THE EFFECT OF COMPETITION FOR AN AUTOMATED SUPPLEMENT FEEDER ON SUPPLEMENT INTAKE BEHAVIOR OF BEEF STOCKER STEERS.

By

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Title of Study: THE EFFECT OF COMPETITION FOR AN AUTOMATED SUPPLEMENT FEEDER ON SUPPLEMENT INTAKE BEHAVIOR OF BEEF STOCKER STEERS.

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Abstract: The objective of this research was to determine the effect of competition for a feeding space at an automated supplement feeder on supplement intake behavior. A 2-yr study was conducted; each yr, 128 mixed-breed beef steers (initial BW = 245 ± 27.5 kg) were randomly assigned to 8 paddocks. One paddock each yr (n = 16 steers) was selected to have continuous access to the feeder for the duration of the 16-wk trial; this paddock was designated the "tester" paddock. The automated feeder had 4 feeding stations that dispensed supplement to individual animals. Steers were limited to 0.50 kg supplement/d. Additional paddocks were commingled with the tester paddock, weekly to increase competition for the feeder. Mean weekly supplement intake and GPS location were recorded for steers in the tester paddock. Additionally, pedometer data were collected in yr 2. Weekly mean supplement intake and time spent near the feeder were regressed on actual feeder stocking density with yr as a random variable. Competition for a feeding station numerically reduced ($P = 0.01$) supplement intake by 4 g/d per steer of additional competition. Steers spent 4.4% of the time within 15-m of the feeder regardless of competition ($P = 0.54$). As competition increased, steers took more steps ($P \leq 0.01$). The objective of the second study was to determine the effects of supplementation level and type on forage intake of steers grazing a high-quality native range. On d 0, steers (n=16, initial BW = 193.7 kg ± 14.3 kg) were randomly assigned to one of 5 dietary treatments, fed once daily in individual stalls for 28-d. Treatments were control (no supplement, n=4), or supplemented with either CSM or DRC, each at either 0.45 kg or 1.81 kg as-fed/d (n=3 for each combination). Some steers had orts, therefore actual mean supplement intake was used in analysis. Results were analyzed with regression, with animal as the experimental unit. Forage intake did not differ by supplement type when offered at 0.45 kg/d yet with increasing supplement forage intake declined for CSM but increased for DRC ($P = 0.01$).

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LITERATURE REVIEW

Introduction

In our current society, sustainability and improving efficiency is one of the top goals within all industries. Over the past decade, producers, regulators, and the general public have had growing concerns about the potential effects of livestock operations on the environment (NASEM, 2016). With these concerns increasing, we must develop new tactics, utilize technologies, and become as efficient as possible in the livestock industry.

Grazing warm-season perennial grass is common in the Southern Great Plains. Grazing system and stocking rate play a significant role on herbage mass and therefore leads to an indirect effect on animal performance (Burns et al., 1989). A common practice to improve forage harvest efficiency and animal performance is supplementation. Supplementation has been shown to improve ADG, overall feed efficiency, and profit. Utilizing precision feeding and supplementation tactics to meet livestock nutrient requirements has the potential to decrease nutrient losses (NASEM, 2016). However, there are limitations in precision feeding systems, such as variability in animal performance, nutrient requirements, composition of feed ingredients, seasonal/climatic effects, logistics, economics, and use of safety margins (NASEM, 2016).

Supplement

A few main reasons for supplementation practices, include conservation of forage, improvement of animal performance, increase economic returns, and managing cattle behavior (Kunkle et al., 2000). Supplementation can be defined as providing additional nutrients to offset specific deficiencies or to meet production demands (Caton and Dhuyvetter, 1997). Supplementation is practiced more often in summer dormancy or decline, or during the fall and winter months, depending on if the forage is a C3 or C4 variety (Caton and Dhuyvetter, 1997). Supplementation can become a substitution for forage when grazed nutrients are removed from animal diets in exchange for a supplement (Caton and Dhuyvetter, 1997). Supplementation and/or substitution can be desirable at specific times, depending on many factors. Of those factors, forage quantity, quality, and production goals are usually the most important, with many other factors to consider as well.

Frequency

Daily supplementation has been shown to maximize forage intake (Beaty et al., 1994). However, it usually requires intensive labor and equipment, and in some situations daily supplemenation has not been shown to improve performance enough to make it cost-effective (Beaty et al., 1994). Ideally, if the frequency of supplementation could be lessened without severely affecting performance, labor and machinery costs would be reduced (Beaty et al., 1994). Beck et al. (2014) reported improved performance whether supplements were fed daily or on alternate days compared to no supplement on growing beef calves grazing warm-season pasture or non-toxic endophyte-infected tall fescue pasture. Beaty et al. (1994) found daily supplementation was favored slightly, but that

supplementation 3 times per wk was a viable management practice. There was also no effect of protein concentration or grain type on those results as long as 0.4 to 0.45% supplementation of BW/ d was achieved per feeding (Beaty et al., 1994). Supplementation of cattle grazing low-quality forages with CSM (7 kg /wk) 3 times or once each week had similar performance compared to cattle fed daily (Adams, 1985). Infrequent supplementation with low-protein grain usually results in lower performance for cattle on low-quality forages (Adams, 1985). Supplements with moderate protein levels may be successfully fed infrequently, which is about 3 times per wk (Adams, 1985).

The balance between protein and energy is essential for cattle grazing low-quality forage to maintain acceptable forage use and livestock performance (Beaty et al., 1994). There is also the chance of animals consuming less than the targeted amount of supplement (Bowman and Sowell, 1997). Since the nutrient composition of grazed forage is often unknown, it can be difficult to formulate nutrient content of the supplement. Overconsumption of supplements can have negative associative effects and not provide the intended results, and under consumption can hurt intended performance (Bowman and Sowell, 1997).

Previous research has been conducted to see if the time of day that supplement is given affects grazing behaviors, however, the research has been variable and overall inconclusive. Other research shows, that the time the supplement is fed could change the amount of time cattle spend grazing because the supplement might be fed at a time that the animal would interrupt normal grazing behavior (Adams, 1985). Scalia et al. (2009) found time of supplementation does affect DMI and grazing behaviors, for cattle

consuming annual ryegrass, but overall ADG was not affected by the time of supplementation. Barton et al. (1992) indicated that for cattle consuming low-quality intermediate wheatgrass forage, time of supplementation did not have an effect on intake, digestion, or digesta kinetics.

Factors and management that affect grazing time

There are many environmental factors that affect grazing behavior including: temperature, wind velocity, and barometric pressure (Krysl and Hess, 1993). Any management or environmental factor affecting grazing activity would have a direct effect on maintenance requirements (Krysl and Hess, 1993; Caton et al., 1997). There is a direct link to the energy expenditure associated with the work of grazing. If less forage is available and it takes more time to acquire the forage, the amount of energy expenditure increases (Caton et al., 1997). Likewise, maintenance requirements can also depend on the production level and energetic expenditure related to the work of grazing, season of the year, and animal breed (Caton et al., 1997).

Stocking rates also affect grazing time and is likely the most crucial grazing management decision a producer can make (Smart et al., 2010). An established relationship between stocking rate, productivity of livestock, and vegetation has been known for a long time (Riewe, 1961; Smart et al., 2010). Research has shown the relationship between ADG and forage allowance is nonlinear, but the relationship between ADG and grazing pressure is linear (Smart et al., 2010). As stocking density increases, animals consume more forage, because it might become limited in the future. It is known that limited forage availability limits intake and therefore would also limit animal weight gain; but also as grazing pressure increases, grazing efficiency also

increases (Combellas and Hodgonson, 1979; Allison et al., 1982; Mazzanti and Lemaire, 1994; Redmon et al., 1995; Poppi, 1996; Smart et al., 2010).

Traditional limiters

Supplement intake limiters allow supplements to be offered by self-feeding. Self-feeding methods assume that an animal is capable of knowing the deficiencies in the basal diet and that they will consume the amount of supplement to meet those deficiencies (Williams et al., 2017). The primary concern or error in self-feeding is that the animal only consumes the desired level of supplement (Williams et al., 2017). Limit feeding of supplements to cattle on pasture to control intake, can be done by adding salt or calcium carbonate to an ad libitum feed supply (Perry et al. 1986, Williams et al., 2017). Salt has been a reliable intake limiter (Perry et al., 1986; Kunkle et al., 2000). When feed is limited, it has been shown to slow the rate of passage, resulting in higher retention time and increased ruminal digestibility and nutrient utilization (DeVries and von Keyserlingk, 2009). Salt levels of 5 or 10% tended to depress ad libitum consumption of corn on brome and orchard grass (Perry et al., 1986). Perry et al. 1986 found a 5% inclusion rate of salt, decreased corn consumption by 22%, and at a 10% inclusion it decreased corn consumption by 29%. Williams et al. (2017) found that salt used as a method of limiting feed intake can sacrifice some potential efficiency. This potential negative result needs further research, and as precision supplementation technology becomes more economical, it may replace salt and other traditional limiters.

Energy Supplementation

Supplemental energy typically decreases forage intake (Cook and Harris, 1968; Kartchner, 1981; Chase and Hibberd, 1987; Horn and McCollum, 1987; Minson,1990;

Sanson et al., 1990; Paterson et al., 1994). This decrease in forage intake can be undesirable or desirable, depending on the situation (Horn and McCollum, 1987). For example, in situations where forage supply is limited, a decrease in forage intake may be desirable (Horn and McCollum, 1987). If maximizing performance is the production goal, then it is often necessary to use an energy supplement instead of small amounts of protein supplement to drive average daily gain (Horn and McCollum, 1987). Reduction in forage intake in response to energy supplementation depends on the basal quality of the forage. (Caton and Dhuyvetter, 1997). The form of supplemental energy also plays a role, as readily digestible fiber usually has a less negative effect on forage intake, whereas grain based supplements increases the efficiency of energy use (National Research Council, 1984). Horn and McCollum (1987) also state that in general, concentrates can be fed in amounts up to about 30 g/kg metabolic BW without significant decreases in forage intake. Supplementation of energy may also affect grazing behavior because it changes how much forage the animal will need to consume to meet its requirements (Caton and Dhuyvetter, 1996).

Energy supplementation also can decrease the digestibility of fibrous particles, such as cellulose and hemicellulose (Chase and Hibberd, 1987). Corn can have a negative effect on the rate of digestible hay disappearance, NDF disappearance, and substitution ratio (Chase and Hibberd, 1987). Reductions in forage intake due to energy supplementation have been attributed to either depression in ruminal pH or a carbohydrate effect (Mould et al., 1983). A carbohydrate effect is when the pH of the rumen has adapted so that the microflora can readily degrade fermentable carbohydrates rather than cellulose (Mould et al., 1983). A declining ruminal pH associated with

increasing dietary starch would shift the ruminal bacteria population towards an increase in amylolytic bacteria and lessen the cellulolytic population, most likely resulting in the reduction of fiber digestion and intake of grazed forage (Horn and McCollum, 1987; Caton and Dhuyvetter, 1997). However, there have been multiple studies showing when ruminants were fed various forages and supplemented with energy sources had mixed ruminal pH levels results. Thus, ruminal pH does not always decrease with grain energy supplementation (Caton and Dhuyvetter, 1997). Bodine and Purvis (2003) saw that when corn was fed alone with no ruminally degradable protein it will exacerbate the potential for reduced intake and digestibility of low quality forages. However, Bodine and Purvuis (2003) saw that when the animal was fed adequate degradable protein along with a corn-based supplement that the animal had enough energy and protein available to allow the animal to achieve greater rates of gain while grazing low quality forages.

Metabolizable energy from concentrates is more efficiently used for maintenance and gain functions than energy obtained from forages (National Research Council, 1984). Therefore, the reductions in forage intake and the marginal changes in total digestible OM intake might be the result of the changing efficiencies in metabolizable energy used. There is evidence that directly providing an energy source will rarely increase the energy status of grazing livestock (Horn and McCollum, 1987). Exceptions include: when supplements are offered frequently at low rates, or with high-quality forages like ryegrass, rye, wheat, or when forage availability is limiting energy intake (Horn and McCollum, 1987; Sanson et al., 1988; McCollum and Horn, 1989). High fiber by-products may be used in energy supplements to offset adverse effects of starch on ruminal fermentation, when the objective is to minimize effects on forage intake (Horn

and McCollum, 1987). The majority of data shows that energy supplementation affects forage intake and digestibility and the extent is variable based on the basal forage diet. Overall, the theory of reduction in ruminal pH associated with energy grain supplementation to the reduction in forage intake and digestibility, should be supported by further research.

Substitution Ratio

Substitution ratio is the unit change in forage intake per unit increase in concentrate intake (Horn and McCollum, 1987). Substitution is desirable when the goal is to extend the supply of available forage or to use high-quality forage nutrients in a more efficient way (Horn and McCollum, 1987; Moore et al., 1999). Substitution ratios are responsive to livestock species and basal forage quality, and need to be considered when comparing substitution ratios to each other (Horn and McCollum, 1987). Substitution ratio is calculated by regressing voluntary forage intake (g/kg metabolic BW) against the amount of supplement fed (g/kg metabolic BW; Horn and McCollum, 1987). When a substitution ratio is negative resulting in a substitution effect, it is related to the increase in forage digestibility (Horn and McCollum, 1987). Other terminology similar to substitution ratio are positive or negative associative effects correlated to a negative or positive intake of forage. Commonly, a negative associative effect is associated with energy supplementation (Horn and McCollum, 1987). Substitution ratio for a specific level of concentrate is positively correlated with forage digestibility (Horn and McCollum, 1987). There are variations in the level of substitution rate as physiological state, activity level, and forage quality and quantity will produce different ratios with any given concentrate at any level (Horn and McCollum, 1987). These deviations can also be

due to the interactions of forages and supplements that either increase or decrease forage consumption and availability of the dietary energy (Moore et al., 1999).

Variable	Substitution Ratio	Interpretation
Forage Quality	↓	An increase in forage mass will result in a less reduction in forage intake with increasing supplement amount
Forage Digestibility	↑	As forage digestibility increases, forage intake due to increasing supplementation will decrease.

Protein Supplementation

Protein supplementation to cattle grazing low quality forages (CP < 7%) has been shown to improve forage intake, utilization, and overall harvest efficiency (Cook and Harris, 1968; Church and Santos, 1981; Caton et al., 1988; Guthrie and Wagner, 1988; McCollum and Horn, 1989; DelCurto et al., 1990; Krysl et al., 1993; Gadberry et al., 2010). Furthermore, when performance is depressed by insufficient forage intake when RDP is deficient, protein supplementation usually improves performance more efficiently than energy concentrates (Hennessey et al., 1983; Lusby and Horn, 1983; Horn and McCollum, 1987; Kunkle et al., 1988; Kunkle and Baldwin, 1988; McCollum and Horn, 1989).

To improve the performance of grazing ruminants, there has to be an increase in energy intake or in the efficiency with which ingested energy is utilized (McCollum and Horn, 1989). When adequate forage is available but the forage is inadequate in crude protein, supplementing the basal diet with protein concentrates have been shown to improve energy status and be favorable from an economical approach (McCollum and Horn, 1989). Inadequate diet protein will suppress forage digestion and intake, along with

reducing the efficiency of metabolizable energy (ME) utilization, because ruminally available protein is the first limiting nutrient for microbial protein production and not energy (Freeman et al., 1992). McCollum and Horn (1989) also state while energy intake may be the primary factor that would limit performance, ruminal protein status appears to be a primary factor in influencing energy intake and utilization.

