N/kg).

If a producer has annual forage nitrate levels that are concerning, and they do not want to risk grazing the forage. There are a few options to still utilize the feed value, although there is a cost associated with each. One option is to ensile the forage. If properly ensiled, the microbial population reduces nitrate to nitrate and then nitrite to ammonia during the fermentation process, and less nitrate is available. However, producers should be cautious and ensure proper ensiling as an incomplete or improper ensiling process can result in nitrite formation, resulting in a forage that is very high risk to feed to cattle. Another option for producers is to bale the forage and then feed the forage in a mixed ration with a low nitrate feed source, resulting in the animal consuming less nitrate.

Rehberger and Hibberd (2000) patented a direct-fed microbial designed to reduce toxicity when cattle consumed high nitrate feeds. Propionibacterium strains were examined to determine what strains were most effective at denitrification when high concentrations of nitrate were available, in order to determine if one of the strains could be fed as a direct fed microbial and reduce the incidence of nitrate toxicity (Swartzlander, 1994). The strain *Propionibacterium acidopropionici* P5 was shown to successfully reduced the toxic effects of a high nitrate diet by reducing peak ruminal and blood nitrite concentrations, and peak methemoglobin when fed as a direct fed microbial and compared to control animals (Swartzlander et al., 1994). This direct fed microbial was patented and is available for purchase and is called Bova-pro (Rehberger and Hibberd, 2000). Treating animals with Bova-pro before turning out onto high nitrate forage is a management strategy available to producers, but the cost needs to be considered before a producer utilizes it.

When grazing high nitrate forages, it is important to allow cattle to be selective. This involved lighter stocking rates, and not overgrazing the forage to a point that the animals are

forced to consume the lower portions of stems. By allowing cattle to be selective, the leaves with less nitrate will be consumed before the stems which contain higher concentrations of nitrate (Wright and Davison, 1964; Chacon et al.1978). Additional energy supplementation can benefit animals on lower quality annual forages (Burrows et al., 1987).

This literature review has emphasized that grazing animals likely can handle a nitrate load greater than traditional recommendations. However, it is important to note there is not enough data available to establish new guidelines. Rather, producers need to be aware of the risk, and weigh the health risk of utilizing the forage with the financial risk of not utilizing the forage to make an informed decision for their operation. More research needs to be done on nitrate toxicity so that recommendations can be given based on individual production scenarios.

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Table 1: Crawford et al., 1961. Fertilizer treatment level and resulting nitrate concentrations in oats (Avena *sativa*)

Fertilization Rate (kg N/ha)	Vegetative Stage (mg NO_3-N/kg DM)	Dough Stage (mg NO_3-N/kg DM)
0	1550	230
224	4140	1610
1121	6210	2760

Table 2: Summary of nitrate toxicity experiments

Study (Author, Year, Treatment)	NO_3 dose reported	mg/kg NO_3-N in diet	Method NO_3 was dosed	# with clinical signs	# Abortions	# Affected (Dead)	% Affected	Animal Type	Animal Weight (kg)	Peak % MtHb
Bradley 1940 75	75 KNO_3/cwt	9231[bc]	Stomach Tube			1 of 1	100%	Beef Calf	91	
Bradley 1940 50	50 KNO_3/cwt	6179[bc]				3 of 3	100%	Beef Calves	145[a]	
Bradley 1940 30	30 KNO_3/cwt	3702[bc]				1 of 1	100%	Beef Calf	211[a]	
Bradley 1940 25	25 KNO_3/cwt	3087[bc]				2 of 4	50%	Beef Calves	151[a]	
Burrows 1987 0	0.3g NaNO3/kg BW	1967[bc]	Through Rumen Cannula	4 of 12			33%	Beef Cows	414[a]	53.1
Burrows 1987 1.6				2 of 8			25%			52.4
Burrows 1987 3.2				0 of 8			0%			27.1
Setchell 1962 ewe	20 g $NaNO_3$	3123[bc]	Stomach Tube			0 of 1	0%	Ewe	42	65
Setchell 1962	20 g $NaNO_3$	3747[bc]				2 of 2	100%	Weathers	35	86[a]
Davison 1964 0	0	0[b]	Topdressed Salt Solution		0 of 5	0 of 5	0%	Dairy Heifers	302[a]	
Davison 1964 440	440mg $NaNO_3$/kg BW	2181[b]			1 of 20	0.00	5%			
Davison 1964 660	660mg $NaNO_3$/kg BW	3301[b]			2 of 20	2 of 20	20%			
Simon 1959 70g	70 g KNO_3	1599[bc]	Through Rumen Cannula	0 of 3			0%	Dairy Heifers	287	
Simon 1959 100 g	100 g KNO_3	2253[bc]			3 of 3		100%		291	
Simon 1959 140 g	140 g KNO_3	2887[bc]				3 of 3	100%		318	
Wang 1961	100 g KNO_3	1647[bc]	Cannula		0	0 of 1	0%	Beef Cows	340[a]	14.9
Lewis 1951a	0 g $NaNO_3$	0[b]	Through Rumen Cannula					3 yr weathers	60[a]	Trace
Lewis 1951a 12	12 g $NaNO_3$	1230[b]								8
Lewis 1951a 17.5	17.5 g $NaNO_3$	1793[b]								15
Lewis 1951a 22.5	22.5 g $NaNO_3$	2305[b]								35
Lewis 1951a 25	25 g $NaNO_3$	2561[b]								60
Hymas 1960 1	928mg NO_3-N/kg BW	8538[b]	Stomach Tube			1 of 1	100%	Dairy Steer	222	
Hymas 1960 2	737mg NO_3-N/kg BW	6780[b]				1 of 1	100%	Dairy Bull	231	

[a] Average of treatment animals.
[b] Calculated using conversion factors from Adams, 1992.
[c] Calculated assuming 2.5% BW DMI.
Burrows treatments- 0, 1.6, 3.2= kg of corn supplemented on a grass hay diet.

Table 3: Suggested dietary nitrate thresholds for various state extension programs

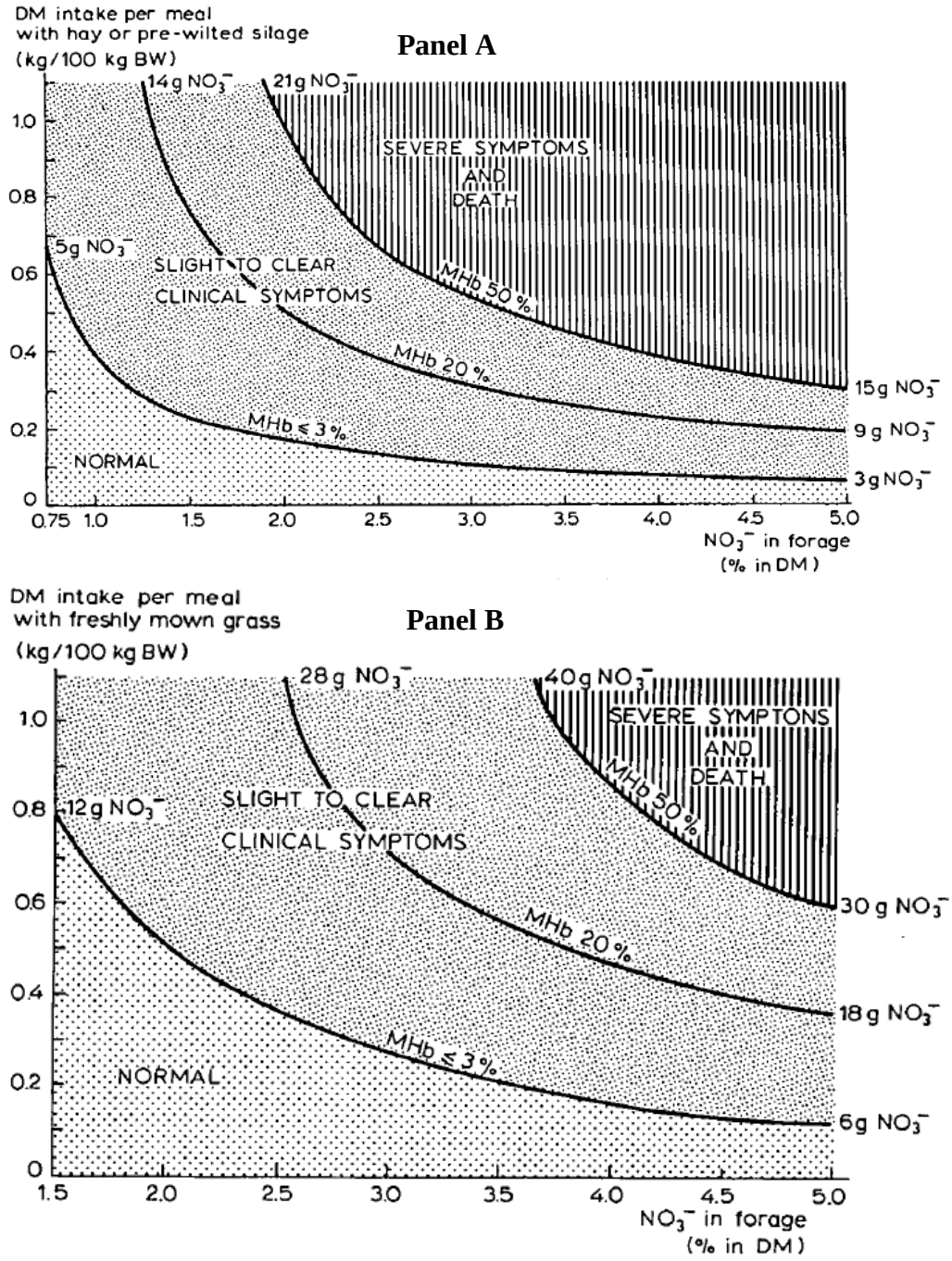
State Extension Program	Author, Year	Safe to feed level	Caution feeding level	Toxic to feed level
		mg NO_3-N/kg of DM equivalent		
Pennsylvania	Adams et al., 1992	< 1000	1000-1700	> 1700
Kansas	Roozeboom et al., 2011	< 1380	1380-2070	> 2070
Nebraska	Rasby et al., 2014	< 1500	1500-2100	> 2100
Oklahoma	Strickland et al., 2017	< 1150	1150-2300	> 2300
Colorado	Whittier, 2014	< 1150	1150-2300	> 2300
Iowa	Ensley and Barnhart, 2012	< 1500	1495-2300	> 2300
UC Davis	Maas, 2001	< 1500	1500-4000	> 4000
Florida	Halsey, 1998	< 1518	1518-4048	> 4048
North Dakota	Stoltenow and Lardy, 2015	< 1500	1500-4500	> 4500

*Calculations done using conversion factors in Adams et al. (1992)
*NE, PA, ND, IA reported in NO_3-N ppm
*UC Davis reported in % NO_3-N
*OK, KS, CO reported in ppm NO_3
*FL reported in % NO_3
*KSU reported <690 safe and <1380 safe in most cases

Figure 1: Predicted methemoglobin formation at various concentrations of nitrate in different forage types. Reprinted from Kemp, 1982.

