

THESIS

EVALUATION OF ROUGHAGE INCLUSION RATE WITHIN A TOTAL MIXED RATION
EFFECTS ON FEEDLOT PERFORMANCE, CARCASS CHARACTERISTICS, AND FATTY
ACID COMPOSITION OF SUBCUTANEOUS ADIPOSE, INTRAMUSCULAR ADIPOSE,
AND MUSCLE TISSUE OF WAGYU CATTLE

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ABSTRACT

EVALUATION OF ROUGHAGE INCLUSION RATE WITHIN A TOTAL MIXED RATION EFFECTS ON FEEDLOT PERFORMANCE, CARCASS CHARACTERISTICS, AND FATTY ACID COMPOSITION OF SUBCUTANEOUS ADIPOSE, INTRAMUSCULAR ADIPOSE, AND MUSCLE TISSUE OF WAGYU CATTLE

The objective of this study was to investigate the effect of roughage inclusion rate of 10, 20, and 30% within a total mixed ration on feedlot performance, carcass composition and marbling distribution in Wagyu cattle (n=41). Upon arrival, cattle were acclimated to bunk style feeding. Initial body weights were collected. Animals were blocked by sex and weight to be assigned to 1 of 3 roughage inclusion rate treatments. Treatments included 1) 10% roughage inclusion on dry matter basis; 2) 20% roughage inclusion and 3) 30% roughage inclusion on dry matter basis. Diets were formulated to be isoenergetic, isonitrogenous, and isoamylolytic. Animals were weighed every 7d. For harvest animals were transported to a commercial abattoir. Hot carcass weight (HCW) was determined at time of harvest, intramuscular adipose, and subcutaneous adipose tissue was collected at harvest and snap frozen for analysis of FA composition. Longissimus muscle area (LMA) and backfat (BF) carcass measurements were determined after 21d storage at 0° C chill. A 1.25 cm cross section of longissimus dorsi at 12th and 13th rib interface was collected for Computer Vision System Ribeye Camera evaluation of marbling distribution. Limited treatment effects were observed. Feedlot performance and carcass characteristics were not significantly affected by roughage inclusion rate ($P>0.05$). Treatment presented a significant effect on Linoleic acid (C18:3) concentration within the longissimus

muscle tissue ($P=0.05$). Concentration of C18:3 increased with increased roughage inclusion rate. Fatty acid profiles for LM, IMF and SQ tissues were similar across treatments. There was not treatment effect for marbling distribution collected via Computer Vision System Ribeye Camera evaluation. Overall, the data suggests that roughage inclusion rate causes very little impact on feedlot performance, carcass characteristics, fatty acid composition and marbling distribution in Wagyu cattle.

Key words: Fatty acid composition, Feedlot performance, Marbling distribution, Roughage inclusion, Wagyu

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CHAPTER I

Review Of Literature

History of the Wagyu Breed

Wagyu cattle were developed with the introduction of cattle across the Korean Peninsula into Japan during the second century (Mukai et al., 1989). Rice cultivation produced a need for draft power. Historically, Wagyu cattle fulfilled that need for draft power leading to selection pressure on strength and endurance. Currently, Wagyu cattle are raised to produce high quality beef with distinctive carcass characteristics that have set them apart from conventional beef breeds. Their ability to deposit greater amounts of marbling (Cameron et al., 1993) with increased concentration of unsaturated fatty acids (Oka et al., 2002) give Wagyu the potential to impact beef production worldwide. This breed has been traditionally raised on small farms in low stress environments; they have been fed a variety of diets and have been developed from a small set of sire lines.

The Wagyu breed transitioned from a source of draft power to beef production when Japanese military leaders fed soldiers beef to increase their strength (Imperial, 2015). Soldiers returned home after military service with an appetite for beef creating a conflict with community elders who upheld Buddhist religious beliefs and cultural standards outlawing beef consumption (Staley, 2004). Returning soldiers consumed beef in secret during fieldwork by heating plow shears over hot coals developing the cooking style of Sukiyaki or ‘plow cooking’ (Staley, 2004). Government efforts during the Meiji Restoration in 1868 finalized the transition of the Wagyu breed towards beef consumption by introducing Western food and habits to Japan (Reserve, 2015). Government encouragement of dairy and beef production influenced genetic modification

to the traditional Wagyu breed by introducing European breeds such as Simmental, Shorthorn, Ayrshire, Brown Swiss, South Devon and Korean genetics to improve upon production characteristics, but was halted in 1910, to create the closed breed found in Japan today (Reserve, 2015). Both the Meiji Restoration and the return of soldiers with an appetite for beef created new opportunities for Japanese agriculture and transitioned Wagyu away from beasts of burden into beef markets.

Japanese production systems are small farm oriented and encourage low stress handling over a long term feeding system. Two sectors make up Japanese beef production, the cow calf producers and feedlot-finishing sector (Hoque et al., 2006). Calves are marketed at eight to ten months of age at specific calf markets (Shojo et al., 2006). Buyers rely primarily on pedigree information to make buying decisions (Shojo et al., 2006). Feedlot operations range in size from less than five animals to more than 1000 (Hoque et al., 2006). Producers develop partnerships with other farms by trading manure as fertilizer for rice straw and harvesting pastures fed directly to cattle (JMAFF, 2007). Due to limited space, production practices are small but intensive (JMAFF, 2007).

The small operation size allows for most animals to be halter broke and daily handling to occur including brushing of the hair coat and individualized feeding (JMAFF, 2007). For the finishing phase, animals are housed in small group pens under well-ventilated barns for the duration of the finishing period (Lake, 2013). To be marketed as Japanese Wagyu all calves must be registered and receive a ten digit identification tag (Lake, 2013). This number remains with the animal for life and follows the carcass through fabrication to serve as source identification of individual cuts. All records are maintained through a centralized database to allow for

traceability and provide access to pedigree and registration information on all animals (Lake, 2013).

The Japanese feeding system is characterized by an extended feeding period with reduced growth rate in comparison to US feeding systems. Calves enter the finishing phase at 8-10 month of age and continue to be fed for 18-20 months (Nishimura et al., 1999). Age at harvest ranges from 24-30 months (Ozawa et al., 2000; Matsuashi et al., 2011). The average final harvest weight for Japanese raised Wagyu is 422 kg (Ozawa et al., 2000). The study conducted by May et al. (1993) matched Japanese feeding style allowing calves to gain 0.9kg/day for 552 days. The Japanese feeding style is also known for the high roughage content, often 25 percent or above (May et al., 1993).

The American Wagyu breed was developed from the import of four Wagyu bulls in 1976 that were crossed with Angus and Hereford females (Lunt et al., 1993a). Additional, purebred genetics entered the U.S. in 1993 and 1994 to total 35 head of imported cattle (Reserve, 2015). No more shipments of live animals were made following the reinstatement of the export ban. The American Wagyu Association, founded in 1990, has grown to include over 500 breeders throughout the US (American Wagyu Association, 2014). In 1990, 20% of Wagyu cattle in the US were considered purebred or full blood. By 2010, the proportion of Wagyu cattle in the US with purebred or full blood genetics increased to 85% (Wagyu International, 2013).

Live Animal Performance

Traditional feeding styles in Japan extend the finishing phase for Wagyu beef. This style contrasts feeding systems in the United States that utilize animals that gain quickly and efficiently. Lunt et al. (1993b) compared Angus and Wagyu calves fed a corn and barley based diet for 522 days. The Wagyu calves consumed 12.3 kg of feed for each kilogram of weight

gained while Angus calves consumed 11.1 kg of feed for each kilogram of weight gained resulting in a difference in feed to gain ratio of 1.2 kilogram of feed consumed for each kilogram of weight gained between the breeds (Lunt et al., 1993b). Shojo et al. (2005) compared conversion capabilities of Wagyu calves on varying diet compositions. Diets were high in roughage and included limited concentrates. Calves fed a forage only diet converted feed at a 4.25 kg/kg feed to gain ratio, while calves supplemented with concentrates converted feed at 2.80 kg/kg feed to gain (Shojo et al., 2005). Robinson et al. (2004) and Lunt et al. (2005) analyzed the effect of endpoint for conventional breeds and Wagyu on conversion capabilities. Angus, Shorthorn, Hereford, and Murray Grey had reduced conversion capabilities when fed to an extended endpoint, 16 months past weaning (Robinson et al., 2004). Specifically, cattle fed to the domestic endpoint, 8 months past weaning, converted feed to gain at 7.1 kg as fed/kg ratio while cattle fed to the Japanese endpoint converted at 11.2 kg as fed/kg feed to gain ratio. These numbers are in agreement with feed to gain ratio of 7.4 kg/kg for continental crossbreds reported by Mir et al. (1998).

Average daily gain values follow patterns similar to conversion ratio discussed previously. Mir et al. (1998) reported 1.52 kg/day gains for continental crossbred steers. Average daily gain decreased with increased Wagyu influence, F1 steers gained at a rate of 0.79 kg/day when fed a grain-based diet for 16 months Mir et al. (1998). Daily gain was increased from 0.49 kg/day to 1.04 kg/day for F1 cross calves on a deferred feeding regimen when fed a concentrate diet for the last 8 months of feeding (Barker et al., 1995). Lunt et al. (1993b) found Angus steers gained .9 kg/day and Wagyu steers gained 0.7 kg/day over a 522-day feeding period.

Long term feeding similar to Japanese production systems with slow growth rates, are not suited to the feeding style of American feedlots. Identifying ideal diet composition is essential to

producing Wagyu beef for American production systems. Studies focused on this topic compare concentrate and roughage diets fed for varying time periods and with both Angus and Wagyu calves. Forage feeding results in slower growth rates for both Angus and Wagyu compared to a concentrate diet. Lunt et al. (2005) reported average daily gain values of 1.25 kg/day for Angus and 1.03 kg/day for Wagyu on a short-term concentrate diet. Similar gain values of 0.78 kg/day for Angus and 0.73 kg/day for Wagyu were seen between the breeds when fed a hay-based diet for 20 months. More importantly feeding style alters the impact on carcass characteristics. More specifically 16 month concentrate feeding resulted in a 12 kg increase in cold left side carcass weight and increased dressing percentage for F1 American Wagyu and Angus cross steers fed a deferred eight month concentrate diet following eight month of forage based diet only (Barker et al., 1995). The deferred feeding style reported by Barker et al. (1995) produced carcasses with reduced incidence of reaching the highest quality and yield grades on the Japanese grading scale. This scale consists of both a yield grade and a quality grade. The yield grade is depicted as a letter A, B, or C with A indicating above average yield and C indicating below average yield (Staley, 2004). The second portion of the grading score indicates quality grade. Numbers 1 through 5 is used to represent a score of multiple factors including beef marbling score, color of lean and adipose tissue as well as the texture of the beef (Staley, 2004). A total of 19% of carcasses on the Barker et al. (1995) study reached above standard A yield grade and no carcasses reached quality grade of 4 or 5 on the Japanese scoring system (Barker et al., 1995).

