Free choice feeding and its impact on feed efficiency and oxidative stress in growing sheep

by

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Abstract

The traditional total mixed ration (TMR) is a staple in the animal feeding industry as it allows, on aggregate, for animals to perform at a high level of efficiency when feeding large numbers of animals together. However, free choice feeding could have performance benefits and help improve the well-being of animals compared to TMR feeding that is used today. With free choice feeding, animals can develop and exercise individual preference of feedstuffs which has been theorized as one potential reason for the improvement in efficiency with free choice feeding. We conducted a study using growing sheep to assess differences in performance and oxidative stress between the two feeding methods. For this study, Polypay lambs (n=23, BW = 29.7 ± 6.6 kg) were utilized with lambs being blocked by body weight and randomly assigned to either a TMR diet (CON) or a free choice diet (FCD). The diets for both TMR and the FCD were of the same primary ingredients, with the free choice group having the macro nutrients (energy and protein) being supplied by the two main components of the TMR, corn and alfalfa, offered separately in pelleted form. For determination of the oxidative health impact of the two feeding systems, blood was collected at the beginning and end of the experiment, and was analyzed for the oxidative stress metabolite malonaldehyde (MDA) and the overall antioxidant capacity (AO). The experiment lasted a total of 42 days. Lambs were fed daily with the refusals targeting 5% to allow for ad libitum consumption. Lambs were weighed weekly for the duration of the experiment and rate of gain was determined using linear regression, with the slope of the regression line being average daily gain (ADG). The heavy block of FCD had a greater (P <0.05) ADG compared to the heavy block of CON (0.23 kg/d vs 0.17 kg/d), but was similar to light blocks of the FCD and CON treatments. For DMI, TDN intake, and the variability of daily intake, there was no significant difference between treatments for any of these variables. For

F:G, although no difference was observed between treatments, a significant treatment by block interaction was observed ($P = 0.04$). Intake of CP was a greater ($P = 0.03$) for CON than for FCD (0.31 kg/d vs. 0.26 kg/d). Sheep in the FCD group showed an initial preference for the alfalfa pellets, but they began to prefer corn relative to alfalfa as the experiment progressed. Measures of oxidative stress showed no differences between treatment groups at the start as well as the end of the experiment. The results of this study showed that, although some benefits to animal performance might occur with FCD, the positive impact on oxidative stress may not be true in all situations, in contrast with previously published literature.

Key words: free choice, performance, oxidative stress

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Chapter 1 - Literature Review

Introduction

Throughout the history of animal agriculture in the United States, many changes have been made to improve the efficiency of animal feeding to allow for quicker timelines to reach harvest weights. In the U.S., these changes include the proliferation of confined feeding operations and the use of pelleted feeds and total mixed rations (Drouillard, 2018; Coffey et al., 2016). Feeding in confinement allow producers to meet the nutritional needs of the animals to achieve the desired growth potential in an efficient manner, (i.e. humans design the plate from which the animal gets to consume). However, this feeding system may be limiting potential animal performance on the individual level, although aggregated performance improves, and negatively impacting oxidative stress burden. Oxidative stress occurs when there is an imbalance of antioxidants and oxidant free radicals within the blood. This reaction that occurs hinders certain natural reactions that occur in the body, leading to issues in ruminants. This topic has become of recent interest in the field and has been shown in multiple studies to have a role performance of ruminants and their overall health (Yuan et al., 2012, Wang et al., 2019, Salinas-Rios et al., 2015, Shibin et al., 2007). One potential way to reduce oxidate stress and improve animal performance is to offer diets in a free choice manner. This free choice allows ruminants to consume their diet (i.e. design their own plate) and gives them full access to however much forage they consume of varying types (Gregorini et al., 2017). This review outlines grazing behavior, development of individual diet preferences, and how animal preference impacts performance. Lastly, the role and interactions of oxidative stress with dietary selection and the multitude of factors that play a role in the expression of oxidative stress (internal and external).

Animal Foraging and Preference

Ruminants have evolved to fill the ecological niche, consuming complex carbohydrates contained in plant material to meet their individual energy demands through the process of reticulo-rumen fermentation that we as humans, or other monogastric species, cannot utilize efficiently. Foraging behavior is a complex process, and is difficult to predict what, when and why animals consume specific plants. One theory to predict this behavior of grazing was coined "optimal foraging theory" (Pyke, 1984). This theory consists of four primary aspects when it comes to ruminants and grazing selection. These four being: 1) where to search, 2) when to feed, 3) which food to consume (i.e. their "diet"), and 4) when to terminate feeding from one location and move on to another or terminate grazing entirely (Smith et al., 2010). Nested within this construct is the idea of energetic efficiency, that ruminants can determine and remember the route of least resistance for capturing maximum nutrition relative to expenditure, and genetic or learned traits and behavior that influence plant selection within a specific grazing patch. Ruminants will choose which forage to consume as they are able to learn from others, previous experience, and the type of supplemental feed or mineral they consume (Provenza et al., 1998). The complexity and variability of foraging behavior indicates that ruminants have a biological understanding of what to consume and when to consume certain forages. Additionally, ruminants have a relationship with plants within their ecological system and they will consume the forages they prefer if there is ample diversity provided (Provenza et al., 1998). As such it is important to consider foraging behavior and diet choice as this plays a key role in grazing-based livestock production (Atwood et al., 2001).

How and Why Animals Consume Feedstuff

As stated previously, ruminants have evolved to consume plants that humans cannot consume and are able to convert these into end products to meet their own energy to maintenance needs through the process of reticulo-rumen fermentation. In their natural environment of grass and rangelands, there is a high degree of dietary choice available to these animals, and as such they can consume a wide range of forages depending on the specific ruminant species, stage of growth, or individual animal, amongst numerous plant-based factors as well. Walker (1994) described "rule-of-thumb" diet selection that common ruminants consume between three plant classifications: grass, forbs, and browse. Over a year long period, sheep roughly consume about 50% grass and the remainder as forbs and browse, goats consume approximately 60% browse, and cattle have a diet of approximately 70% grass (Walker, 1994). While there is some overlap between species, particularly cattle and sheep, each of the major domesticated ruminants fall into distinct groupings in terms of diet composition with goats being more traditionally "browsers", sheep considered "intermediate" grazers and cattle being "tall grass" grazers. There are multiple other factors that affect an animal's selection of forage when grazing, including body size, time it takes to consume the forage, types of forages present, the energy that is expended when grazing, location relative to body of water, and many others impact the consumption of specific and preferred forages by ruminants (Milne, 1991).

Numerous studies have examined the behavior of animals and why they consume specific forages (Milne, 1991; Walker, 1994; Launchbaugh and Provenza, 1991). Milne (1991) reported some of the major factors that impact grazing animals' diet selection, starting with animal factors of body size and experience of grazing. Starting with body size, larger ruminant species tend to select plant parts higher in cell walls (i.e. structural carbohydrates) and will digest fibrous materials more efficiently due to larger rumen capacity and the forage being retained in the rumen for a longer period. Smaller ruminant species, are not able to digest dense energy material as efficiently and have evolved to select a diet that is higher in cell contents (i.e. more rapidly fermented) which lends to a smaller rumen to body size ratio and smaller retention periods (Milne, 1991). These concentrate selectors also typically have larger salivary glands, relative to body weight, to provide more buffering capacity within the rumen, more evenly distributed and larger ruminal papillae, and only 1-2 orders of omasal laminae compared to the 3-4 orders with grass or roughage selectors.

Within species, the role of previous or learned experience can also influence an individual animals diet selection (Milne, 1991; Provenza, 1996). This learning process includes animals will learn from either observation of “peers”, adverse feedback interactions, or experiences at a young age as they develop “familiar” foods. Launchbaugh and Provenza (1991) hypothesized that diet selection through the process of learning has three elements involved: 1) a parental/guardian figure as a social model, 2) caution when sampling of new forages, and 3) they develop their preferences based on gastrointestinal feedback (Launchbaugh and Provenza, 1991). They hypothesized that if an animal is exposed to these three core elements, they will learn and be able to develop their own preference leading to diverse consumption of forage. With the parental/guardian figures, this can also include learned behavior from “peer” animals. For example, Costa et al. (2016) examined if naive dairy heifers were grouped with dairy heifers that have been accustomed to grazing pasture, they would learn quicker and have altered behaviors compared to naïve heifers grazing separately. They used 63 pregnant naïve Holstein heifers having no time spent on pasture grouped with or without 21 heifers with prior experience. They found that cattle grazing with no prior experience co-grazed with those who had experience required less time to initially begin grazing and demonstrated less agnostic social behavior upon turnout compared to naïve heifers grazed alone. Costa et al. (2016) demonstrated that grazing behavior, and therefore indirectly animal performance, can be modified by who animals graze with.