Panel A: The nitrate content (x-axis) and the dry matter intake (y-axis) of preserved grass (hay or pre-wilted silage) in relation to the formation of methaemoglobin in cows.

Panel B: The nitrate content (x-axis) and the dry matter intake (y-axis) of freshly mown herbage in relation to the formation of methaemoglobin in cows.



CHAPTER II. Management and Risk of Nitrates in Annual Forages: Results of Beef Cattle Producer Survey[1]

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ABSTRACT

When using annual forages, a major challenge beef cattle producers face is the possibility of nitrate toxicity. To understand how often producers cope with and test for high nitrates in annual forages, we distributed a survey through the "UNL Beefwatch" newsletter to 1182 subscribers. Most survey respondents (n = 107/115; 93%) were from the midwestern U.S. and indicated nitrate toxicity when feeding annual forages is important to them (70%). The majority of the respondents (80%) indicated use of annual forages in three or more years out of five, but reported that they do not regularly test nitrate in pasture or hay. However, 38% reported they have experienced nitrate toxicity with annual forages. Producers were more (P = 0.02) likely to test annual forages fed as hay (53%) than grazed (38%). However, there was a tendency (P = 0.09) for more producers to respond that they have had toxicity issues with annual forage pasture (34%) than with hay (24%). Producers were not more likely to test annual forage pasture (P = 0.28) and or hay (P = 0.94) if they previously experienced a nitrate toxicity issue. Past experience with toxicity did not influence the likelihood that they would graze (P = 0.31) or feed hay (P = 0.28) that tested high in nitrate in the future. Though producers indicated concerns about nitrate toxicity in annual forages, most have not experienced issues and those that have do not appear to make different management decisions based on that experience.

Keywords: annual forages, nitrate toxicity, survey

INTRODUCTION

A reduction in perennial pasture in the North Central Region of the U.S. has increased the need for alternative feed sources for beef operations. Annual forages provide an avenue to fill this need. Wright and Wimberly (2013) noted a 530,000 ha decrease in grassland when they assessed land use in the western Corn Belt from 2006 to 2011. A survey by Asem-Hiablie et al. (2016) indicated that 19% of operations in the Midwest and northern plains regularly utilize small grains as a grazing resource. A challenge when utilizing this feed source is that annual forages have greater potential for accumulating nitrate than perennial forages (Crawford et al., 1961). Currently, no information is available to understand the frequency that beef producers experience nitrate toxicity when using annual forages as hay or pasture, the frequency that they test annual forages for nitrates and their resulting management of annual forages. Therefore, a survey of beef producers was conducted to gather information regarding nitrate toxicity and nitrate testing of annual forages.

MATERIALS AND METHODS

A survey was distributed to producers through the monthly "UNL BeefWatch" newsletter published online and emailed to subscribers (n = 1182). The survey consisted of 16 multiple choice questions, 13 of which asked about production decisions made regarding testing and use of annual forage pasture or hay, and the remaining three addressed demographics. There were 115 respondents; most (93%) were located in the midwestern U.S., with 4% from the southwest, 2% from the Western regions of the U.S. and 2% of respondents from outside of the U.S.

Statistical Analysis

Chi-Square analysis in SAS was used to compare 1) the frequency of testing hay to the frequency of testing pasture and 2) the frequency of issues when feeding hay to frequency of

issues when grazing pasture by comparing the proportion that responded with very frequently or frequently to those that responded occasionally, rarely, or never.

Binomial analysis is SAS was used to determine 1) if those that have experienced issues with nitrate toxicity in hay or pasture differed from those that have not experienced issues in their frequency of testing those forages, and 2) if those that reported experienced issues with nitrate toxicity in hayed or grazed annual forages differ in their use of hay or pasture that tested high in nitrates. For all analysis, effects were considered significant at $P \leq 0.05$ and a tendency when $P > 0.05 \leq 0.10$.

RESULTS AND DISCUSSION

The size of operation varied with the majority (51%) managing 100-500 cows, and the bulk of the remaining operations being smaller (19% managing 50-99 cows and 15% managing 1-49 cows). However, a small proportion of operations were relatively large with 5% managing 500-999 cows and 5% managing over 1000 cows. Only 4% did not own any cows. For stocker and backgrounding operations, 50% of respondents managed over 100 calves, 8% managed 50-99 calves, 23% managed up to 50 calves, and 19% did not manage any calves. There were 103 producers that answered both questions on if they own or manage cows and if they own or manage stockers. Of those 103 respondents, 76% owned or managed both cows and stocker/backgrounded calves, 20% managed only cows, and 4% managed only stocker/backgrounding cattle.

Of the producers responding to the survey, 85% used annuals as a forage source three or more years out of five (Figure 1). The majority (70%) of these producers responded that the issue of nitrate toxicity in annual forages was very important or important to them (Figure 2). The survey respondents were more likely ($P = 0.02$) to respond that they tested annual forage put up

as hay than annual forage that was grazed for nitrate concentrations (Table 1). Of these respondents, only 53% very frequently or frequently tested their annual forage hay and 38% very frequently or frequently tested annual forages that were going to be grazed. However, when asked about the frequency that their annual forages tested high in nitrates the majority (90%) responded that they never, rarely, or occasionally test high (Figure 3). Thus, despite the perceived importance, the majority of annuals used for forage by the respondents appear not to be tested and those that are tested often do not contain elevated nitrate concentrations.

Interestingly, most (62%) of the producers that responded to the survey had not experienced nitrate toxicity when grazing or feeding hayed annual forages (Figure 4). There was a tendency for producers to report that they have had issues more ($P = 0.09$) with pasture than with hay (Table 1). However, the majority of producers also responded that they rarely, or almost never used the forage if a pasture (14%) or hay (36%) tested high (Table 1). Therefore, despite the relatively low likelihood of testing and low incidence of toxicity, it does appear that producers are concerned about the potential for toxicity and use the test results to make decisions.

This may suggest that their system or environment is one in which nitrate accumulation is more likely to occur. However, there was not a difference ($P = 0.28$) between producers that have had issues with nitrate toxicity in grazed annual forages (n = 34) and those that have not (n = 76) in their likelihood to test pasture (47% vs. 36%, respectively; Table 2). There was also no difference ($P = 0.94$) in the likelihood producers that have experienced issues with nitrate toxicity in hay (n = 24) would test hay for nitrates (54%) when compared to those reporting that they have not had an issue with hay in the past (n = 90; 53%; Table 2). This indicated that with both fresh and hayed forages, if a producer experiences nitrate toxicity, the experience does not

influence them to implement regular testing of annual forages for nitrate content as a prevention strategy.

When comparing producers that had experienced nitrate toxicity in the past when grazing or feeding hay to those that have not, there was not a significant difference ($P \geq 0.28$) in the likelihood that they would graze or feed hay that tested high in nitrates in the future (Table 2). Thus, experiencing nitrate toxicity also did not affect if a producer would or would not utilize hayed annual forage high in nitrate in the future.

The majority of respondents whom suspected nitrate toxicity when grazing (65%) responded that they consulted at veterinarian for diagnosis, while 50% that suspected nitrate toxicity with hay consulted a veterinarian.

CONCLUSION

Although the majority of producers responded that the issue of nitrate toxicity was important to them, relatively few (39%) had experienced issues with nitrate toxicity. Many producers (45%) did not frequently test annual forages for nitrate concentrations, and when they did, they reported that the majority of the time the forages did not contain high in nitrates. However, when nitrate analyses indicated elevated, and potentially toxic, concentrations in the annual forage, the majority of producers (86% and 64% for pasture and hay, respectively) responded that they were not likely to use the forage. Additional data collection aimed at determining why producers do not test annual forages for nitrates on a regular basis would improve advisor understanding of their decision-making processes and assist with development of improved guidelines to provide better management recommendations.

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Table 1: Differences in beef cattle producer responses regarding testing, use, and toxicity of annual forage pasture vs. hay.

	Grazing		Hay		
	n	%	n	%	*P*-value
Frequently test annual forage for nitrate[1]	114	n = 43 (38%)	115	n = 61 (53%)	0.02
Experienced an issue with nitrate toxicity when using annual forage[2]	110	n = 34 (31%)	24	n = 24 (21%)	0.09
Use forage that test high in nitrate[3]	108	n = 15 (14%)	40	n = 40 (36%)	< 0.01
Consulted vet for diagnosis is suspected nitrate toxicity[2]	97	n = 63 (65%)	46	n = 46 (50%)	0.04

[1] Responded frequently or very frequently

[2] Responded yes

[3] Responded almost always, usually or often

Table 2: Effect of beef producers' previous experience with nitrate toxicity on their response regarding frequency of testing and use of annual forages that contain potentially toxic nitrate concentrations.