When protein supplementation has failed to improve performance, it usually also failed to improve forage intake, illustrating their correlation. (McCollum and Horn, 1989; DelCurto et al., 1990; Moore et al., 1999). The response shown between forage intake and protein supplementation can be highly variable at varying forage CP levels. The potential magnitude of forage intake due to supplementation is dependent on the forage CP. As forage CP decreases, it is more likely that a protein supplement will increase intake (McCollum and Horn, 1989). There is only a small increase in forage intake when the forage contains 10-11% or higher CP (McCollum and Horn, 1989). This leads to the idea that an increase in forage intake is related to the CP of the forage and not the CP of the total diet (McCollum and Horn, 1989). However, there is evidence that in native range forages, intake responses are not solely dependent on forage CP (McCollum and Horn, 1989).

Caton et al. (1988) found protein supplementation at 150% of animal 1996 NRC requirements suggested level did not substantially alter hindgut fermentation or digesta flow in ruminants consuming low-quality forages. McCollum and Horn (1989) provided a review of 10 Oklahoma studies that fed a low amount of all-natural high-protein supplements (0.45/kg/hd/d, 38% - 44% CP) to growing cattle grazing range or bermudagrass in the summer increased ADG 0.14 to 0.22 kg/hd (Lusby, 1989). They also

reported similar reports from various regions in Australia. Most of those studies utilized cottonseed meal or soybean meal as their protein source. A protein supplement that has a greater potential of ruminal escape may improve the conversion of the supplement depending on the forage quality (McCollum and Horn, 1989).

McCollum and Horn (1989), provided an overview of the mechanisms of action when protein supplementation occurs.

1. "Correction of ruminal N deficiency which increases rate and in some instances, extent of digestion, and increase forage (energy) intake.
2. Increased non-ammonia nitrogen (NAN) flow to the small intestine via microbial protein or undegraded feed protein. Improved N status may stimulate feed intake and energy utilization.
3. Correction of an amino acid deficiency or imbalance at the tissue level which may stimulate forage intake and increase ME utilization.
4. Increased supply of amino acids which promote tissue deposition and enhance energy utilization.
5. Increased supply of glucogenic amino acids and recycled N which may stimulate forage intake and ME utilization."

These effects do not occur independently; therefore, it is difficult to determine causes and effects (McCollum and Horn, 1989). All of these mechanisms improve energy status, by causing the increased intake and/or more efficient digestion and utilization of ME (McCollum and Horn, 1989). Even with the results being the same, targeting any of these mechanisms specifically would require different supplement formulation strategies (McCollum and Horn, 1989).

There is almost always a decline of intake and performance as forage CP decreases, and it is usually attributed to a ruminal N deficiency. Part of an animal's daily energy requirement is protein and a bypass protein source can serve as a form of energy (Horn and McCollum, 1987). Many protein bypass studies have shown that forage intake is not directly caused by the ruminal influences, of passage rate and digestion, but that there are many functions occurring (Egan and Moir, 1965; Egan, 1977; Egan and Doyle, 1985; Barry et al., 1982; Lindsay et al., 1982; Moberg et al., 1989). Most protein supplementation studies have shown either rate of passage, rumen fill, or both are increased when protein supplements are fed (Guthrie and Wagner, 1988; Kryl et al., 1987; Egan and Doyle, 1985; McCollum and Galyean, 1985; Stokes et al., 1989; Hannah et al., 1991). Fleck et al. (1988) reported increased digestibility and intake of low-quality grass hay, but there was no change in the rate of passage with protein (soybean meal; **SBM**) supplementation. Stokes et al. (1989) also observed higher forage intake along with more extensive hay digestion, faster passage rates, and increased N flow into the small intestine in steers consuming grass hay supplemented with protein (SBM). Hannah et al. (1991) showed supplements that provided adequate protein to cattle that are grazing low-quality range type forage can improve their performance because of enhanced forage intake and digestion. McCollum and Gaylean (1985) showed supplementing cottonseed meal increased particulate rate of passage and is a major factor associated with increased low-quality prairie hay intake. Williams et al. (2017) saw an increase in weight gain due to protein supplementation in steers grazing dormant tallgrass prairie. Church and Santos (1981) found supplementation from CSM will increase consumption of straw, but that a liquid supplement containing a non-protein N compound did not show the same results.

Further, Church and Santos (1981) found an increase in intake did not result in an increase in digestibility, which was in agreement with other studies (Chicco et al., 1972; Jones et al., 1976).

With rate and extent of digestion playing a role in intake regulation, enhanced nutrient flows may be a more crucial ruminal response affecting intake and performance in grazing livestock (Egan, 1977; McCollum and Horn, 1989). The nutrient flow could be improved by reducing the total feed required to meet a nutrient deficiency or limiting the quantities of costly feed ingredients in the supplement formulations (McCollum and Horn, 1989). There is also research to be conducted in identifying the specific compound in protein supplements that stimulates ruminal activity, to promote that specific mechanism (McCollum and Horn, 1989).

Technology

Trials using automated feeders

With new technology and systems to automate feeding, it is important to understand potential animal variation, behavior responses, and the effect of competition in those systems. Precision supplementation feeders could have added benefits including reducing labor and improving animal welfare by ensuring that individual animals are consuming feed (DeVries et al., 2003; Reuter et al., 2016; Reuter and Moffet, 2016). The use of an automated precision supplementation feeder could potentially decrease labor and costs while improving overall performance and economics of the herd. The use of a precision feeder would also determine exact supplement intake and allow for daily feed consumption, resulting in fewer nutrient losses. Williams et al. (2017) reported that while

supplementation often leads to desired improvements in performance, hand-feeding individuals is laborious, costly, and impractical in commercial settings.

Reuter et al. (2016) describes the SmartFeed system (C-Lock, Inc., Rapid City, SD) as a stainless steel feed bin suspended on 2 weigh cells, with an RFID reader, and an adjustable framework that can be used to limit access of the feeder to 1 animal at a time. Reuter et al. (2016) reported that the herd tended to visit the feeder as a group, and would displace one another to consume supplement, rather than visiting the feeder individually throughout the day. Research has shown that within extensive grazing systems, herding behavior will often result in behavior synchronization, such as the entire group will drink, eat, and ruminate at the same time (Miller and Wood-Gush, 1991; Rook and Huckle, 1995; DeVries et al., 2004). This research is in agreement with the results observed by Reuter et al. (2016).

Reuter et al. (2016) also saw that RFID tags were sometimes read less than 1 s apart, resulting in the conclusion that there was competition for space at the SmartFeed feeder. Competition at an individual feeder will cause behavior changes as compared to a commercial setting, where the producer feeds in a trough, minimizing the competition (Reuter et al., 2016). Reuter et al. (2016) states that additional research is needed to understand the effect of competition at automated feeders, and the suspected behavior responses of that competition. Aggressiveness and dominance of the cattle, the type of cattle, the stocking rate, paddock type, and environment can also play a major role in the behavior and effect of competition at an automated feeder (Reuter et al., 2016).

Devries et al. (2003) validated the use of another automated feeding system that uses a radio frequency electronic monitoring system. The GrowSafe system (GrowSafe

Systems Ltd.; Airdrie, AB, Canada) is adequate for measuring the feeding behavior of free stall barned dairy cattle (DeVries et al., 2003). The GrowSafe system provides a reasonable estimate of when cattle are present in the feed alley as well as appropriate meal ciritia (DeVries et al., 2003). Allwardt et al. (2017) evaluated the use of the Insentec Roughage Intake Control system (Insentec, Marknesse, the Netherlands) for individual feed and water intake, by direct observation along with time-lapse video. Allwardt et al. (2017) found the Insentec to be a useful instrument in monitoring individual animal intakes along with the ability to restrict water and feed intake. Chapinal et. al. (2007) also found the Insentec system to be accurate in monitoring feed and water intake, along with other feeding behaviors.

Stocking densities/competition

DeVries and von Keyserlingk (2009) conducted a behavior study utilizing an Insentec system (Insentec, Marknesse, the Netherlands) and looked at the effect of competition for feed on the feeding behavior of growing dairy heifers. DeVries and von Keyserlingk (2009) were able to record individual feed intake for all of the trial animals and evaluated the number of visits, duration of visit, and the amount eaten per visit. This data was also used to calculate DMI. DeVries and von Keyserlingk (2009) found competition for feeding space changes the feeding behavior of growing dairy heifers. Competition increased the number of visits with shorter feeding times, especially during their peak feeding times (right after delivery of feed). DeVries and von Keyserlingk (2009) concluded competition altered the heifer's feeding pattern, reduced their overall access to feed, and increased day to day variation in feeding behavior (DeVries and von Keyserlingk, 2009).

Research has been conducted in the feedlot and dairy industry on feeding space on feeding behaviors. Within dairies, most findings show that if feeding space is limited, there is increased competition for the feeding space, and this results in some cattle modifying their feeding behaviors to avoid aggressive interactions (Miller and Wood-Gush, 1991; DeVries et al., 2004). Research also lead to the conclusion that increased feeding competition could result in reduced intake and increased feeding rate. (Friend et al., 1977; Shaver, 1997; Shaver, 2002; DeVries et al., 2004; Huzzey et al., 2006). DeVries et al. (2004) found that when dairy cattle are allowed more feeding space, an increase from 0.5 m to 1.0 m, it allowed the animals more space to themselves and reduced the frequency of aggressive behaviors. Huzzey et al. (2006) found increasing stocking density at the feed bunk increased the frequency that cows were displaced, especially for subordinate cows using a post-and-rail feed barrier. A hypothesis for these behavioral responses is that individual animals in excessively large group sizes, struggle to remember the social hierarchy of the group, which results in aggressive behavioral interactions (Kondo et al., 1989). Kondo et al. (1989) also show that dominance hierarchy varies with different ages, breeds, and environmental times. Therefore, work on behavioral projects should be conducted in all environments with all types of cattle to obtain optimal results.

Increased competition at feed bunks has shown to decrease the amount of time an animal spends eating, increase the time animals stand waiting to access the feed, and increase the rate at which animals are displaced from the feeding area (Huzzey et al., 2006). However, Proudfoot et al. (2009) found that individual cattle's behaviors can be unreliable and variable, especially from dominance to subordinate with many variables

affecting the potential behavior outcome of a specific animal to a group of animals. Grant and Albright (2001) concluded if feeding behavior could be altered positively, decreasing within-group variation and overall group intake, it would result in less variable animal performance. If animals were receiving the same nutrients, rather than dominant animals receiving more nutrients than others, the herd would be more efficient (Grant and Albright, 2001). Alternatively, improvement in feed delivery utilizing a precision automated feeding system may be utilized to improve efficiency of the entire herd (Reuter et. al., 2016).

Forage DMI

Estimation of intake:

Forage intake is one of the most essential components determining performance by grazing ruminants (Lippke, 2002). Intake can be estimated by estimating fecal output and digestibility of the diet consumed (Lippke, 2002; Dove et al., 2005). Digestibility traditionally has been determined as the difference between feed intake and fecal output. Estimation of fecal output can be determined using internal or external markers (Lippke, 2002; Lund et al., 2007). A wide range of markers that can be used, all of which have positive and negative attributes. To be considered an acceptable marker it must display the characteristics developed by Faichney (1975); it must be strictly non-absorbable, it must not affect or be affected by the GI tract or its microbial population, it must be physically similar to or intimately associated with the material it is to mark, its methods of estimation in digesta samples must be specific and sensitive, and it must not interfere with other analyses. (Waller et al., 1980; Lippke, 2002; Dove et al., 2005). There are probable errors in the collection of internal and external markers. While these errors are

present and discussed later in the review, markers are still the best way to estimate intake of grazing animals while meeting the requirements and allowing for ease and precision of measurement (Lippke, 2002).

External markers:

Titanium Dioxide (**TiO_2**) is an external marker that offers advantages over previously used markers as TiO_2 can be added to food animal's diets legally and without fear of potential carcinogenic effects, unlike chromium oxide (**Cr_2O_3**; Peddie et al., 1982; Titgemeyer et al., 2001; Myers et al., 2004). Errors in collecting external marker data include but are not limited to; collection of feces, the recovery rate of markers, and administration of the marker (Lippke, 2002). Total collection of feces in a grazing environment has distinct challenges (Lippke, 2002). The labor requirement is high, not only for the actual collection but for the training and management of docile animals (Lippke, 2002). Moreover, having to empty the fecal collection bags once or twice daily is likely to affect the animal's grazing behavior and thus their potential intake (Lippke, 2002). Another error in total fecal collection is the potential for feces to escape the collection bag (Lippke, 2002). Unless animals are continuously observed, the type of errors and the magnitude is often unknown. (Lippke, 2002). A problem with Cr_2O_3 and TiO_2 is that the marker moves through the digestive tract independently of undigested particles of the diet (Lippke, 2002). The positive aspect of this is that these markers can measure total intake and not just intake of a specific feedstuff. However, it also means the recovered fecal concentrations of that marker will show strong diurnal variation (Lippke, 2002).

Depending on the rate of passage of potential undigested residues, the first appearance of any external marker in feces has shown to be around 6 to 15 h after dosing (Lippke, 2002). Waller et al. (1980) found there was no advantage, regardless of the particulate marker, to extending the sampling interval beyond 24-hr or taking more than one sample per day. However, the animals were fed hourly, and results could be different for animals fed once or twice daily. Hafez et al. (1988) found that the concentration of TiO_2 from rectal fecal samples in the morning corresponded very well with the concentration from the total fecal collection data, when TiO_2 was dosed in the morning. He also found that the samples that were taken in the evening showed lower and less correlated concentration values compared to their total fecal collection samples.

Internal markers:

Ruminants can efficiently digest fibrous feed materials better than monogastrics. These fibrous plant materials are comprised mostly of cellulose, hemicellulose, and lignin, as well as other cell wall and cell content components (NASEM, 2016). When potentially digestible fiber is removed from a feedstuff, the remaining neutral detergent fiber (**NDF**) portion is indigestible NDF (**iNDF**; Palmonari et al., 2016; Lippke et al., 2000; Lund et al., 2007, NASEM, 2016). This portion is in theory, indigestible by microorganisms even after an infinite amount of time. The iNDF is mostly comprised of lignin and a portion of cellulose (NASEM, 2016). The iNDF can be used to predict OM digestibility, total tract digestibility, ruminal fill, DMI, and ruminal passage rate (Lippke et al., 2000; Krizsan et al., 2012; Palmonari et al., 2016).

An estimate of iNDF can be obtained by conducting *in situ* procedures by incubating samples in permeable bags for 288 h (Krizsan et al., 2012; Krizsan and

Huhtanen, 2012; Krizsan et al., 2013; Palmonari et al., 2016; Weld and Armentano, 2016; Ciriaco et al., 2018). Some errors with this procedure are that it relies on the availability of ruminally cannulated animals and is limited by the characteristics of the bags used, which are not always representative of what happens in the rumen (Krizsan et al., 2012; Palmonari et al., 2016). The bags do not allow for various passage rates, which can alter estimations of digestion. This procedure can also be done in vitro but is extremely difficult to mimic (Cochran et al., 1986).