Individual Animal Preference

As ruminants have preferred forages, it is important to examine how individual plant characteristics influence animal preference. Preference is perhaps most evident at the animal level when examining palatability and toxicosis. Starting with palatability, this was defined by Baumont (1996) as certain "dietary characteristics" that will stimulate certain selective responses by the animal consuming it. The determination of palatability of a forage with animals can be examined by the temperature of the food, texture of the food, smell, taste, experience and appearance (Baumont, 1996). Palatability is important as it can influence performance as animals will not consume forages, or under consume, that they do not prefer and performance can be negatively impacted. Burritt and Provenza (1997) demonstrated these key aspects of preference by looking at the combination of familiarity, toxicosis and novel location in feeding behavior of sheep. They trained one group lambs (n =14) to avoid a novel food (wheat) by pairing it with lithium chloride and a second group (n = 14) was offered wheat without the lithium chloride. They then moved half of the lambs in each treatment group to novel environments and offered them combinations of novel food (oats), familiar food (wheat), with half the lambs being adverse, and a familiar food (alfalfa). They found that lambs in novel environments still preferred the familiar but adverse food in wheat rather the novel oats. They also hypothesized that animals exposed to novel feeds in familiar environments can expedite intake once moved to a new location by offering familiar foods in novel environments thereby preventing declining intake.

Provenza et al. (1996) hypothesized that flavor and nutrient availability of a range of different diets will impact preference. To test this, they offered cross bred lambs (*n*=34) three diets with the same ingredients at different concentrations across two separate experiments. The diets provided the animals with varying amounts of digestible energy (DE) and digestible protein (DP), as well as varying flavors and nutrient quality. The ingredients examined across the experiments were barley, grape pomace, alfalfa, and soybean meal. Diet 1 consisted of primarily grape pomace (40%) and barley (35%), diets 2 had higher concentration of barley (40%) and grape pomace was lowered (32%), and diet 3 had the highest concentration of barley (46%) and the lowest concentration of grape pomace (20%). To examine the impact of flavor on animal preference, different flavors were added to each of the diets given with diet 1 having no flavor, and diets 2 having 1 % of oregano added and diet 3 having 1% onion powder added.

In the first experiment, the objective was to determine if flavor had a role in diet selection. They wanted to see if the lambs (*n* = 24) were first given each of the three diets separately and then offered together, the lambs would select based on flavor or nutrient availability. This experiment lasted for a total of 23d. All diets (~ 2 kg) were first given separately for 3d to allow for the lambs to adapt to them. After the adaptation period, they were then offered the diets *ad lib* from (1 – 23 d). Lithium chloride (LiCl), an anti-herbivory drench, was added to the diets on d 9 as to determine if this would change the preference. Following this they were then given all diets all together from d 10-16 with two diets receiving a flavoring and one not. Then for d 17-18 they received alfalfa pellets and from d 19-23, they were given all diets with no flavoring. The results of the first experiment showed that when offered individually, diet 1 had the highest consumption of feed (1,907 g/d) with diet 3 having the lowest (1,580 g/d). When offered together, diet 3 had the highest intake overall on d 1-23.

The second experiment that was performed was to examine how important flavor and nutrients had on overall food preference. In this experiment, the lambs (*n* = 10) were given the same diets in the first experiment (diets 1,2,3) in the same ingredient percentages. They were offered for the same time length of 9d for adaptation, each diet being offered for 3d each. Diet 1 contained no flavor and was offered from d 1-3 to all lambs. From d 4-6 diet 2 was offered with 5 lambs being offered it with 1% onion powder (Trt1) and the other 5 being offered diet 2 with 1% oregano added (Trt 2). On d 7-9, diet 3 was fed with Trt 1 receiving it with an addition of 1% oregano and Trt 2 receiving it with an addition of 1% onion powder. To examine preference, all diets were offered together for 3d. Trt 1 received diet 1, diet 2 (1% onion), and diet 3 (1% oregano) at the same time. Trt 2 received diet 1, diet 2 (1 % oregano), and diet 3 (1% onion). The results of this experiment showed similar results to experiment 1 where when offered separately, diet 1 had the highest overall consumption (1,907 g/d) and diet 3 having the lowest (1,580 g/d). When offered all at the same time, diet 3 had the highest overall consumption.

Within both experiments, Provenza et al. (1996) found that the consumption of diet 3 when offered all together, was due to this diet being the easiest to digest in their rumen, and having the most nutrients available for their consumption of energy and protein ratio. This paper shows that a variation of diets will allow animals to be able to consume more various flavors and nutrients. Looking at Scott and Provenza (1999), they expanded on this by first examining if sheep would have a preference of diets that were high in protein or high in energy, and if reducing these components caused animals to compensate by changing their diet preference. To do this they had cross bred lambs (*n* = 24) divided into the two experiments. In experiment 1, 24 lambs were housed in individual pens and offered ground barley as the energy macronutrient, and ground alfalfa as the protein macronutrient. Each was provided in a separate container and were feed ad libitum. This was done for a total of 25 d, the data presented looked at day 16-25. This was done because they saw the least amount of variation between these days. Significance was declared at a $P < 0.05$. Results of experiment 1 showed a variation in consumption between barley and alfalfa. Barley having a consumption of 221 to 991 g/lamb and alfalfa having a consumption of 51 to 558 g/lamb. Overall, they saw a higher consumption of barley compared to alfalfa (616 g barley to 334 g alfalfa).

Experiment 2 was performed by taking the lambs in experiment 1 and dividing them into two treatment groups. The lambs were first ranked from the highest consumption of alfalfa and barley to the lowest. After this they were then assigned into the two treatment groups at random, Treatment group 1 (Trt1) being the basal diets that were the preferred diets, diets of similar proportions to exp 1, with a dilution of grape pomace (10%). Treatment group 2 (Trt 2) consisted of basal diet that had 10% of only alfalfa when being consumed *ad lib* and was diluted with grape pomace (25%). Trt 1 had only 5 diets, Trt 2 had 6 diets. The diets were fed at ad libitum from d 26-42 of trial. From d 26-32 basal diet intake was monitored and recorded. From this they performed three 2 d trials (d 33-42) examining if the lambs preferences have changed due to the diets. This was done by taking away the original basal diet and replaced with two alternatives. These alternatives were given for only 15 minutes at a time. The first trial (d 33-34) they were provided with barley and alfalfa, trial two (d 37-38) separate diets of barley and alfalfa (80%), both diluted with grape pomace (20%), were given, and the third trial (d 41-42) they fed diets of food that was high in barley (80% and 20 % grape pomace), and a diet high in alfalfa (70% , 16 % grape pomace, 14 % cornstarch). They concluded that when animals develop preference for certain feedstuffs then are offered “novel” diets, feed intake decreases as animals require time to adapt to the new diet.

Results of experiment two showed that Trt1 had a higher consumption of protein (236g) than that of Trt2 (208g). Similarly, they saw the same with energy consumption, with Trt1 consuming about 4.73 Mcal/kg and Trt2 consuming 4.15 Mcal/kg. Looking at the results of the three trials performed within this, they saw that the first trial had a higher consumption of barley in both groups with no significant difference being seen. Trials two and three showed that the preferred diet group had a higher consumption of barley and the low-alfalfa group had a higher consumption of alfalfa when given those diets. Scott and Provenza (1999), stated that these differences could be due to a list of factors. First looking at that while animals tend to select the diets that provide the most output with little input, they still select a variety of nutrients or diets as one cannot fulfill their needs. Additionally animals tend to have different nutrition requirements based on their physiological state, and within similar stages of growth still deviate at the individual level. Therefore, animals will then develop their own personal preference of diets.

Nutritional Aspects of Animal Preference

When, where, and what animals consume can play a critical role in their performance, particularly when offered a varied diet within a pasture. However, today much of our grazing and feeding systems offer limited diversity in an effort to minimize variability and maximize performance. Some research, on the contrary, has indicated that this lack of dietary diversity may in fact be limiting animal performance (Garrett et al., 2021). As such, some researchers have been exploring grazing systems where pastures offer a diversity of forages and compared to a mono-species pasture to allow for easier management and provides animals with diverse forages to allow for a wider range of nutrient availability (Garrett et al., 2021). With mono-species grazing systems, animals may be deficient in some nutrients and health benefits. Beck and

Gregorini (2020) hypothesized that when allowed to select their own diets, animals can meet their own needs and provide some level of self-medication through the plant secondary compounds. It has also been hypothesized that even with animals being able to select and consume a diet of their own choosing, certain nutritional aspects play a critical role in allowing them to reach that constant homeostasis and impact the individual preference of that animal. A large one that is important regarding animal performance and feed preference is the level of protein vs. energy available in the diet. Studies have been done to examine the effect of varying protein vs. energy levels on animals' preference and performance (Villalba et al., 2002, Atwood et al., 2001, Araboui et al., 2025, Garrette et al., 2021, Moya et al., 2014).