	Previously experienced toxicity		No previous issue		
	n	%	n	%	p-value
Test annual forage hay for nitrates[1]	24	n=13 (54%)	90	n=48 (53%)	0.94
Test annual forage pasture for nitrates[1]	34	n=16 (47%)	75	n=27 (36%)	0.28
Use annual forage hay that tested high[2]	24	n=11 (46%)	86	n=29 (34%)	0.28
Use annual forage pasture that tested high[2]	33	n=3 (9%)	72	n=12 (17%)	0.31

[1] Responded frequently or very frequently

[2] Responded almost always, usually or often

Figure 1: Producer responses (n = 108) to the question "How often do you use annuals as a source of forages for cattle?"

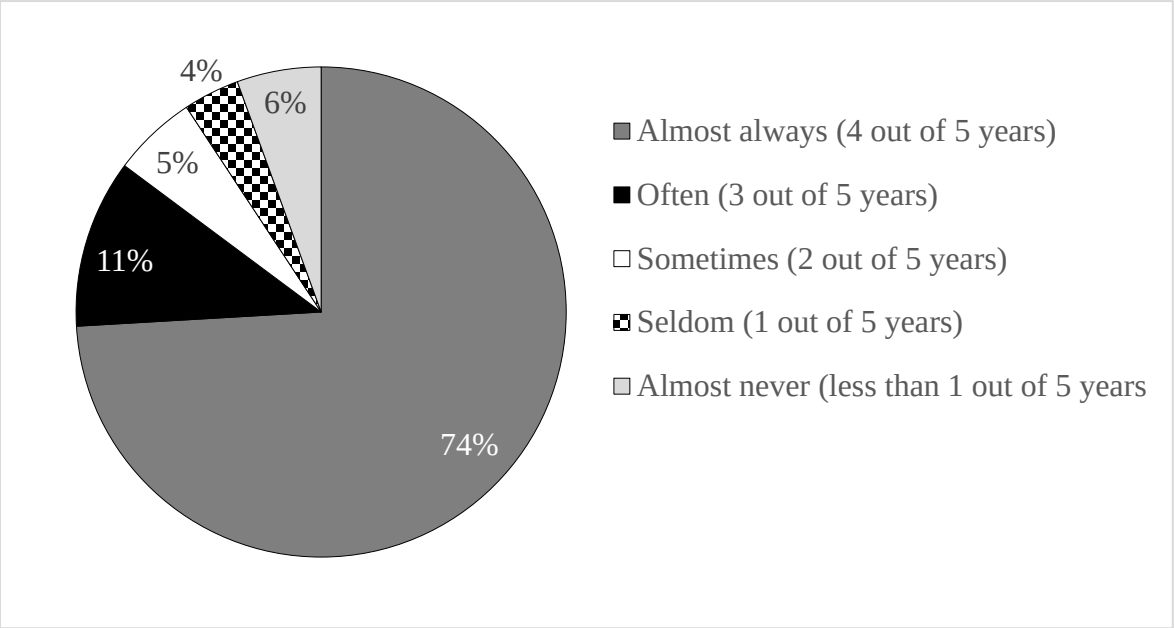


Figure 2: Producer responses (n = 114) to the question "How important is the issue of nitrates in annual forages and the potential for toxicity to you?"

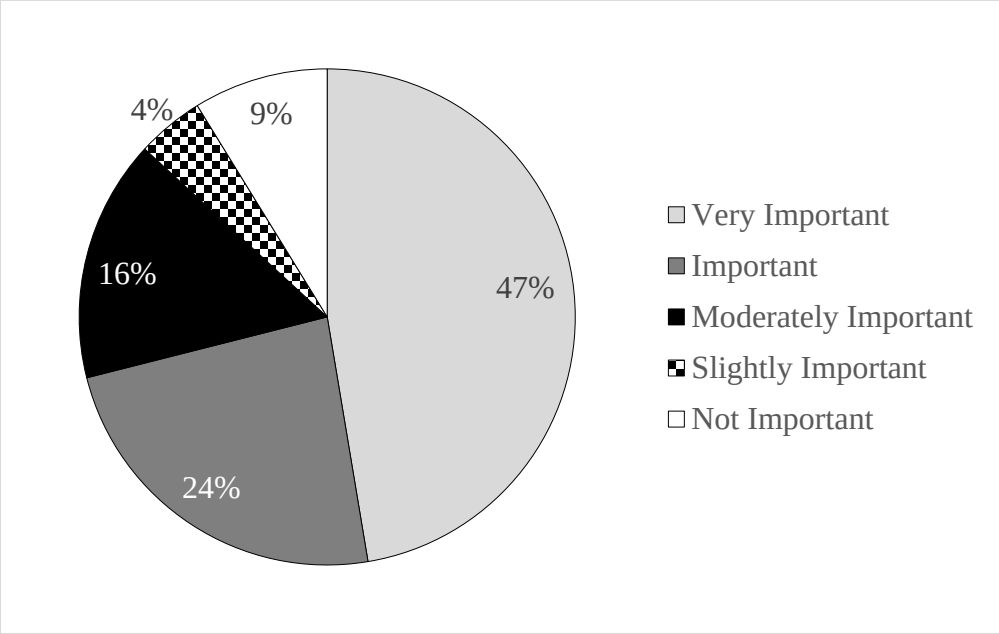


Figure 3: Producer responses (n = 110) to the question "How often do your annual forages test high for nitrates?"

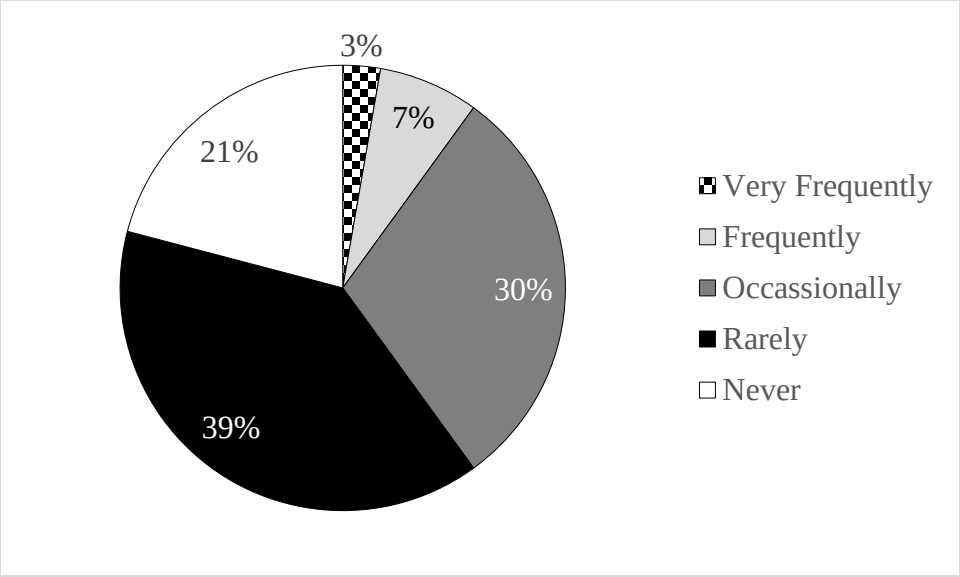
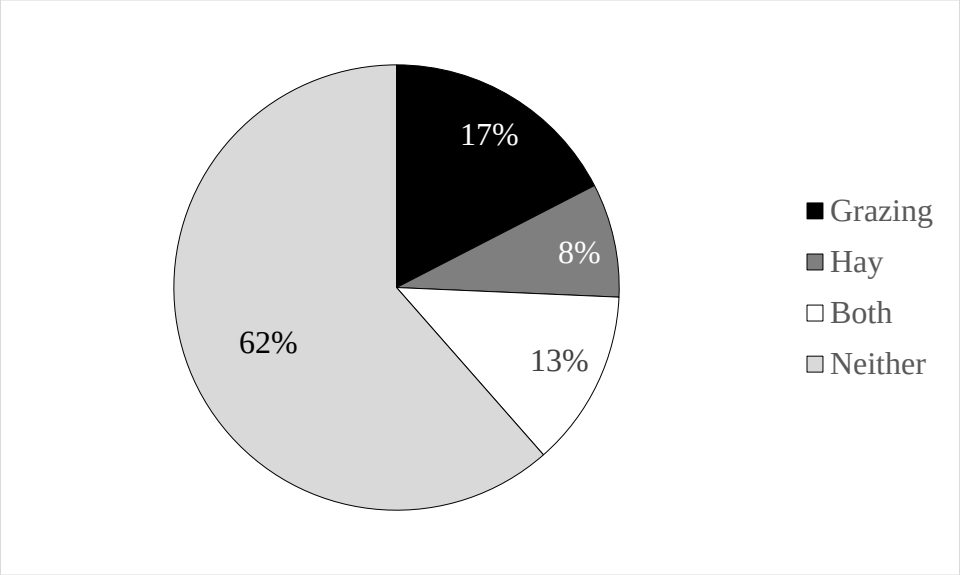


Figure 4: Proportion of producers (n = 109) that have experienced toxicity only when grazing annual forages (grazing), only when feeding annual forage hay (hay), both when grazing annual forages and feeding annual forage hay (both), or have not experienced issues when using annual forage pasture or hay (neither).