Extending the feeding period from 12 to 20 months resulted in an increase of extractable lipid from 7.8 percent to 20.4 percent for Wagyu steers fed a forage-based diet (Lunt et al., 2005). Increased muscling was also seen for Wagyu steers fed over an extended time on forage-based diets (Lunt et al., 2005).

Carcass Quality and Composition

Carcass quality and composition are primary indicators of value for Wagyu beef in both American and Japanese markets. Quality grade and yield grade are influenced by factors such as fat content, distribution, and degree of muscling and how that impacts yield, and marbling characteristics.

Wagyu beef is known for its increased capacity to deposit intramuscular adipose or marbling. Beef marbling score (BMS) for Wagyu steers corresponds to total extractable ether. The Japanese BMS is a 12-point scale and Wagyu steers average marbling score of 6 (Ozawa et al., 2000). Beef Marbling Score of 1 corresponds to 5.1% extractable ether and BMS of 12 corresponds to 34.0% extractable ether. Lunt et al. (1993a) reported a significant difference in ether extract of totally trimmed rib eye steaks of 14.50% and 18.93% for Angus and Wagyu steers respectively. In agreement with Lunt et al. (1993a) May et al. (1993) reported a 4% increase in extractable ether for Wagyu steers over Angus steers. Fat content for purebred and crossbred steers allowed free access to a concentrate based diet increased gradually up to 20 months of age and then rapidly increased thereafter (Nishimura et al., 1999). For 32-month-old steers 18% crude fat was present (Nishimura et al., 1999). The ability for Wagyu to marble is beneficial to improving quality grades. For all breeds raised in the U.S. only 2-3% of carcasses grade Prime and have a 8.56% ether-extractable fat or greater (Cameron et al., 1993). On the other hand, 90.8% of Japanese raised Wagyu carcasses produced had a BMS of 2 or greater corresponding to 8.3% or more extractable ether (Cameron et al., 1994).

Diet composition affects carcass composition leading to differences in quality and yield grades. Marbling capabilities of Wagyu beef are diminished when fed concentrate diets, Lunt et al. (2005) reported 14.1% extractable lipid for steers fed 16 months on concentrate and 20.4%

extractable lipid for steers fed a forage-based diet for 20 months. The same study reported greater average daily gain was greater for concentrate diets regardless of time on feed or breed (Lunt et al., 2005). Concentrate feeding for 16 months did produce carcasses with greater marbling scores, meat color, brightness score, firmness, and fat luster compared to a deferred feeding method (Barker et al., 1995).

Subcutaneous adipose influences carcass yield and is often trimmed off in excess as not desired by consumers. Mir et al. (1989) report values of 7.7 mm subcutaneous fat thickness for continental crossbred steers. Crossbred steers fed to a final weight of 475 kg on a high concentrate or high roughage diet produced carcasses with 12.9 mm and 13.2 mm back fat respectively (Mears et al., 2001). American Wagyu fed to Japanese end point or 522 days on feed produced carcasses with 3.71 cm subcutaneous fat (Lunt et al., 1993b).

The degree of muscling as well as fat deposition dictate carcass yield. Hoque et al. (2006) reported lower carcass weights of 432 kg for Wagyu steers compared to 532 kg for continental crossbred steers. Barker et al. (1995) reported cold left side carcass weight of 218.3 kg for purebred Wagyu steers. Similarly, Lunt et al. (1993b) recorded yield estimates of 70.25 and 67.67 percent for Wagyu and Angus fed for 522 days respectively. Mir et al. (1998) reported yield percentages for continental crossbred steers of 60.3%.

Unique Attributes

Long term feeding styles used to finish Wagyu beef produce carcasses with excessive levels of external fat in Angus steers. Wagyu cattle are more likely to deposit large quantities of intramuscular fat without the increased level of external fat and are better adapted to long term feeding styles.

For conventional beef breeds, subcutaneous fat makes an increased contribution to total fat compared to intramuscular fat and body cavity fat as increased fattening occurs (Berg et al., 1979). Wagyu beef is characterized by their ability to marble without depositing excessive external fat (May et al., 1993). Xie et al. (1996a) compared North American Wagyu with Angus steers to determine differences in fat deposition patterns. Wagyu steers had higher marbling scores per centimeter of subcutaneous fat thickness and lower subcutaneous fat thickness per 100 kilograms of carcass weight than Angus steers (Xie et al., 1996a). Wagyu calves produced 1.56 cm subcutaneous fat thickness and Angus calves produced 1.96 cm subcutaneous fat (Xie et al., 1996a). The Wagyu breed may be able to make a positive contribution to North American gene pool due to their ability to deposit more intramuscular fat and less external fat.

Consumption of cholesterol, monounsaturated and saturated fatty acids are important for providing calories, essential fatty acids and transporting fat-soluble vitamins. Public concern about the impact of fat consumption and the impact on coronary heart disease has generated attention to consumption of specific fatty acids. Wagyu beef contains higher percentages of Palmitoleic Acid C16:1 and Oleic Acid C18:1 and lower percentages of Stearic Acid C18:0 and Palmitic Acid C16:0 than Angus steers (Elias Calles et al., 2000). May et al. (1993) reported significant differences between Angus and Wagyu steers of 6.34% C 16:1 in SQ fat and 4.79% C16:1 in IMF fat for Wagyu steers and 5.50% C16:1 in SQ fat and 3.76% C16:1 in IMF fat for Angus steers. Wagyu steers have 5.03% more C18:1 in SQ adipose and 5.44% more C18:1 in IMF fat than Angus steers (May et al., 1993). Angus beef contains more saturated fatty acid, than Wagyu beef. May et al. (1993) reported 30.15% SQ and 30.16% IMF C16:0 and 11.33% SQ and 13.81% IMF C 18:0 for Angus steers. Wagyu beef contained 26.68% SQ and 10.83% IMF C18:0 (May et al., 1993). Tissue type is also important to consider for fatty acid profile. Subcutaneous

adipose contained greater concentrations of Myristoleic Acid C14:1, C16:1 and C18:1 unsaturated fatty acids compared to intramuscular fat and kidney, pelvic, and heart fat (Oka et al., 2002). This is in agreement with Sturdivant et al. (1992) who reported 2.21% C14:1 in subcutaneous adipose tissue and 1.23% C14:1 in intramuscular tissue of ½ to 7/8 crossbred Wagyu steers. This in combination with the increased percentage of C18:1 and C16:1 in subcutaneous adipose tissue results in a greater monounsaturated fatty acid to saturated fatty acid MUFA:SFA ratio of 1.46 for subcutaneous adipose tissue (Sturdivant et al., 1992). Oka et al. (2002) reported a weak, negative, correlation of -0.22, -0.14, -0.09 between body weight and MUFA percentage in subcutaneous, intramuscular, and kidney, pelvic, and heart fat, respectively. A weak, positive, correlation of 0.22, 0.22, and 0.15 exists between wither height and saturated fatty acids in subcutaneous, intramuscular, and kidney, pelvic, and heart fat respectively (Oka et al., 2002).

Rational for Current Experiment

Commonly Wagyu are fed a 25% roughage diet (Lunt and Smith, 1993a) for periods of time often over 500 days (Lunt and Smith, 1993a). The increased maturity excludes high quality Wagyu beef from markets requiring younger age of harvest Specifically, Japan requires a less than 30 months of age for imported beef (FSIS, 2013). Few studies have focused on variation in time on feed and the impact on quality attributes Wagyu beef are known for. Time on feed could be altered by altering diet composition, starting calves on feed at earlier ages, or a combination of both.

Carcass quality is the primary factor of marketability for Wagyu beef. The unique sweet and fatty aroma due to high marbling creates the demand (Ueda et al., 2007). Carcass yield and development are different from conventional breeds such as Angus. Lunt et al. (2005) compared

growth and carcass traits of Wagyu steers and Angus steers on both an American concentrate based diet and Japanese forage based diet. Regardless of feeding style Wagyu calves produced lighter carcasses and exhibited lower gain rates (Lunt et al., 2005). Marbling score and quality grade were increased for Wagyu steers. Extractable lipid averaged at 20% for Wagyu steers and 14% for Angus steers allowing for more specific comparison of fat percentages (Lunt et al., 2005). One key difference between Wagyu and Angus is the time line of fat deposition. After 16 months of age, Angus steers stopped depositing intramuscular fat, but Wagyu steers did not exhibit a decrease in intramuscular fat deposition (Harris et al., 1995). Angus steers on long fed diets produced more subcutaneous fat and subsequently higher yield grades compared to long term fed Wagyu steers (Lunt et al., 2005).

In contrast to elevated fat deposition, Wagyu are lighter muscled compared to other breeds. Wagyu sired calves dressed 1.5% lower than Piedmontese-sired calves and overall produced 13% less retail cuts (Greenwood et al., 2006). Due to elevated fat content Wagyu beef does not experience as much cooking loss, but overall protein percentages are lower (Ueda et al., 2007).

The carcass characteristics compared between breeds are under genetic control. Heritability for average daily gain, carcass weight and ribeye area were higher for Wagyu breeds than conventional breeds. Hereford and Angus reported low heritability of 0.13 and 0.18 respectively for average daily gain (Hirooka et al., 1996). Robinson et al. (2004) compared gain rates to show that Japanese breeds gained the least daily along with Korean breeds and domestic market animals. Subcutaneous fat measurements and marbling measurement traditionally have a positive correlation for conventional breeds. Hirooka et al. (1996) reported correlation value of 0.38 for the relationship between subcutaneous fat and intramuscular fat measurements.

All information gathered will build data about the breed for further improvement. The composition of fatty acids impact on health benefits of beef along with improvement of flavor make this factor a strong selection component for the breed. Fatty acid heritability has been estimated between 0.31 and 0.73 (Ishi et al., 2013). Stronger selection decisions for desired fatty acids will magnify breed improvement.

The initial objective of this research consisted of correlating live animal ultrasound images to post mortem carcass evaluation. As a cost effective method for accurate prediction of carcass variables, live animal ultrasound would provide a valuable tool for identifying quality of Wagyu beef prior to harvest (Wilson et al., 1992). This objective was unsuccessfully achieved due to the fact that image quality decreased rapidly with increased body weight for the Wagyu calves on the study. Image clarity declined to a point that prevented the identification of anatomical landmarks required to accurately measure rib eye area, backfat thickness, and marbling percentage in a consistent and repeatable fashion. Unfortunately live animal ultrasound did not provide a tool for monitoring carcass growth or serve as a prediction tool.

Wagyu beef contains higher percentages of monounsaturated fatty acids C16:1 and C18:1 and lower percentages of saturated fatty acids than Angus steers (Elias Calles et al., 2000 and May et al., 1993). This has a positive influence on meat quality by lowering melting point of adipose tissue and increasing beefy flavor (Matsushashi et al., 2011). Identifying fatty acid content of Wagyu beef for this study is valuable to identify dietary effects as well as compare values to other breeds for purebred genetics. Previous studies conducted by Mir et al. (1999) and May et al. (2003) utilized crossbred genetics. Additionally the fatty acid profile of Wagyu beef may be valuable in identifying health attributes of the meat.