Villalba et al. (2002) looked at whether sagebrush intake will be affected by variation in supplemental energy or protein levels. The experiment used lambs (n =24) and kids (n = 24) over a period of time. They first had an acclimation period to the supplements and diets over 14 days. Trial one was repeated for 9 days, with trial two and three being repeated for 7 days each. To examine how macronutrients (protein and energy) would impact the amount of sagebrush consumed, they performed three trials. Of those three, two looked at macronutrient influence on consumption and the third trial examined how charcoal would impact sagebrush consumption. For the supplements, the high energy supplement consisted primarily of barley grain, while the high protein was primarily alfalfa hay. One treatment received only the high protein supplement, a second treatment received only the high energy supplement, and the last treatment was offered a choice between either high protein or energy supplement. They found that in both trials, the protein only treatment and choice treatment had higher consumption of sagebrush compared to that of the energy only treatment. They concluded that the increase of sage brush with the protein and choice group was due to the balancing act of protein to energy, rather than the total amount of energy that was consumed.

Atwood et al. (2001) examined this free-choice preference in feedlot fed beef steers offered free choice feeding of primary ingredients and mixed ration diets, there will be higher intake levels and efficiency when free choice feeding was offered. Thirty-one steers and heifers were used in the experiment, with the control treatment (*n* =16) being offered a diet that was a mixed diet made of four primary ingredients and the second treatment (*n* = 15) being offered the same four ingredients but in separate bunks for consumption. To determine feed intake, each ingredient intake level was measured multiple times a day. To examine animal efficiency, they determined average daily gain (ADG), dry matter intake (DMI) of the diets, and the overall cost of each ingredient in the diet. With respect to DMI, they found that the animals given the mixed ration had a higher amount of overall intake. However, protein and energy consumption did not differentiate between the two treatments, but the free choice group consumed different ratios between protein and energy each day. With respect to production efficiency, they found that there were no differences in terms of gain between the two groups with TMR treatment having an ADG of 0.89 kg/d and choice treatment having an ADG of 0.92 kg/d. They did find the mixed group had higher cost/day compared to the free choice group. They concluded that the similar rate of gain but lower cost/day was due to the steers being able to effectively meet their individual needs. This study therefore demonstrates that animals can effectively select the ratio of protein and energy when given a choice, and that it may be economically advantageous to do so in small group settings.

Arbaoui et al. (2025) examined the impact of animal preference on rumen function and production when beef cattle are fed diets that are not mixed and total mixed rations. Their hypothesis was that when animals were fed a TMR diet compared to providing the forage and concentrates separate would impact methane yield, decrease the risk of ruminal acidosis from occurring, and that animal production would improve on TMR compared to the free selection. They conducted one experiment in a commercial facility to examine animal performance using crossbred male calves in two pens where the treatments were applied. The first pen was the control pen (n = 58) with the diet consisting of concentrate and barley straw offered freely and the second pen (n = 58) was the treatment pen where the diet offered was a TMR of barley and straw. A second trial was conducted using 18 steers from this commercial trial (9 per treatment) who were selected for digestibility and rumen fermentation analysis. For animal production, the weights were taken at the start of the trial and continued to be taken every fourth week on the commercial trial, however it should be noted that this experiment lacks true replication and instead relied on pseudo replication. For animal performance, intake, and gas production in the rumen, they found no significant difference between the two diets being fed on these parameters ($P > 0.10$). Further, there was no difference in CH_4 gas production between the two treatments, indicating that feeding a TMR alone does not result in improved emissions relative to letting animals design their own plane of nutrition. The authors did observe a significant decrease in ruminal pH (5.87 vs. 5.58, for free choice and TMR diets, respectively) which they suggested increased the risk of ruminal acidosis in TMR fed cattle.

Similar to Arbaoui et al. (2025), Garret et al. (2021) examined diets that varied in diversity would impact lamb performance, rumen characteristics and total antioxidant status. The difference between a varied and diverse diet entails that a varied diet is when the feed was offered (AM vs. PM), while diverse diets provided free choice selection of several fresh cut forages. The studied utilized Coopworth rams (n = 21; 7 per treatment) over 20 days and sorted them into treatment groups of differing diets. The forage in the diet consisted of alfalfa, ryegrass, chicory, and plantain. The three treatments were varied (VAR), diverse (DIV), and single forage (SFA). VAR was done in two increments, in the morning lambs were offered rye grass and plantain while in the afternoon they were offered alfalfa, and chicory. DIV was given all diets at one time, and SFA was only given alfalfa. When examining the ADG of the VAR diet, these lambs had a higher ADG by 28% compared to the DIV diet and more than 67% compared to the SFA diet. Subsequently, this was similar when looking at the FCE between all diets with VAR being 63% and 30% higher than the SFA and DIV diets, respectively. They acknowledge these differences were possibly due to the difference in nutrient composition between the DIV/VAR diets and the SFA diet. The VAR lambs were also observed to spend less time eating and more time ruminating compared to lambs offered the other diets ($P \leq 0.03$). Similarly, VAR and DIV lambs had significantly higher total antioxidant status compared to SFA fed lambs ($P < 0.05$).

Lastly, Moya et al. (2014), examined how ruminal pH, animal performance, intake behavior and blood characteristics heifers would be impacted when animals were given a list of feedstuffs in a free diet choice and a TMR diet. In this study, continental crossbred heifers (n = 79) were used over a 53-d period to determine how the feeding of barley grain (BG), corn silage (CS), and corn distillers' grain (DG) in a free choice diet and TMR. They utilized a total of four treatments: 1) the control fed a TMR consisting of barley grain (BG) and corn silage (CS), 2) free choice diet of BG and CS (BGCS), 3) free choice diet of BG and distillers grains (BGDG), and 4) free choice diet of CS and DG (CSDG). Feed behavior was collected by using an electronic bunk system that measured the amount the specific heifer was consuming at that time when it came to the specific treatment they were on. For pH of the rumen and how different diet methods and composition of diets impacted the levels, they found that heifers offered the CSDG diet had the highest ruminal pH compared to treatments ($P < 0.01$). Additionally, all free choice treatments groups had higher pH values compared to those on the TMR diet ($P < 0.05$). They suggested that this could possibly be due to the CS and how rumination is stimulated due to the higher amounts of fiber. Intake for CSDG was observed to be higher compared to BGCS and BGDG, but was similar to the TMR diet. In terms of performance, the BGDG treatment had similar ADG to the TMR treatment, in addition to the highest G:F ratio compared to all other treatments. Overall, Moya et al. (2014), determined that animals put on a free choice diet can perform equal or even better compared to animals put on a TMR diet while also not impacting their rumen to allow for improved wellbeing.

Oxidative Stress

When feeding, many factors are at play in the internal parts of the animal that would impact the animal's health, with oxidative stress (OS) being one of those factors. OS occurs when there is an imbalance of oxidants and antioxidants in the blood stream leading to many biochemical reactions which can hinder the body, such as DNA damage. OS occurs primarily when the animal undergoes activities causing an increase in oxygen demand, leading to an increase in metabolic demand and oxidant levels. These oxidants are classified as reactive oxygen species (ROS) and are composed of two species, free radical and non-radical species. Free radical ROS species have a single electron that isn't paired with that specific molecule which promotes this electron to be transferred through reduction or oxidation reactions (Halliwell and Gutteridege, 2007). ROS of interest include the production of superoxide anion ($\bullet O^-$), hydrogen peroxide (H_2O_2), and hydroxy radicals ($OH^\bullet$). The imbalance that causes OS is when there is a higher concentration of ROS molecules compared to antioxidant molecules. Some oxidative/antioxidative markers include malondialdehyde (MDA), superoxidase dismutase

(SOD), reduced glutathione (GSH), catalase (CAT), intracellular thiol groups (SH), and thiobarbituric acid reactive substances (TBARS). The health implications of oxidative stress are variable but includes a variety of common animal health issues including sepsis, mastitis, enteritis, pneumonia, and other diseases (Celi 2010). At the cellular level oxidative stress impacts many macromolecules (lipids, proteins, etc.) as well and may negatively impact animal performance (Sordillo and Aitken, 2008). The main causes that research has shown to increase OS would be environmental stressors and when there are inflammatory and metabolic events occurring (Celi and Gabai, 2015).

Causes of Oxidative Stress

Physiological Factors

In the animal there are countless biological processes that occur every second. Many of these processes are needed for the animal to function and maintain a homeostatic environment. However, there are times when certain physiological factors that are happening internally can cause an increase in OS leading to oxidative damage. This oxidative damage includes oxidative modification of macromolecules at a cellular or an individual level (Lykkesfeldt and Svendsen, 2007). Beck and Gregorini (2020) discuss the “how” with some of the more important oxidants that can be present including superoxide dismutase, hydrogen peroxide, NADPH oxidase, and Fe^{+}.

First, the production of superoxide ($\bullet O^{-}$) occurs in mammalian cells, specifically the mitochondria. Mammalian cells being a eukaryotic cell which is important in the role of cell function and stability (Ganguin et al., 2023). The produced $\bullet O^{-}$ is converted, with the enzyme mitochondrial superoxide dismutase, to hydrogen peroxide (H_2O_2). H_2O_2 is then released from the mammalian cell and is used within the cytosol and nucleus of the cell with redox signaling.