CHAPTER III. Nitrate Concentrations of Annual Forages Submitted by Producers to a Commercial Forage Testing Laboratory in Nebraska[1]

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ABSTRACT

Annual forages are used as both a source of pasture and hay and are more prone to accumulating nitrate than perennial forages. To quantify the nitrate concentration of various annual forage species used by producers, samples submitted to Ward Laboratories Inc. (Kearney, NE) for nitrate analysis were compared. Samples were sorted by "fresh" (< 26% DM; n =443) and "dry" (>84% DM; n = 1514) categories, and then by species. When small grains, millet, and sorghum x sudangrass were evaluated, fresh forage had greater ($P < 0.01$) nitrate content than dry forages. Within the fresh samples, brassicas accumulated more nitrate ($P < 0.01$) than small grains, millet, sorghum x sudangrass, and mixtures. However, small grains, millet, sorghum x sudangrass, and mixes were not different from each other ($P > 0.05$). Brassicas exceeded 2,100 mg NO_3-N/kg DM in 48% of samples and were 3 to 5 times more likely to exceed this threshold than other species. For dry forages, sorghum x sudangrass did not differ from oats/pea mixtures ($P = 0.78$) and both exceeded the 2,100 mg NO_3-N/kg DM threshold in 11.5 and 8% of samples, respectively. The millets contained less nitrate than sorghum x sudangrass ($P < 0.01$), but did not differ ($P = 0.19$) from oats/pea mixtures. Dry small grains contained the least nitrate ($P < 0.05$) and exceeded 2,100 mg NO_3-N/ kg DM in only 2.5% of samples. Of the annual forage samples submitted to a commercial laboratory in Nebraska, 48% of the fresh brassica samples, 23% fresh annual grasses, and 5% of the dry annual grasses were considered at risk for causing nitrate toxicity.

KEYWORDS: nitrate content, annual forages, nitrate toxicity

INTRODUCTION

Nitrate toxicity has long been recognized in ruminant animals with the general guidelines for threshold nitrate concentrations developed in the 1940s and 1960s (Bradley et al., 1940; Crawford et al., 1966). Several factors can cause increased dietary nitrate concentrations. Annual forages are prone to accumulate high concentrations of nitrate simply because annual species accumulate more nitrate than perennial species (Crawford et al., 1961). Additionally, these annual forages are often fertilized, or grown in cropping systems that leave residual N in the soil which can increase the likelihood of nitrate accumulation (Crawford et al., 1961). Stage of maturity influences accumulation, and immature forages accumulate more than mature forages (Crawford et al., 1961; Bolan and Kemp, 2003). The objectives of this study were to better understand: 1) which annual forage species accumulate the most nitrate, 2) if forages harvested fresh (likely grazed) or dry (hayed) were more likely to contain elevated amounts of nitrate, 3) how often the nitrate concentration would be considered toxic using the guidelines set forth by Bradley et al. (1940), and 4) differences in nitrate content when brassicas and small grains are grown in in the same fields.

MATERIALS AND METHODS

Laboratory assessment

Annual forage samples (n = 1957) submitted by producers to Ward Laboratories (Kearney, NE) for nitrate analysis during 2016 and 2017 were summarized. Samples were initially sorted into “fresh” (< 26% DM) and “dry” (>84% DM) categories. The fresh samples (n = 443; 18.2% DM; SD ± 4.6%) were classified into five species groups based on their label: 1) brassica [turnip (Brassica *rapa*), radish (Raphanus *sativus*), collard (Brassica *oleracea*); n = 63], 2) mixture (cover crop mix or multiple annual forage species; n = 34), 3) small grain [oat (Avena

sativa), rye (Secale *cereal*), triticale (Triticosecale), wheat (Triticum *aestivum*), barley (Hordeum *vulgare*); n = 70], 4) millet [pearl (Pennisetum *glaucum*), foxtail or German (Setaria varidis); n = 40], or 5) sorghum/sudan [cane, milo or sorghum (Sorghum *bicolor*), sorghum-sudangrass (Sorghum *ssp. drummondii*) sudangrass *(*Sorghum *sudanense*); n = 236]. The dry samples (n = 1514; 87.0% DM; SD ± 2.2%) were sorted into four species groups based on their label: 1) oats/pea mix [oats/pea mix (Avena *sativa* and Pisum *sativum* mix); n=60], 2) small grain (oats, rye, barely, triticale, wheat; n = 595), 3) sorghum x sudangrass (cane, sorghum, sudangrass, milo, sorghum x sudangrass; n = 532), and 4) millet (pearl, foxtail, German; n = 327). Samples were analyzed to evaluate species differences within moisture type in average nitrate-nitrogen (NO_3-N) concentration. Within moisture type, the proportion of the samples in each species category that fell into nitrate toxicity recommendation ranges was also evaluated. These nitrate toxicity ranges were: 1) Safe (<1400 mg NO_3-N /kg DM), 2) Marginal (1400-2100 mg NO_3-N /kg DM), 3) Caution (2100-5000 mg NO_3-N /kg DM), 4) Toxic (>5000 mg NO_3-N /kg DM). Bradley et al. (1940) recommended that 2100 mg NO_3-N/kg DM be set as the threshold above which toxicity may occur. Nebraska Extension (Rasby et al., 2014) suggests that below this threshold the forage is safe to feed and above this it must be mixed with other feeds in a ration. Additionally, for the species with samples in both fresh and dry groups (sorghum x sudangrass, millet and small grains), nitrate concentration was compared to evaluate differences among moisture types.

Field evaluation

Additionally, six fields planted to a small grain-brassica mixture in late summer were sampled as fresh forage in late fall and evaluated to determine if species accumulation of nitrates differed when grown under identical conditions. These mixtures included oats or cereal rye planted with turnips and/or radishes. Samples were obtained by randomly selecting individual

species throughout the field, clipping small grains to ground level, and pulling the whole brassica plant up and separating the top (leaves + stem) from the root. All samples were dried in a 60 °C forced air oven and ground to a 1-mm screen in a Wiley mill (Thomas Scientific, Swedesboro, NJ). Nitrate-nitrogen was determined on a dry matter basis (mg NO_3-N /kg DM) using a nitrate ion selective electrode. One gram of dried, ground sample was continuously mixed in 40 ml of pH 7 distilled water at room temperature with a rocker for 30 minutes before measuring. A standard curve was developed using known nitrate standards to calibrate the electrode prior to sample analysis (Anderson and Case, 1999).

All data were using the GLIMMIX procedure of SAS 9.3 (SAS Inst. Inc., Cary, NC). To determine effect of species on nitrate concentration within moisture type the model included species as a fixed effect. To evaluate the differences of fresh or dry forages the model included moisture type and species, and their interaction as fixed effects. To determine what proportion of the samples in each species would fall into the nitrate toxicity categories, a multinomial distribution with a cumulative logit link function was used to conduct pairwise comparisons of species within moisture type using the odds ratio function and confidence interval of 95%. The model for the grass and brassica sampled from the six fields, included species as a fixed effect and location as a random effect. For all analysis, effects were considered significant at $P \leq 0.05$ and a tendency when $P > 0.05 \leq 0.10$.

RESULTS

Fresh Forages (<26% DM)

From the fresh forages in the commercial lab dataset, there was a significant effect ($P < 0.01$) of species on nitrate concentration. Brassicas contained the most ($P < 0.01$) nitrate (Table

1). The cover crop mixtures, sorghum x sudangrass, millet, and small grains did not differ ($P > 0.05$) in nitrate concentration.

Dry Forages (>84% DM)

In the dry samples from the commercial lab data set, sorghum x sudangrass and oats/pea did not differ ($P = 0.78$) for nitrate concentration (Table 1). Millet contained less ($P < 0.01$) nitrate than sorghum x sudangrass but did not differ ($P = 0.19$) from oats/pea. Small grains contained the least nitrate ($P < 0.05$) compared with other species.

Fresh vs. dry

There was no interaction ($P = 0.51$) of moisture type and species when nitrate concentration was compared across millet, small grains and sorghum x sudangrass. Fresh samples (1,321 mg/kg NO_3-N) had greater ($P < 0.01$) nitrate content than dry samples (637 mg NO_3-N /kg DM). Across moisture type, sorghum x sudangrass and millet were not different ($P = 0.10$), but were greater ($P < 0.05$) than small grains.

Fresh small grains vs. brassicas when grown under identical conditions

The six field collections with fresh small grain and brassica mixes agreed with the dataset from the commercial laboratory with small grains (161 mg NO_3-N /kg DM) containing less ($P < 0.01$) nitrate than brassicas tops and roots. Within brassica species, there was no difference ($P \geq 0.77$) between the top and roots. However, radish top (9,248 mg NO_3-N /kg DM) tended ($P = 0.06$) to have greater nitrate than turnip tops (5,932 mg NO_3-N /kg DM) whereas radish roots (9,073 mg NO_3-N /kg DM) were numerically, but did not statistically different ($P = 0.12$) from turnip roots (6,354 mg NO_3-N /kg DM) in nitrate content.

Frequency samples exceeded toxicity guidelines

Of the fresh samples in the commercial lab dataset, the frequency that each of the species would be considered safe, marginally safe, fed with caution, and toxic are illustrated in Figure 1. Brassicas exceeded the caution or toxic thresholds in 47.6% of samples and the odds ratio indicated that they were 3 to 5 times more likely to be above this threshold than the other species categories (Table 2). The other species did not differ in the frequency they fit into the caution and toxic categories. The remaining species ranged from 20-28% of the samples fitting into the caution or toxic categories, indicating that there is still a reasonably high likelihood that these fresh annual forage samples could be considered toxic using current guidelines.

For dry samples, figure 2 depicts how often each species fell into each guideline category. Small grains only exceeded the caution threshold in 2.5% of samples and the odds ratio indicated that they were 2 to 3.6 times less likely than the other species to contain nitrate in the caution or toxic category (Table 3). Both millet and oats/pea mixtures exceeded the caution threshold in 8% of samples and did not differ in the likelihood that they would contain nitrate in the caution or toxic category. Sorghum x sudangrass exceeded the caution threshold in 11.5% of samples and was 1.6 fold more likely to exceed the caution threshold than millet (Table 3).

DISCUSSION

There were nearly three times as many dry samples submitted for analysis compared to fresh samples (n = 1514 vs. n = 443). This indicates producers submit more hayed forages for analysis than fresh forages, an observation that was also made in a survey of producers conducted by Lenz et al., (unpublished data).