Cochran et al. (1986) also described a way of predicting digestibility of different diets using different internal markers. They used iNDF, iADF, acid detergent lignin, and acid detergent fiber incubation cellulase (**ADFIC**). However, Cochran et al. (1986) found the relationship between in vivo dry matter digestibility (**DMD**) and DMD determined by iADF and iNDF ratio was highly variable. Caution should be exercised in using iADF and iNDF as internal markers, particularly with immature, freshly harvested, or grazed forage. Cochran et al. (1986) determined that both an internal and external fecal marker should be used to explain any observed differences between iADF or iNDF. Cochran et al. (1986) defined that in-vivo acid detergent lignin and ADFIC were least acceptable for use as internal markers in all diets evaluated.

Few studies have been conducted to analyze iNDF for consistency within forage type and source. The iNDF varies among forage types and should not be estimated by a simple regression equation, as it is strictly related to feeding behavior, rumen fill, and digestion kinetics (Palmonari et al., 2016). Lund et al. (2007) had an incubation period to 504 h; however, there is little evidence that those results were different from 288 h, as the rate of digestion approaches 0 past h 288.

Potential errors:

A considerable error of estimating digestibility is that of obtaining a sample representative of the diet actually being consumed by grazing or browsing animals. (Lippke, 2002; Dove et al., 2005). In monocultures, samples hand-selected by a trained researcher are likely to be accurate (Lippke et al., 2000, Lippke, 2002) However, in paddocks with increased biodiversity, diet samples can be more accurate if the animal retrieves the forage diet themselves, avoiding human error. Animals with rumen cannulas or esophageal-fistulated animals can obtain a more accurate sample by letting them collect the diet sample. In this method, rumen cannulated animals are allowed to graze for a short period of time followed by rumen evacuations. Samples are then taken out of the rumen, to represent the forage selected by trial animals. These samples are often referred to as masticate (Bodine et al., 2001; Wheeler et al., 2002; Martinez-Pérez et al., 2013). There are flaws in this sampling technique also. Usually, when the animal obtains the sample, the collection is only conducted once or a few times and only over a time-span of a few minutes (Lippke, 2002). This may not be an accurate sub sample of forage because the research trial may have been conducted over days, weeks, or months (Lippke, 2002). In addition, the diet selected by the cannulated animal may differ from the diet selected by the test animals (Lippke, 2002). This variation could be from the cannulated animals being different sexes, of different physiological states, or managed differently from the test animals (Lippke, 2002). However, this is still believed to be the most accurate system to estimate dry matter intake.

The grind size of samples has also been a variable that many researchers have not explicitly researched. The most typical grind sizes are 1mm and 2mm for digestibility

studies. Vanzant et al. (1998) recommends 1.5 - 3.0 mm screen for concentrates and 1.5 - 5.0 mm screen for forages. However, many researchers have done 1.0 mm (Kartchner, 1981; Cochran et al., 1986; Highfill et al., 1987; Köster et al., 1996; Lippke et al., 2000; Davis et al., 2014) and 2.0mm (Caton et al., 1988; Guthrie and Wagner, 1988; Hannah et al., 1991; Freeman et al., 1992; Bodine et al., 2001; Wheeler et al., 2002; Martínez-Pérez et al., 2013) for both concentrate and forages.

Equations:

Various studies have used equations to estimate forage intake, fecal output, or digestibility of a diet, as those factors are dependent upon each other. Some studies have calculated forage intake by using estimated fecal output from an external marker and an internal marker to estimate forage indigestibility (Bodine et al., 2001; Wheeler et al., 2002; Bodine and Purvis., 2003; Ebert et al., 2016). The following equations have been used throughout literature:

Kartchner et al. (1981) and Galyean Lab Manual Equations:

$$DMI\ \left(\frac{kg}{d}\right) = Fecal\ Output\left(\frac{kg}{d}\right) \div indigestibility(\%)$$

$$Fecal\ Output = Marker\ Consumed\ (g) \div Marker\ concentration\ in\ feces$$

$$Indigestibility\ (\%) = 100 - (100 - \frac{iNDF\ Feed}{iNDF\ Feces})$$

Lippke et al. (2002) equations:

$$Digestibile\ DM = 1 - (Fecal\ Output\ \times Intake^{-1})$$

Rearranged into:

$$Intake = Fecal\ Output\ \times (1 - Digestibile\ DM^{-1})$$

$$Fecal\ Output = Dose\ of\ External\ Marker \times (Marker\ concentration\ in\ feces)^{-1} \times Exponential\ Passage\ Rate$$

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CHAPTER II

THE EFFECT OF COMPETITION FOR AN AUTOMATED SUPPLEMENT FEEDER ON SUPPLEMENT INTAKE BEHAVIOR OF BEEF STOCKER STEERS

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ABSTRACT: The objective of this research was to determine the effect of competition for a feeding space at an automated supplement feeder on supplement intake behavior. A 2-yr study was conducted; each yr, 128 mixed-breed beef steers (initial BW = 245 ± 27.5 kg) were randomly assigned to 8 paddocks. One paddock each yr (*n* = 16 steers) was selected to have continuous access to the feeder for the duration of the 16-wk trial; this paddock was designated the "tester" paddock. The automated feeder had 4 feeding stations that dispensed supplement to an individual animal. Steers were limited to 0.50 kg supplement/d. Additional paddocks were commingled with the tester paddock, weekly to increase competition for the feeder. This resulted in a stocking density from 4 to 32 steers per feeding station. For yr 2, one feeding station was disabled, resulting in a stocking densities of 5.3 to 42.7 steers per feeding station. Mean weekly supplement intake and GPS location were recorded for steers in the tester paddock. Additionally, pedometer data were collected in yr 2. Weekly mean supplement intake and time spent near the feeder

were regressed on actual feeder stocking density with yr as a random variable. Tester steers consumed an average of 0.29 kg supplement/d. Competition for a feeding station reduced ($P = 0.01$) supplement intake by 4 g/d per steer of additional competition. Steers spent 4.4% of the time within 15-m of the feeder regardless of competition ($P = 0.54$). As competition increased, steers took more steps ($P \leq 0.01$).

Key words: Precision Supplementation, Stocking Density, Stocker Steers, Technology

INTRODUCTION

In many grazing systems, a supplement is traditionally provided during the late summer to cattle either daily or 3 times per wk. Daily supplementation has been shown to maximize forage intake (Krysl et al., 1993), but it requires additional labor and equipment, and has not been shown to improve performance enough to make it cost effective (Beaty et al., 1994). Precision supplement feeders could reduce labor and improve animal welfare by ensuring individual animal feed intake (DeVries et al., 2003; Reuter et al., 2016; Reuter and Moffet, 2016). Precision supplementation could result in improving the effectiveness of supplement programs, because overconsumption and under consumption are often seen in bunk feeding systems (Bowman and Sowell, 1997). The overconsumption of supplements can have a negative cost association and not provide the intended results, and under consumption can hurt intended performance (Bowman and Sowell, 1997). Further, it allows for additional herd management, such as limit feeding individual animals (Bowman and Sowell, 1997).

However, such technology can be expensive and could potentially cause changes in animal behavior, especially when competition for the feeder increases. Huzzey et al. (2006) showed that higher stocking densities at conventional feed bunks decreased the

amount of time that the animal was eating and increased the time animals stood waiting to access feed.

Reuter et al. (2016) analyzed the variation in daily intake of a salt-limited supplement by grazing steers utilizing a SmartFeed device (C-lock Inc., Rapid City, SD). Reuter et al. (2016) reported that the herd tended to visit the feeder as a group, and would displace one another to consume supplement, rather than visiting the feeder individually throughout the day. Reuter et al. (2016) also saw that RFID tags were sometimes read less than 1 s apart, indicating intense competition for space at the feeder. Therefore, the objective of this study was to determine if feeder stocking density would affect the behavior of grazing steers.

MATERIALS AND METHODS

All procedures used in this experiment conformed to the FASS Ag Guide (FASS, 2010) and were approved by the Oklahoma State University Institutional Animal Care and Use Committee (#AG-16-9).

Location and Pasture

The experiment was conducted over 2 yr; May 29 to September 18, 2018 and June 4 to September 25, 2019 (two, 16-wk periods). The research pasture was a warm-season perennial grass-dominated, primarily composed of Bermudagrass (*Cynodon dactylon)* and Yellow bluestem *(Bothriochloa ischaemum*) located at the Oklahoma State University Bluestem Research Range, 14.5 km southwest of Stillwater, OK. This pasture was not fertilized during the experiment or within 6 mo. prior to the start of yr 1. The 80.9 ha pasture used for the experiment was divided into 8 paddocks by electric fence, in

an arrangement that included a common area accessible to all paddocks (Figure 1). The automated feeder was located in the common area for the duration of the experiment.

Each paddock had access to ad libitum water sourced from Payne County rural water district. Cattle were excluded from all other sources of surface water in the paddocks by electric fence. Mineral (FortiGraze Stocker Altosid mineral; Livestock Nutrition Center, LLC. Chickasha, OK) was provided in a covered ground feeder (Dura-Bull Mineral Feeder; Pride of the Farm, Houghton, IA) near the water tank in each paddock. Mineral was offered a weekly rate of 0.59 kg/wk per steer during the experiment and access was not restricted. Supplement used throughout this experiment contained 80.5% cottonseed meal, 7.0% soybean meal, 7.4% wheat midds, 4.8% molasses, 0.25% Maxibond (MAXIBOND, Karnatake, India), and 0.14% monensin sodium (Rumensin 90; Elanco, Greenfield, IN) with 88.2% DM, 42.9 % CP, and 77.8% TDN. The supplement was pelleted into a 9.53 mm pellet and was produced in an 7.3 metric ton batch. A new batch was made each yr.

Forage mass was determined by a calibrated rising plate meter (Model EC-20; Jenquip, Feilding, New Zealand). Measurements were conducted weekly, beginning on d -5. In each paddock, 30 plate meter readings were taken in random locations every wk to estimate the forage biomass available (Thompson et al., 2019). Forage nutritive value was determined by hand clipping ten, 0.09-m² quadrat, forage samples to ground level in 7 d intervals (Williams et al., 2018). The plate meter was calibrated by regressing the forage mass clipped on the forage height recorded by the rising plate meter. The equation; $forage\ mass\ (g) = dry\ forage\ weight\ (g) \div 0.96 \div 1000 \times 107639$ was used to predict forage mass ($P < 0.01$; Figure 2). The average forage nutritive value in yr

1 was 38.6 % DM, 11.6% CP, and 61.6% TDN, yr 2 the nutritive value was 39.8% DM, 9.3% CP, and 64.1% TDN. Contrary to our expectation, forage nutritive value was consistent throughout the trial in each yr, possibly due to unseasonal rainfall. Precipitation at this site for 268 d (January 1st to September 25th) was 704 mm for yr 1 and 1528 mm for yr 2. The 10 yr average for the same amount of d at this location was 974 mm (2009-2019 data, OK Mesonet "Marena," 2.7 kn southwest of research station; Williams et al.,2018).

Automated Feeder

The feeder used in this experiment was a prototype feeder (Super SmartFeed, **SSF**; C-Lock, Inc, Rapid City, SD) that consisted of a large feed bin (1800 kg capacity) that dispensed feed into 4 feeding stations. Each feeding station was accessible to 1 animal at a time and was controlled independently. The dispense of feed was triggered by the presence of an RFID tag on each animal. If the animal was eligible for feed, supplement was dispensed in 30 s intervals, until the limit imposed by the researchers was met. The limit imposed for this experiment was 0.50 kg/d per steer. Supplement limitations were reset for all animals at 0000 h daily. Data were uploaded to a cloud database every h. The SSF was validated every mo by making the feeder drop 8 motor chain movements in each of the 4 feeding stations. Each set of 8 motor movements was repeated 5 times for each feeding station. Each feed dispense set was scooped out of the tray, weighed, and recorded. Also, test EID tags were used to validate how much supplement each steer was allowed to have. A test EID tag was put up to EID reader until the feeder dispensed feed, and was held up to the reader until no more supplement was dispensed. The SSF feeding station scales and supplement dispense was calibrated after

each validation according to manufacturer's instructions. The 8 motor movement validation showed a mean in supplement for tray 1 to be 0.59 ± 0.07 kg (CV: 12%), tray two 0.60 ± 0.24 kg (CV: 40%), tray three 0.69 ± 0.17 kg (CV: 25%), and tray four 0.63 ± 0.15 kg (CV: 23%). The test tag validation showed a mean of 0.54 ± 0.01 kg (CV: 2%).

Animals and Acclimation

Mixed breed beef steers (*n* = 418, initial BW = 228.6 ± 28.7 kg), approximately 8-10 months of age, were sourced from sale barns in Oklahoma and Arkansas. Steers were preconditioned by the owner at a private facility for approximately 30 d before arrival on the research location. Prior to the start of the experiment, steers were exposed to the automated feeder in groups of 40 to 45 animals. The 4 stall dividers on the SSF were not set down to allow animals to explore the feeder. During this acclimation period, each group was held with the feeder in a 1 ha pen with hay for 1 wk. Once a steer consumed 0.50 kg of supplement for 3 d, the steer was removed from the group and classified as a trained steer. Trained steers were removed from the group to lessen competition for the feeder, and allow other steers to be trained. If a steer did not visit the SSF feeder during that period of time, they were considered a “non-feeder”. Due to the lack of sufficiently trained steers, for yr one, 5 non-feeder steers and in yr two, 6 non-feeder steers were used in the experiment and were randomly allocated along with the feeder steers.

After the acclimation period for all animals, each yr, 128 steers (*n* = 256 total, initial BW = 245 ± 27.5 kg) were randomly assigned to one of the 8 paddocks. Steers in one paddock each yr (*n* =16 steers) were selected to have continuous access to the feeder for the duration of the 16 wk trial; this paddock was designated the “tester” paddock. The tester paddock steers had access to the automated feeder for the duration of the

experiment. To increase competition for the feeder, each wk additional paddocks were commingled with the tester paddock and given access to the feeder. This resulted in a theoretical feeder stocking density of either 4, 8, 16, or 32 steers per feeding station in various weeks. For yr 2, one feeding station was disabled, resulting in a theoretical stocking density of 5.3 to 42.7 steers per feeding station. When steers were commingled, they were also rotated among paddocks to keep pasture stocking rate at 0.63 ha per steer for the entirety of the 112 d experiment. In 28-d intervals, steers were gathered horseback with trained stock dogs at approximately 0500, driven 0.8 km to a working facility, and weighed individually on a validated scale. Steers were then immediately driven back to the experimental area and sorted to assigned paddock. When steers did not have access to the feeder, they were fed the same supplement in feed bunks at 0630 h and at a rate of 0.50 kg/steer/d.