Redox signaling is when the H_2O_2 regulates certain modifications within certain proteins to assist with many naturally occurring biological responses (Cobley et al., 2015). While this is an important process to the regulation of many proteins and enzymes while under normal function, when in excess there is an impact on performance and health (Zhong et al., 2023, Xing et al., 2021, Liang et al., 2024).

Zhong et al. (2023) examined how elevated levels of hydrogen peroxide would impact health and performance in squabs. These treatments consisted of control with no injection, negative control with an injection of normal saline solution, low dose (2.0 mmol/ kg BW H_2O_2), medium dose (2.5 mmol/ kg BW H_2O_2), and a high dose group (3.0 mmol/ kg BW H_2O_2). With sampling collection of serum and duodenum for OS markers and for changes in intestinal tract. After this they were euthanized for duodenum collection. With OS marker identification within the serum and duodenum of the squabs, they examined for protein carbonyls (PCO), 8-oxo-deoxyguanosine (8-OHdG), and MDA using Elisa Kits for pigeons.

They found that through all the variables examined, there was a negative impact with the addition of H_2O_2. Starting with the development of the duodenum, they found that with the addition of higher amounts of H_2O_2 there was higher damage to the duodenum in villi development. Further crypts arrangement was irregular and squabs with elevated H_2O_2 also showed signs of lymphocyte infiltration. Those squabs also had growth performance results mirrored that of duodenum damage with reduced performance with increasing dosage of H_2O_2. The authors observed a decreased in performance for the medium and high doses by 17.38% and 22.04 % respectively compared to the control and negative control groups after 7 d. Regarding the ROS markers within the serum, overall ROS was significantly different in all H_2O_2 dosage groups compared to both control and negative control. The authors suggested that these differences occurred due to an overdose of ROS molecules leading to damage of the internal environment.

Environmental Factors

When looking at the causes of OS, one factor to examine is the environment that the animal is in. While little research has been done examining possible environmental factors impacting OS on animals while grazing, one stressor that has been determined is heat stress (Celi and Gabai, 2015). Heat stress occurs when the animals internal body temperature is high due to external/internal factors increasing body heat while the animal is not effectively dissipating that excess heat. When an animal is in heat stress, ROS production will increase or the antioxidant amount in the animal will have decreased leading to OS in the animal (Celi and Gabai, 2015, Bernabucci et al., 2002, Trana et al., 2007, Chauhan et al., 2014). For example, Bernabucci et al (2002) hypothesized that the levels of certain OS markers in dairy cattle will increase during the summer and spring months during the transition period. There were 2 treatments, so to speak, cattle who gave birth in the spring (n = 11) or the summer (n = 9). With blood samples they measured the packed cell volume (PCV), measured percentage of red blood cells in a sample, and centrifuged samples and separated the plasma from them. The OS markers examined included TBARS, SOD, GSH and SH. Results showed that when dairy cattle are under heat stress or in an extremely hot environment while on the transition period, they experience higher levels of TBARS, SOD, GSH and SH before and after calving in the summer compared to the spring (Bernabucci et al., 2002).

Another environmental factor that may influence OS would be parasitic infection. When infected with a parasite, the body will produce and consume a large amount of oxygen to combat the infection (Pawłowska et al., 2023). Specifically, the phagocytic cells will go into what is called oxygen shock. This being when cells are mass producing oxygen cells to produce the ROS molecules $•O^-$ and H_2O_2. Silva et al. (2017) examined dairy cows were infected with the lungworm *D. viviparus* and how this would impact levels of OS. 22 dairy cows that were infected with the *D. vivparus* and sorted them into sampling groups of mild clinical signs and severe clinical signs. The clinical signs that were used to determine mild and severe were cough and nasal secretions. Blood collections were done on d 0 and d 10. They found that for OS indicators (TBARS, SOD, ROS, and CAT), the animals that were showing severe clinical symptoms had higher TBARS, SOD, ROS, and CAT. OS compared to those animals who were showing mild signs of dictyocasulosis. A reasoning for this difference in markers could be that with the severe infection animals' lipid peroxidation will increase leading to alterations to the CAT and SOD levels as ROS levels are increasing to rid the animal of the parasite.

Dietary Factors

Dietary factors that impact OS would be a diet that is composed of high carbohydrates and fats as the body will be put under a high metabolic load (Celi 2015). On the contrary, when animals are under-nourished, they can experience an increase in OS as this under-nourishment will increase the amount of ROS molecules potentially increasing the risk of some diseases and disorders, such as inflammation of the gastrointestinal tract that can impair nutrient utilization (Ponnampalam et al., 2022). Ponnampalam et al. (2022), stated that higher performing animals of things will need higher nutrient demand to keep up. From all of this we see that there is an impact that the diet of animals will increase or decrease ROS species present in their blood stream. One factor that contributes to the impact of this would be plant secondary metabolites (PSM). From Acamovid and Brooker (2004), PSM are phytochemicals that can range from having no nutritional value to having some but are helpful in providing animals with certain beneficial aspects. One benefit includes the metabolization of proteins within the rumen as PSM chemical structure is similar in comparison to some amino acids found in the ruminants (Acamovid and Brooker, 2004). PSM has a very wide range of interactions within the animal itself. These PSM come in wide range of compounds (phytate, protease inhibitors, lectins, alkaloids) but one we will be focusing on and discussing is tannins. Tannins can be identified within two classifications, the first being hydrolysable tannin, and the second being condensed tannins (CT). While both have some positive and negative effects regarding animals and feeding, we see that CT have a more positive impact on animals' wellbeing in relation to OS. A good amount of research has been performed to examine the impact that CT will have on the animal regarding performance and OS (Batchu et al., 2021, Dey and De, 2014).

Batchu et al. (2021), examined how the condensed tannin *Lespedeza cuneata,* would impact goats with regards to certain physiological responses, meat quality, and the microbes in their guts. This study was performed by taking uncastrated Spanish goats (n = 36) and were separated into three groups, one eating grounded *Lespedeza cuneata* (SER), the others eating bermuda grass hay (BG) with one group being dewormed (BG-DW). At the end of the trial, they took half of the goats and put them on a transport truck to induce stress (TS) for measurements while the other half was not put on a transport tuck and left in pens (NTS). Each group contained 12 goats and were fed for a total of 56 d. The control group received dewormer as they were primarily comparing the results from the SER group and the BG group. They selected to focus normetanephrine/metanephrine as there is a correlation between cortisol levels and metanephrine. The results showed that the SER group had higher cortisol levels when under the TS treatment compared to the BG-DW and BG ($P < 0.01$). Regarding the normetanephrine/metanephrine levels, the SER had lower levels when the TS was applied compared to the other groups. A possible reasoning for this difference in the cortisol levels is that the BG-DW group was dewormed leading to less parasitic infection causing lower levels of cortisol to be produced. Regarding the normetanephrine/metanephrine, they suggested that these levels were found as epinephrine has short half-life leading to the production of these two compounds to be relatively quick. From this we can see that tannins, while having some negative effects, can overall improve the wellbeing of animals leading to a more efficient and productive animal.

Impact of Oxidative Stress

Oxidative stress can have a large impact on a few factors including cellular level damage, biological factors, and can ultimately impact the performance of animals leading to lower quality products. First examining the negative impact OS has on animal performance, Ponnampalam et al. (2022) shows us that when farm animals are put under oxidative stress from a list of internal and external factors, there will be an impact on animal performance and quality. Looking at this list again, we will see that there is a wide range of impacts that the free ROS will have. These being damage to the membrane, nucleic acid, protein oxidation, and damage to other small molecules that are located within the body. This damage can impact key processes that help animals convert feed into energy and allow for basic internal maintenance. This can lead to poor performance, poor feed efficiency, and health risks.

Pannampalam et al. (2022) goes into detail regarding the oxidation of lipids, proteins, DNA, and carbohydrates and how this will negatively impact the animal. Starting with lipids, lipids are a very important macro molecule that help with a vast number of functions that occur within the body ranging from the internal help of producing hormones to protecting the body from certain extreme environmental conditions. As such, the oxidation of these compounds can lead to the damage of blood cells and muscle tissue (Pannampalam et al., 2022). Protein and carbohydrate oxidation have a similar impact in that when oxidation occurs the overall shape and function will change making it so the function of them will either change or be lost entirely, leading to damage to the cell and thus the animal. DNA oxidation will have an impact on the cells leading to possible alteration of expression causing damage to the cell and possible infection.

The oxidation of these molecules does not just have an impact on the cells themselves. This damage will cause a chain of events leading to an animal having a decrease in performance causing lower economic returns. Many studies have recently examined how OS impacts animals in terms of performance throughout many sectors of the industry (Yuan et al., 2012, Wang et al., 2019, Salinas-Rios et al., 2015, Shibin et al., 2007). These papers examined dairy cattle, sheep, pigs, and chicken performance with various impacts on oxidative stress and the performance of these animals.