The majority of fresh species, exceeded the traditional threshold of 2,100 mg NO_3-N/kg DM in 20-28% of their samples, and the majority of dry species exceeded this threshold in 8-

11% of their samples. The exceptions were fresh brassicas, in which a much larger proportion (48%) of the samples exceeded 2,100 mg NO_3-N/kg DM and dry small grains which only exceeded this threshold in 2.5% of samples. Lenz et al., (unpublished) reported that although producers in the Midwest region are concerned about nitrate toxicity, only a small percentage regularly test forages for nitrates. Since most producers appear to not test forages, the submitted forages may only be representative of nitrate levels when the producer is concerned about increased concentrations. About 10% of the producers that submit samples for analysis indicated that the annual forages "very frequently" or "frequently" test high in nitrates with 29% indicating that they "occasionally" test high in nitrates (Lenz et al., unpublished data). The data from the survey supports the data from the commercial lab for the frequency that these forages contained high concentrations of nitrate.

Genetics and management differences likely account for the difference in species accumulation. A review by Garnett et al. (2009) discusses differences in root systems and the influence on nitrate accumulation. Root size, length, surface area, and present transporters affect nitrate uptake. Available N and internal regulations influence the activity of N transporters as well (Garnett et al., 2009). Brassicas are often included in nitrate accumulator lists due to their tendency to accumulate nitrate (Maynard et al., 1976; Provin and Pitt, 2003). Both the commercial laboratory data set and the in field comparison suggest that there is an increased risk for brassicas to contain potentially toxic concentrations of nitrate.

Based on survey data, the majority of producers have not experienced an issue with nitrate toxicity when utilizing annual forages as a feed source. However, the majority of producers responded that if they test a forage and results find high nitrate concentrations, they typically do not utilize the feed (Lenz et al., unpublished). Unfortunately, the decision to not

utilize a forage can be costly as a producer must then find and purchase another feed resource. Therefore, understanding the risk of toxicity and management strategies to reduce the risk are important to a profitable operation.

Lenz et al., (unpublished data) reported that producers experience toxicity more often in grazed forages than hayed (31 vs. 21% of producers, respectively) which would support the data that fresh samples accumulate higher concentrations of nitrate. However, it is important to note that the incidence of toxicity in the survey data was not extremely elevated despite the fact that fresh samples in the commercial laboratory data set contained twice as much nitrate as the dry forages and were in the caution or toxic category over twice as often. When weighted based on the number of samples received by the commercial lab, 27% of fresh and 6.7% of dry samples were above 2,100 mg NO_3-N/kg DM. When only the grass samples that were in common (small grains, millet and sorghum x sudangrass) are considered, 23% fresh annual grasses and 5% of the dry annual grasses were above 2,100 mg NO_3-N/kg DM. The increased nitrate concentrations in fresh forages can be expected as forage maturity impacts nitrate concentrations. Immature, vegetative forage contains more nitrate than mature forage (Crawford et al., 1961) and fresh forages are often grazed in vegetative stages while hayed forages are harvested at a more mature stage in order to increase yields.

The guidelines on nitrate toxicity were first developed by Bradley et al. (1940) in which they orally dosed cattle with nitrate salts. Most studies since then have provided a nitrate salt through a drench or top-dressed on feed rather than measuring nitrate in the forage itself. Kemp (1982) found that fresh forages have a lower risk of nitrate toxicity, but few, if any guidelines on nitrate toxicity take this data into account.

The difference in the risk of toxicity when utilizing fresh, grazed forages compared to dried hays observed by Kemp (1982) may be partially explained by the following factors. When grazing, cattle selectively choose to consume the leaf portion of a plant before consuming the stem (Chacon et a., 1978) which contains the greatest concentration of nitrate in a plant (Crawford et al., 1966). Thus, grazing at lower stocking rates to allow for selectivity can reduce toxicity potential, and cattle can adapt to more nitrate in the diet as they consume for forage. In an in vitro study, Geurink et al., (1979) found a substantial difference in the rate nitrate was available when provided in a fresh plant cell compared to a dry plant cell. Within 20 minutes, 80% of the total nitrate was available when a grass hay was submerged in water. At the same time, only 30% of the nitrate was available when fresh chopped grass or turnips were submerged. Additionally, energy supplementation has been shown to reduce the risk of toxicity (Burrows et al., 1987; Sapiro et al., 1949) and annual forages, particularly brassicas and late summer planted small grains are highly digestible (Coblentz and Walgenbach, 2014; Contreras-Govea and Albrecht, 2006; Villalobos and Brummer, 2015). The additional energy in the diet promotes bacterial growth. Additionally, a lower pH favors nitrite reduction rather than nitrate reduction, which may help reduce the buildup of nitrite that is the underlying cause of nitrate toxicity (Tillman et al., 1965; Lewis et al., 1951).

CONCLUSION

Annual forages have the potential to accumulate substantial concentrations of nitrate. Using 2,100 mg NO_3-N/kg DM as the nitrate toxicity threshold, 48% of the fresh brassica samples, 23% fresh annual grasses, and 5% of the dry annual grasses samples submitted to a commercial laboratory in Nebraska would have been considered at risk for causing toxicity.

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Table 1: Effect of species and moisture classification on nitrate concentration (mg NO_3-N/kg DM) of samples submitted by producer to a commercial laboratory for analysis.

	Brassica	Millet	Oat-pea	Small Grains	Sorghum/sudan	Cover crop mix	SEM[3]	*P*-value
Fresh[1]	4,060[a] n = 63	1,391[b] n = 236	-	1,008[b] n = 70	1,564[b] n = 236	1806[b] n = 34	419	< 0.01
Dry[2]	-	617[bc] n = 327	789[ab] n = 60	469[c] n = 595	824[a] n = 532	-	120	< 0.01

[a-c] Values within row without the same superscript differ ($P \leq 0.05$).
[1]Fresh refers to samples with < 26% DM
[2]Dry refers to samples with > 84% DM
[3]Greatest SEM from species estimates reported

Table 2. Odds ratio (95% confidence interval1) of the likelihood that fresh samples in a species category contained greater than 2100 mg NO_3-N/kg DM relative to the likelihood of another species using multivariable logistic regression analysis

	Millet	Small grains	Sorghum/Sudan	Mix
Brassica	0.20 (0.09 to 0.46)	0.21 (0.11 to 0.42)	0.27 (0.16 to 0.47)	0.31 (0.15 to 0.65)
Millet		1.04 (0.44 to 2.42)	1.36 (0.65 to 2.83)	1.56 (0.64 to 3.76)
Small grains			1.31 (0.75 to 2.30)	1.50 (0.71 to 3.16)
Sorghum/sudan				1.15 (0.62 to 2.12)

[1]Confidence interval range that includes 1 indicates no difference in likelihood.

Table 3. Odds ratio (95% confidence interval[1]) of the likelihood that dry samples (>84% dry matter) in a species category contained greater than 2100 mg NO_3-N/kg DM relative to the likelihood of another species using multivariable logistic regression analysis

	Oat-pea mix	Small grains	Sorghum/Sudan
Millet	1.024 (0.474 to 2.213)	0.445 (0.288 to 0.689)	1.584 (1.096 to 2.290)
Oat-pea mix		0.435 (0.201 to 0.941)	1.546 (0.741 to 3.229)
Small grains			3.556 (2.450 to 5.162)

[1]Confidence interval range that includes 1 indicates no difference in likelihood.

Figure 1. Distribution within risk of toxicity categories of fresh (< 26% dry matter; n= 443) annual forage samples submitted to a commercial laboratory for analysis of nitrate content (mg NO_3-N per kg of DM).