Little information is known about the effect of increased roughage inclusion on marbling distribution with Wagyu beef. Under traditional methods, Wagyu beef are fed high roughage diets for an extended period of time as described by Lunt et al. (2005). This experiment manipulates the roughage concentration of the diets in an effort to identify a threshold of maximum daily gain to potentially shorten days on feed without sacrificing carcass quality, as well as reduce age at harvest to increase access to markets with age restrictions.

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CHAPTER II

Feedlot performance of Wagyu cattle fed diets with 10, 20, and 30% roughage inclusion

Introduction

Improvement of feedlot performance in Wagyu cattle is key to achieving efficient production. To make improvements it is necessary to understand how Wagyu cattle compare to conventional feedlot breeds in feedlot performance and how variation in diets will effect feedlot performance for Wagyu steers and heifers through the measurement of body weight, dry matter intake, average daily gain, and gain to feed conversion.

Lunt et al. (1993) compared Angus and Wagyu calves fed a corn and barley based diet for 522 days. The Wagyu calves consumed 12.3 kg of feed for each kg of weight gained while Angus calves consumed 11.1 kg of feed for each kg of weight gained resulting in a difference in feed to gain ratio of 1.2 kg of feed consumed for each kg of weight gained between the breeds (Lunt et al., 1993). In addition to feed to gain, Lunt et al. (1993) reported values for average daily gain of 0.9 kg/head/day for Angus steers and 0.7 kg/head/day for Wagyu steers. Harvest weights were significantly different between the breeds. Angus steers weighed 713.4 kg at harvest compared to Wagyu steers weighing 648.6 kg at harvest.

Average daily gain varied by 0.07 kg/head/day between Piedmontese and Wagyu with Piedmontese gaining 1.70kg/head/day and Wagyu gaining 1.62 kg/head/day. Wagyu steers averaged 678 kg at harvest and Piedmontese steers were 14 kg heavier or 692 kg at harvest (Café et al., 2009).

Mir et al. (1998) compared conversion capabilities of cross bred steers with increased influence of Wagyu genetics fed over a two year period with increasing concentrate inclusion.

Feed to gain ratios increased and dry matter intake decreased with increasing Wagyu influence, but there was no difference between 75% Wagyu steers and cross-bred steers with no Wagyu influence (Mir et al., 1998). Differences were seen between crossbred steers and Wagyu cross steers for average daily gain, but there were no differences were observed with increasing Wagyu influence (Mir et al., 1998).

Lunt et al. (2005) and Gotoh et al. (2009) compared Wagyu cattle and other breeds over extended feeding period. Body weight and hot carcass weight increased linearly from 6 to 24 months regardless of breed when fed decreasing percentage of roughage (Gotoh et al., 2009). Angus steers had greater final weight than Wagyu steers regardless of forage or concentrate diet fed and Japanese or U.S. endpoint (Lunt et al. 2005). Wagyu fed decreasing roughage described by Gotoh et al. (2009) showed steady growth of Longissimus dorsi area up to 26 months but other muscles showed decreased growth after 14 months. For Wagyu and Angus steers fed to both Japanese and U.S. endpoints on either a high roughage or high concentrate diet described by Lunt et al. (2005). Wagyu steers had 20 % extractable lipid at the 20-month endpoint when fed hay while Angus steers had 12% extractable lipid. For the Angus steers intramuscular fat increased up to 16 months on feed while Wagyu steers continued to show increased intramuscular fat with increased time on feed past 16 months (Lunt et al., 2005).

Therefore, the objectives of this experiment were to determine the effect of roughage inclusion of 10%, 20% and 30% on body weight, average daily gain, dry matter intake and gain to feed conversion in full blood Wagyu steers and heifers.

Materials And Methods

Animals

Experimental procedures conducted with animals were approved by the Colorado State University Animal Care and Use Committee prior to initiation of the experiment. The experiment utilized 42, 24±3 m Wagyu calves from a single supplier. There were 12 heifers and 30 steers. All animals were provided by Emma Farms Wagyu (Colorado Wagyu Emma Farms Cattle Company, Walden CO), due to limited availability of feeder heifers, number of each sex are unbalanced. Upon arrival all calves were housed in a standard feedlot pen and fed a high forage starter ration to acclimate to bunk feeding. Calves were transitioned to treatment rations over 14 d. Calves were randomly distributed into three treatment groups, blocking by sex and BW and then randomly assigned to one of 7 treatment allotted 5x33 m feedlot pen in groups of 2 animals. The resulting design consisted of 3 treatment groups, and 21 pens, with each treatment consisting of 7 pens of 2 animals apiece. Calves were transitioned to treatment rations over a 14 d period.

Diets

Three total mixed rations (TMR) were formulated to contain 10, 20 and 30 percent roughage. The rations consisted of corn silage, alfalfa hay or grass hay, cracked corn, dry distillers grains, soybean meal, limestone, soybean oil, and molasses based liquid mineral supplement (Archer Daniels Midland Co., Chicago, IL). All rations were formulated to be isocaloric, isoamylolytic and isonitrogenous, and to meet or exceed NRC (2000) requirements for growing beef animals (NRC, 2000). Diet composition including ingredient and chemical composition is summarized in Table 2.1. Fatty acid composition of diets is summarized in Table 2.2. Total lipid were extracted according to the procedures of Folch et al. (1957) for composite samples of treatment rations collected weekly throughout entire feeding trial. Folch-extracted

samples were transmethylated according to the method of Park and Goins (1994) and evaporated on a rotary evaporator under a gentle stream of nitrogen at 25° C. Dried fatty acid methyl esters were reconstituted with 2µL of hexane and analyzed by an Agilent 6890 series gas chromatograph (Agilent Technologies, Santa Clara, CA) equipped with flame ionization detector and a 100 m x 0.25mm (i.d.), fused silica capillary column (SP-2560, 0.2µm film thickness, Supelco, Bellefonte, PA). A triacylglycerol of tridecanoic acid (13:0, 1.0mg) was used as the internal standard. Oven temperature was maintained at 175° C for 40 min, and then increased to 240° C at 10° C/min. Injector and flame-ionization detector temperatures were 245° C/min. Helium was the carrier gas at a split ratio of 50:1 and a constant flow rate of 0.8 mL/min. Fatty acid peaks were recorded and integrated using GC ChemStation software (version A.09.03, Agilent Technologies). Retention times were compared with known FAME standards to identify individual FA (Nu-Check Prep, Inc., Elysian, MN, and Matreya Inc., Pleasant Gap, PA). Trial was initiated on May 1, 2014 calves were fed once daily at 0800 with ad libitum access to water. Feed was initially offered at the daily rate of 15 kg (AF basis) per animal and quantity was adjusted daily until trace amounts of uneaten feed remained at time of subsequent feeding. Calves were weighed at 7d intervals and weighing was initiated at 0100. Feed refusals were measured at 7d intervals prior to feeding and weight collection.

Endpoint Selection

Projected finish weights were set based on literature review to 590 kg for heifers and 680 kg for steers. The first six calves were harvested and animal owners were not satisfied with marbling levels. The weight endpoint was eliminated and endpoint was determined to be a visual fat covering evaluation. Endpoint selection as based on visual evaluation of frame size, evenness of fat cover and weight by an owner representative. The change in endpoint caused an

unforeseen extension to the feeding component of the trial. Calves were consolidated due to lack of pen space into fewer pens on October 31, 2014. Final weights for the initial feeding phase were collected over a two-day period. Weight was first collected at d 6 of the weight interval and again at d 7 of the weight interval as normal. Final weights were determined by the average of these two weights. Calves remained within original treatment groups and sexes were not combined. Pens had no more than 4 head per pen after consolidation. Initial weights were collected for the second phase of feeding and 7 d interval weights continued to be collected along with feed refusals as conducted in phase 1. Calves remained on feed until January 30, 2015 to conclude the feeding trial. Weights were collected consecutively over two days and averaged to determine final weight. One heifer was removed from the study when she was discovered to be pregnant.

Statistical Analysis

Differences in feedlot performance were analyzed using the MIXED procedure in SAS (SAS institute Inc., Cary, NC) which fits generalized mixed models. Backward model selection was utilized to include all significant factors ($\alpha=0.05$). All significant factors were used in the final statistical model to analyze feedlot performance. Factors that were available to the model included treatment, sex, initial weight, initial age, and days on feed. Differences in body weight were analyzed using the REG procedure to calculate a predicted 168-day weight. This was conducted to account for variation in days on feed due to initial endpoint selection based on weight prior to modification of design. Values for average daily gain and gain to feed were calculated using results of regression analysis and further analyzed using the MIXED procedure. Feedlot performance data collected up to pen consolidation or 168 days on feed was utilized for analysis. Data collected for feedlot performance following pen consolidation was not analyzed

for presentation due to collapse of experimental unit and variation of harvest date within the consolidated pens.

Results And Discussion

This experiment was designed to explore the variation in feedlot performance in Wagyu cattle due to the alteration of roughage inclusion rate within a total mixed ration to 10%, 20% or 30%. Treatments were designed to maintain isocaloric, isonitrogenous, and isoamylolytic nature of the diet and allow for variation of roughage. All feedlot data corresponds to the 168 days on feed prior to pen consolidation.

The experimental data indicate that Wagyu feedlot performance including average daily gain, total body weight, dry matter intake and gain to feed conversion is not significantly affected by roughage inclusion rate.

Information regarding treatment means and variation are presented in Table 2.3 as an overview of the study. Feedlot performance statistics are presented in Table 2.4. Predicted initial weight and predicted 168d weight represent values calculated by regression of weekly weight collection during feeding trial. Actual initial weight varied between the treatments by 8.12 kg ($P=0.95$) and did not influence other weight factors. There was no significant difference in harvest weight ($P=0.27$). Predicted initial weight and predicted 168d weight represent estimated values of growth based on a regression of all weekly collected weights for 168 days on feed. Cattle continued to be on feed past 168 days, but consolidation of pens as well as variation in harvest date within consolidated pen confounded the analysis of weight gain. Predicted initial weight values are higher than actual initial weight but there were no significant differences between treatment groups ($P=0.66$). Treatment group did not result in significant variation for predicted 168 d weights ($P=0.46$). Numerically, ADG was lowest for 20% roughage inclusion

rate treatment at 0.92 kg/day and highest for 30% roughage inclusion rate treatment of 1.45 kg/day. Treatment groups did not significantly differ for ADG or dry matter intake ($P=0.53$, 0.35 respectively). Gain to feed ratio followed the same numeric pattern as ADG. The 20% roughage inclusion rate produced the lowest conversion ratio while 30% roughage inclusion rate produced the highest conversion ratio, although not significantly different ($P=0.50$). Total DMI for 168 days on feed was also not affected by treatment group ($P=0.27$).