Yuan et al. (2007) examined how OS would impact the performance of weanling pigs over a 26-d period. They had Landrace x Yorkshire weanling pigs (*n* =24) be divided into three groups, 8 pigs in each group, two of them (group 1 and 3) were fed a diet consisting of fresh fish oil, while the other group (group 2) was fed the same diet with the addition 5.0% oxidized fish oil. Group 3 additionally had an injection of diquat dibromide monohydrate. They found that groups 2 and 3, which were the OS representative groups, had lower BW on d 14, compared to the control group. ADG and ADFI, measured as g/head/d, showed a similar relationship from d 0-14 between all three groups with group 1 having a ADG of 362.86 g/hd/d, group 2 with 303.04 g/hd/d, and group 3 with 213.56 g/hd/d. ADFI with group 1 having 510.66 g/hd/d, group 2 having 449.69 g/hd/d, and group 3 with 338.46 g/hd/d. For OS markers they found that there were higher MDA levels in both plasma and liver samples in groups 2 and 3. The plasma showing an MDA concentration of 2.73 nmol/ml within group 2 and group 3 showing 3.57 nmol/ml and the liver samples showing concentrations of 1.41 nmol/mg protein for group 2 and 2.04 nmol/mg protein for group 3. Looking at the antioxidant enzymes the results showed that group 1 had the highest concentration of these enzymes, group two and three having a decreasing amount. The authors hypothesized that these differences may be due to the fish oil being heated at a temperature of 37°C for 60 h while in the presence of certain metal cations had a production of certain oxidant products which resulted in a higher production of certain oxidant compounds. This leads to poor performance and poor internal maintenance as the body will be in OS and unable to counter this due to higher oxidants to antioxidants as seen by the results described prior in both the plasma and liver of the animals.

Positive Role of Oxidative Stress

While so far focusing on the negative aspects of oxidative stress, primarily being poor health and poor performance, there are more positive aspects of maintenance of positive oxidative stress status. Many studies have examined the impact of OS on animals rather than looking at the impact of it on the product the animals produce. When examining ROS and OS within the animal at a low concentration, we see that there is an overall benefit in a few regards. With human consumption, some of these being a higher concentration of certain antioxidants enzymes, vitamin E and β-carotene, B-vitamins, and levels of α-tocopherol within the meat.

All these chemicals are very important in the process of managing and improving the OS levels within animals and will in turn benefit the consumer. Daley et al. (2010) provides us with a review of some of the antioxidant enzymes, and Vitamin E/α-tocopherol and how it relates to beef cattle that are primarily fed/finished on grass. First with vitamin E/α-tocopherol, these biochemicals are very beneficial in the process of reducing the overall amount of the free radicals that contribute to OS, primarily hydrogen peroxide (Daley et al., 2010). They provide additional benefits as well, being the reduction of nitrosamines, and enhancement of the immune system for the consumer and beef cattle. Continuing with Daley et al. (2010), for antioxidant enzymes, they discuss the enzyme Glutathione (GT) and its relevance in grass finished beef for the consumer. GT has shown to be able to reduce the amount of ROS molecules within meat allowing for more health beneficial meat. Meat in general contains many of the fatty acids. Similarly, we see that meat will be affected in terms of nutrient composition based on how the animals are finished in terms of what system they are put under. Recent research has looked at how feeding animals a feeding method impacts composition of antioxidant activity in the final products (Duckett et al., 2009).

Ducket et al. (2009) provides an example for how changing management can influence the human health benefits of the final production through improved OS status. Their main purpose of this paper was to examine how different stocking rates and the finishing systems of either a concentrate (CONC) or put on pasture (PAST), and how it impacted a list of meat characteristics. This study was performed over a 3-year period and used Angus-cross steers (n = 198). At the start of each year for this study, steers were randomly allocated into the different growth rates of low (0.23kg/d), medium (0.45 kg/d), and high (0.68 kg/d) on a dry lot for the months from December till mid-April. After the stocking phase was finished, they were then again randomly allocated to the two finishing systems of CONC and PAST. After the finishing period they were sent off for slaughter and were examined for fatty acid composition, proximate estimation nutrients, cholesterol levels, fat soluble and B vitamins, and some antioxidants.

Looking at the finishing system and its impact on the overall nutrient composition and fatty acid composition, they saw varying results between them. The finishing systems were the CONC, which was a diet that consisted of high corn silage, timothy hay, soybean meal and hulls, and a mineral mix. The PAST consisted of a wide range of plants present in the pasture. Looking at the results of the two finishing systems, they saw that the PAST had higher antioxidant vitamins present in the meat. Looking at the proximate analysis of the two systems, they found a significant difference ($P = 0.001$) between the CONC finish and the PAST finish regarding the finishing system and its impact on the levels of certain vitamins and minerals. From the PAST finish, they saw that the levels of α-tocopherol and β-carotene were higher overall than the CONC cattle. PAST had α-tocopherol levels of 773.43 µg/100g and β-carotene levels of 28.53 µg/100g compared to the CONC finish with α-tocopherol levels of 199.23 µg/100g and β-carotene levels 28.53 µg/100g. A similar result can be seen with the B-vitamin levels (measured as Riboflavin and Thiamine) with the PAST group being overall higher in both measurements. One possible reason for these difference in levels of fat soluble vitamins and B-Vitamins is the cattle finished on pasture have a wider range of those plant secondary compounds and are able to consume a wider range of nutrients. Ducket et al (2009) shows us that cattle put on a pasture finish, have a wider range of health benefits in their meat compared to when put on a grain finish.

Conclusion

In this literature review, we examined how forage theory shows that when animals are given free choice feeding compared to a TMR, they can help with the mitigation of oxidative stress in their blood to improve overall well-being and see higher efficiency in terms of overall performance and production. Within foraging theory, examining how and why animals will consume certain feedstuffs, development of individual preference when it comes to feed, and how certain nutritional aspects will play a role in the selection of animal's preferences. Following this, we examined oxidative stress metabolites and the factors that influence this production and how we see its impact on animal performance.

Looking at the overall idea of animals having a free choice of what they can consume, we can see that they do have an innate ability to develop their own preference in terms of foraging. Shown by Walker (1994), each main domesticated ruminant has a diet selection of a specific type of forage. Sheep have a main consumption of grass with a mixture of browse and forbs, goats with a primary consumption of browse, and cattle with grass. The selection of these specific forages being due to the body size the ruminants have and having experience of grazing. Milne (1991) shows that with different body sizes of ruminants, they select different parts of the plants to graze. Regarding experience of grazing, we saw Costa et al. (2016) provide a clear example of ruminants that are inexperienced to grazing, graze with those that will take up to it alot easier leading to easier consumption and adaptation. From here we learn the development of preference is associated with physical factors in relation to the rumen capacity and past experience and learning from other ruminants while grazing. Within these preferences of forages, we saw that while there are certain forage types they prefer, there are additional characteristics that play a key role in the selection as well. We discussed that the palatability of the forage will impact the preference of animals as its a major component. Looking at multiple papers regarding this palatability (Baumont 1996, Buritt and Provenza ; (1997), Scott and Provenza; (1999), Provenza et al. (1996), we can determine that there is a clear connection between animals and palatability impacting the overall selection and preference of the forages they were consuming. One more factor to relate with free choice feeding would be the macronutrients that are being consumed. The primary macronutrients that we discussed in this paper were protein and energy. As such we can see that from the studies discussed in this section, there is a clear sign that animals are able to balance their own ration of energy and protein for maximum efficiency in gain. As such we can see that this would be a improvement in economic return in regard to small-scale operations.

We saw that oxidative stress is when we have an imbalance of antioxidants and oxidants in the blood stream leading to the production of harmful biochemicals that are then released into the blood stream impacting cells and other biological processes that occur in the body naturally. These can be impacted by many factors including dietary, environmental, and physiological changes. From these factors we see overall that with this increase in oxidative stress, the performance and efficiency of animals and feed consumption would decrease. Leading to lower economic returns. The impact of this stress will impact many naturally occurring biological processes that will hinder the fermentation in the rumen. We see that a large for this to be mitigated is through the process of free choice feeding and proper feed management.

Free choice feeding on feed efficiency and oxidative stress should be studied more as we see that there is a positive relationship between well being and performance when we look at free choice compared to a TMR diet. This allows producers to possibly save large amounts of economic inputs for higher outputs, focusing on smaller scale operations.