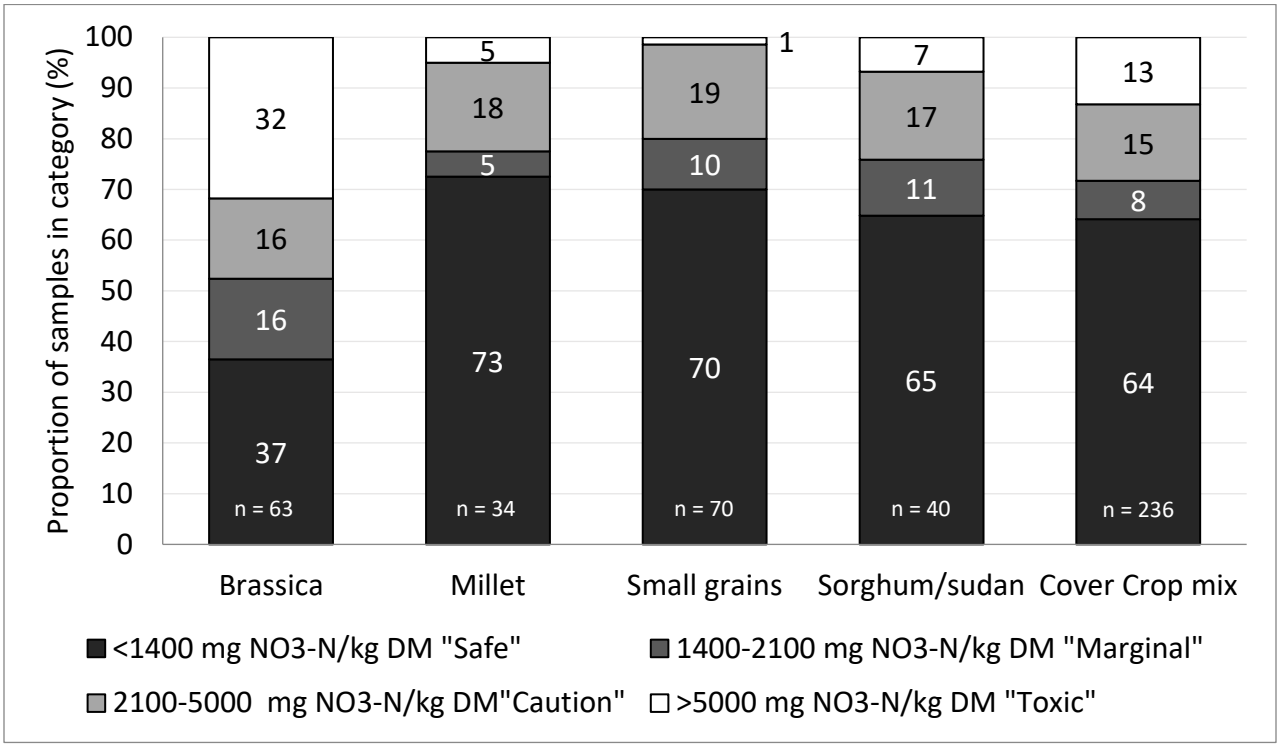
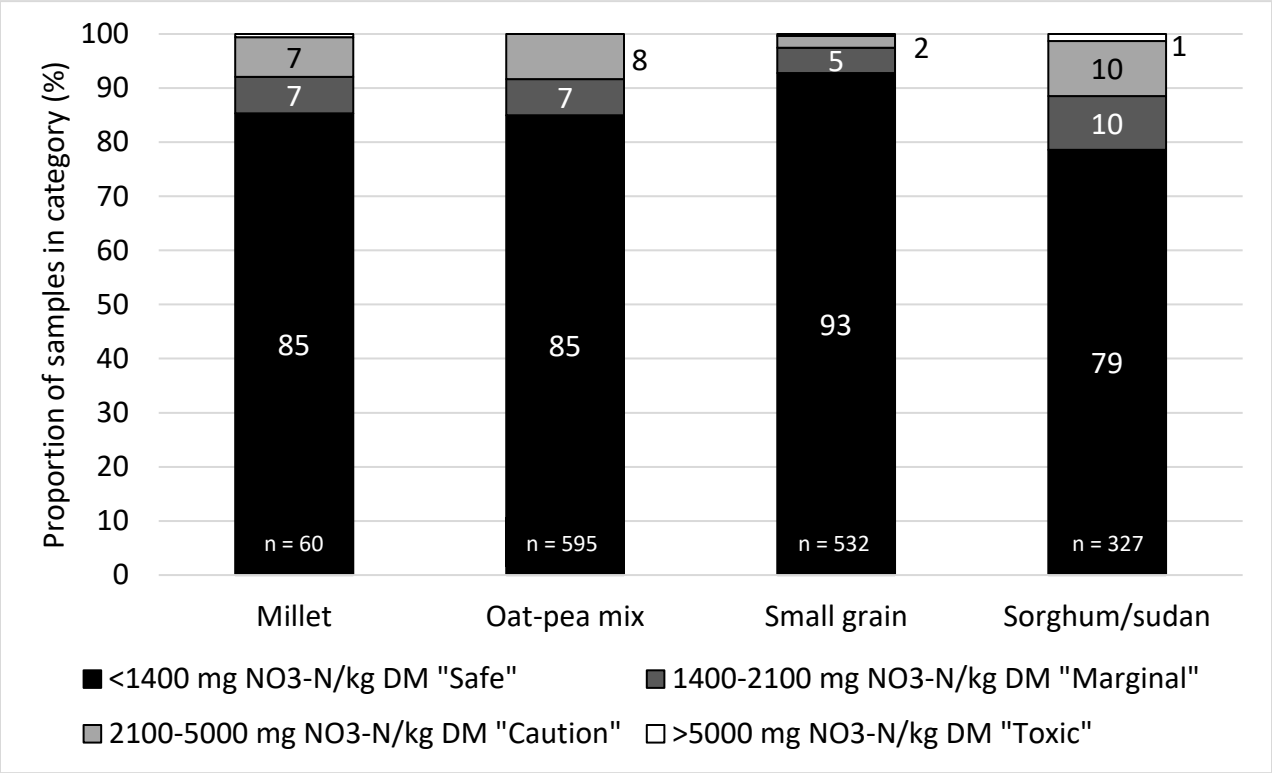


Figure 2: Distribution within risk of toxicity categories of dry (> 84% dry matter; n = 1514) annual forage samples submitted to a commercial laboratory for analysis of nitrate content (mg NO_3-N per kg of DM).



CHAPTER IV. Nutritive Value Change during the Fall of Late-summer Planted Oats, Turnip, and Radish[1]

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[1]Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the USDA. The authors have no conflict of interest. USDA is an equal opportunity provider and employer.

Abbreviations: ADF, acid detergent fiber (ADF); CP, crude protein (CP); DM, dry matter (DM); IVOMD, In vitro organic matter digestibility; NDF, Neutral detergent fiber ; S, sulfur; TESC, total ethanol soluble carbohydrates

ABSTRACT

The change in nutritive value of late-summer planted oats (*Avena sativa* L.) and brassicas (*Brassica* spp.) during the fall in the midwestern U.S. is not well documented. A mixture of 'Jerry' oats , 'Purple Top' turnip (Brassica *rapa* ssp. *Rapa* L.), and 'Daikon' oilseed radish (Raphanus *sativus* L.) was drilled-planted following corn (*Zea mays* L.) silage harvest in late August/early September in southcentral Nebraska. Forage samples were hand-harvested in early November, December, and January. At each harvest, in vitro organic matter digestibility (IVOMD) of turnip (87%) and radish (86%) tops (leaves + stem) was not different ($P \geq 0.09$), but with greater IVOMD than oats (75%) ($P < 0.01$). Within a forage type, IVOMD in November and December was not different ($P \geq 0.17$), but decreased ($P < 0.01$) from December to January. However, oats IVOMD appeared to decline more (9% units) than turnip and radish tops (5% units). In both years, crude protein (CP) of oats (16% CP) was less ($P < 0.01$) than both turnip (24%) and radish (27%) tops. The CP content decreased ($P < 0.01$) 2 to 3% units from November to December in both years, but in yr 1, CP content increased ($P < 0.01$) 4% units from December to January, whereas, in year 2, CP continued to decrease ($P < 0.01$) 2% units from December to January. The color of the forage mixture changed green to brown following several freezes in the month of November. However, this was not indicative nutritive value as much of the forage nutritive value was retained through January. Thus, delayed grazing of these cool-season forage mixtures late into the fall and early winter seems an option for cattle producers in the midwestern U.S.

Keywords: Brassicas, Forage, Nutritive value, Oats

Core Ideas

- Late-summer planted oats, turnips, and radishes are highly digestible in the fall
- Oats, turnips, and radishes maintain a high nutritive value through early winter
- Brassica tops are more comparable to a concentrate than a roughage
- The sulfur content of brassicas remains elevated through the fall
- Unlike radish, turnip roots retain high ethanol soluble carbohydrates into winter

INTRODUCTION

Late-summer planted cover crops can fit into some cropping systems such as after wheat (*Triticum aestivum* L.) or early harvested corn silage. An established cover crop provides the producer with agronomic and conservation benefits, and by utilizing ruminant livestock to graze the forage produced, the cost of establishing the cover crop can be offset (Drewnoski et al., 2018). There are a variety of plant species a producer can use as a cover crop to meet the needs of their operation. Small grains and brassicas are two forage types that can be planted in mid to late summer and grown through the fall in the Midwest. Coblentz and Walgenbach (2010b) compared fall-grown oats, winter wheat, and spring triticale forage yields over two years, and observed a range of 1,312 lb/ac to 5,933 lb/ac produced by the final harvest date in November, with oats consistently yielding the highest (2,439 to 5,933 lb/ac), and wheat the lowest (Coblentz and Walgenbach, 2010b). Brassicas can attain similar or greater yields as small grains (Lauriault et al., 2009). For example, turnip and radishes grown in northern Colorado yielded between 2,300 and 6,360 lb/ac with planting date being the main factor influencing biomass production (Villalobos and Brummer, 2017).

Mid to late summer planted spring small grains and brassica have been shown to be highly digestible (80 to 90% in vitro true digestibility) and have moderate CP (15 to 20% CP)

content in early fall (Coblentz and Walgenbach, 2010b; Villalobos and Brummer, 2015; Villalobos et al., 2017). Compared to spring-planted oats, oats planted and grown during fall have been observed to have greater digestibility and contain more water-soluble carbohydrates (Coblentz and Walgenbach, 2010a). Due to yield potential and the high nutritive value observed in these forages, small grain and brassica cover crops can be a cost-effective strategy to grow calves in the fall, or to extend the grazing season of cows and reduce feed costs (Drewnoski et al., 2018).

Initiating grazing later may allow for increased forage yields. However, typically delaying grazing will result in increased forage maturity and decreased digestibility. Previous research suggests that brassica forage digestibility is not affected by maturity as much as other grasses and legumes used as forage (Smith and Collins, 2003; Villalobos and Brummer, 2015; Wiedenhoeft and Barton, 1994). Relatively stable NDF and CP were noted by Wiedenhoeft and Barton (1994) in Maine, when these measures were taken on brassicas planted early, mid, and late summer, and then harvested through early December. Villalobos and Brummer (2015) evaluated the effect of harvest date (mid-October or mid-November) in Fort Collins, Colorado, on mid-summer planted brassicas and found little differences in nutritive value of brassicas due to maturity in the fall. However, the extent to which the nutritive values of these forages change during the late fall, has not been well established and thus the feasibility of their use as stockpiled forages has yet to be fully evaluated. Therefore, the objective of this study was to evaluate how the nutritive value composition and changes of oats, turnips, and radishes during fall in the midwest U.S.

MATERIALS AND METHODS

Management and sampling of forages

A 124 ac (year 1) and a 131 ac (year 2) irrigated corn silage pivot located at the U.S. Meat Animal Research Center near Clay Center, Nebraska was utilized to evaluate the forage yield and nutritive value of a mixture of 84 lb/ac of Jerry oats (Avena *sativa*), 2.0 lb/ac of daikon radish (Raphanus *sativus*), and 1.5 lb/ac of purple top turnips (Brassica *rapa* ssp. *rapa*) planted on September 8th and August 25th in year 1 and 2, respectively, using an 8-in. row spacing. Nitrogen was split applied via pivot with 48 lb N/ac total (year 1) and 40 lb N/ac total (year 2). The pivots were split into quarters (North, South, East, and West). Biomass was sampled in early November with 4 random 4 ft^2 areas were sampled in each quarter. The turnips and radishes within this area were pulled up so that root biomass could be included and oats were clipped at ground level. The samples were separated by species with the brassica tops being separated from the root and samples were dried in paper bags, in a 140°F forced-air oven (Model LBB2-21-1, Despatch, Minneapolis, MN) until a constant weight was obtained.