Initial weights for the cattle on this study were heavier than initial weights reported by Barker et al. (1995), Lunt et al. (1993& 2005), and Mir et al. (1998). The listed studies utilized weaned calves of approximately 8-9 months of age. Initial age was much older 24 ± 3 m at the initiation of the feeding trial. Harvest weights for this study were comparable to the results presented by Ozawa et al. (2000) of 654 ± 6 kg for steers fed 75% concentrate and 25% roughage and Barker et al. (1995) of 671.18 ± 13 kg for steers continuously fed grain based diets. Conversely Mir et al. (1999) reported lower harvest weights of 415 kg for steers with increasing Wagyu influence fed 35% barley and 65% barley silage. This reduced final weight is a result of a selection for harvest at 460 kg. When compared to Angus cattle, Wagyu were lighter weight at harvest. Lunt et al. (2005) compared Angus and Wagyu steers at various days on feed as well as grain or forage based diets. Angus steers were consistently heavier (Lunt et al., 2005). Harvest weights are comparable to steers fed for 20 months on a forage based diet of 603.4 kg and 663.1kg for Wagyu and Angus steers respectively (Lunt et al., 2005). Few comparisons have been made with Angus cattle that focus on forage inclusion rate without other treatment factors to make comparisons to this study.

During the 168-day feeding trial treatment groups 10%, 20%, and 30% roughage gained 1.27, 0.92, and 1.45 kg/day ($P=0.53$) respectively. These results are in agreement with Mir et al.

(1998) who reported 0.9-1.2 kg/day ADG values for steers with increasing Wagyu influence. Conversely ADG values from this study were greater than values reported by Barker et al. (1995), Lunt et al. (2005), and Ozawa et al. (2000). Barker et al. (1995) reported ADG of 0.79 kg/day and Ozawa et al. (2000) reported ADG as low as 0.57 kg/day and an overall ADG of 0.71 kg/day. Average daily gain is only comparable to steers fed 8 months on a corn-based diet as reported by Lunt et al. (2005) of 1.03 kg/day. There is also a similarity between Angus steers within this same treatment group of 1.25 kg/day (Lunt et al., 2005). Average daily gains were reduced with increased time on feed regardless of diet (Lunt et al., 2005). In comparison to this current study, long fed Wagyu and Angus had reduced ADG values that presented in the current study.

Treatment did not have an effect on dry matter intake ($P=0.35$). The 10% roughage inclusion group consumed 9.98 kg/day, the 20% roughage inclusion group consumed the least per day at 9.38 kg/day and the 30% roughage inclusion group consumed 9.92 kg/day. Dry matter intake values were greater than values presented by Yamada et al. (2009) and Mir et al. (1998). Dry matter intake for steers of increasing Wagyu influence consumed 7.5 ± 2 kg/day (Mir et al., 1998). Dry matter intake varied from 7.74 kg/day, 4.73 kg/day, and 1.93 kg/day of silage and 4.97 kg/day, 5.86 kg/day, and 6.68 kg/day of concentrate for the high medium and low roughage diets, respectively (Yamada et al., 2009). Combining both concentrate and silage, steers consumed 12.71 kg/day, 10.56 kg/day, and 8.61 kg/day on the high medium and low roughage rations. Both the high and medium rations contain more forage than the current treatment formulations. The low roughage diet corresponds to the current study the most. Comparing the dry matter intake for the low roughage group of 8.61 kg/day to the DMI of the current study of 9.98 kg/day, 9.38 kg/day, and 9.92 kg/day shows a difference of $1.2 \pm .5$ kg/day between the two

studies (Yamada et al., 2009). Moore et al. (1990) compared crossbred steers fed 35% roughage of various sources. For steers fed alfalfa, the most comparable to the current study, DMI of 5.9 kg/day was observed. The large difference in DMI for Moore et al. (1990) and the current study can be attributed to the difference of 300kg in initial body weight.

Gain to feed ratio was the lowest for the 20% roughage group. Although not significantly different from the other treatment groups, the numerical decrease in conversion reflects the decreased average daily gain exhibited by the 20% roughage treatment. Gain to feed values were similar to values presented by Wertz et al. (2002) of 0.138 kg/kg for two year old Wagyu heifers and 0.157 kg/kg for Wagyu heifer calves. Angus conversion ratios are also similar at 0.130 kg/kg for two-year-old Angus heifers and 0.173 for Angus heifer calves (Wertz et al., 2002).

Implications

No significant differences were observed in feedlot performance for Wagyu cattle fed 10%, 20%, or 30% roughage within a total mixed ration. Cattle on this trial were older and all experienced similar background feeding prior to feedlot entry. More variation due to treatment may be apparent when fed to cattle started on feed at a younger age. Although not significant, average daily gain was numerically highest for 30% roughage inclusion rate at 1.45 kg/day. There was not a benefit of feeding any of the three treatment diets over the other. This can result in economic implications of feeding Wagyu cattle. Feeding the higher roughage diets without compromising feedlot performance can reduce feed costs.

Table 2.1. Ingredient and nutrient composition of treatment diets of 10, 20, and 30% roughage inclusion on dry matter basis fed to Wagyu cattle during finishing phase

Item	Treatment ¹		
	10%	20%	30%
Alfalfa hay, kg	-	15.00	25.00
Grass hay, kg	5.00	-	-
Corn silage, kg	10.00	9.80	10.00
Cracked corn, kg	49.80	46.62	43.77
Dry distillers grains, kg	27.20	17.29	-
Soy bean meal, kg	-	-	9.05
Soy bean oil, kg	2.00	4.60	5.50
Ground limestone, kg	1.00	1.00	1.00
Urea, kg	-	0.69	0.68
Mineral premix ² kg	5.00	5.00	5.00
Theoretical Composition			
EE, mg/kg	0.069	0.069	0.069
CP, %	14.04	14.00	14.00
ME, Mcal, kg	3.12	3.07	2.99
NEm, Mcal/kg	2.13	2.06	1.97
NEg, Mcal/kg	1.43	1.42	1.35
TDN, %	86.50	83.71	79.92
Chemical composition, DM basis			
CF, %	5.25	6.03	6.31
CP, %	14.88	14.80	15.89
ASH, %	2.77	4.25	4.12
DM, %	20.32	20.02	25.02

¹ 10%= roughage inclusion of 10% on dry matter basis; 20%= roughage inclusion of 20% on dry matter basis; 30%= roughage inclusion of 30% on dry matter basis

² 30% CP Hay Treat R400 (ADM Alliance Nutrition, Quincy, IL)

Table 2.2. Fatty acid composition of treatment diets of 10, 20, and 30% roughage inclusion fed to Wagyu cattle during finishing phase

Fatty Acid ^{1,3}	Common Name	Treatment ²		
		10%	20%	30%
C12:0	Lauric Acid	ND	ND	ND
C14:0	Myristic Acid	ND	ND	0.018
C16:0	Palmitic Acid	10.67	14.05	16.73
C16:1	Palmitoleic Acid	0.36	0.74	0.99
C18:0	Stearic Acid	1.79	2.55	3.20
C18:1	Oleic Acid	45.61	42.08	37.42
C18:2	Linoleic Acid	36.38	34.50	31.91
C18:3	Alpha-linoleic Acid	4.62	5.61	9.36
C20:0	Arachidic Acid	0.19	0.15	0.10
C20:1	Eicosenoic Acid	0.14	0.12	0.08
C22:0	Behenic Acid	0.06	0.04	0.05
C22:1	Erucic Acid	0.06	0.07	0.07
C24:0	Lignoceric Acid	0.14	0.11	0.09
C24:1	Nervonic Acid	ND	ND	ND

¹ Adjusted Diet Fatty Acid by weight percentage or g/100g.

² 10%= roughage inclusion of 10% on dry matter basis; 20%= roughage inclusion of 20% on dry matter basis; 30%= roughage inclusion of 30% on dry matter basis.

³ Analysis performed on composite sample of mixed feed samples collected throughout the feeding trial.

Table 2.3. Summary statistics of Wagyu cattle fed diets with 10, 20, and 30%, roughage inclusion during finishing phase

Item	Mean	SD	Min	Max
Initial d0 BW, kg	547.6	62.06	431.82	634.09
Harvest BW, kg	675.2	53.27	584.09	804.55
Predicted initial BW, kg ²	544.5	58.48	446.62	620.50
Predicted final BW, kg ²	753.0	169.15	596.30	954.79
Age at d0	720.8	76.96	594.00	794.00
Age at harvest, d	873.4	83.46	720.50	1036.00
Days on feed, d	130.4	54.64	33.00	168.00
Total DMI, kg ¹	1208.6	479.74	407.54	1797.04
ADG, kg/d ¹	1.2	0.86	0.43	4.66
G:F ¹	0.12	0.077	0.05	0.44
HCW, kg	425.6	32.90	371.36	487.95
Dressing percentage, %	62.2	1.40	59.38	65.34
Rib eye area, cm ²	102.1	8.93	77.40	114.49
Back fat thickness, cm	1.4	0.44	0.76	2.28
Calculated yield grade	3.5	0.61	2.72	4.71

¹ Values measured over first 168 d of feeding period

² Values calculated from regression analysis of weekly body weights

Table 2.4. Least square means of feedlot performance in Wagyu cattle during 168d feeding trial fed diets with 10, 20, and 30% roughage inclusion

Item	Treatment ¹			SEM	P-value ²
	10%	20%	30%		
No. of Pens	7	7	7		
No. of steers	10	10	10		
No. of heifers	4	3	4		
Days on feed	126.0	120.9	144.3	21.38	0.72
BW, kg					
Initial	523.4	531.5	528.2	18.99	0.95
Harvest	691.1	674.0	689.4	8.04	0.28
Predicted, d0 ³	546.1	547.3	540.0	5.96	0.66
Predicted, d168 ³	767.5	700.5	791.0	52.41	0.46
ADG, kg/d ³	1.27	0.93	1.46	0.332	0.53
DMI, kg/d ³	9.98	9.38	9.93	0.319	0.35
G:F ³	0.12	0.10	0.15	0.030	0.50

¹ 10%= roughage inclusion of 10% on dry matter basis; 20%= roughage inclusion of 20% on dry matter basis; 30%= roughage inclusion of 30% on dry matter basis

² Probability that treatment means are not different.

³ Values calculated from regression analysis of weekly body weights

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CHAPTER III

Carcass characteristics and fatty acid composition of subcutaneous adipose, intramuscular adipose and muscle tissue of Wagyu cattle fed diets with 10, 20, and 30% roughage inclusion

Introduction

Understanding dietary impacts on Wagyu carcass quality is necessary to modify production practices to maintain carcass quality. Carcass quality factors that are of importance are yield, quality grade, adipose distribution, as well as adipose composition.