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Chapter 2 - Free Choice Feeding and its Impact on Feed Efficiency and Oxidative Stress in Growing Sheep

Abstract

The objective of this study was to examine the effect of diet choice on feed efficiency and oxidative stress in growing lambs. Previous research has indicated that providing animals free-choice in selecting diet components may improve performance and overall animal wellbeing compared to feeding a complete pelleted diet. A 42-d experiment was conducted. Polypay lambs (n = 23; initial BW = 29.9 ± 6.6 kg) were housed in raised individual pens and were provided two different diet feeding methods, free choice diet (FCD) and a complete pelleted diet (CON). From d -14 to -1, lambs were fed a pelleted control diet and allowed to acclimate to the facilities. On d 0, lambs were weighed and randomly assigned within heavy (FCD-H/CON-H) or light (FCD-L/CON-L) weight block to one 1 of 2 treatment diets: 1) Pelleted control diet (CON) formulated to meet vitamin and mineral requirements and containing 46% ground corn, 28% alfalfa, and 16% soybean meal on an as-fed basis , or 2) Free-choice diet (FCD) of pelleted alfalfa and pelleted ground corn offered for ad-libitum intake and similarly formulated to meet vitamin and mineral requirements. Blood samples were collected via jugular venipuncture on d 0 and 42 and analyzed for oxidative stress markers. Performance data were analyzed using treatment, block, and treatment by block interaction as fixed effects. Oxidative stress data was measured with treatment, day, and initial body weight as fixed effects. Significance was declared at $P < 0.05$. The FCD-H block had a greater ($P < 0.05$) ADG than the CON-H block (0.23 kg/d vs. 0.172 kg/d). TDN intake saw no difference ($P > 0.05$) between the CON (1.22 kg/d) and FCD (1.29 kg/d) groups. The results of this study indicate that offering lambs free-choice in selecting from primary diet ingredients may positively improve animal performance without impacting feed efficiency.

Introduction

The method in which diets are fed to animals can have a large impact on the overall performance and emphasizes the responses of “average” animals (Atwood et al., 2001). In the industry today, the common method by which animals in confinement are fed is a total mixed ration (TMR) which did not exist a century ago. TMR allows animals to reach their necessary level of energy to allow for their body to be maintained at a stable level while allowing excess energy consumed to be used for performance or production of certain products (Schingoethe, 2017). While this allows farms to provide the appropriate plane of nutrition for animals on the aggregate, TMR feeding can also have consequences for animal health when not properly formulated, such as ruminal acidosis. This can negatively impact their performance and overall well-being, but animals may have the ability to modulate this impact when given dietary choice (Phy and Provenza, 1998). Phy and Provenza (1998) showed that animals offered high grain diets will seek feed ingredients that attenuate acidosis. On the contrary, a free-choice diet allows animals freedom to select their own diet and may improve performance efficiency at the individual level compared to the TMR and result in similar planes of nutrition and fermentation profiles (Atwood et al., 2001; Moya et al., 2011).

Some research has been conducted exploring the idea of allowing animals free choice to consume their own diets and the resulting implications on performance and overall well-being (Villalba et al 2002, Atwood et al. 2001, Araboui et al. 2025, Garrett et al. 2021a, Moya et al. 2014). “Diet diversity” results when animals are given a diverse composition of forage on pasture or given feedstuffs offered separately to allow for individual consumption and preference. The evolution of ruminants as free-ranging animals with a diverse selection of plant species and plant parts from which to choose has resulted in a complex behavior where animals have learned an innate preference in their diet that is impossible to appease with TMR feeding. Beck and Gregorini (2021) hypothesized that when animals are allowed to have this diet diversity, they consume large amounts of plant primary and secondary compounds (PPC/PSC) to meet their own nutritional and health needs. The authors referred to this as design theory, or the conscious choice of the manager to design a feeding system to maximize choice with animal well-being being a central meta-requirement. Animal well-being is a concept that is hard to measure and can come in many forms. Some research has focused on traditional animal science interests such as ruminal pH level (Moya et al., 2011), whereas others such as Beck and Gregorini (2020) focus on oxidative stress markers. Oxidative stress (OS) is when the body has an imbalance between oxidants and antioxidants in the blood. This can affect many biochemical reactions that occur in the body in a negative way. When an animal has an elevated OS burden, they have a higher production of reactive oxygen species (ROS), which are the main biochemical compounds that cause many negative reactions to occur within the animal's body. The production of these molecules is influenced by certain physiological factors, environmental factors, and the diet that the animals are consuming (Beck and Gregorini, 2020). As such, it is theorized that dietary choice or diversity may alleviate an animal's OS burden through various mechanisms, such as modulating the intake of particular nutrients (e.g. energy) thereby minimizing risks associated with overconsumption. The objective of this research is to examine how offering growing lambs a free choice diet would impact the overall feed efficiency and OS levels compared to lambs fed a traditional TMR.

Material and Methods

The procedures performed in this experiment were all approved by Kansas State Institutional Animal Care and Use Committee (IACUC protocol #5026).

Animals and Treatments

This project was conducted at the Kansas State University Sheep and Meat Goat Center located in Manhattan, KS. The experiment was conducted from July 29 (d 0) to September 9 for a total of 42 d. Prior to the experiment, female Polypay lambs (n = 23; initial BW = 29.7 ± 6.6 kg), were randomly assigned to individual raised feeding pens (2 m × 4 m). All lambs were given ad libitum access to water and allowed 2 weeks to acclimate to their pens prior to d 0. During the acclimation, they were offered an 18% crude protein (CP) pellet formulated to meet all vitamin and mineral needs (Table 1). Feed was provided using clip on feeders (28 × 10 cm) to allow for easy collection of refusals and to minimize water contamination of feed. For the duration of the experiment, pens were cleaned twice daily at approximately 0800 and 1700.

On d 0, lambs were individually weighed and then blocked by BW (heavy [H] or light [L]) into one of two treatment groups: 1) Control group fed the 18% crude protein pellet containing primary ingredients of ground corn and alfalfa (CON, n = 13), or 2) Free choice group fed corn and alfalfa offered in separate feeders (FCD, n = 10). The corn and alfalfa were each ground and pelleted to be offered individually (final pellets contained approximately 90.25% ground corn or alfalfa) with each containing salt, dicalcium phosphate, vitamin/trace mineral premix along with ammonium chloride to minimize risk of urinary calculi (Table 1). Diet refusals were weighed each morning for both treatment groups, and >5% orts were targeted to allow ad libitum feed consumption for both the alfalfa pelleted diet and corn pelleted diet in the FCD group and the complete pelleted diet in the CON group. These diets were produced by the Kansas State University Research Feedmill in Manhattan, KS.

Weekly for the duration of the experiment, unshrunk individual body weights were measured at 0700 h on a calibrated scale prior to feeding. Average daily gain (ADG) was calculated as the slope of the regression line of body weight regressed on day of the experiment. Three lambs were removed from the study for health reasons unrelated to experimental diets.

Blood and Laboratory Analysis

At the beginning and end of the trial, blood samples were collected via jugular veni-puncture using 10-mL heparinized blood collection tubes (Valley Vet Supply, Marysville, Ks). After collection, tubes were centrifuged at 2,000 g at 4°C for 15 minutes. Serum was transferred into 2-mL microcentrifuge tubes and stored in a -80°C freezer until analysis. Serum samples were later analyzed for the oxidative stress marker maldonaldyde (MDA) and total antioxidant capacity using commercial assay kits (Cayman Analytic, Ann Arbor, MI).

Feed samples were collected for both the CON and FCD diets on a weekly basis from the start of the experiment to the end. The samples were dried 48 hours in a 55°C oven to determine DM. Feed samples were then ground and then composited prior to analysis. Samples were analyzed at a commercial laboratory (Dairy One, Ithaca, New York) for CP, acid detergent fiber (ADF), neutral detergent fiber (NDF), non-fiber carbohydrates (NFC), and calculated total digestible nutrients (TDN; Table 2).

Statistical Analysis

The experiment was analyzed as a randomized block design with individual animal as the experimental unit. All analyses were conducted using R (R Core Team, 2025; version 4.5.0). Animal performance and efficiency metrics of interest included ADG, average dry matter intake (DMI), variability in daily intake, gain:feed, and feed:gain. These parameters were analyzed using the lm model in R, with the model including fixed effects of treatment, block, and the treatment by block interaction. To examine intake behavior between treatments, the coefficient of variation in daily dry matter intake was utilized and analyzed using the model previously described. Blood parameters were analyzed using a repeated measures analysis with the lme model in R. The model included fixed effects of the treatment, day, and the treatment by day interaction as well as initial body weight as a covariate. Significance was declared at $P \leq 0.05$ and tendencies at $0.05 < P \leq 0.10$.

Results

Dry Matter Intake and Intake Behavior

There were significant differences between treatment, blocks, or their interaction for dry matter intake (DMI) ($P > 0.24$, Table 3). With intake behavior, there was variation from the start of the trial compared to the end of the trial when it came to the consumption of the corn pellets and the alfalfa pellets for some of the lambs in the FCD group (Figure 1). Over the entire experiment, sheep provided the FCD consumed more corn (0.95 kg/d) than alfalfa (0.78 kg/d). Intake behavior was examined in 7-d increments to assess consumption of corn and alfalfa in FCD lambs, and a significant difference was observed between ingredients over time ($P < 0.01$; Figure 2). Lambs ate significantly more alfalfa in week 1 (0.86 kg/d vs. 0.45 kg/d, for alfalfa and corn, respectively), but no difference was observed in week 2 between ingredients. From weeks 3 through 6, corn consumption was significantly greater than alfalfa ($P < 0.01$). No difference was observed between blocks on feed intake behavior. Looking at the CP and TDN levels that were consumed within each of the treatment groups over the experiment period, with only numerical differences, the FCD had a higher amount of TDN being consumed (1.29 kg/d) and the CON being lower (1.22 kg/d). With CP, the CON group was higher ($P < 0.05$) compared to the FCD group, with the CON consuming 0.309 kg/d and the FCD group consuming 0.257 kg/d of protein. Variability in daily intake was determined as the coefficient of variation in daily intake for each animal. Between the two treatments, no difference was observed in daily intake variation, with the FCD group having a CV of 21.3% and the CON group having a CV of 17.5%.