Samples for nutrient analysis were collected in early November, December, and January in both years. Each species (oats, radish, and turnip) was collected at random within each quarter and put into separate bags according to species type. They were kept in a portable cooler with ice for transport to the lab. Once at the lab brassicas were separated into tops (leaves + stem) and roots and all samples were stored frozen at 25°F. In year 1, there was not enough brassica root sample collected to conduct analysis of nutritive value. In year 2, sample size was increased to allow analysis of brassica root.

Lab Analysis

Samples were freeze-dried (Virtis Freezemobile 25ES, Life Scientific Inc., St. Louis, MO) and ground through a 1-mm screen using a Wiley mill (Thomas Scientific, Swedesboro,

NJ). Freeze-dried samples were analyzed for corrected DM (212°F), OM, S, CP, NDF, ADF, TESC, and IVOMD.

Organic matter was determined by placing samples in a muffle furnace for 6-h at 1,112°F (AOAC, 1999: method 4.1.10). Sulfur and CP analysis was determined using a combustion chamber (TruSpec N Determinatr, Leco Corporation, St. Joseph, MO; AOAC, 1999; method 990.03). Neutral detergent fiber and ADF analysis were analyzed sequentially (Ankom 200, ANKOM Technology Corp., Macedon, NY) with heat resistant alpha-amylase added for NDF (Van Soest et al., 1991). Ethanol soluble carbohydrate analysis was conducted using the procedure described by Dubois et al. (1956) with a modification from the Hall et al. (1999). In vitro OM digestibility was determined within 48 h using the method described by Tilley and Terry (1963), modified by adding urea to the McDougall's buffer (McDougall, 1948) at a rate of 1 g urea/L buffer solution, to ensure adequate N was available for microbes in the rumen fluid (Weiss, 1994). The after incubation the Whatman 541 filter paper (22 µm pore size) plus samples was placed in crucibles and heated in a muffle furnace for 6-h at 1,112°F (AOAC, 1999: method 4.1.10). Blanks were included in the in vitro run to adjust for any feed particles that might have come from the inoculum. There was only 1 run conducted for forage samples collected in year 1 and a separate run conducted for forage samples collected in year 2. Five hay standards with known in vivo (total tract) digestibility (51-60% range) were used to adjust IVOMD values. These adjustment values resulted in 4.2 and 4.6 percentage units added to IVOMD in year 1 and 2 respectively.

Statistical Analysis

Forage yield and nutritive value was analyzed using the GLIMMIX procedure of SAS 9.3 (SAS Inst. Inc., Cary, NC). When evaluating the quality of oats and brassica tops over both

years, the pivot quarter nested within year was the experimental unit. The model included fixed effects of species, date, year, and their interactions. A random residual statement with species by quarter nested within year as the subject and a covariance structure of ANTE(1) was used (best fit determined by AICC). Table 2 provides a summary of the analysis of variance. The three-way interaction was only significant ($P < 0.01$) for TESC and there was tendency for a three way interaction for ADF ($P < 0.10$). The two-way interaction of date by species was significant ($P < 0.01$) for TESC, ADF, IVOMD, and S and there was a tendency for ($P = 0.07$) a two way interaction for NDF. The two-way interaction of harvest date by year was significant for TESC, ADF, NDF, CP and S. The two-way interaction of year by species was significant ($P < 0.01$) for NDF, ADF, IVOMD, CP and S.

When comparing brassica roots and tops in year 2, the model included the fixed effect of date and sample type. There was a plant part by date interaction ($P < 0.01$) for TESC, ADF, NDF, CP, and S. For all analysis, effects were considered significant at $P \leq 0.05$ and a tendency when $P > 0.05 \leq 0.10$.

RESULTS

The weather during the growing season and sampling period is shown in Table 1. Year 1 was cooler during both the late summer growing and fall sampling period than year 2.

Forage Yield

Yield (DM basis) of the oats, radish, and turnip mixture (including brassica roots) was measured in early November. There was a year effect ($P < 0.01$) with yield in year 1 being less ($P < 0.01$) than year 2 (3351 and 4589 lb/ac, respectively; SEM = 327 lb/ac). There was a year by species interaction ($P < 0.01$) for the proportion of total DM that each component comprised. However, in both years oats made up the greatest ($P < 0.01$) proportion (57%), turnip tops (22%)

comprised the second greatest amount, radish tops the third greatest amount (13%) whereas the turnip root (5.0% of DM) and radish root (2.8 % of DM) did not differ ($P \geq 0.30$) and made up the least amount of DM (SEM ± 1.09%).

Forage Quality

Total Ethanol Soluble Carbohydrate Content

Total ethanol soluble carbohydrates (Figure 1) of turnip top, radish top and oats peaked during December in year 1, ranging from 17 to 22%, and dramatically decreased ($P < 0.01$) to 5 - 6% in January. In year 2, this trend was the same for oats and turnip tops ($P < 0.01$), but radish tops did not differ ($P = 0.78$) in TESC from November (10.6%) to December (10.2 %). Like year 1, oats TESC and turnip and radish tops decreased ($P < 0.01$) from December to January in year 2.

In year 2, the TESC content of radish (32.4%) and turnip (50.0%) root was significantly greater ($P < 0.01$) than the above ground plant parts (7.8 and 13.8% for radish top and turnip top, respectively) across all dates and TESC content of turnip root was greater ($P < 0.01$) than radish root (Table 3). Additionally, turnip root maintained TESC content better from December to January than radish root, as radish root decreased from 43.6 to 17.4% TESC, whereas turnip root in December and January did not differ ($P = 0.24$; 52.5 vs 50.8%).

In both years, these data suggest that following initial frost, photosynthesis continued to occur, and soluble carbohydrates continued to increase through the month of November. Then due to weathering during the month of December, much of the soluble carbohydrates are lost in the oats, turnip top, radish top and radish root with the turnip root maintaining high TESC content into early January.

Fiber content

Over the fall, NDF and ADF of oats was greater ($P < 0.01$) than both radish and turnip tops (Table 4). The NDF and ADF of both radish and turnip tops in November and December were quite low being more similar to a concentrate than to forage. In both years, the ADF and NDF content of all species increased ($P < 0.01$) in January.

The brassica roots had extremely low fiber content. In year 2, the ADF and NDF content of radish and turnip roots was significantly less ($P < 0.01$) than the above ground plant parts (tops) at all dates (Table 3). In November, NDF of radish root and turnip root did not differ ($P \geq 0.33$); in December both decreased (due to the gain in TESC) but turnip root had less ($P \leq 0.05$) ADF and NDF than radish root, in January radish root had a large increase in ADF and NDF (due to a loss of TESC) whereas as turnip root remained unchanged ($P \geq 0.49$) resulting in radish root having significantly more ($P < 0.01$) ADF and NDF than turnip root. In South Dakota, turnip roots harvested in October following an August planting date had a 13.7 and 11.8% DM NDF and ADF respectively (Smart and Pruitt, 2006) and similarly, a NDF of 11.92% in Colorado (Villalobos, 2015). The turnip roots harvested in this study had comparable fiber content with NDF ranging from 14-18% DM and ADF ranging from 10-12% DM.

Digestibility of the forages

In each month, the turnip and radish tops did not differ ($P \geq 0.09$) in digestibility and both were more ($P < 0.01$) digestible than oats (Table 4). Within species, the digestibility in November and December did not differ ($P \geq 0.17$) but decreased from December to January ($P < 0.01$). The digestibility of oats appeared to decline more (10% unit decline) than turnip and radish leaves (5% unit decrease); however, the digestibility of oats in January was still high (67 % IVOMD).

The digestibility of oats was less in year 2 than year 1 (69% vs 80% IVOMD, respectively) when the forage was planted earlier in the summer but digestibility of turnip and radish top (85-87% IVOMD) did not differ ($P \geq 0.25$) among year within species. A decrease in oats forage digestibility due to increased maturity in the fall has been observed by others (Colbentz and Walgenbach, 2010b) as has the lack of a decrease in digestibly with maturity in brassicas (Villalobos and Brummer, 2015).

In year 2 across all dates, turnip and radish roots were more digestible ($P < 0.05$) than turnip and radish tops (Table 3). In November, the turnip root (95.4% IVOMD) was ($P = 0.04$) more digestible than the radish root (92.5% IVOMD). In December, the digestibility of both brassica roots slightly increased but radish root (93.8%) increased more than turnip (95.7%) resulting in no difference in digestibility ($P = 0.14$) between the two. In January, the digestibility of both radish root (88% IVOMD) and turnip root (94% IVOMD) decreased but the change in radish root was much greater than the turnip root resulting in the turnip root being more ($P < 0.01$) digestible that the radish root. The high digestibility of the brassica roots has been reported by others. Koch et al. (2002) planted brassicas in mid-July to mid-October over four years. The turnip roots ranged from 85.7% to 89.1% in vitro DM digestibility when harvested in October, and 82.8% to 86.5% when harvested in November in Powell, WY.

Crude Protein

The CP of oats and turnip and radish tops was less ($P < 0.01$) in year 2 when the forage was planted earlier than in year 1. The CP content of oats (21 and 10%, for year 1 and 2, respectively) was lower ($P < 0.01$) than radish and turnip top in both years (Table 3). The CP of the radish (29%) and turnip (28%) top did not differ ($P = 0.27$) in year 1, but in year 2 the CP of radish top (24%) was greater ($P < 0.01$) than turnip top (20%). The CP content of all species

decreased from November to December in both years (5% units in year 1 and 2% units in year 2) but in year 1, CP content increased from December to January (5% units) whereas CP continued to decrease from December to January in year 2 (2% units).