Wagyu and Angus steers fed a corn and barley based diet for 522 days Lunt et al., (1993) reported significant decrease of 41.10 kg in hot carcass weight for Wagyu steers which was a direct reflection in the difference in final weight between Angus and Wagyu steers. No differences in other yield grade factors including adjusted fat thickness, rib eye area and kidney, pelvic and heart fat between the breeds were seen. Japanese grading standards include cold left side weight and a yield estimate. Angus steers produced larger carcasses than Wagyu, but yield estimate was lower than Wagyu (Lunt et al., 1993). Overall yield grade favored Wagyu steers based on the Japanese grading scale (Lunt et al., 1993). Barker et al., (1995) reported an increase in the dressing percentage for concentrate fed F1 Wagyu and Angus crossbred steers compared to roughage fed group. Live weight did not differ for the two feeding styles. The variation in dressing percentage was attributed to accelerated gastrointestinal growth (Barker et al., 1995). There were no differences in Japanese yield estimates between the two groups and rib eye area was only numerically greater for the linear fed group (Barker et al., 1995). Lunt et al. (2005) compared the impact of breed as well as diet and endpoint on carcass characteristics. Rib eye

area was significantly increased for both Wagyu and Angus steers fed either a hay based or concentrate diet to an extended endpoint of 16 or 20 months. Additionally, yield grade was increased for Angus steers fed to the extended endpoints of 16 and 20 months on feed compared to the shorter endpoints of 8 and 12 months on feed (Lunt et al., 2005).

Quality grade was also discussed by Lunt et al. (1993), Barker et al. (1995), and Lunt et al. (2005). For Wagyu and Angus steers fed to both US and Japanese endpoint a numerical increase in quality grade was seen for Angus steers at the shorter US endpoints (Lunt et al., 2005). This was reversed for the extended Japanese endpoint; Wagyu steers had numerically higher quality grades (Lunt et al., 2005). These results were in agreement with the previous study conducted by Lunt et al. (1993). Marbling score was numerically a full degree higher for Wagyu steers. The lack of significance was attributed to the high variation within the breed (Lunt et al., 1993). On the Japanese grading scale significant differences were seen for beef marbling score, texture score and firmness score. Wagyu expressed favorable values for all three factors compared to their Angus counterparts, resulting in better quality grades for the Wagyu steers (Lunt et al., 1993). When comparing feeding regimes Barker et al. (1995) presented significant increases in beef marbling score for linear fed steers than deferred fed steers of 4.74 and 3.30 respectively. Additionally, the linear fed group presented significantly increased values for all quality grade values on the Japanese grading system resulting in improved quality grades for linear fed Wagyu steers (Barker et al., 1995).

Lunt et al. (1993) reported that Wagyu were more likely to qualify for the highest marbling grade compared to Angus steers, but variation within the breed provides opportunities to select sire lines with increased propensity to deposit intramuscular adipose (Lunt et al., 1993). Wagyu continue to increase intramuscular adipose with continued time on feed compared to

Angus (Gotoh et al., 2009 and Lunt et al., 2005). Wagyu steers showed a 20% increase in IMF percentage within the longissimus dorsi over German Angus steers fed by Japanese standards for 26 months (Gotoh et al., 2009). Wagyu continued to increase intramuscular adipose past 16 months on feed while Angus steers no longer increased intramuscular adipose after 16 months on feed (Lunt et al., 2005). Marbling scores were not significantly different regardless of breed, diet or endpoint, but extractable lipid provided better illustration of the differences in intramuscular fat content between Angus and Wagyu steers. Hay fed Wagyu had 20% extractable lipid and hay fed Angus had 12 % extractable lipid when fed to the extended 20 months on feed (Lunt et al., 2005). In addition to the increased extractable lipid, Angus cattle deposited more subcutaneous adipose regardless of diet or endpoint when compared to Wagyu (Lunt et al., 2005). Barker et al. (1995) reported an increase in 6th rib fat thickness for concentrate fed Wagyu compared to grass fed Wagyu of 3.14 cm and 3.09 cm respectively.

Wagyu are known for significantly higher ratios of monounsaturated fatty acids (MUFA) to saturated fatty acids (SFA) than domestic breeds. This is valuable because increased consumption of MUFA paired with decreased consumption of SFA is believed to prevent increases and possibly decrease blood cholesterol (Boylston et al., 1995). Wagyu have higher lipid content than other breeds with consistently higher content of unsaturated fatty acid (Boylston et al., 1995). This is in agreement with Sturdivant et al. (1992) and May et al. (1993) who reported significant decreases in 16:0 and 18:0 fatty acids for Wagyu compared to Angus steers. The percentage of unsaturated fatty acids 14:1, 16:1, 18:1, and 18:12 were significantly higher compared to Angus steers (Boylston et al., 1995). May et al. (1993) compared fatty acid composition within tissue type. Wagyu consistently produced more 16:1 and 18:1 fatty acid for subcutaneous and intramuscular tissues compared to Angus and produced significantly less 18:0

and 16:0 fatty acids regardless of tissue type compared to Angus (May et al., 1993). For all tissue types the differences in fatty acids resulted in significant differences in MUFA:SFA ratio with Wagyu having consistently higher ratios (May et al., 1993). Although increased MUFA:SFA ratio is desirable for human nutrition, Wagyu have higher propensity to lay down adipose than other breeds (Boylston et al., 1995) and may create an issue with total fat as dietary recommendations suggest that less than 30%, but not less than 20%, of the dietary energy is derived from fat (Lee et al., 2001).

The majority of the research completed at this time has utilized crossbred Wagyu genetics ranging from F1 to $\frac{3}{4}$ and $\frac{7}{8}$ bloodlines. Research conducted with full blood Wagyu will be valuable to clarify variation presented in the literature for carcass characteristics. Little is known about the dietary effect of adipose deposit size. Gotoh et al. (2009) compared fleck size of marbling deposit between Wagyu and German Angus. This study reported 37.0 cm² size flecks for Wagyu and 6.7cm² size flecks for German Angus. There were no differences in the number of flecks between the breeds and distribution was not different for the breeds (Gotoh et al., 2009). Additionally Kuchida et al. (1999) compared image analysis to grader assigned beef marbling scores. Beef marbling standards assigned by examiners differed by -1 to +2 from the beef marbling score based on the marbling percentage estimated by image analysis (Kuchida et al., 1999). Image analysis provides an objective measurement to understand variation in adipose distribution as well as reduce grading error.

Therefore the objectives of this experiment were to determine the effect of roughage inclusion rates of 10, 20, and 30% on carcass quality characteristics and fatty acid composition of full blood Wagyu steers and heifers.

Materials And Methods

Animals

Experimental procedures conducted with animals were approved by the Colorado State University Animal Care and Use Committee prior to initiation of the experiment. The experiment utilized 42, 24±3 m Wagyu calves from a single supplier. There were 12 heifers and 30 steers. All animals were provided by Emma Farms Wagyu (Colorado Wagyu Emma Farms Cattle Company, Walden CO), due to limited availability of feeder heifers animal numbers are unbalanced. Upon arrival all calves were housed in a standard feedlot pen and fed a high forage starter ration to acclimate to bunk feeding. Calves were transitioned to treatment rations over 14 d. Calves were randomly distributed into three treatment groups, blocking by sex and BW and then randomly assigned to one of 7 treatment allotted 5x33 m feedlot pen in groups of 2 animals. The resulting design consisted of 3 treatment groups, and 21 pens, with each treatment consisting of 7 pens of 2 animals apiece. Calves were transitioned to treatment rations over a 14 d period.

Diets

Three total mixed rations (TMR) were formulated to contain 10, 20 and 30 percent roughage. The rations consisted of corn silage, alfalfa hay or grass hay, cracked corn, dry distillers grains, soybean meal, limestone, soybean oil, and molasses based liquid mineral supplement (Archer Daniels Midland Co., Chicago, IL). All rations were formulated to be isocaloric, isoamylolytic and isonitrogenous, and to meet or exceed NRC (2000) requirements for growing beef animals (NRC, 2000). Diet composition is summarized in Table 2.1. Fatty acid composition of diets is summarized in Table 2.2. Total lipid were extracted according to the procedures of Folch et al (1957) for composite samples of treatment rations collected weekly throughout entire feeding trial. Folch-extracted samples were transmethylated according to the

method of Park and Goins (1994) and evaporated on a rotary evaporator under a gentle stream of nitrogen at 25° C. Dried fatty acid methyl esters were reconstituted with 2µL of hexane and analyzed by an Agilent 6890 series gas chromatograph (Agilent Technologies, Santa Clara, CA) equipped with flame ionization detector and a 100 m x 0.25mm (i.d.), fused silica capillary column (SP-2560, 0.2µm film thickness, Supelco, Bellefonte, PA). A triacylglycerol of tridecanoic acid (13:0, 1.0 mg) was used as the internal standard. Oven temperature was maintained at 175° C for 40 min, and then increased to 240° C at 10° C/min. Injector and flame-ionization detector temperatures were 245° C/min. Helium was the carrier gas at a split ratio of 50:1 and a constant flow rate of 0.8 mL/min. Fatty acid peaks were recorded and integrated using GC ChemStation software (version A.09.03, Agilent Technologies). Retention times were compared with known FAME standards to identify individual FA (Nu-Check Prep, Inc., Elysian, MN, and Matreya Inc., Pleasant Gap, PA). Trial was initiated on May 1, 2014 calves were fed once daily at 0800 with ad libitum access to water. Feed was initially offered at the daily rate of 15kg (AF basis) per animal and quantity was adjusted daily until trace amounts of uneaten feed remained at time of subsequent feeding. Calves were weighed at 7 d intervals and weighing was initiated at 0100. Feed refusals were measured at 7 d intervals prior to feeding and weight collection.

Endpoint Selection

Projected finish weights were set based on literature review to 590 kg for heifers and 680 kg for steers. The first six calves were harvested and animal owners were not satisfied with marbling levels. The weight endpoint was eliminated and endpoint was determined to be a visual fat covering evaluation. Endpoint selection as based on visual evaluation of frame size, evenness of fat cover and weight by an owner representative. The change in endpoint caused an

unforeseen extension to the feeding component of the trial. Calves were consolidated due to lack of pen space into fewer pens on October 31, 2014. Final weights for the initial feeding phase were collected over a two-day period. Weight was first collected at d 6 of the weight interval and again at d 7 of the weight interval as normal. Final weights were determined by the average of these two weights. Calves remained within original treatment groups and sexes were not combined. Pens had no more than 4 head per pen after consolidation. Initial weights were collected for the second phase of feeding and 7 d interval weights continued to be collected along with feed refusals as conducted in phase 1. Calves remained on feed until January 30, 2015 to conclude the feeding trial. Weights were collected consecutively over two days and averaged to determine final weight. One heifer was removed from the study when she was discovered to be pregnant.

Tissue Collection and Processing

Calves were transported 55 km to a commercial packing company (Innovative Foods, LLC., Evans, CO) where they were humanely harvested. Adipose tissue samples were collected between ribs 12 and 13 immediately after the carcass was separated. Tissue samples were immediately dissected into subcutaneous adipose and intramuscular adipose. Samples were snap frozen in liquid nitrogen. Hot carcass weight was determined prior to cooler storage. After 21 d at 0°C carcasses were cross-sectioned between ribs 12 and 13; circumferences of LM were traced and BF measurements were made at the lateral three-quarter point over the LM. During fabrication process a 1.27cm cross section from the anterior face of the short loin was collected for imaging. Steaks were frozen on a sheet tray at -20°C for 18±6 h prior to vacuum packaging and stored at -20°C. All samples collected on the harvest floor were stored at -80°C.