Animal Performance and Feed Efficiency

Feed to gain (F:G) and gain to feed (G:F) were used to determine the feed efficiency between the FCD and CON groups. There was a significant treatment by block interaction for F:G ($P = 0.04$; Table 3). However, upon mean separation, there was no difference observed among the treatment × block combinations ($P > 0.10$). The CON had a F:G of 8.57 kg/kg and the FCD treatment had a F:G of 8.29 kg/kg. G:F similarly demonstrated a significant treatment by block interaction (P = 0.04) without statistical significance among groups upon mean separation ($P > 0.10$). Numerically, G:F of the CON was 0.119 kg/kg and the FCD was 0.124 kg/kg. For ADG there was a significant treatment × block interaction ($P = 0.01$). Looking at the treatments in their weight blocks for ADG, the FCD-H had a greater ADG than the CON-H (P < 0.05) but did not differ from the FCD-L or CON-L.

Oxidative Stress

Concentration of the oxidative stress marker MDA were not different between treatments at d 0 or d 42 ($P > 0.05$). For total antioxidant capacity, there were no significant differences between the two treatment groups.

Discussion

Dry Matter Intake and Intake Behavior

At d 0, there was an overall higher consumption of alfalfa compared to the corn (Figure 2). By week 3, there was a shift with intake behavior resulting in corn becoming the preferred feed ingredient compared to alfalfa pellets. Primarily, this occurred in animals 1, 5, 6, and 8, where they initially consumed mostly alfalfa pellets and then changed their intake mostly to corn pellets (Figure 1). However, this response was not observed for all lambs, such as lamb 2 where alfalfa pellets remained the preferred feedstuff for the duration of the study. Interestingly, on aggregate for all lambs in the FCD treatment, alfalfa consumption varied very little on a weekly basis across the experiment ranging from 0.67 kg/d to 0.89 kg/d. Corn consumption, in comparison, slowly increased over the course of the experiment beginning at 0.45 kg/d in week 1 until peaking at 1.2 kg/d in week 4. These results are similar to those of Atwood et al. (2001) with beef steers offered 4 different feedstuffs (barley, corn silage, alfalfa, and rolled corn). On aggregate for that study, steers preferred the barley and corn silage, yet on the individual animal level there were marked differences in preference. Additionally, we observed that our lambs were able to achieve similar levels of energy intake when offered the free choice diet compared to lambs offered the complete pellet, albeit protein consumption was lower for the FCD lambs. The variability between the energy and protein ingredient preference combined with similar performance levels indicates that lambs were able to consume a diet to meet their individual needs, at least in comparison to lambs offered the complete pellet in this study, in agreement with previous studies (Kyriazakis and Oldham, 19930; Villalba and Provenza, 1999; Villalba et al., 2002; Gregorini et al., 2017). Villalba et al. (2002) found when animals were consuming sage brush with the offering of a protein and energy supplement, they had a higher consumption of protein supplements. They hypothesized that this was an attempt by the animal to balance out the low protein content that was present in the sage brush. This built on the work of Provenza et al. (1996), who hypothesized that this intake behavior is a result of an animals preference to select the diet that is more easily digestible and having the highest energy to protein ratio. This is in alignment with the current study with the FCD lambs consuming diets that allowed for the easiest digestibility and with the highest energy to protein ratio.

Previous research is inconsistent on the impacts of FCD on diet intake. Atwood et al. (2001) showed that between groups of cattle, one fed a TMR and the other fed a free choice diet, DMI intake was greater in the TMR group but the growth rate was the same. On the contrary, Nocek et al. (1986) in a study utilizing dairy cows found that cows fed forage and grain separately had lower intake relative to TMR-fed cows. Similar to Nocek et al. (1986), Atwood et al. (2006) found that lambs offered free choice feed stuffs had greater intake than TMR-fed lambs. There are several potential reasons for the disparity between this study and others, including duration, housing design, animal growth potential, type of diet ingredients or method of offering the feedstuffs. However, this research agrees with others that performance is not sacrificed regardless of change in intake.

We observed no difference between the FCD and CON groups for variability of daily intake. This goes against the work of Garrett et al. (2021b), who reported that lambs offered multiple forages or a single forage had an intake CV 21% lower when offered a diet containing multiple forages. However, the diets offered in our study were markedly different than the forages offered by Garrett et al. (2021b). In a study of beef steers offered similar ingredients, Moya et al. (2014) found that intake was commonly lower in beef steers offered ingredients free-choice and intake behavior was variable depending on the type of ingredients offered. Therefore, the impact of free-choice feeding on diet intake and intake variability may be due to the type of ingredients offered, rather than the method of feeding.

Animal Performance and Feed Efficiency

Some proponents of diet diversity and free choice feeding have hypothesized that free-choice diets encourage reductions in DMI, and improved and feed efficiency (Nocek et al., 1986), although we did not observe reductions in DMI for FCD compared to CON. Within this study, we observed that when offering lambs either a TMR or a free choice diet, both groups largely had a similar performance and similar feed efficiency, similar to Atwood et al. (2001) and Araboui et al. (2025). However, we observed that, within the heavy weight block, lambs fed a FCD had greater ADG than of CON lambs. Although Atwood et al. (2001) reported no difference in performance in free choice fed beef steers and those fed a TMR, they found there was a lower daily feeding cost for a free choice diet compared to the TMR diet due to numerical differences in both intake and performance that favored free choice fed steers. A similar economic analysis was not practical in out study, considering the duration of the study and small sample size utilized would not yield accurate economic results or interpretations. Moya et al. (2014) showed as well that beef steers that offered a free choice diet will have performance similar to those fed a TMR and also maintain a ruminal fermentation profile similar to those fed a TMR. Garrett et al. (2021a) showed that lambs fed a varied forage diet, had better performance compared to those fed a single forage. Also, in contrast with our study, Garrett et al. (2021a) found that feed efficiency was improved when lambs were offered a diverse diet compared to a single forage. However, CON lambs in our experiment were not offered a single dietary ingredient as in that study, but rather a pelleted feed designed to meet macro and micro-nutrient needs, which may explain the disparities between the two experiments. Overall, we observed there may be some performance benefit when feeding free choice diets, in agreement with other experiments. However, improvements in feed efficiency may be driven, in part, by the contrasting diets offered rather than solely by the feeding method.

Oxidative Stress

We examined OS in this study as there has been some research showing its negative impact on the overall performance of animals, although the direct causal link is still unclear

(Yuan et al. 2012; Wang et al. 2019; Salinas-Rios et al. 2015; Shibin et al. 2007). We hypothesized that providing diets free choice would reduce potential markers in oxidative stress relative to a TMR, by increasing the lamb's ability to select their individual diet (Beck and Gregorini, 2020). Beck and Gregorini (2020) stated that when animals are able to "design" their individual diet, there may be some benefit on oxidative stress status via several mechanisms including balancing their diet to meet their individual nutrient need (e.g., not overconsuming any particular nutrient). However, the results of our experiment do not support our original hypothesis for the markers of malondialdehyde and total antioxidant capacity. We observed no significant difference between the treatment groups for the variables that were measured. Malondialdehyde, a marker of lipid peroxidation, has been observed in previous studies to be elevated in lambs with body weights similar to our lambs when undergoing environmental stress (Wang et al., 2019), which might be expected in our study because lambs were housed in individual pens for an extended period of time. However, we observed no benefit of feeding method in altering MDA concentration. Garrett et al. (2022) found that total antioxidant status was improved when lambs were fed a diet of diverse forages compared to a solely ryegrass diet, again contrary to the results of our experiment. The type of diet offered for both the CON and FCD groups was markedly different between experiments, as was the production environment, which may in part explain the differences in the results between the studies.

Conclusion

Free choice feeding is a method that has been theorized as a way to improve performance and reduce the overall OS within ruminant animals. We observed that the feeding of a free choice diet to growing lambs yielded similar G:F and DMI compared to feeding a TMR. However, we did observe some positive impact with respect to ADG of the heavy block of FCD lambs compared to the heavy block of CON lambs. Additionally, we observed that lambs allowed to select their individual diet consumed more TDN than lambs fed a TMR. Furthermore, there was considerable variability across days and individual animals for ingredient preference. Although no benefit was observed on OS markers, the results of this experiment indicate that TMR feeding is not always the most biologically efficient feeding strategy, albeit logistically simpler. Further research is still needed to examine how free choice feeding varies across different diets and stages of productivity.