Animal Behavior Data Collection

The location of tester steers was recorded for both yr with a custom-built global positioning system (**GPS**) collar (i-gotU GT-600; Mobile Action Technology Inc., New Taipei City, Taiwan; Knight et al., 2018; Bailey et al., 2018). The GPS devices were replaced with new units every 28 d when the steers were weighed. The GPS units were programmed to record location every 5 min. The standard error (**SE**) for the GPS units were 10 m for each point. This was determined by placing 7 GPS units in a specific location on a fence, and allowing data to be collected while the GPS unit did not move. Additionally, in yr 2, step data were collected via IceQube pedometers (IceRobotics; Edinburgh, United Kingdom).

Laboratory Analysis

All forage samples were dried to a constant weight at 60° C in an oven for 72 h and ground to pass a 1 mm screen in a cutting mill (Pulverisette 19; Fritsch Milling and Sizing, Inc, Pittsboro, NC) and stored for chemical analysis. Protein was determined by dry combustion analysis using a Carbon Nitrogen (**CN**) analyzer (TruSpec CN analyzer; LECO, St. Joseph, MI). The ADF and NDF were analyzed in an ANKOM 2000 analyzer according to manufacturer's instructions (Van Soest et al.,1991; ANKOM Technology, Macedon, NY). In addition, 12 mL of alpha amylase and 20 g of sodium sulfate were added to the NDF solution during the NDF analysis (ANKOM Technology, Macedon, NY). TDN was determined using the equation; $TDN\ (\%) = 88.9 - (0.779 \times ADF\ \%)$ (Kuehn et al., 1999).

Statistical Analysis

Weekly mean supplement intake and time spent near the feeder were regressed on actual feeder stocking density with yr as a random variable. Week was the experimental unit, month was considered a random blocking effect, with previous density also in the model. A Williams crossover design method was used to develop the sequence of weekly stocking densities for both yr. This design method was used to balance potential residual or carryover effects for each of the 4 wk sequences. This resulted in 4 observations of each of the 4 feeder stocking densities (16 wk). Regression models in R (R Core Team, 2017, v. 3.4.3) were used for statistical analysis of all variables of interest. Data were processed with the tidyverse package (Wickham, 2017). Weather data from the Oklahoma Mesonet were retrieved with the okmesonet package (Allred et. al., 2014).

Other packages used were readxl (Wickham and Bryan, 2019), Rgooglesheets (Bryan and Zhao, 2018), and stringr (Wickham, 2019).

RESULTS AND DISCUSSION

Supplement Intake and Feeding Behavior

Approximately 31% of the steers did not voluntarily use the feeder; therefore, effective competition was less than the study design (Figure 3). Tester steers consumed an average of 0.29 kg supplement/d, and individual supplement intake variability was observed in both yr (Figure 4 and 5). Overall, tester steers consumed less feed in yr 2, than in yr 1 (0.21 kg vs. 0.37 kg respectively). Animals vary in supplement intake, and competition has been observed to be at least a partial cause for this variability (Bowman and Sowell, 1997). Bowman and Sowell (1997) showed similar results to the current experiment, with greater levels of competition for a small trough space, resulting in more non-feeding animals. Bowman and Sowell (1997) results also showed that lower competition resulted in more variation of individual intake, and the chance of animals consuming less than the targeted amount of supplement. This indicates that a balance in the competition for feed will provide optimal feed intake results. This is because overconsumption of supplements can have a negative cost association and not provide the desired results, and under consumption can decrease intended performance (Bowman and Sowell, 1997).

Competition for a feeding station reduced tester steer supplement intake by 4 g/d per steer of additional competition ($P = 0.01$; Figure 6). DeVries and von Keyserlingk (2009) results also concluded that competition altered feeding patterns, reduced overall access to feed, and increased day to day variation in feeding behavior in dairy heifers.

When feed intake is limited, it has been shown to slow the rate of passage, resulting in higher retention time (DeVries and von Keyserlingk, 2009).

Mean supplement intake for all steers did not decrease as stocking density of the feeder increased ($P = 0.34$, Figure 7). In the southern Great Plains, nutritive value of warm-season perennial grasses decline in late summer (Bodine and Purvis, 2003; McMurphy et al., 2011). However, this decrease in quality did not occur either yr of this study. We hypothesize that, had there been a decrease in forage quality, steers may have searched for supplement more aggressively and thus consumed more supplement. Had the steers had inadequate diet protein, steers would have experienced suppressed forage intake, reduced efficiency of metabolizable energy utilization, and would have required additional supplementation (Freeman et al., 1992). This could have led to the SSF being utilized more intensively and/or a higher supplementation intake from the steers. Had the steers sought out supplement more aggressively, potentially the stocking density of the SSF would have been closer to the study design. Had stocking density increased, there may have been more of an effect on the tester steers supplement intake behavior.

GPS

Steers spent an average of 4.4% of the time within 15 m of the feeder regardless of competition ($P = 0.54$; Figure 8). As stocking density increased, steers took approximately 108 more steps per steer of additional competition ($P \leq 0.01$; Figure 9). Previous wk stocking density did not have an effect on the number of steps taken ($P = 0.85$), suggesting that walking more was not a learned behavior from the wk before. Further, the steer's lying bouts, the amount of times they laid down and got back up, did have a tendency to decrease ($P = 0.09$; Figure 10).Previous research has shown that

within extensive grazing systems, as the herd gets larger, a synchronization of behavior will occur. This synchronization results in the entire herd drinking, eating, and ruminating at the same time (Miller and Wood-Gush, 1991; DeVries et al., 2004). Which could explain as the group got larger, steers took more steps to stay together as a herd. However, the amount of time the tester steers laid down in h, did not significantly decrease as stocking density increased ($P = 0.11$; Figure 11).

Proudfoot et al. (2009) found that individual cattle behaviors can be highly unreliable and variable, with many aspects affecting potential behavior outcomes. Knowing that animal behaviors are different, Grant and Albright (2001) concluded that if feeding behavior could be altered in a beneficial way, and decrease within-group variation and overall group intake, it would result in individual animal performance being less variable. If animals were receiving the same nutrients, rather than dominant animals receiving more nutrients than others, the herd would be more efficient as a whole(Grant and Albright, 2001). A hypothesis for these behavioral responses is that individual animals in excessively large group sizes, struggle to remember the social hierarchy of the group, which results in aggressive behavioral interactions (Kondo et al., 1989). Kondo et al. (1989) results show that dominance hierarchy occurs at different ages, breeds, and environmental times. Therefore, to fully comprehend behavioral data, all types and kinds of animals should be researched.

APPLICATIONS:

This data illustrates that in environments similar to this experiment, the automated feeder can be stocked with at least 20 animals per feeding station, with a slight effect on supplement intake. Animal aggressiveness and dominance of the cattle, stocking rate,

paddock type, and environment can also play a role in the behavior and effect of competition at an automated feeder (Reuter et al., 2016). Further research is needed to characterize potential effects of the stocking density of similar feeders. Also, future research on the economics and the efficiency of this technology is warranted.

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Figure 2.1: The 80.9 ha pasture used for the experiment was divided into 8 paddocks by electric fence, in an arrangement that included a common area accessible to all paddocks. The black square represents the common area where the automated supplement feeder was stationed and the red dot represents the automated feeder.

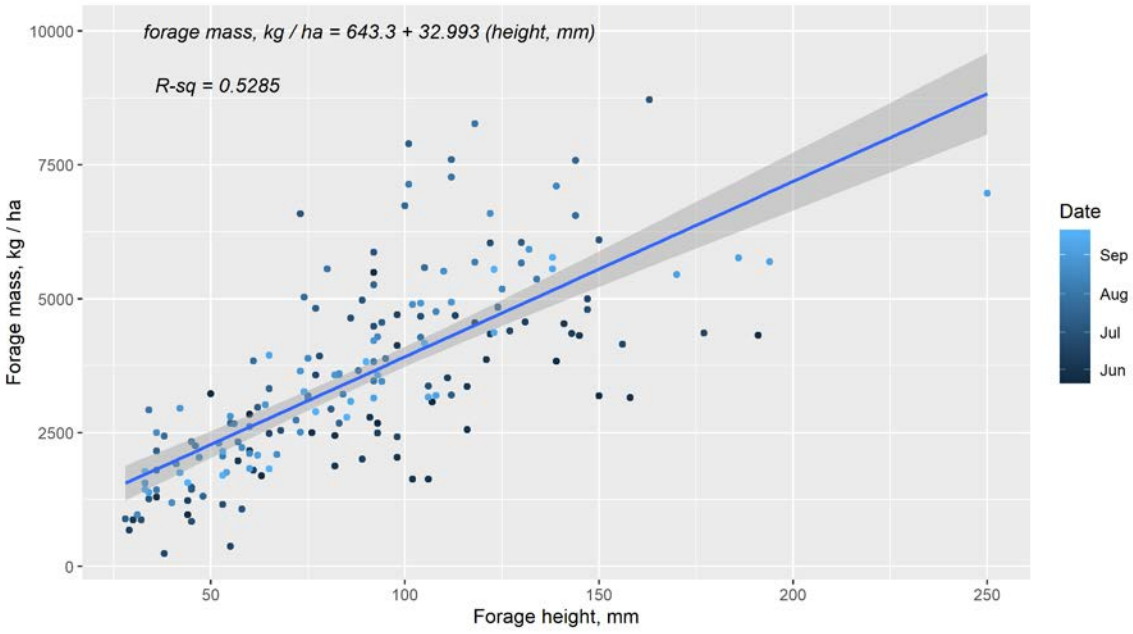

Figure 2.2: Forage calibration model, as height increased, forage mass increased. The plate meter was calibrated by comparing the forage mass clipped to the forage mass height recorded by the rising plate meter.

Figure 2.3: The number of steers that consumed supplement, per feeding station on the stocking density. Approximately 31% of the steers did not voluntarily use the feeder; therefore, effective competition was less than the study design and effective competition was used in analysis.

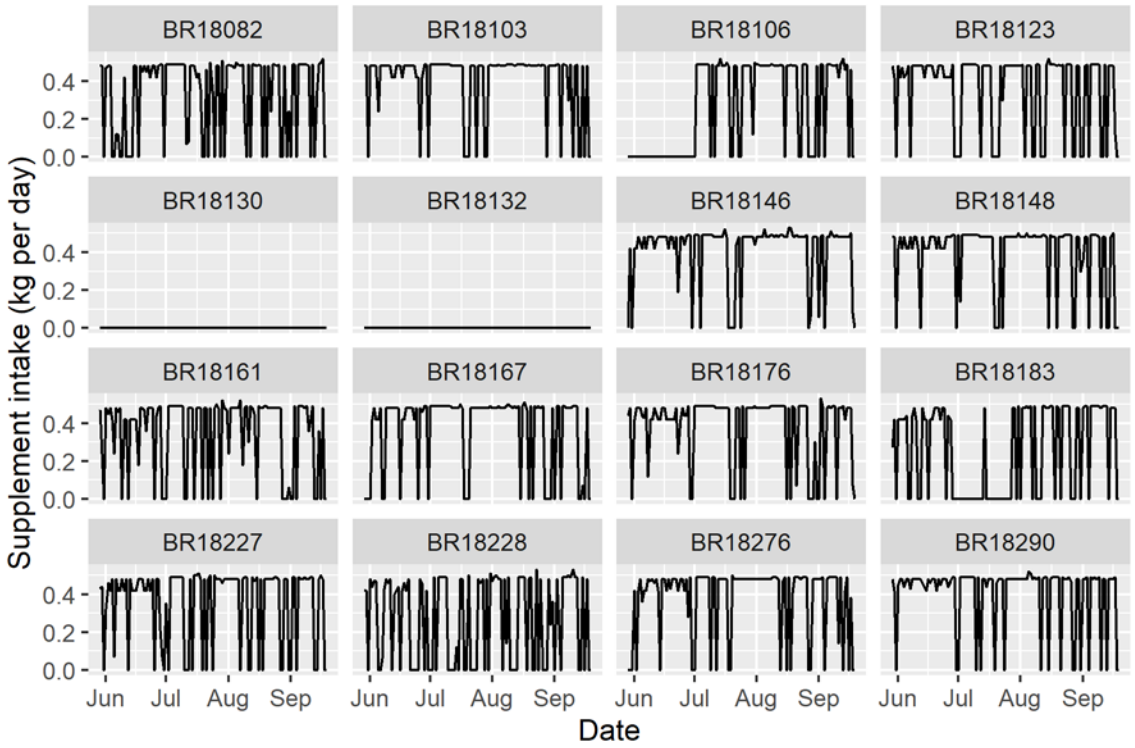

Figure 2.4: Daily supplement intake for each tester steer in yr 1 for the duration of trial. Each panel represents one tester steer (n = 16).

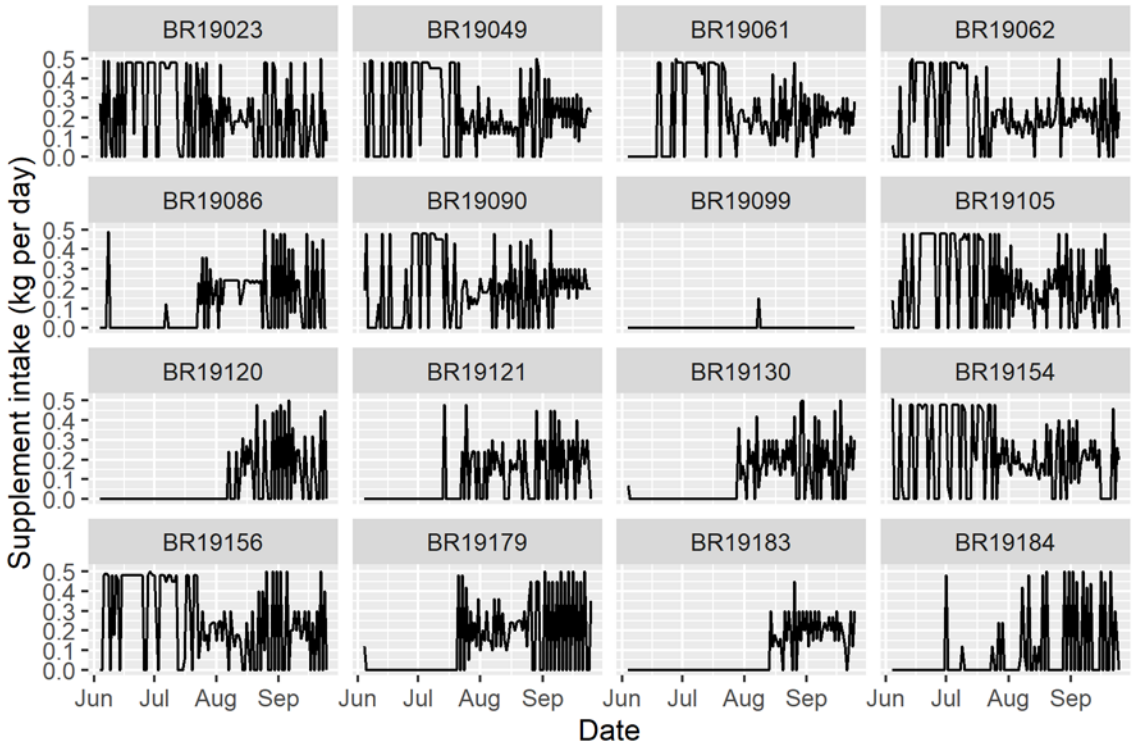

Figure 2.5: Daily supplement intake for each tester steer in yr 2 for the duration of trial. Each panel represents one tester steer (n = 16).