Samples of adipose and muscle tissue were homogenized for nutrient analysis. Subcutaneous and composition samples were ground using a robot coupe blender. Sample was cut into 2x2 cm cubes and submerged in liquid nitrogen. During blending liquid nitrogen is added to blender at 45 s intervals to keep samples frozen. Intramuscular adipose samples were diced finely with scalpels to prevent loss of product. 1 g samples of subcutaneous and composition samples were oven dried at 100° C for 24 h. Moisture content of dried samples was determined by weight loss after cooling in a desiccation chamber at 25° C for 1 to 2 h. To determine ash content, 2 g samples of subcutaneous and composition samples were dried in muffle furnace at 600° C for 18h. After cooling for 30 to 45 m samples were transferred to desiccation chamber for 1 to 2 h. Percent ash was determined by weight loss after cooling as a percentage of initial weight. Total lipid were extracted with duplicate samples run every ten from subcutaneous adipose, intramuscular adipose, and composite samples according to the procedures of Folch et al (1957). Folch-extracted samples were transmethylated according to the method of Park and Goins (1994) and evaporated on a rotary evaporator under a gentle stream of nitrogen at 25° C. Dried fatty acid methyl esters were reconstituted with 2µL of hexane and analyzed by an Agilent 6890 series gas chromatograph (Agilent Technologies, Santa Clara, CA) equipped with flame ionization detector and a 100 m x 0.25mm (i.d.), fused silica capillary column (SP-2560, 0.2µm film thickness, Supelco, Bellefonte, PA). A triacylglycerol of tridecanoic acid (13:0, 1.0mg) was used as the internal standard. Oven temperature was maintained at 175° C for 40 min, and then increased to 240° C at 10° C/min. Injector and flame-ionization detector temperatures were 245° C/min. Helium was the carrier gas at a split ratio of 50:1 and a constant flow rate of 0.8 mL/min. Fatty acid peaks were recorded and integrated using GC ChemStation software (version A.09.03, Agilent Technologies). Retention times were

compared with known FAME standards to identify individual FA (Nu-Check Prep, Inc., Elysian, MN, and Matreya Inc., Pleasant Gap, PA).

Frozen steaks were stored at -20°C until all samples were collected for imaging. Prior to imaging, steaks were thawed at 0°C for 3 d within vacuum packaging. All steaks were removed from packaging 30 m before imaging to allow for color to bloom. Images were taken using Computer Vision System Ribeye Camera (Research Management Systems, Fort Collins, CO) to objectively measure rib eye area, shape, marbling percentage, fat thickness, and lean and fat color.

Statistical Analysis

Differences in carcass characteristics were analyzed using the MIXED procedure in SAS (SAS institute Inc., Cary, NC) which fits generalized mixed models. Backward selection was utilized to include all significant factors ($\alpha=0.05$) in the final statistical model to analyze carcass performance. Factors that were available to the model included treatment, sex, initial weight, initial age, and days on feed. Fatty acid analysis was performed with the MIXED procedure. Backward model selection was also conducted. Tissue type was also included as a factor for the final model. The final model consisted of treatment, tissue type, and the treatment \times tissue type interaction.

Results And Discussion

Experimental data indicates that carcass characteristics for Wagyu cattle are not significantly affected by roughage inclusion rate.

A summary of treatment means is presented in Table 3.1 as an overview of carcass characteristics as well as feedlot performance. Carcass characteristic statistics are presented in Table 3.2. Harvest weight was collected 12 \pm 3 h prior to transport to harvest. No differences

were seen between the treatment groups ($P=0.27$). Numerically, the 10% roughage inclusion treatment were the heaviest at 691.05 kg and 20% roughage inclusion treatment were the lightest to harvest at 673.95 kg. Hot carcass weight was no significantly different ($P=0.58$) between the treatment groups. This similarity is observed in dressing percentage as well. All treatment groups were in the low 60% for dressing percentage and no differences were seen ($P=0.77$). Back fat thickness as well as rib eye area was collected immediately following ribbing of the carcass and all measurements were collected from the right side. No differences were observed for back fat or rib eye area ($P=0.22, 0.14$, respectively). The 20% roughage inclusion treatment had the largest rib eye area of 107.15 cm. for back fat the 10% roughage treatment had the greatest fat coverage of 1.68cm.

Harvest weights for this study were similar to weights reported by Barker et al. (1995) and Yamada et al. (2009). In comparison to harvest weights presented by Lunt et al. (2005) there were only similarities between steers fed hay based ration for 20 months. Harvest weights were lighter for both Angus and Wagyu steers fed either corn or hay at shorter feeding lengths (Lunt et al., 2005). Mir et al. (1998) reported harvest weights of 450 kg for steers on feed 175 days. The feeding period is similar to the current study, but initial weights were also much lower than the current study. In comparison to Angus steers, harvest weights were lighter for the Wagyu steers of the current study (Gorocica-Buenfil and Loerch 2005; Quinn et al., 2011). This is in agreement with Lunt et al. (2005) who reported increased harvest weights for Angus steers compared to Wagyu steers regardless of diet type or time on feed.

Hot carcass weights varied from 416.32 kg to 424.94 kg for the current study. Hoque et al. (2006), Ozawa et al. (2000), and Xie et al. (1996b) reported carcass weights ranging from 409 kg to 431.63kg for Wagyu steers similar to values observed on the current study. In contrast,

Lunt et al. (2005), Mir et al. (1998), and Wertz et al. (2002) reported hot carcass weights ranging from 250 kg to 350 kg for Wagyu steers. Dressing percentages values were not reported by Lunt et al. (2005), but Mir et al. (1998) reported dressing percentages of 58.3% for 75% Wagyu steers. The 10% difference in dressing percentage between values reported by Mir et al. (1998) and the current study are a direct reflection of the differences in hot carcass weights reported by the two studies. Hot carcass weights for Angus steers reported by Gorocica-Buenfil and Loerch (2005) and Quinn et al. (2011) were lower than the weights of Wagyu steers on the current study. This is not in agreement with the data presented by Lunt et al. (2005). Angus steers consistently produced heavier carcasses than their Wagyu counterparts regardless of diet or time on feed (Lunt et al., 2005). Hot carcass weights were heavier for Wagyu heifer calves than their Wagyu counterparts, but were lower for two-year-old Wagyu heifers and two-year-old Angus heifers of same breeding style (Wertz et al., 2002).

No differences in dressing percentage were observed between treatment groups ($P=0.77$). Values are lower than values reported by Barker et al. (1995) of 65.05% for Wagyu and Angus cross steers. In comparison, steers with increasing Wagyu genetics had dressing percentage values of 57.6% for steers with 75% Wagyu influence (Mir et al., 1998). Dressing percentage values for Angus steers were similar (Gorocica-Buenfil and Loerch, 2005; Quinn et al., 2011; Sexten et al., 2012).

Twelfth rib back fat was not significantly different between treatment groups ($P=0.22$). Values ranged from 1.28 cm to 1.68 cm, with 10% roughage inclusion rate having the most and then back fat decreasing with increasing roughage inclusion rate. Similar values for back fat were presented by Xie et al. (1996a), Lunt et al. (2005), Oka et al. (2002), Mir et al. (1998), Xie et al. (1996b), and Ozawa et al. (2000). Lunt et al. (2005) and Ohsaki et al. (2009) reported back

fat values of 2.71 cm and 2.97 cm respectively. Description of the measurement location is unclear. The elevated measurements may be due to variation in grading practices of Japanese grading systems and American grading systems as Japanese measurements are collected at the 6th rib rather than the 12th rib. Back fat for Wagyu cattle on the current study are comparable to Angus back fat measurements of 1.5 cm, 1.38 cm, and 1.11 cm reported by Quinn et al. (2011), Gorocica-Buenfil and Loerch (2005), and Sexten et al. (2012). Lunt et al. (2005), Wertz et al. (2002) and Xie et al. (1996b) made direct comparisons between Wagyu and Angus carcass performance. Angus steers produced carcasses with greater back fat than Wagyu steers. Wertz et al. (2002) reported Angus heifers with 2.71 cm of back fat and Wagyu heifers with 1.90 cm of back fat. Similarly, Xie et al. (1996b) reported 1.96 cm back fat for Angus heifers and 1.56 cm back fat for Wagyu steers. Lunt et al. (2005) reported the greatest quantity of back fat for Angus steers fed corn-based diet for 16 months of 2.51 cm and 1.53 cm for Wagyu steers within the same group.

Rib eye area varied greatly within the literature for Wagyu cattle. Oka et al. (2002) and Ozawa et al. (2000) reported values of 42.1 cm² and 48.7 cm² for rib eye area respectively. Xie et al. (1996b) reported rib eye area of 101.4 cm². For the current study rib eye area ranged between 98.67 cm² and 107.15 cm². These values are similar to values presented by Wertz et al. (2002), Xie et al. (1996a), and Xie et al. (1996b) who reported rib eye area of 93.0 cm², 101.4 cm², and 95.5 cm² respectively. In comparison to Angus cattle rib eye area was larger for Wagyu cattle on the current study. Sexten et al. (2012) reported rib eye area of 79.74 cm² and Quinn et al. (2002) and Gorocica-Buenfil and Loerch (2005) reported similar values of 62 cm² rib eye area. Rib eye area was numerically greater for Wagyu compared to Angus (Wertz et al., 2002). For both Lunt et al. (1993) and Lunt et al. (2005) Angus had numerically greater rib eye area

compared to their Wagyu counterparts. The data from this study suggests that Wagyu have greater rib eye area than Angus cattle, but this is not supported by other literature comparisons of Wagyu and Angus cattle (Lunt et al., 1993; Lunt et al., 2005).

Fatty Acid Composition

The percent inclusion of C10-C24 fatty acids (FA) in i.m. adipose (IMF) and s.c. adipose (SQ) are quantified and compared (Table 4.6, and Table 4.7, respectively). Intramuscular FA composition did not differ between treatments. There is a tendency ($P=0.07$) for linolenic acid (C18:3) concentration to increase with increasing percentage of forage inclusion rate. Conjugated linoleic acid isomer C18:2t10c12 tends to increase with decreasing forage inclusion rate ($P=0.09$). No differences were detected in FA composition of SQ tissue. There was a tendency for linolenic acid (C18:3) concentration to be increased with decreasing forage inclusion ($P=0.08$).

Comparison of FA by treatment across tissue types is presented in Table 1.8. Intramuscular adipose contained the greatest concentration of Lauric acid (C12:0) ($P=0.05$). Subcutaneous adipose contained the greatest concentration of Lauroleic acid (C12:1) and the least Myristic acid (C14:0) ($P=0.05$ and 0.01 , respectively). Differences were seen between tissue types for Myristicoleic acid (C14:1) ($P=0.001$). Subcutaneous adipose contained the greater concentration than IMF. No differences were detected between SQ and IMF for Palmitic Acid (C16:0), Palmitoleic acid (C16:1), or $P=0.63$ and 0.38 , respectively). Intramuscular adipose contained a greater concentration of Heptadecanoic Acid (C17:0) than SQ ($P=0.001$). No differences were seen for Heptadecenoic Acid (C17:1), Stearic Acid (C18:0), or C18:1 t-6,8 ($P=0.45$, 0.05 , and 0.04 , respectively). Subcutaneous adipose contained the greatest concentration of Elaidate Acid (C18:1, t-9) ($P=0.04$), C18:1t-10 ($P=0.02$), and Vaccenic Acid

(C18:1 t11) ($P=0.03$). Intramuscular adipose contained the greatest concentration of Oleic Acid (C18:1c9) as well as *Cis*-Vaccenic Acid (C18:1c11) ($P=0.05$, 0.01, respectively). The greatest concentration of Linoleic Acid (C18:2) was found in IMF compared to SQ ($P=0.01$). No difference was detected between treatment groups for Linolenic acid (C18:3) ($P=0.06$).