In November of year 2, the CP content of radish root and turnip root were less ($P = 0.01$) than turnip top which was less ($P = 0.02$) than radish top (Table 3). In December, CP content of turnip root increased whereas radish root, radish top and turnip top remained relatively stable. This resulted in CP of radish root and turnip root not differing ($P = 0.84$) but being less ($P \leq 0.02$) than turnip top which was less ($P < 0.05$) than radish top. From December to January, the CP content of turnip root and radish root remained unchanged while turnip top tended ($P = 0.06$) decrease and radish top did decrease ($P < 0.01$) resulting in turnip root not differing ($P = 0.25$) from turnip top and radish root ($P = 0.24$) which were less ($P \leq 0.05$) than radish top.

Sulfur content

Across all dates the S content of oats was less ($P < 0.01$) than that of radish and turnip (Table 4). The S content of oats did not differ ($P = 0.62$) between November (0.30%) and December (0.29%) as well as not differing between ($P = 0.23$) December and January (0.25%; Table 2). For both turnip and radish top the S content did not differ ($P \geq 0.30$) between November (0.92% and 0.81%, respectively) and December (0.90 and 0.78%, respectively) but decreased ($P < 0.01$) from December to January (0.63 and 0.60% respectively). When comparing the S content, radish top was greater ($P < 0.01$) than turnip top in November and December but did not differ ($P = 0.26$) in January.

In November and December of year 2, the S content of radish top and root did not differ ($P \geq 0.12$) and were greater ($P < 0.01$) than turnip top which was greater ($P < 0.01$) than turnip

root (Table 3). In January, radish root was greater ($P \leq 0.02$) than radish top, turnip top and turnip root which did not differ ($P \geq 0.13$).

The high S content of the brassica tops and roots in this study agreed with previous research. In New Zealand, S concentrations reported were high, as kale had 0.85% S, rapeseed 0.61% S, and turnips 0.69% DM (Sun et al., 2012). The recommended maximum tolerable level of S is suggested to be 0.5% when cattle are consuming a high-fiber diet, thus intake of brassicas alone could potentially cause polioencephalomalacia (Drewnoski et al., 2014). This is consistent with previous case studies that observed polioencephalomalacia when grazing Brassicaceae family forages. In a case study 1% death incidence was reported on cattle grazing a diet with an estimated S content of 0.44-0.61% DM. The diet was composed of turnips, rape, and grass, with sulfur concentrations of each being 0.63, 0.91, and 0.19% DM respectively (Gould, 2000). Although there was a decrease in S over the fall in the present study, brassicas in January still contained extremely high levels of S (tops and roots greater than 0.5%). Given the much lower concentrations in the oats and the higher NDF (greater levels of NDF in the diet have been shown to decrease risk of toxicity; Drewnoski et al., 2014) including a grass in mixtures with brassicas for grazing would be recommended.

CONCLUSIONS AND RECOMMENDATIONS

Digestibility and CP content of brassica are greater than oats when planted in late-summer although oats were still high in nutritive value. Turnip and radish tops and roots are more comparable to a concentrate than roughage as they were highly digestible and low in fiber. The digestibility of all species decreased over the fall, with the largest decrease occurring during the month of December. However, all forages were still highly digestible in early January. Minimal changes in CP content were observed over the fall. Thus, even though the forage

changes color from green to brown after hard frosts, the brown forage still has good feed value, suggesting that color is not a good indicator of feeding value.

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Table 1. Weather during late-summer, posting planting of forage and during the fall sample collection period.

	Year 1				Year 2			
	September[1]	October	November	December	September[1]	October	November	December
Mean high temperature, °F	77.3	69.1	47.6	38.1	80.5	69.8	55.9	42.2
Mean low temperature, °F	54.3	42.3	22.1	22.9	57.4	44.2	33.5	24.5
Mean monthly temperature, °F	65.8	55.7	34.85	30.5	69.1	57	44.7	33.3
Total monthly Precipitation, inches	5.47[2]	1.00	0.13	0.54	1.57	1.15	2.14	2.54

[1] Year one forages were planted on August 25th thus September includes the 5 days (August 26th-31st) post planting that were in August. In year 2 the forage was planted on September 8th thus monthly mean does not include September 1-8th.
[2] A 3.2 inch rain event occurred three days post planting.

Table 2: Analysis of variance for nutrient content of oats (Avena *sativa*) and brassica tops [daikon radishes (Raphanus *sativus*) and purple top turnips (Brassica *rapa*)] sampled in early November, December, and January over a two year period.

	Yield	TESC	NDF	ADF	IVOMD	CP	S
				$P > F^{‡}$			
Year	NS	*	*	*	*	*	*
Date	NS	*	*	*	*	*	*
Species	*	*	*	*	*	*	*
Date × year	NS	*	*	*	NS	*	*
Date × species	*	*	**	NS	*	NS	*
Year × species	*	NS	*	*	*	*	*
Date × year × species	NS	*	NS	**	NS	NS	NS

† Yield, total ethanol soluble carbohydrates (TESC), neutral detergent fiber (NDF), acid detergent fiber (ADF), crude protein (CP), and sulfur (S) was analyzed on a dry matter basis. In vitro organic matter digestibility (IVOMD) was analyzed on an organic matter basis.

‡NS (P >0.10); *($P \leq 0.05$); **($P > 0.05 \leq 0.10$)

Table 3. Nutrient content of brassicas, daikon radishes (Raphanus *sativus*) and purple top turnips (Brassica *rapa*) sampled in early November, December, and January in year 2.

	Radish						Turnip							
	Top			Root			Top			Root				*P*-value
Nutrient†	Nov.	Dec.	Jan.	Nov.	Dec.	Jan.	Nov.	Dec.	Jan.	Nov.	Dec.	Jan.	SEM	Sample × Date
TESC,%	10.6^{cd}	10.2^{d}	2.7^{e}	36.1^{b}	42.6^{b}	17.4^{c}	15.3^{cd}	17.8^{c}	8.2^{d}	45.8^{ab}	52.5^{a}	50.8^{a}	3.8	<0.01
NDF,%	30.5^{c}	28.1^{c}	46.9^{a}	19.9^{e}	16.8^{ef}	28.1^{c}	25^{d}	23.5^{d}	37.5^{b}	18.3^{ef}	13.8^{g}	14.6^{fg}	1.4	<0.01
ADF,%	22.7^{bc}	20.5^{c}	35.5^{a}	13.1^{ef}	13.8^{e}	24.3^{b}	16.9^{d}	16.6^{d}	25.7^{b}	12.3^{ef}	10.4^{f}	11.5^{ef}	1.1	<0.01
IVOMD,%	88.8^{de}	87.0^{e}	82.2^{f}	92.5^{bc}	93.9^{ab}	88.5^{de}	89.6^{cd}	89.4^{d}	83.8^{f}	95.5^{ab}	95.7^{a}	94.0^{ab}	1.1	0.12
CP,%	25.7^{a}	24.9^{a}	21.3^{b}	15.8^{c}	15.9^{cd}	17.8^{bc}	21.3^{ab}	20.3^{b}	17.8^{bc}	11.5^{d}	15.6^{cd}	15.7^{cd}	2.1	<0.01
S,%	1.10^{ab}	0.98^{cd}	0.77^{fg}	1.17^{a}	1.03^{bc}	0.89^{de}	0.96^{cd}	0.79^{ef}	0.71^{fg}	0.75^{fg}	0.65^{g}	0.69^{fg}	0.042	<0.01

$^{a-g}$ Values within row without the same superscript differ ($P \leq 0.05$).

†Total ethanol soluble carbohydrates (TESC), neutral detergent fiber (NDF), acid detergent fiber (ADF), crude protein (CP) and sulfur are reported on a dry matter basis. In vitro organic matter digestibility (IVOMD) is reported on an organic matter basis.

Table 4. Two year mean nutrient content of oats (Avena *sativa*) and brassica tops [daikon radishes (Raphanus *sativus*) and purple top turnips (Brassica *rapa*)], sampled in early November, December, and January.

	Oats			Radish			Turnip				*P*-value
Nutrient†	Nov.	Dec.	Jan.	Nov.	Dec.	Jan.	Nov.	Dec.	Jan.	SEM	Date × Species
CP,%	17.9^{e}	13.8^{f}	15.8^{e}	27.9^{a}	25.8^{b}	25.6^{b}	24.9^{bc}	22.8^{d}	23.4^{cd}	1.07	0.26
IVOMD,%	79.0^{d}	77.0^{d}	67.4^{e}	88.0^{ab}	87.0^{b}	81.6^{c}	89.8^{a}	87.9^{ab}	83.5^{c}	1.01	<0.01
NDF,%	48.9^{b}	48.2^{b}	66.2^{a}	26.5^{e}	26.9^{e}	44.6^{c}	21.5^{f}	23.7^{f}	37.4^{d}	0.99	0.07
ADF,%	27.1^{c}	25.7^{c}	38.2^{a}	19.9^{d}	18.9^{d}	31.0^{b}	15.2^{e}	15.9^{e}	25.4^{c}	1.12	0.23
Sulfur,%	0.303^{d}	0.286^{de}	0.246^{e}	0.920^{a}	0.896^{a}	0.634^{c}	0.812^{b}	0.776^{b}	0.596^{c}	0.026	< 0.01

[a-f] Values within row without the same superscript differ ($P \leq 0.05$).

†Neutral detergent fiber (NDF), acid detergent fiber (ADF), crude protein (CP) and sulfur are reported on a dry matter basis. In vitro organic matter digestibility (IVOMD) is reported on an organic matter basis.

Figure 1: Total ethanol soluble carbohydrate (TESC) content of oats (Avena *sativa*) and brassica tops (daikon radishes (Raphanus *sativus*) and purple top turnips (Brassica *rapa*) in Early November, December, and January of year 1 and 2. Bars without the same letter differ ($P \leq 0.05$).