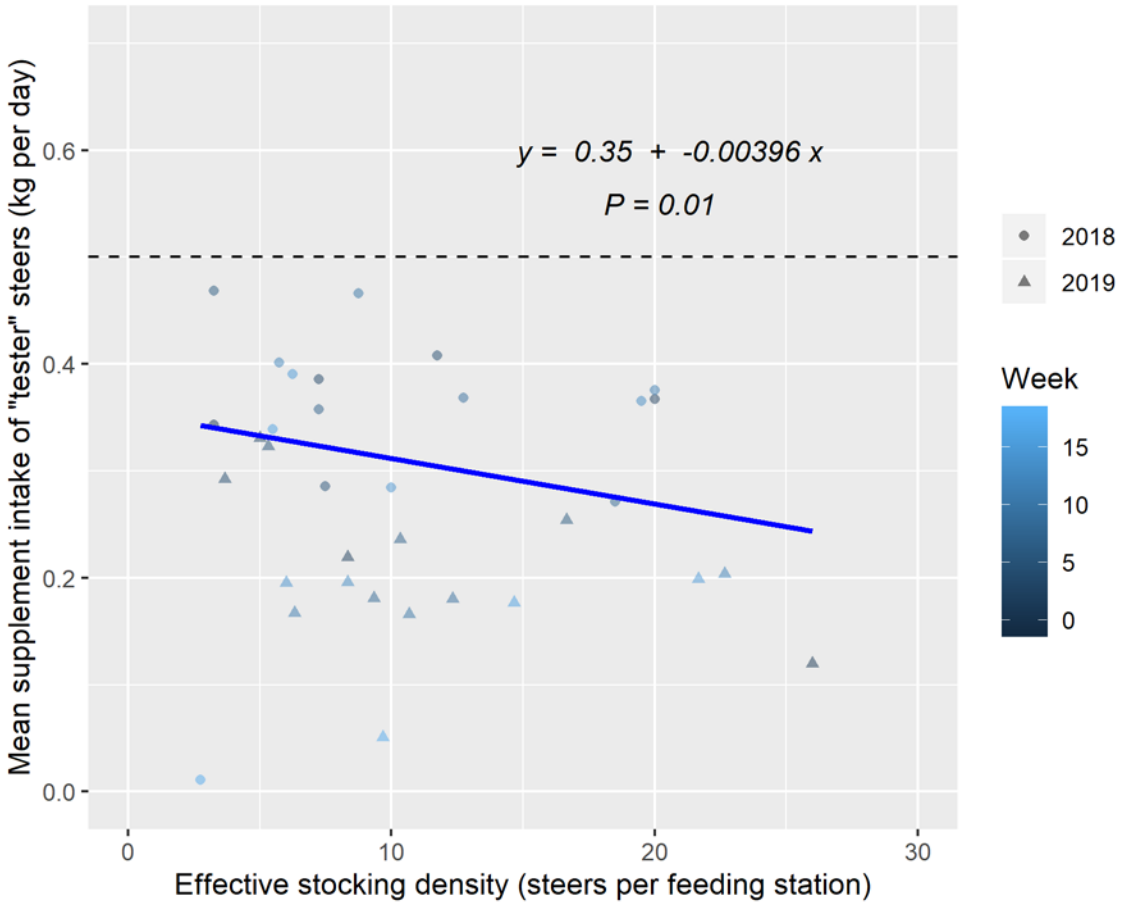

Figure 2.6: Mean supplement intake for the tester steers on the stocking density of the feeder. Competition for a feeding station reduced tester steer supplement intake by 4 g/d per steer of additional competition (*P* = 0.01).

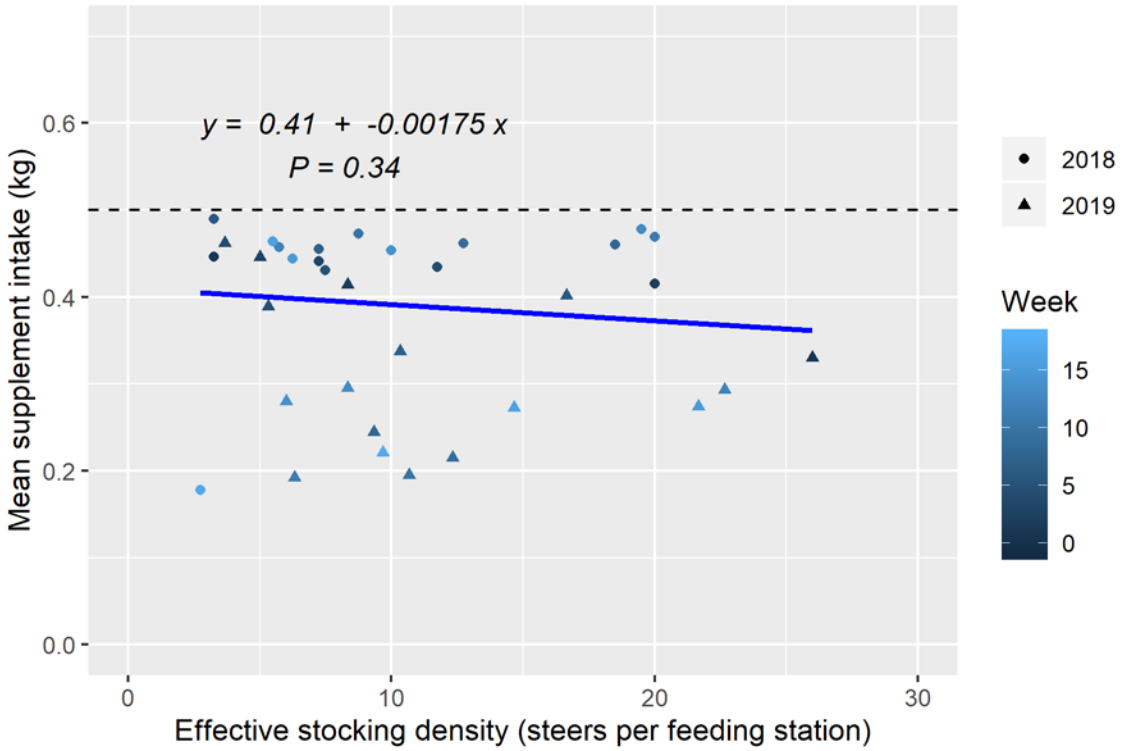

Figure 2.7: Mean supplement intake for all of the trial steers on the stocking density of the feeder. Mean supplement intake for all steers did not decrease as stocking density of the feeder increased (P = 0.34).

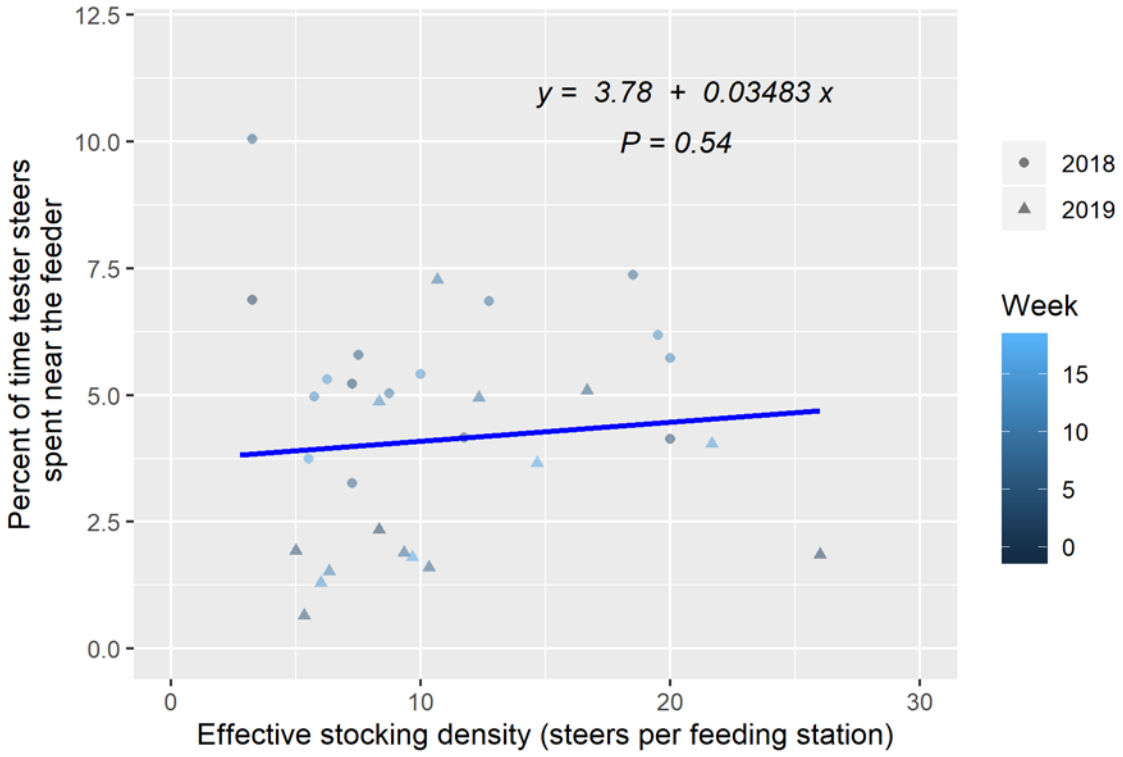

Figure 2.8: The percent of time that the tester steers spent near the feeder, on the stocking density of the feeder. Steers spent an average of 4.4% of the time within 15 m of the feeder regardless of competition (P = 0.54).

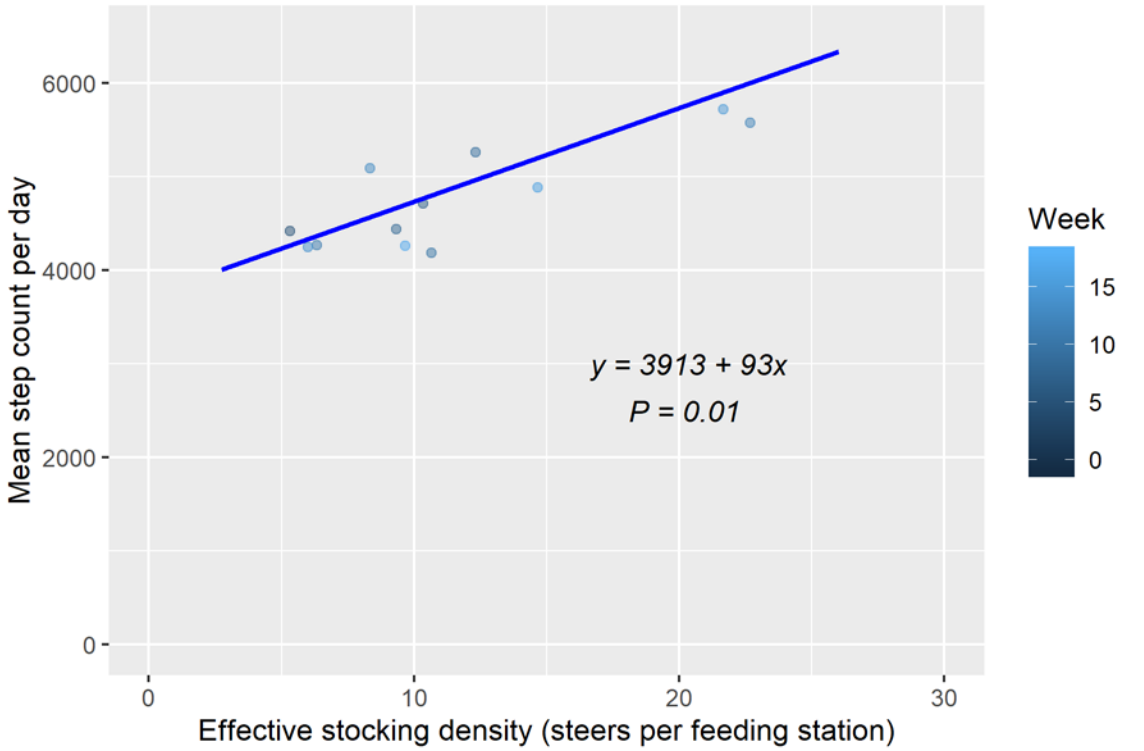

Figure 2.9: Mean daily steps taken by tester steers on stocking density per feeding station. As stocking density increased, steers took approximately 108 more steps per steer of additional competition ($P \leq 0.01$). Previous wk stocking density did not have an effect on the number of steps taken ($P = 0.85$), suggesting that walking more was not a learned behavior from the wk before.

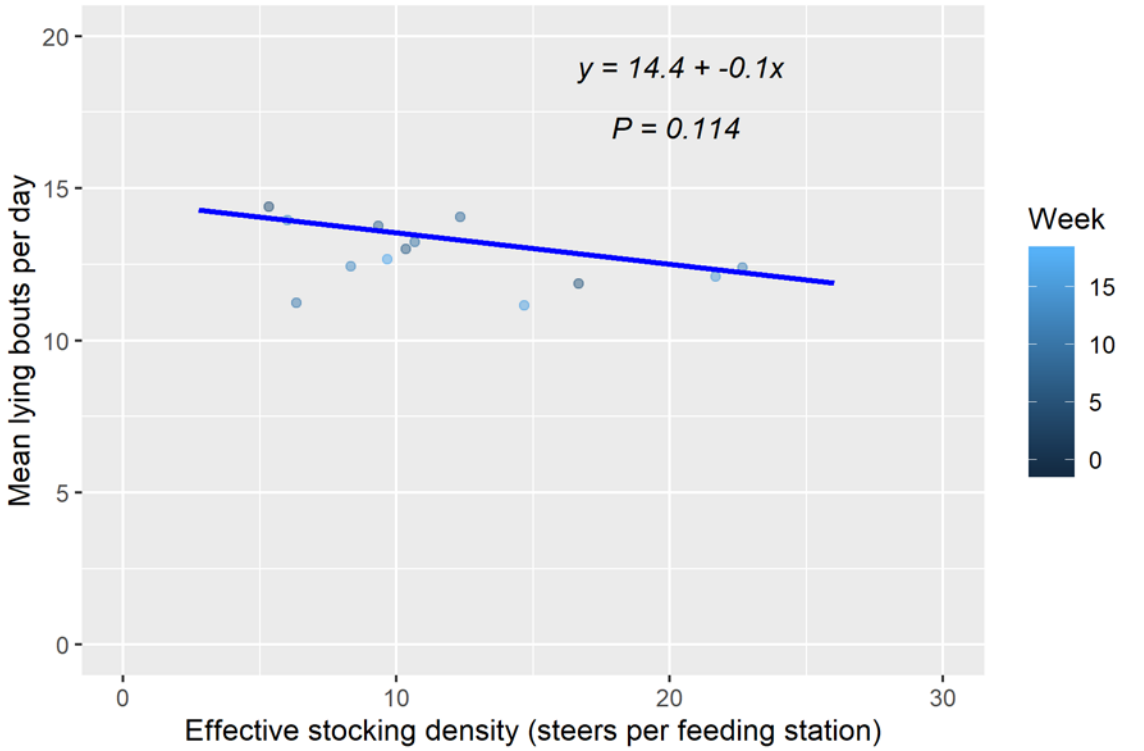

Figure 2.10: The steer's lying bouts, the amount of times they laid down and got back up, did have a tendency to decrease (P = 0.09).