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Table 1 Diet composition of experimental diets

Ingredient (% of DM)	Mixed Ration, %	Alfalfa Pellets, %	Corn Pellets, %
Ground corn	46.25	---	90.25
Alfalfa pellets	28.00	90.25	---
Soybean meal, 44% CP	16.00	---	---
Liquid molasses	4.00	---	---
Calcium carbonate, 38% Ca	1.75	1.75	1.75
Ammonium chloride	1.00	1.00	1.00
Salt	2.50	2.50	2.50
Calcium phosphate dibasic, 18.5% P	0.25	0.25	0.25
Vitamin premix[i]	0.25	0.25	0.25

[i] Vitamin premix contained: Zinc Sulfate, Calcium Carbonate, Zinc Oxide, Manganese Sulfate, Sodium Molybdate, Mineral Oil, Ethylenediamine Dihydriodide, Sodium Selenite, Manganous Oxide, Ferrous Sulfate, Cobalt Sulfate.

Table 2 Ingredient composition of experimental diets

Ingredient[1] (% of DM)	Mixed Ration	Alfalfa Pellets	Corn Pellet
CP	20.8	21.8	10.5
NDF	15.7	41.4	10.2
ADF	12.1	32.2	3.6
TDN	82.0	59.0	88.0
DM (% as is)	80.6	70.9	78.0

[1]CP - Crude Protein, NDF – Neutral Detergent Fiber, ADF – Acid Detergent Fiber, TDN – Total Digestible Nutrients, DM- Dry Matter

Table 3 Animal Performance and Feed Efficiency

	CON		FCD		P-values			
Item	Light[i]	Heavy	Light	Heavy	SEM	Trt	Block	Trt × Block
No. observations (*n*)	7	6	4	6				
Alfalfa pellet intake (kg/hd/d)	---	---	0.74	0.78				
Corn pellet intake (kg/hd/d)	---	---	0.85	0.98				
DMI (kg/d)	1.52	1.58	1.59	1.76	0.08	0.24	0.34	0.61
ADG[ii] (kg/d)	0.199^{ab}	0.172^{a}	0.185^{ab}	0.230^{b}	0.02	0.09	0.80	0.01
F:G (kg/kg)	7.82	9.32	8.79	7.78	0.44	0.52	0.48	0.04
G:F (kg/kg)	0.13	0.11	0.12	0.13	0.01	0.50	0.63	0.04
CP intake (kg/d)	0.30	0.32	0.25	0.27	0.02	0.03	0.51	0.88
TDN intake (kg/d)	1.20	1.24	1.23	1.35	0.06	0.38	0.38	0.58
NDF intake (kg/d)	0.23^{a}	0.24^{a}	0.41^{b}	0.43^{b}	0.03	0.001	0.36	0.80
Daily intake variability[iii] (%)	18.1	16.8	20.2	21.4	2.20	0.26	0.77	0.71

[i]Light and heavy weight blocks based on initial body weight.

[ii]ADG = Average Daily Gain; DMI= Dry Matter Intake; F:G = Feed to Gain ratio; G:F = Gain to Feed ratio

[iii] Daily Intake Variability was calculated using the coefficient of variation in daily intake for each animal

a,b = means not bearing a common superscript differ, $P < 0.05$.

Table 4 Oxidative stress markers in plasma [i]

	CON		FCD			*P*-Value			
Item	d 0	d 42	d 0	d 42	SEM	Trt	Day	IBW[ii]	Trt × Day
AOC[i] (mM)	0.286	0.233	0.253	0.226	0.027	0.47	0.19	0.85	0.52
MDA (mM)	5.11	4.50	4.48	5.29	0.58	0.89	0.95	0.42	0.17

[i] AOC = Antioxidant Capacity, MDA = Malondialdehyde.

[ii] IBW = Initial Body Weight

Figure 1. Daily intake of each animal within FCD treatment

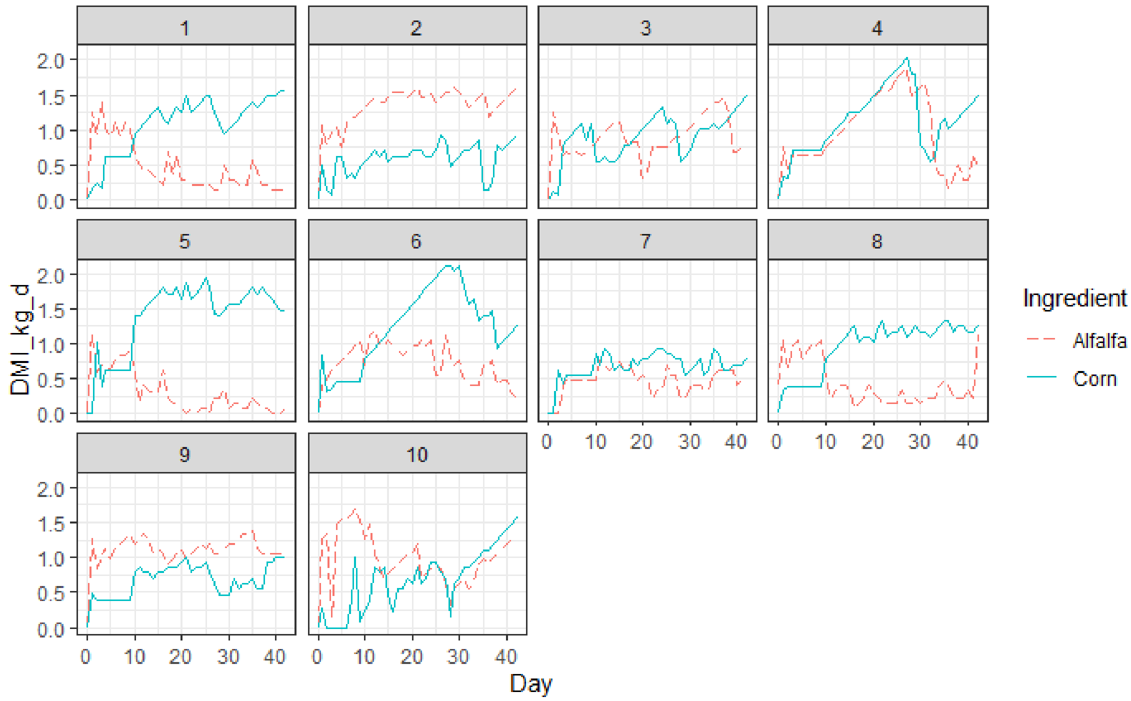
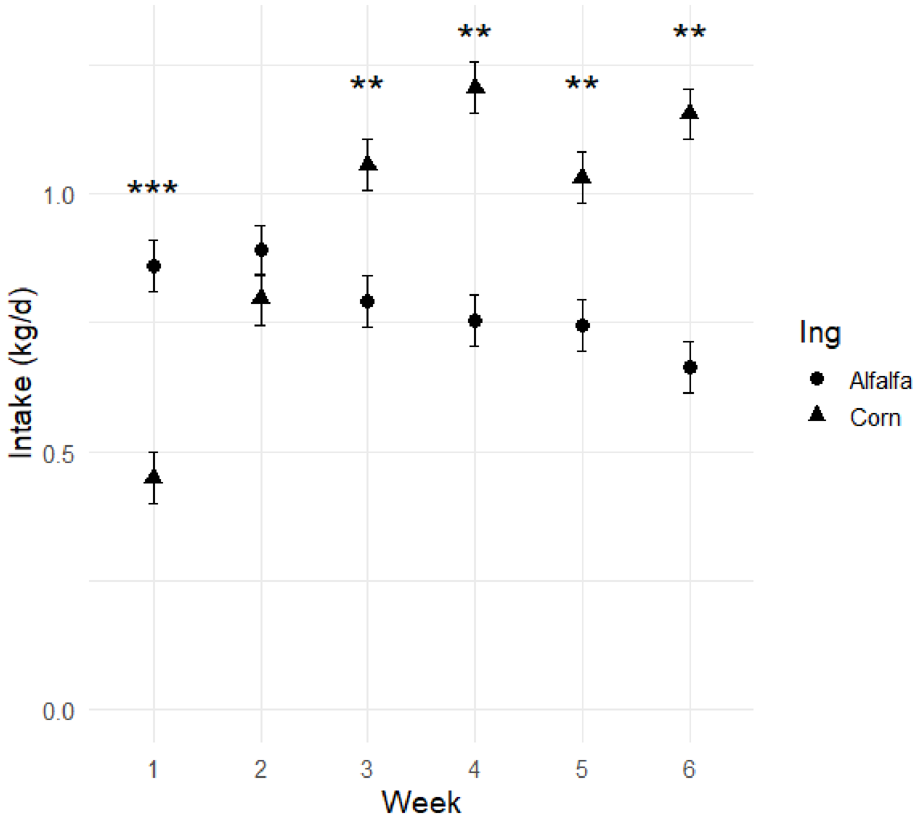


Figure 2. Ingredient consumption by week within FCD treatment[i]



[i] ** = $P < 0.01$ of difference between the intake of the specific ingredients of within week, points that have *** = $P < 0.001$ of difference of intake of those ingredients within week**.**