Subcutaneous adipose contained the greatest concentration of both Conjugated Linoleic Isomers, C18:2c9t11 and C18:2t10c12, Eicosenoic Acid (C20:1), and Lignoceric Acid (C24:0) ($P=0.02$, 0.001, 0.04, and 0.05, respectively). No difference was detected in concentration of (C20:2), Lignoceric acid (C20:4), Eicosapentaenoic Acid (C20:5) and Docosahexaenoic Acid (C22:6) ($P>0.05$).

Mean concentrations of saturated FA (SFA), unsaturated FA (UFA), trans FA, as well as n-3 and n-6 FA are presented in Table 1.9. No differences were observed for SFA, UFA, MUFA, and Omega 3 fatty acids ($P>0.05$). Intramuscular adipose had greater concentration of PUFA and Omega 6 fatty acids ($P=0.05$). Subcutaneous adipose contained greater concentrations of trans fatty acids as well as conjugated linoleic acids ($P=0.03$ and 0.02, respectively). No differences were seen for ratios of MUFA and PUFA to SFA ($P>0.05$). Omega 6 to Omega 3 ratio was more favorable for SQ compared to IMF as the ratio was lower ($P=0.04$).

No significant treatment effects of fatty acid composition were seen for SQ and IMF. Treatment showed a tendency for Linolenic Acid C18:3. Few studies report concentration of C18:3 within skeletal muscle for Wagyu cattle. French et al. (2000) reported values of 0.71 g/100g C18:3 for grass fed crossbred steers. Leheska et al. (2008) also reported an increase in C18:3 concentration with grass fed beef. Concentration of C18:3 increased with increasing rate of roughage inclusion. Values of C18:3 are less than those reported by French et al. (2000), Leheska et al. (2008) and Nuernberg et al. (2005). Limited comparison is available for C18:3

within skeletal muscle. Oka et al. (2002) and Chung et al. (2006) compare C18:3 within SQ tissues and concentrations are similar. Concentrations of C14:0 and C18:0 are comparable to values presented by May et al. (1993), Elias Calles et al. (2002) and Xie et al. (1996a) for skeletal muscle. Concentration of C16:0 reported in the literature are elevated compared to concentrations within SM (Elias Calles et al., 2000; May et al., 1993; Xie et al., 1996a and 1996b). This pattern remains true for SQ and IMF concentrations of C14:0, C18:0, and C16:0. Oleic acid concentration is correlated to positive beef palatability and increased consumption of oleic acid can reduce risk factors for metabolic diseases in humans (Smith et al., 2006). Wagyu express elevated levels of Oleic acid compared to conventional feedlot breeds (May et al., 1993; Oka et al., 1992; Sturdivant et al., 1992). French et al. (2002) reported C18:1 concentrations of 39.74 g/100g for concentrate fed and 40.58 g/100g for grass fed crossbred steers. While May et al. (1993) reported values of 45.22%, 50.25% and 50.25% for SM, IMF, and SQ concentration of C18:1 for Wagyu cattle. Oleic acid concentrations presented are similar to reported Wagyu values and support the statement that Wagyu produce more oleic acid than conventional beef breeds. No differences were detected for Conjugated linoleic acid between treatment groups. De La Torre et al. (2006) reported CLA *c9,t11* concentrations of 0.77% for Charolais bulls fed 30% roughage. Alfaia et al. (2009) reported 81.34 mg/g for CLA *c9,t11* in Alentejano bulls. Grass fed crossbred steers expressed increased levels of CLA *c9,t11* compared to concentrate fed steers (French et al., 2000). CLA isomers are not delineated apart from C18:2 by Chung et al. (2006), Oka et al. (2002), and Ohaski et al. (2009). Concentrations of CLA *c9,t11* are comparable to values presented by French et al. (2000), Alfaia et al. (2009), and De La Torre et al. (2006). Linoleic acid concentrations are comparable to values presented for C18:2 undifferentiated by Chung et al. (2006), May et al. (1993), Ohaski et al. (2009), and Oka et al. (2002). Differences in

FA composition were significant by tissue type. Skeletal muscle contained the greatest concentration of SFA. This is in agreement with values presented by Ellias Calles et al. (2000) who reported SFA concentrations of 45.7% for longissimus dorsi muscle and 41.1% for SQ. Xie et al. (1996b) reported elevated levels of SFA within the longissimus dorsi muscle than SQ. Oka et al. (2002) reported values of 46.07% and 38.02% SFA within IMF and SQ respectively. Intramuscular fat was not significantly different from SQ and does not support findings of Oka et al. (2002). Monounsaturated ratios are greater for IMF and SQ than SM. This is supported by results presented by Ellias Calles et al. (2000) and Xie et al. (1996a). Oka et al. (2002) reported significantly greater concentrations of MUFA in SQ than IMF. Tissue type was statistically significant for total PUFA. Skeletal muscle contained greater concentration of PUFA than IMF and SQ. This is supported by results presented by Ellias Calles et al. (2000) and Xie et al. (1996a and 1996b). Oka et al. (2002) reported greater concentrations of PUFA in SQ compared to IMF and does not support findings of the current study that IMF contains greater PUFA. No differences were observed for MUFA: SFA ratio. Skeletal muscle expressed greater levels of MUFA and SFA than SQ and IMF. Proportions were not different for tissue types. Xie et al. (1996a) reported 1.40 and Xie et al. (1996b) reported 1.21 MUFA:SFA ratio for longissimus dorsi tissue. Chung et al. (2006) reported MUFA:SFA ratio of 1.41 and 1.18 of SQ for Wagyu and Angus steers respectively. MUFA:SFA ratios are greater than values reported in the literature. The ratio of PUFA: SFA is greatest for SM and not different for SQ and IMF. Xie et al. (1996a) reported PUFA:SFA ratio of 0.10 and 0.06 for longissimus dorsi muscle and SQ. Elias Calles also reported greater PUFA:SFA ratios for longissimus dorsi than SQ tissues.

Carcass Imaging

Comparisons of carcass characteristics obtained using Computer Vision System Cold Camera (CVS; Research Management Systems, USA, Inc., Fort Collins, CO) are presented in Table 3.10. Treatment did not account for significant variation in carcass characteristics except for ribeye area. The 20% roughage inclusion treatment group produced the largest rib eye area of 100.99 cm² followed by 30% roughage inclusion treatment at 94.83 cm² and 87.85 cm² for 10% roughage inclusion treatment (P=0.05). No differences were observed for ribeye length of rib eye width between the treatment groups (P=0.824, 0.164 respectively). Numerically, Marbling degree increased with roughage inclusion rate although not significantly (P=0.76). Distribution and size of marbling was not effected by treatment. The 10% roughage inclusion treatment had mean marbling size of 2.77 mm² and a maximum marbling size of 153.33 mm². The mean marbling size for 20% roughage inclusion was 2.57 mm² and maximum marbling size of 181.90 mm². Both mean marbling size and maximum marbling size was numerically greater for the 30% roughage inclusion treatment of 2.97 mm² and 231.62 mm² respectively. No differences were detected for values of distance between marbling deposits. The 10% treatment group had the numerically greatest distance between marbling while the 20% and 30% treatment groups were similar at 27mm. Mean distance between marbling was greatest for 20% treatment group at 4.57 mm (P=0.45). The 30% roughage inclusion treatment had the numerically lowest mean distance between marbling deposits of 4.06mm.

Limited information is available utilizing imaging technology to analyze carcass characteristics of Wagyu cattle. Comparisons with the Computer Vision System Cold Camera utilized for this study such as data presented by Cannell et al. (2002) and Vote et al. (2003) have been limited to validation of quality grade factors as well as comparison of image values to

eating experience such as tenderness. Gotoh et al. (2009) utilized a computerized image analysis system working with oil red stained samples of longissimus dorsi muscle. Digital images of carcass cross sections were taken between the 6th and 7th rib by Osawa et al. (2008) of Japanese black steers. Both studies compared marbling fleck size, the number of marbling flacks as well as distribution patterns within the muscle tissue. Osawa et al. (2008) describe distribution in terms of overall coarseness of marbling fleck and coarseness of maximum marbling fleck. Conclusions regarding the number of adipose cells as well as amount of adipose within each deposit can be made by comparing marbling flack number and size. Greater fat content is reflected by greater marbling fleck area as well as the proportion of marbling area within the ribeye area, but does no directly correlate to number of marbling flecks (Gotoh et al., 2009).

Consumers utilize marbling as an indicator of quality and nutritional value (Pena et al., 2013). Intramuscular fat content is moderately correlated with number of marbling flecks, but the proportion of the largest flecks doesn't correlate to IMF content allowing for greater IMF contents to be reached without being negatively perceived by consumers (Pena et al., 2013).

Values of ribeye area varied greatly within the literature for Wagyu cattle depending on the system of measurement used. Oka et al. (2000) reported values of 42.1 cm² and 48.7 cm² for rib eye area respectively. Xie et al. (1996b) reported ribeye area of 101.4 cm². Rib eye area acquired from image analysis for the current study range between 87.85 cm² and 100.99 cm². These values presented y Wertz et al. (2002), Xie et al. (1996a) and Xie et al. (1996b) who reported ribeye area of 93.0 cm², 101.4 cm², and 95.5 cm² respectively. In comparison to Angus, cattle rib eye measurements are greater for Wagyu cattle. This is in agreement with data presented by Wertz et al. (2002), Lunt et al. (1993) and Lunt et al. (2005). Length and width measurements for ribeye area are not commonly discussed when describing ribeye area or

dimensions. Discussion of image analysis of Wagyu cattle is limited within the literature. Factors such as visible lean area and visible fat area are not discussed. Marbling degree is often assessed through the assignment of USDA quality grades by Japanese quality grades or by Japanese quality grade standards. Xie et al. (1996a) reported marbling score of 538.2 for Wagyu compared to 478.2 for Angus steers. In comparison, days on feed caused an increase in marbling score for Wagyu steers from 450.7 to 458.5 as reported by Xie et al. (1996b). Wagyu carcasses are also graded based on Japanese grading standards. Limited comparisons can be made for marbling score between the U.S. system and Japanese system. Mir et al. (1998) reported increased marbling score with increased Wagyu genetics. Similar results are presented by Ozawa et al. (2000) who reported beef marbling score values ranging from 5.4 to 10.2 on the Japanese scale. Wagyu cattle produced greater marbling scores than their Angus counterparts. Lunt et al. (2005) reported marbling scores of 612.5 to 897.5 for Wagyu steers. This is in agreement with values observed with the current study.