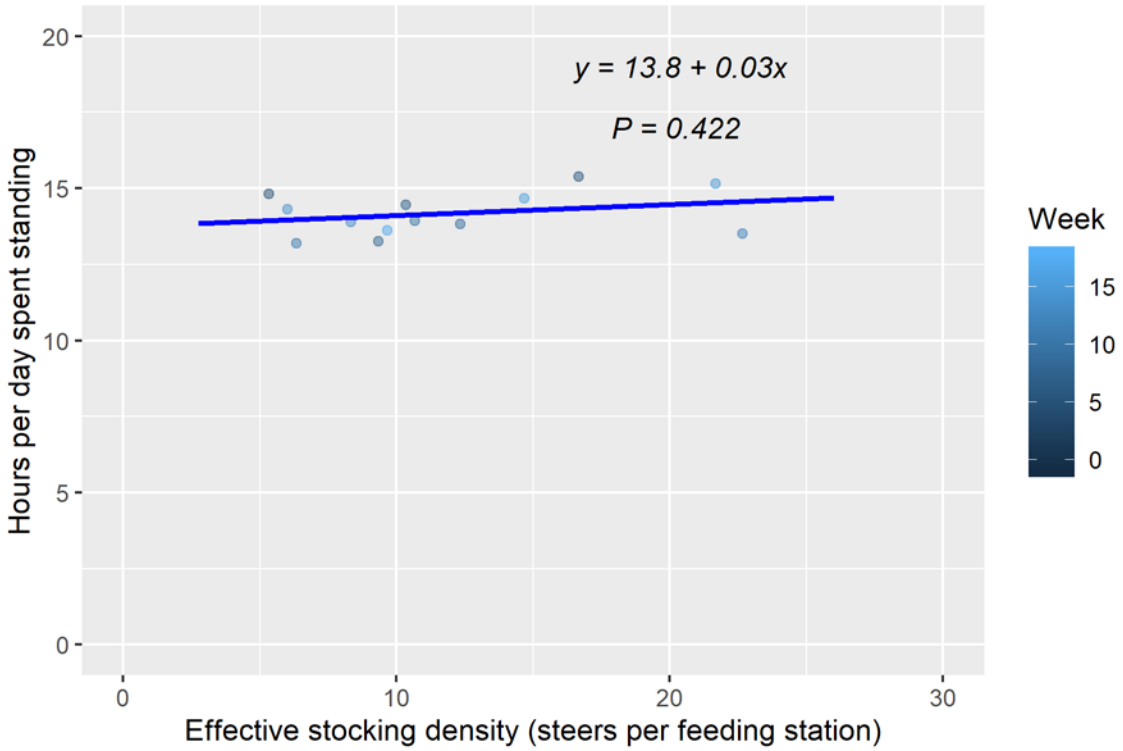

Figure 2.11: The amount of time the tester steers laid down in h, did not significantly decrease as stocking density increased (P = 0.11).

CHAPTER III

EFFECTS OF AN ENERGY OR PROTEIN SUPPLEMENT ON FORAGE DRY MATTER INTAKE FOR STEERS GRAZING SUMMER NATIVE RANGE

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ABSTRACT: The objective of this study was to determine the effects of supplementation level and type on forage intake of steers grazing a high-quality native range. This study was conducted in late May through the end of June. On d 0, steers (*n* =16, initial BW = 193.7 kg ± 14.3 kg) were randomly assigned to one of 5 dietary treatments, fed once daily in individual stalls for 28 d. Treatments were control (no supplement, *n* = 4), or supplemented with either cottonseed meal or dry rolled corn, each at either 0.45 kg or 1.81 kg as-fed/d (*n* = 3 for each combination). Beginning on d 7, steers were orally administered titanium dioxide boluses (10 g /steer) once daily for 21 d. Fecal samples were collected from the rectum once daily for 14 d, analyzed for Ti concentration via a handheld X-ray fluorescence analyzer, and total fecal output was estimated. Diet digestibility was estimated after a 288-h *in situ* incubation of forage and fecal samples to determine indigestible NDF. Some steers had orts, therefore actual mean supplement intake was used in analysis. Results were analyzed with regression, with animal as the experimental unit. Mean estimated forage DMI of unsupplemented animals

was 1.7% BW. Contrary to expectation, forage intake did not differ by supplement type when offered at 0.45 kg/d yet with increasing supplement forage intake declined for CSM but increased for DRC (P = 0.01).

Key Words: DMI, iNDF, Titanium Dioxide, Native range.

INTRODUCTION

There has been extensive research conducted regarding the effects of supplementation on the DMI of poor quality (CP > 7%)forages. It is well known that protein supplementation increases forage intake and the digestibility of low quality forage (Cook and Harris, 1968; Caton et al., 1988). Also well documented is that providing a supplemental level of energy typically decreases forage intake when forage CP is < 7% (Horn and McCollum, 1987; Paterson et al., 1994). Chase and Hibberd (1987) found that energy supplementation can decrease digestibility of fibrous particles including cellulose and hemicellulose. Corn has been shown to negatively affect the rate of digestible hay disappearance, NDF disappearance, and substitution ratio (Chase and Hibberd, 1987). However, energy supplementation is also often used effectively in grazed wheat pasture systems to assist in the metabolism of the excess CP available in the forage (Horn and McCollum, 1987).

Protein supplementation to cattle grazing warm-season in late summer or low quality forages has been shown to improve forage intake, utilization, and overall harvest efficiency (Guthrie and Wagner, 1988; McCollum and Horn, 1989; Gadberry et al., 2004).

Furthermore, when performance is depressed by insufficient forage intake, protein supplementation usually improves performance more efficiently than energy concentrates

(Hennessey et al., 1983; Horn and McCollum, 1987; McCollum and Horn, 1989). However, little research has been conducted to analyze the effects of energy or protein supplementation on native range forages when the nutritive value is of moderate or better quality. There are times that producers may need to adjust forage intake to conserve forage mass, improve forage utilization, animal performance, or economic return (Horn and McCollum, 1987; Kunkle et al., 2000). Supplementation can also be used to manage cattle behavior, which can be favorable for specific environments and management types (Kunkle et al., 2000). Therefore, the objective of this study was to determine the effects of feeding 2 different levels of a protein or energy supplementation on forage substitution rate (supplement for forage).

MATERIALS AND METHODS

All procedures used in this experiment conformed to the FASS Ag Guide (FASS, 2010) and were approved by the Oklahoma State University Institutional Animal Care and Use Committee (#AG-16-9).

Location and Pasture

The grazing paddock used in this experiment was located at the Oklahoma State University Bluestem Research Range (36° 04’ N, 97° 11’ W; 302 m elevation) 14.5 km southwest of Stillwater, OK. The experiment was conducted from May 23rd to June 19th, 2019. The paddock (24.3 ha) was primarily made up of warm-season native grass Big Bluestem (*Andropogon gerardi*), Little Bluestem (*Schizachyrium scoparium*), Indian Grass (*Sorghastrum nutans*), and Switchgrass (*Panicum virgatum*). There was a small amount of forbs and eastern red cedars (*Juniperus virginiana*) in the pasture. The average

nutritive value was 48% DM, 5.6% CP, 36.4% ADF, 67.5% NDF, and 60.6% TDN. Forage quality was measured weekly by hand-clipping 10, 0.09 m^2 quadrats. Samples were oven dried at 55° C for 72 h, weighed, and stored for later proximate chemical analysis (Williams et al., 2018) to characterize whole plant chemical composition. Forage mass was measured by a calibrated rising plate meter weekly (Model EC-20; Jenquip, Feilding, New Zealand).

Animals and Acclimation

Mixed breed beef steers (n = 170, initial BW = 214.1 kg ± 18.4 kg, approximately 8-10 m of age), were sourced from sale barns in Oklahoma and Arkansas. Steers were preconditioned by the owner at a private facility for approximately 30 d before arrival to the research location. Trial animals (n = 16, initial BW = 193.7 kg ± 14.3 kg) were selected based on willingness to eat supplement in the individual stanchion barn and overall acceptable disposition. After trial animals were selected, steers were randomly assigned treatments on d 0, and adapted to the paddock and supplement for 14 d. Treatments were control (no supplement, n = 4), or supplemented with either cottonseed meal (**CSM**) or dry rolled corn (**DRC**), each at either 0.45 kg or 1.81 kg/d (as-fed basis; n = 3 for each combination). Cattle were fed using individual feeding stanchions inside of a barn to monitor intake and measure any orts. Steers were observed while feeding and after 45 min steers were released from stanchions. If present, orts were weighed back

after the 45 min. The 4 control steers did not go into the stanchions. After supplementation all steers were managed in a single group in the research paddock.

Digestibility

Beginning on d 7 at 0700 h, steers were orally dosed with a gelatin bolus containing 10 g titanium dioxide (**TiO_2**; Titgemeyer et al., 2001). Steers were bolused for 7-d before fecal samples were collected beginning on d 15 (Titgemeyer et al., 2001). Cattle continued to receive TiO_2 boluses until the end of the fecal sampling period on d 28 (Titgemeyer et al., 2001). Fecal grab samples were collected once daily from the rectum from d 15 to d 28 and weighed individually. Approximately 75 g of feces were removed from the sample, composited within animal, and frozen. Any leftover feces were weighed, dried at 55º C in a forced-air oven for 96 h, allowed to air-equilibrate, and weighed again to determine DM percentage (Lippke, 2002; Dove et al., 2005). At the end of the 14-d fecal collection period, frozen composites were thawed, mixed, and dried at 55º C in a forced-air oven for 96 h. Fecal composites and daily fecal samples were ground to pass a 2 mm screen in a cutting mill (Pulverisette 19; Fritsch Milling and Sizing, Inc, Pittsboro, NC) and stored for future analysis.

Rumen evacuations were conducted on d 15 of the trial. Rumen evacuations were used to minimize the potential human error when collecting multi-specie forage samples (Lippke, 2002). Animals can better select what they would potentially consume, more accurately than a human can, therefore producing a more precise forage feed sample (Lippke, 2002). Cannulated steers (n = 2; 609 kg ± 31 kg) were kept on a hay forage diet for the duration of trial. Cannulated steers were restricted from feed and water for 12 h

before rumen evacuations, and evacuations were conducted at 0500 h. All rumen contents were removed and stored in a 55-gallon trash bin. Animals were then turned out to the research paddock to graze for 1 h, without access to water. After 1 h, cannulated steers were brought back up to the working facilities and all solid masticate was removed from the rumen (Bodine et al., 2001; Martinez-Pérez et al., 2013). Solid masticate was transferred into aluminum pans, weighed, and placed into a conventional oven at 55º C and stirred every h for 6 h. After 6 h, masticate was stirred every 12 h for a total drying time of 96 h (Bodine et al., 2001; Martinez-Pérez et al., 2013).

To determine indigestible neutral detergent fiber (**iNDF**), 4.00 g DM of masticate from the rumen evacuations and fecal composites from the 16 trial steers were placed into a 10 × 20 cm *in situ* forage bag (R1020; ANKOM Technology, Macedon, NY). The 4.00 g DM was determined by using the Vanzant et al. (1998) equation; $SS{:}SA = sample\ size(mg) \div (bag\ width\ [cm] \times bag\ length\ [cm] \times 2)$. For this experiment we used 10 mg/cm² for the SS:SA value (Vanzant et al., 1998).

In situ bags were placed into cannulated steers at 0700 h and incubated in the rumen for 288 h (Krizsan et al., 2013; Palmonari et al., 2016). After 288 h, *in situ* bags were removed, put into an ice bath, and were washed 5 times in room temperature water. Bags were then dried at 55º C in a forced-air oven for a minimum of 96 h, allowed to air-equilibrate in a desiccator, and weighed (Vanzant et al., 1998). Digested masticate and fecal *in situ* samples were composited within animal, into a single sample, and stored until further analysis (Köster et al., 1996).

Laboratory Analysis

All forage samples were dried to a constant weight at 55° C for 72 h and ground to pass through a 1 mm screen in a cutting mill (Pulverisette 19, Fritsch Milling and Sizing, Inc, Pittsboro, NC) before storage for chemical analysis. Chemical analysis was done on all forage samples and the undigested masticate sample. Protein was determined by dry combustion analysis using a CN analyzer (TruSpec CN analyzer: LECO, St. Joseph, MI). The ADF and NDF were analyzed in an ANKOM 2000 analyzer according to manufacturer's instructions (Van Soest et al.,1991; ANKOM Technology, Macedon, NY). In addition, 12 mL of alpha amylase and 20 g of sodium sulfate were added to the NDF solution during the NDF analysis (ANKOM Technology, Macedon, NY). Total digestible nutrients was determined using the equation; $88.9 - (0.779 \times ADF)$; (Kuehn et al., 1999).

The TiO_2 concentration was determined using a handheld X-ray fluorescence (**XRF**) analyzer (Delta Professional; Olympus Cooperation, Waltham, MA; Thompson et al., 2019). The iNDF value was calculated by placing the digested, composited masticate and fecal matter into Ankom F57 fiber bags, and analyzed as previously mentioned for NDF (ANKOM 2000 analyzer; ANKOM Technology, Macedon, NY). The Ankom F57 fiber bags were then ashed at 600° C for 6 h to correct iNDF value for organic matter inclusion (ANKOM Technology, Macedon, NY).

Equations Used

Forage intake was calculated using equations that estimate fecal output from the TiO_2 concentration in the feces, and the estimated forage digestibility from the iNDF of

the feed and the feces (Galyean, 1997; Bodine et al., 2001; Gadberry et al., 2004; Ebert et al., 2016). The equations used can be found in Table 1, and an example can be found in Appendix 1.

Statistical Analysis

Data were analyzed with analysis of variance, with animal as the experimental unit. Randomly, 2 control steers were made 0 kg/d supplement intake of either CSM or DRC treatments. Some steers had orts, therefore actual mean supplement intake was used in analysis rather than designed intake. The model included cannulated steer as a random variable. The reduced model was used for analysis because the quadratic model was not significant. Two trial steers, 1 from the 0.45 kg CSM and 1 from the 1.81 kg CSM treatments were removed from data analysis because both steers were removed from trial on d 22 due to morbidity. Analysis and figures were produced from R (R Core Team, 2017, v. 3.4.3). Additional packages used in R were; tidyverse (Wickham, 2017), readxl (Wickham and Bryan, 2019), Rgooglesheets (Bryan and Zhao, 2018), stringr (Wickham, 2019), ggplot2 (Wickham, 2016), and emmeans (Lenth, 2019).

RESULTS AND DISCUSSION

Mean estimated forage DMI of unsupplemented animals was 1.7% BW (Figure 1). Contrary to expectation, corn supplemented steers exhibited greater forage DMI as a percentage of BW than CSM supplemented steers ($P = 0.01$; 2.2 vs. 1.6 % of BW, respectively). Contrary to expectation, forage intake did not differ by supplement type when offered at 0.45 kg/d yet with increasing supplement forage intake declined for CSM but increased for DRC ($P = 0.01$).

A majority of research with this type of forage, is typically conducted when the forage is considered low quality, instead of moderate to high quality. Based on previous low quality forages and wheat pasture research, for this trial, the hypothesis was either no benefit in feeding any supplement, or energy supplementation would decrease forage DMI and protein supplementation would increase forage DMI. McCollum and Gaylean (1985) reported that supplementing CSM increased the rate of passage of particulates, which was associated with increased intake of low-quality prairie hay. Kartchner (1981) found that cows supplemented with protein, had increased intake of dormant winter range while barley-grain supplementation decreased forage intake. Chase and Hibberd (1987) noted that energy supplementation decreases the digestibility of fibrous particles, such as cellulose and hemicellulose, and has a negative effect on substitution ratio, which was not seen in this study. Mould et al. (1983) suggested that reductions in forage intake due to energy supplementation have been attributed to either depression in ruminal pH or a carbohydrate effect, which was also not seen in this study.

McCollum and Horn (1989) showed the relationship that as forage CP decreases, it is more likely that a protein supplement will increase intake. However, there is evidence that in native range forages, intake responses are not solely dependent on forage CP (McCollum and Horn, 1989). In support, Judkins et al. (1985) did not see an increase in forage due to protein supplementation, on moderate to low quality native range forage.

The whole plant forage in this study was relatively high in TDN, but low in CP. Historical data indicates that the CP % could have been higher, especially at peak growing season (Williams, 1953; Chase and Hibberd, 1987). In dormant native range, CP often leaches out of the forage, causing it to not be as nutritious (Williams, 1953; Chase

and Hibberd, 1987). The CP in the forage in the current trial could have been lower because of the unseasonable amount of rainfall received in this location. This location received 1085 mm of rain from January 1 to June 19, 2019. The 10 yr average for this location was 636 mm. This amount of unseasonable rainfall could have resulted in CP leaching from the forages (Guilbert and Mead, 1931). However, even if the CP had leached from the forage, the CSM supplement should have met that deficiency and caused an increase in forage DMI. Forage DMI was similar between the control, 0.45 kg CSM, and DRC treatments. These results indicate that steers had adequate dietary protein available. Inadequate diet protein would have suppressed forage digestion, forage intake, and reduced the efficiency of metabolizable energy utilization (Freeman et al., 1992). The undigested masticate sample nutritive value from chemical analysis was 10.2% CP, 43.2% ADF, 75.9% NDF, 55.3% TDN, 1.15 NEm Mcal/ kg, and 1.02 NEg Mcal/kg. With the masticate being higher in CP, ADF, and NDF but a lower TDN.

The values in this study are based on internal and external markers and the assumptions and potential error associated with markers. Estimates of fecal output are dependent on TiO_2 recovery and forage and supplement digestibility are dependent on iNDF recovery, both of which affect the estimates of forage DMI. However, TiO_2 offers advantages over previously used markers as it can be added to food animal's diets legally and without fear of potential carcinogenic effects, unlike chromium oxide (Peddie et al., 1982; Titgemeyer et al., 2001; Myers et al., 2004). Errors in collecting external marker data include but are not limited to; collection of feces, the recovery rate of markers, and administering the marker, among others (Lippke, 2002). Therefore, it is important to note

that while this technique is widely accepted, it's not always accurate, but is the most researched and practical way of estimating intake in present science.

IMPLICATIONS

These results indicate that supplementation did not increase forage DMI when the forage was of moderate to high quality. While our results do not match previous research conducted, it is important to note that this forage source when it is considered to be high quality has not been researched extensively. Previous research has only been conducted for this forage source while it is considered low quality. Future research and repeatability of the research is warranted to fully comprehend the potential benefits, detriments, and the economics of supplementation when high quality forage is available.

ACKNOWLEDGEMENTS

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Table 3.1.

Eq. 1. $iNDF\ (\%), DM, OM\ of\ digested\ sample =$ $iNDF\ (\%) from\ ANKOM\ analysis \div DM\ \%\ of\ sample \div OM\ \%\ of\ sample$ (ANKOM Technology, Macedon, NY)
Eq. 2. $iNDF\ (\%)\ feces = \frac{iNDF\ \%\ of\ digested\ feces, DM, OM}{100} \times (1 -$ $insitu\ disapperance\ of\ feces, g\ DM)$
Eq. 3. $iNDF\ (\%)\ feed = \frac{iNDF\ \%\ of\ digested\ feed, DM, OM}{100} \times (1 -$ $insitu\ disapperance\ of\ feed, g\ DM)$
Eq. 4. $Indigestibility\ (\%) = 100 - 100(\frac{iNDF\ (\%)\ feed}{iNDF\ (\%)\ feces})$ (Galyean, 1997)
Eq. 5. $Fecal\ output\ \left(\frac{kg}{d}\right) = Marker\ consumed, DM\ (g) \div$ $Marker\ concentration\ in\ feces, DM\ \left(\frac{g}{kg}\right)$ (Kartchner, 1981; Galyean, 1997)
Eq. 6. $Fecal\ supplement\ output\ \left(\frac{kg}{d}\right) = Supplement\ intake\ (kg, DM) \times$ $Supplement\ indigestibility\ (\%) \div 100$ (Kartchner, 1981; Galyean, 1997)
Eq. 7. $Fecal\ output\ adj.\ for\ supplement = Fecal\ output, DM\ \left(\frac{kg}{d}\right) -$ $Fecal\ supplement\ output\ \left(\frac{kg}{d}\right)$ (Kartchner, 1981; Galyean, 1997)
Eq. 8. $DMI\ \left(\frac{kg}{d}\right) = Fecal\ output\ \left(\frac{kg}{d}\right) \div Indigestibility(\%)$ (Galyean, 1997)
Eq. 9. $Forage\ DMI\ \left(\frac{kg}{d}\right) = DMI\ \left(\frac{kg}{d}\right) - Daily\ supplement\ intake\ \left(\frac{kg}{d}\right)$ (Kartchner, 1981)

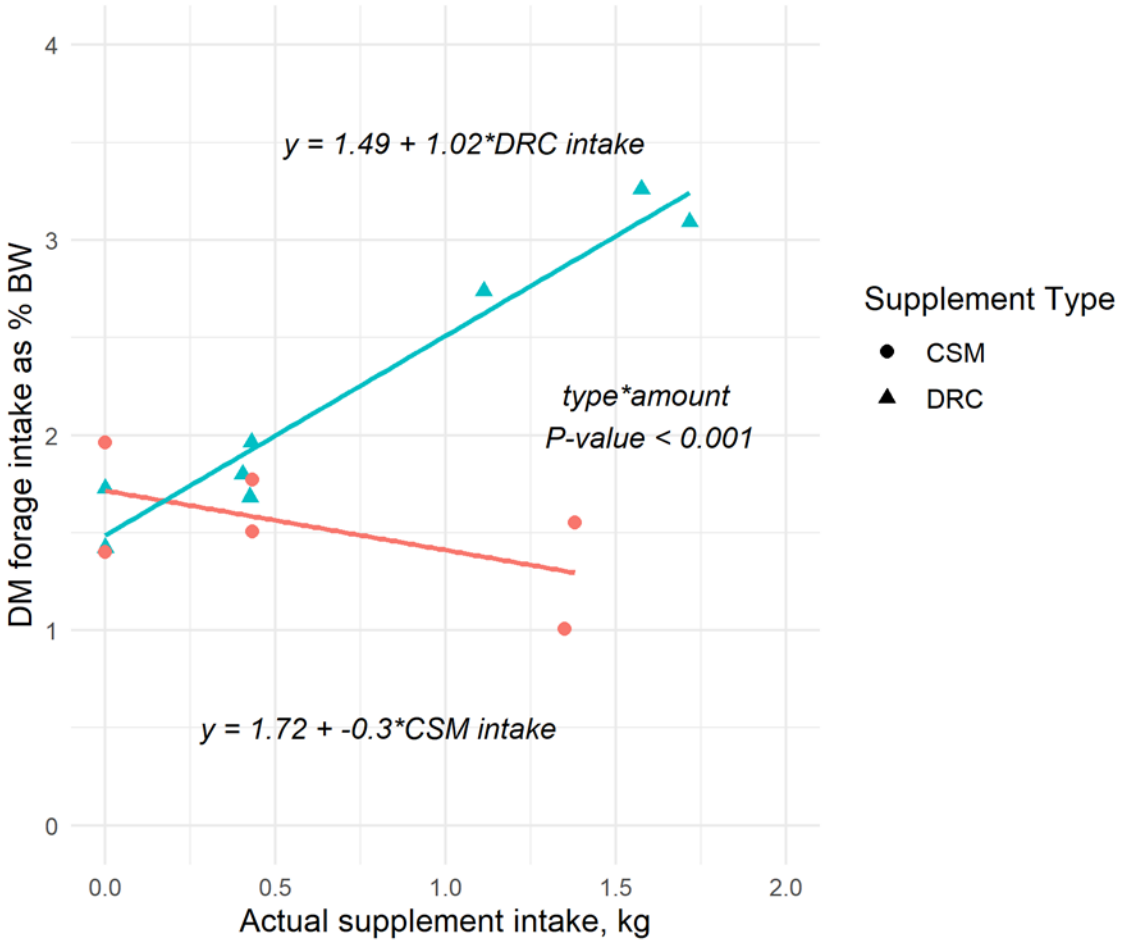

Figure 3.1: Forage DMI as a percent of BW, on actual supplement intake (kg). Corn supplemented steers exhibited greater forage DMI as a percentage of BW than cottonseed meal supplemented steers (P = 0.01).

Footnote: CSM- Cottonseed meal, DRC- Dry- Rolled Corn, None-No supplement

APPENDICES

Appendix 1. Equation Example

Eq. 1 $iNDF\ (\%), DM, OM = \ iNDF\ (\%) \div DM\ \%\ of\ sample \div OM\ \%\ of\ sample$

Ex. 1. $iNDF\ (\%), DM, OM = \ 71.2\ (\%) \div 91.8\ \%\ of\ sample \div 96.0\%\ of\ sample$

Answer 1: iNDF%, Dry Matter, Organic Matter = 80.8%

Eq. 2. $iNDF\ (\%)\ feces = \frac{iNDF\ \%\ of\ digested\ feces, DM, OM}{100} \times (1 - insitu\ disapperance\ of\ feces, g\ DM)$

Ex. 2. $iNDF(\%) feces = \frac{80.8\%}{100} \times (1 - 0.3843, g\ DM)$

Answer 2: iNDF (%) feces = 49.7%

Eq. 3. $iNDF\ (\%)\ feed = \frac{iNDF\ \%\ of\ digested\ feed, DM, OM}{100} \times (1 - insitu\ disapperance\ of\ feed, g\ DM)$

Ex. 3. $iNDF\ (\%) feed = \ (77.8\%)/100 \times (1 - 0.77, g\ DM)$

Answer 3: iNDF (%) feed = 17.9%

Eq. 4. $Indigestibility\ (\%) = 100 - (100 - \frac{iNDF\ (\%)\ feed}{iNDF\ (\%)\ feces})$

Ex. 4. $Indigestibility\ (\%) = 100 - (100 - \frac{17.9\%}{49.7\%})$

Answer 4: Indigestibility (%)= 35.9%

Eq. 5. $Fecal\ Output\ (kg/d) = Marker\ Consumed, DM\ (g) \div Marker\ concentration\ in\ feces, DM\ (g/kg)$

Ex. 5. $Fecal\ Output\ \left(\frac{kg}{d}\right) = 5.99\ g\ Ti, \frac{DM}{d} \div 4.4235\ \left(\frac{g}{kg}\right)\ Ti, DM$

Answer 5: Fecal Output, DM (kg/d) = 1.2768

Eq. 6. $Fecal\ Supplement\ Output\ \left(\frac{kg}{d}\right) = supplement\ intake\ (kg, DM) \times supplement\ indigestibility\ (\%) \div 100$

Ex. 6. $Fecal\ Output\ adj.\ for\ supplement\ intake\ (kg/d) = 0.4034\ (kg, DM) \times 27.82\% \div 100$

Answer 6: Fecal Supplement Output (kg/d) = 0.11228

Eq. 7. $Fecal\ Output\ adj.\ for\ supplement = Fecal\ Output, DM\ \left(\frac{kg}{d}\right) - Fecal\ Supplement\ Output\ \left(\frac{kg}{d}\right)$

Ex. 7. $Fecal\ Output\ adj.\ for\ supplement = 1.2768, DM\ \left(\frac{kg}{d}\right) - 0.11228\ \left(\frac{kg}{d}\right)$

Answer 7: Fecal Output adj. for supplement= 1.1646

Eq. 8. $DMI\ \left(\frac{kg}{d}\right) = Fecal\ Output\left(\frac{kg}{d}\right) \div indigestibility(\%)$

Ex. 8. $DMI\ \left(\frac{kg}{d}\right) = 1.1646\left(\frac{kg}{d}\right) \div 36.5\ \%$

Answer 8: DMI (kg/d) = 3.0224

Eq. 9. $Forage\ DMI\left(\frac{kg}{d}\right) = DMI\left(\frac{kg}{d}\right) - daily\ supplement\ intake\ \left(\frac{kg}{d}\right)$

Ex. 9. $Forage\ DMI\left(\frac{kg}{d}\right) = 3.0224\left(\frac{kg}{d}\right) - 0.4034\left(\frac{kg}{d}\right)$

Answer 9: DMI forage intake (kg/d) = 2.62

Appendix 2. R Code

```
library(tidyverse)
library(googlesheets)
library(stringr)
library(ggplot2)
library(emmeans)
gs <- gs_title("1902_Database")

##########
## Cattle weight
##########
weight<- gs_read(gs, ws = "animals", range = "a1:k17") %>%
  select(tag, average)%>%
  mutate(average= average/2.205)

##########
## Forage calculations and analysis
##########
DM_intake <- gs_read(gs, ws = "clean_R")%>%
  left_join(weight)%>%
  mutate(DMI_forage_BW=(DMI_forage_kg/average)*100)%>%
  mutate(DMI_BW=(Total_DMI_kg/average)*100)%>%
  filter(tag != "19134") %>%
  filter(tag != "19164") %>%
    filter(grind == 1) %>%
    filter(cannula == 1) %>%
    mutate(supp_amount_sq = average_supp_intake_kg_DM *
average_supp_intake_kg_DM) %>%
    mutate(supp_type = ifelse(tag %in% c("19054", "19073"), "DRC",
supp_type)) %>%
    mutate(supp_type = ifelse(tag %in% c("19080", "19085"), "CSM",
supp_type))

final_anova <- lm(data = DM_intake, DMI_forage_BW ~
average_supp_intake_kg_DM*supp_type + supp_amount_sq*supp_type)
final_anova_red <- lm(data = DM_intake, DMI_forage_BW ~
average_supp_intake_kg_DM*supp_type)
anova(final_anova, final_anova_red)
final_anova_red2 <- lm(data = DM_intake, DMI_forage_BW ~
average_supp_intake_kg_DM + supp_type)
anova(final_anova_red, final_anova_red2)

summary <- summary(final_anova_red)
summary
intercept_drc = summary$coefficients[[1]] + summary$coefficients[[3]]
intercept_csm = summary$coefficients[[1]]
slope_csm = summary$coefficients[[2]]
slope_drc = summary$coefficients[[4]] + slope_csm

drc_eq <- paste("y = ", round(intercept_drc, 2), " + ",
                round(slope_drc, 2), "*DRC intake", sep="")
drc_eq

interaction_p <- summary$coefficients[[16]]
```

```r

csm_eq <- paste("y = ", round(intercept_csm, 2), " + ",
                round(slope_csm, 2), "*CSM intake", sep="")
csm_eq

##########
## Plotting results
##########
ggplot(DM_intake, aes(x =  average_supp_intake_kg_DM, y =
DMI_forage_BW, color= supp_type, shape= supp_type)) +
  geom_point(size = 2.1) +
  ylab("DM forage intake as % BW") +
  xlab("Actual supplement intake, kg") +
    xlim(0, 2) +
    ylim(0, 4) +
    geom_smooth(method = "lm", se=F) +
    annotate(geom = 'text',
             x = 0.75,
             y = 0.5,
             hjust = 0.45,
             label = csm_eq,
             fontface = 'italic') +
    annotate(geom = 'text',
             x = 1,
             y = 3.5,
             hjust = 0.45,
             label = drc_eq,
             fontface = 'italic') +
    annotate(geom = 'text',
             x = 1.6,
             y = 2.1,
             label = paste("type*amount \nP-value < 0.001"),
             fontface = 'italic') +
        theme_minimal()+
  theme(legend.text = element_text(size= 10)) +
  theme(legend.title = element_text(size=12))+
  theme(axis.title = element_text(size=12))+
  labs(shape= "Supplement Type")+
  labs(color=NULL)+
  ggsave("DMI_forage_BW Actual supplement intake.png", height = 5,
width = 6)
```

Appendix Table 3. Raw data:

Animal Tag #	Supplement Type	Grind Size, mm	Cannula Animal	iNDF, %, DM, OM	Average Insitu Sample In, g	Average Insitu Sample Out, g	iNDF % of DM, feces	Ti g/kg	Ti Consumed, g	Average Supplement Intake, kg, DM
19054	none	1	1	81.22	4.0031	2.5547	0.51833	5.4445	5.99	0
19054	none	1	2	81.15	4.0013	2.4307	0.49297			0
19054	none	2	1	79.97	4.005	2.6302	0.52517	4.991	5.99	0
19054	none	2	2	76.82	4.0066	2.2762	0.43644			0
19073	none	1	1	76.92	4.004	2.2004	0.4227	3.8985	5.99	0
19073	none	1	2	75.23	4.0039	2.3507	0.44166			0
19073	none	2	1	76.04	4.004	2.4002	0.45581	4.058	5.99	0
19073	none	2	2	74.28	4.0062	2.0774	0.38519			0
19080	none	1	1	83.85	4.0021	2.5713	0.53874	3.8585	5.99	0
19080	none	1	2	82.68	4.0045	2.3816	0.49173			0
19080	none	2	1	84.47	4.004	2.5843	0.54517	3.4925	5.99	0
19080	none	2	2	85.08	4.0066	2.2118	0.46968			0
19085	none	1	1	81.52	4.0041	2.3306	0.47452	5.037	5.99	0
19085	none	1	2	81.35	4.0048	2.3935	0.48618			0
19085	none	2	1	84.68	4.0035	2.5917	0.54816	4.8455	5.99	0
19085	none	2	2	84.47	4.0038	2.2106	0.46641			0
19111	DRC	1	1	80.78	4.0027	2.4645	0.49739	4.4235	5.99	0.4034
19111	DRC	1	2	79.02	4.0047	2.2062	0.43531			0.4034
19111	DRC	2	1	81.54	4.0038	2.6005	0.5296	4.4415	5.99	0.4034

19111	DRC	2	2	78.26	4.0025	2.3028	0.45027			0.4034
19116	DRC	1	1	80.88	4.0027	2.4853	0.50217	4.572	5.99	0.4248
19116	DRC	1	2	76.08	4.0044	2.3932	0.45467			0.4248
19116	DRC	2	1	76.83	4.0053	2.725	0.52271	4.526	5.99	0.4248
19116	DRC	2	2	75.52	4.0047	2.2639	0.42693			0.4248
19118	DRC	1	1	81.45	4.0033	3.0281	0.61605	3.042	5.99	1.5748
19118	DRC	1	2	79.71	4.0037	3.0346	0.60413			1.5748
19118	DRC	2	1	84.43	4.0073	3.0374	0.63995	3.546	5.99	1.5748
19118	DRC	2	2	82.4	4.0054	2.7663	0.5691			1.5748
19125	DRC	1	1	81.83	4.0051	2.853	0.58292	2.9415	5.99	1.1114
19125	DRC	1	2	79.49	4.0043	2.6871	0.53341			1.1114
19125	DRC	2	1	79.63	4.006	2.8325	0.56304	2.6935	5.99	1.1114
19125	DRC	2	2	74.25	4.0044	2.6136	0.48463			1.1114
19127	DRC	1	1	77.4	4.0044	2.994	0.57873	2.6095	5.99	1.7164
19127	DRC	1	2	77.85	4.002	2.9869	0.581			1.7164
19127	DRC	2	1	82.39	4.0055	2.9838	0.61372	2.4985	5.99	1.7164
19127	DRC	2	2	78.1	4.0046	2.8235	0.5507			1.7164
19133	CSM	1	1	79.31	4.0044	2.2539	0.4464	3.112	5.99	0.4323
19133	CSM	1	2	75.78	4.0035	2.2441	0.42476			0.4323
19133	CSM	2	1	79.23	4.006	2.4472	0.48402	3.178	5.99	0.4323
19133	CSM	2	2	75.56	4.0065	2.1102	0.39799			0.4323
19134	CSM	1	1	73.87	4.005	2.1082	0.38885	3.9255	5.99	N/A
19134	CSM	1	2	70.21	4.0034	1.9704	0.34557			N/A
19134	CSM	2	1	71.22	4.0029	2.3376	0.41589	4.451	5.99	N/A
19134	CSM	2	2	71.84	4.0057	2.1356	0.38303			N/A
19147	CSM	1	1	78.75	4.006	2.4613	0.48385	4.1205	5.99	0.4323
19147	CSM	1	2	80.34	4.0054	2.3265	0.46661			0.4323

19147	CSM	2	1	85.68	4.0068	2.4742	0.52907	4.194	5.99	0.4323
19147	CSM	2	2	81.14	4.0043	2.2175	0.44935			0.4323
19150	CSM	1	1	74.61	4.0041	2.0667	0.38507	3.719	5.99	1.3487
19150	CSM	1	2	73.03	4.0054	2.1505	0.39207			1.3487
19150	CSM	2	1	72.45	4.0047	2.111	0.38193	4.057	5.99	1.3487
19150	CSM	2	2	71.15	4.0061	1.9927	0.35394			1.3487
19151	CSM	1	1	76.24	4.0046	2.2871	0.43544	3.0275	5.99	1.379
19151	CSM	1	2	73.25	4.0059	2.278	0.41653			1.379
19151	CSM	2	1	75.42	4.0046	2.3976	0.45152	3.233	5.99	1.379
19151	CSM	2	2	74.79	4.0034	2.1154	0.3952			1.379
19163	DRC	1	1	81.15	4.0024	2.5359	0.51414	3.4365	5.99	0.4291
19163	DRC	1	2	81.72	4.0028	2.5597	0.52259			0.4291
19163	DRC	2	1	85.05	4.0042	2.5054	0.53217	3.1965	5.99	0.4291
19163	DRC	2	2	82.64	4.008	2.3624	0.48707			0.4291
19164	CSM	1	1	67.34	4.0038	2.0847	0.35062	3.723	5.99	N/A
19164	CSM	1	2	66.81	4.0031	1.9043	0.31785			N/A
19164	CSM	2	1	65.19	4.0037	2.1293	0.34672	3.8275	5.99	N/A
19164	CSM	2	2	64.56	4.0049	1.9517	0.3146			N/A
Masticate	-	-	-	77.8	4.006	0.9332	-	-	-	-
CSM	-	-	-	77.02	4.0149	0.6259	-	-	-	-

Appendix 4: iNDF Protocol

Indigestible NDF (iNDF) or Indigestible ADF (iADF) Protocol

Purpose:

The purpose of this assay is to determine the indigestible portion of Neutral Detergent Fiber (**NDF**) or Acid Detergent Fiber (**ADF**) of a feed substance. The NDF is the residue remaining after digesting in a detergent solution and the residues are predominantly hemicellulose, cellulose, and lignin. The ADF is the residue remaining after digesting with H2SO4 and CTAB and the residues are predominantly cellulose and lignin. Therefore, the indigestible NDF (**iNDF**) is the indigestible portion of the hemicellulose, cellulose, and lignin. Further, the indigestible ADF (**iADF**) is the indigestible portion of the cellulose and lignin.

Materials Needed:

Desiccator
Desiccant bag
Heat Sealer
Permeant Marker
Digestion Instrument- ANKOM Fiber analyzer (ANKOM 2000)
Filter Bags- ANKOM F57 Fiber Bags
ANKOM R1020- 10 X 20 cm *in situ* forage bags
ANKOM NDF or ADF solution
Alpha Amylase
Sodium Sulfate
Drying oven (set to 55°C)
Drying oven (set to 105°C)
Ash Oven (Capable of over 500°C)
Ruminally cannulated animals or in vitro set up to mimic the rumen (Daisy)

Procedure:

1. Obtain samples that are needing iNDF or iADF on. This could be forage, feed, masticate, and/ or fecal matter.
2. Add 4.00 g DM of sample (masticate, diet, and/or fecal matter) into a 10 x 20 cm *in situ* forage bag (R1020, ANKOM Technology, Macedon, NY). The 4.00 g DM is determined by using the Vanzant et al. (1998) equation; $SS{:}SA = sample\ size(mg) \div (bag\ width\ [cm] \times bag\ length\ [cm] \times 2)$. For this experiment the goal was a 10 mg/cm² for the SS:SA value (Vanzant et al., 1998).

3. Place *in situ* bags into a mesh laundry bag, and into the ventral sac of a rumenally cannulated steer and allow to incubate in the rumen for 288 h (Krizsan et al., 2013; Palmonari et al., 2016). The mesh laundry bag is used for ease of recovery of *in situ* bags inside the rumen. This is when in vitro methods could be utilized instead of in vivo. Should in vitro methods be used, more research is necessary for this protocol to guarantee correct methods are utilized.
4. After 288 h, *in situ* bags should be removed, and immediately placed into an ice bath. The ice bath should be in a container or bucket that can hold the *in situ* bags and should be filled with half ice and half water. This is used to "shock" the rumen microbials and stop all potential digestion.
5. Rinse *in situ* bags in room temperature water until the water is completely clean and clear. However, with fecal matter, completely clear water might be impossible. Therefore, count how many washes it takes to clean the feed matter, and wash all *in situ* bags the same amount of times. Usually, about 5 complete washes will get the bags clean.
6. Bags should then be laid out evenly, onto an oven safe tray. The tray with the bags should be transferred into a 55ºC forced-air oven for a minimum of 96 h. The amount of drying time should be adjusted based on oven capacity and amount of *in situ* bags. Bags need to be completely dry, and considered to be at DM.
7. Take *in situ* bags out of the oven and allow *in situ* bags to air-equilibrate in a desiccator. Once equilibrated, weigh bags using an analytical balance (Vanzant et al., 1998).
8. After *in situ* bags are weighed, bags can be cut open and digested *in situ* samples can be composited. Once bags are cut open, they cannot be re-weighed, so guarantee weights are correct and recorded. Composites are normally within animal within period, into a single sample. Check with P.I. before compositing samples, to guarantee composites are done correctly. Samples should be stored in a dry, room-temperature place until further analysis (Köster et al., 1996).
9. Composited samples should be added to ANKOM F57 bags at 0.45-0.50 g, according to ANKOM directions. Proceed to follow ANKOM directions for either NDF or ADF. Running these protocols will result in the iNDF or iADF for the substance, because the digestible portion of the substance was digested completely in the rumen, leaving only the indigestible portion left.
10. After completion of the ANKOM instructions on NDF or ADF, ash the Ankom F57 fiber bags at 600°C for 6 h to correct iNDF value for organic matter inclusion (ANKOM Technology, Macedon, NY). *Note: Place F57 bags into an oven safe crucible to catch all ash.
11. Utilize ANKOM ADF, NDF, and CF excel spreadsheet to do calculations and the equations below to calculate specific values as needed for this assay. Spreadsheet and other instructions can be found at this website: https://www.ankom.com/analytical-methods-support/fiber-analyzer-a2000

Equations:

Eq. 1. $iNDF\ (\%), DM, OM\ of\ digested\ sample =$ $iNDF\ (\%) from\ ANKOM\ analysis \div DM\ \%\ of\ sample \div$ $OM\ \%\ of\ sample$ (ANKOM Technology, Macedon, NY)
Eq. 2. $iNDF\ (\%)\ feces = \frac{iNDF\ \%\ of\ digested\ feces, DM, OM}{100} \times (1 -$ $Insitu\ disapperance\ of\ feces, g\ DM)$
Eq. 3. $iNDF\ (\%)\ feed = \frac{iNDF\ \%\ of\ undigested\ feed, DM, OM}{100} \times (1 -$ $Insitu\ disapperance\ of\ feed, g\ DM)$

Notes: Specific aspects of this protocol are subject to change based on new research. Aspects may need to be adjusted for different parameters. The steps used in this protocol are from various sources and results are subjective on the project and the accuracy of each of the steps.

Hazards of this Assay:

Always use caution when around livestock, and while in a laboratory and when operating laboratory equipment. Various accidents can occur while running this protocol. To guarantee your safety, make sure you have been properly trained to be in a laboratory and to run the specific equipment necessary for this protocol.

Citations:

Köster, H. H., R. C. Cochran, E. C. Titgemeyer, E. S. Vanzant, T. G. Nagaraja, K. K. Kreikemeier, G. St. Jean. 1997. Effect of increasing proportion of supplemental nitrogen from urea on intake and utilization of low-quality, tallgrass-prairie forage by beef steers. J. Anim. Sci, 75(Issue 5):1393–1399, https://doi.org/10.2527/1997.7551393x.

Krizsan, S. J., F. Jančík, M. Ramin, and P. Huhtanen. 2013. Comparison of some aspects of the in situ and in vitro methods in evaluation of neutral detergent fiber digestion. J. Anim. Sci., 91(Issue 2):838–847. https://doi.org/10.2527/jas.2012-5343.

Palmonari, A., A. Gallo, M. Fustini, G. Canestrari, F. Masoero, Charles J. Sniffen, and A. Formigoni. 2016. Estimation of the indigestible fiber in different forage types. J. Anim. Sci., 94(Issue 1):248–254. https://doi.org/10.2527/jas.2015-9649.

Vanzant, E. S., R. C. Cochran, E. C. Titgemeyer. 1998. Standardization of in situ techniques for ruminant feedstuff evaluation, J. Anim. Sci.,76 (Issue 10): 2717–2729. https://doi.org/10.2527/1998.76102717x.

VITA

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