Imaging allows for in depth description of marbling distribution including size of marbling flecks, as well as distance between flecks. No differences were observed for distribution characteristics. Numerically maximum marbling fleck size increased with the increase of roughage inclusion and maximum distance between flecks decreased with roughage inclusion. Data presented by Gotoh et al. (2009) discussed marbling fleck area, proportion of area accounted for by marbling and the total number of flecks. Wagyu steers had the greatest marbling fleck area of 37.0 cm² compared to Belgian Blue at 1.9cm² and German Angus at 6.7cm². The marbling present accounted for 35.8% of ribeye area for Wagyu cattle and 6.1% in Angus cattle (Gotoh et al., 2009). Although Wagyu had the greatest intramuscular fat content over Angus, Belgian Blue, and Holsteins, they had fewer number of marbling flecks (Gotoh et

al., 2009). The difference in fat content was accounted for by larger fleck area rather than a greater number of flecks and may be a consequence of fusion of marbling flecks as fat deposition increased (Gotoh et al., 2009). Osawa et al. (2008) discussed marbling distribution in terms of overall coarseness of marbling particles as well as fat area ratios. Muscle area of longissimus muscle was much smaller at 52.3 cm² than values reported within the current study. Fat area ratio was reported at 39.3% (Osawa et al., 2008). Overall coarseness of marbling particles index was 19.6 and coarseness of maximum marbling particle index was 4.8 (Osawa et al., 2008). In comparison to other breeds marbling fleck size was greater for the present study. Albrecht et al. (2006) reported marbling fleck size of 1.59mm² for Angus, 1.31 mm² for Galloway, and 1.32 mm² for Holstein-Friesian at 24 months of age. Marbling fleck size increased with age for all breeds (Albecht et al., 2006). The Wagyu steers were 24 months of age at initiation of the feeding trial and may explain the increase in marbling fleck size. Number of marbling flecks for Angus, Belgian Blue, and Holstein are similar between Gotoh et al. (2009) and Albrecht et al. (2006), but proportion of marbling fleck area percentage values were lower for all breeds than values presented by Gotoh et al. (2009).

Implications

Minor differences were observed in carcass characteristics, fatty acid composition or skeletal muscle, subcutaneous adipose and intramuscular adipose, or image analysis of carcass characteristics. No differences were observed in carcass weight, backfat thickness, dressing percentage or yield grade. Linoleic acid concentration was decreased in 10% roughage inclusion rate treatment, but no difference was observed between the 20% and 30% roughage treatments. The diet analysis shows a stepwise increase in linoleic acid of 4.619 g/100g for 10% roughage, 5.611 g/100g for 20% roughage and 9.355g/100g for 30% roughage concentrations within the

feed. The variation in availability of the Linoleic fatty acid follows the same pattern observed within the tissues of increasing linoleic acid concentration with increasing roughage inclusion. The elevated levels of linoleic acid within the diet allow for increased integration within the tissue. Concentrations of reported fatty acids were comparable to reported figures and support the claim that Wagyu produce greater concentrations of oleic acid which can have positive effect on reducing metabolic disease risk factors in humans (Smith et al., 2006). Ratios of saturation of fatty acid are in agreement with values reported by Elias Calles et al. (2000), Xie et al. (1996a), Oka et al. (2002), and Chung et al. (2006). The limited effect of treatment on fatty acid composition does not highlight benefits or disadvantages of roughage inclusion rate within the diet for modifying fatty acid composition. Roughage inclusion rate resulted in no treatment effects for carcass imaging characteristics involving marbling amount, distribution or size of marbling flecks. Ribeye area measured via carcass imaging was affected by treatment, but did not follow a pattern regarding roughage rate inclusion. Ribeye values obtained from carcass imaging do agree with values presented by Xie et al. (1996a and 1996b), Lunt et al. (1993 and 2005), and Wertz et al. (2002) and supports the statement that rib eye area is greater for Wagyu cattle than Angus cattle when fed for extended periods of time (Lunt et al., 1993). No discernable treatment effects were observed for marbling amount, distribution, and fleck size. Consumers infer taste, tenderness, juiciness, and nutrition from intrinsic and extrinsic factors of color, size, visible leanness, and price and labeling (McIlveen and Buchanan, 2001). Grunert et al. (1997) concluded that fat content and color are most important to quality evaluation by customers, but consumers avoid obvious fat due to perceptions of reduced nutritional value (Chambers and Bowers, 1993). Number of marbling flecks is moderately correlated to IMF content, but large particles do not serve as a good representation of IMF content (Pena et al., 2013). Finer marbling

fleck size allows for increased marbling content within the muscle tissue without being identified by consumers as a non-desirable product (Chambers and Bowers, 1993). Although no treatment differences were observed, marbling degree values reached prime quality grades and support that Wagyu are known for marbling ability and are more likely to reach highest quality than Angus cattle (Lunt et al., 1993). Marbling fleck size, although not effected by treatment was greater than values reported by Albecht et al. (2006) and Osawa et al. (2008). The increased coarseness may be due to fusion of marbling flecks (Gotoh et al., 2009) and may be perceived negatively by consumers (McIlveen and Buchanan, 2001). Treatment groups did not identify a superior roughage inclusion rate for carcass characteristics, fatty acid composition or marbling distribution.

Table 3.1. Ingredient and nutrient composition of treatment diets of 10, 20, and 30% roughage inclusion on dry matter basis fed to Wagyu cattle during finishing phase

Item	Treatment ¹		
	10%	20%	30%
Alfalfa hay, kg	-	15.00	25.00
Grass hay, kg	5.00	-	-
Corn silage, kg	10.00	9.80	10.00
Cracked corn, kg	49.80	46.62	43.77
Dry distillers grains, kg	27.20	17.29	-
Soy bean meal, kg	-	-	9.05
Soy bean oil, kg	2.00	4.60	5.50
Ground limestone, kg	1.00	1.00	1.00
Urea, kg	-	0.69	0.68
Mineral premix ² ,kg	5.00	5.00	5.00
Theoretical Composition			
EE, mg/kg	0.069	0.069	0.069
CP, %	14.04	14.00	14.00
ME, Mcal/kg	3.12	3.07	2.99
NEm, Mcal/kg	2.13	2.06	1.97
NEg, Mcal/kg	1.43	1.42	1.35
TDN, %	86.50	83.71	79.92
Chemical composition, DM basis			
CF, %	5.25	6.03	6.31
CP, %	14.88	14.80	15.89
ASH, %	2.77	4.25	4.12
DM, %	20.32	20.02	25.02

¹ 10%= roughage inclusion of 10% on dry matter basis; 20%= roughage inclusion of 20% on dry matter basis; 30%= roughage inclusion of 30% on dry matter basis

² 30% CP Hay Treat R400 (ADM Alliance Nutrition, Quincy, IL)

Table 3.2 Fatty acid composition of treatment diets of 10, 20, and 30% roughage inclusion fed to Wagyu Cattle during finishing period

Fatty Acid ^{1,3}	Common Name	Treatment ²		
		10%	20%	30%
C12:0	Lauric Acid	ND	ND	ND
C14:0	Myristic Acid	ND	ND	0.018
C16:0	Palmitic Acid	10.67	14.05	16.73
C16:1	Palmitoleic Acid	0.36	0.74	0.99
C18:0	Stearic Acid	1.79	2.55	3.20
C18:1	Oleic Acid	45.61	42.08	37.42
C18:2	Linoleic Acid	36.38	34.50	31.91
C18:3	Alpha-linoleic Acid	4.62	5.61	9.36
C20:0	Arachidic Acid	0.19	0.15	0.10
C20:1	Eicosenoic Acid	0.14	0.12	0.08
C22:0	Behenic Acid	0.06	0.04	0.05
C22:1	Erucic Acid	0.06	0.07	0.07
C24:0	Lignoceric Acid	0.14	0.11	0.09
C24:1	Nervonic Acid	ND	ND	ND

¹ Adjusted diet fatty acid by weight percentage or g/100g.

² 10%= roughage inclusion of 10% on dry matter basis; 20%= roughage inclusion of 20% on dry matter basis; 30%= roughage inclusion of 30% on dry matter basis.

³ Analysis performed on composite sample of mixed feed samples collected throughout the feeding trial.

ND = Not detected

Table 3.3. Summary statistics of Wagyu cattle fed diets with 10, 20, and 30% roughage inclusion during finishing phase

Item	Mean	SD	Min	Max
Initial d0 BW, kg	547.6	62.06	431.82	634.09
Harvest BW, kg	675.2	53.27	584.09	804.55
Predicted initial BW, kg ²	544.5	58.48	446.62	620.50
Predicted final BW, kg ²	753.0	169.15	596.30	954.79
Age at d0	720.8	76.96	594.00	794.00
Age at harvest, d	873.4	83.46	720.50	1036.00
Days on feed, d	130.4	54.64	33.00	168.00
Total DMI, kg ¹	1208.6	479.74	407.54	1797.04
ADG, kg/d ¹	1.2	0.86	0.43	4.66
G:F ¹	0.12	0.077	0.05	0.44
HCW, kg	425.6	32.90	371.36	487.95
Dressing percentage, %	62.2	1.40	59.38	65.34
Rib eye area, cm ²	102.1	8.93	77.40	114.49
Back fat thickness, cm	1.4	0.44	0.76	2.28
Calculated yield grade	3.5	0.61	2.72	4.71

¹ Values measured over first 168 d of feeding period

² Values calculated from regression analysis of weekly body weights

Table 3.4 Least square means of carcass characteristics of Wagyu cattle fed diets with 10, 20, and 30% roughage inclusion during finishing phase

Item	Treatment ¹			SEM	P-value ²
	10%	20%	30%		
No. of Pens	6	7	6		
No. of steers	8	10	8		
No. of heifers	4	2	4		
Days on feed	117.80	170.14	154.00	38.40	0.58
Initial Wt., kg	523.3	531.5	528.2	18.99	0.95
Harvest Wt., kg	691.1	674.0	689.4	8.04	0.28
HCW, kg	424.9	416.3	417.1	7.23	0.58
Dressing %	61.91	61.82	61.43	0.580	0.77
Back fat thickness, cm	1.69	1.52	1.28	0.174	0.22
Ribeye area, cm ²	98.67	107.15	99.13	3.265	0.11
Calculated yield grade	3.98	3.27	3.45	0.239	0.11

¹ 10%= roughage inclusion of 10% on dry matter basis; 20%= roughage inclusion of 20% on dry matter basis; 30%= roughage inclusion of 30% on dry matter basis

² Probability that treatment means are not different.

Table 3.6 Influence of 10, 20, and 30% roughage inclusion diets fed during finishing phase on subcutaneous adipose tissue fatty acid composition of Wagyu cattle

Item ²	Common Name	Treatment ¹			SEM	P-value ³
		10%	20%	30%		
C10:0	Capric Acid	0.042	0.041	0.042	0.011	0.54
C12:0	Lauric Acid	0.078	0.075	0.081	0.001	0.12
C12:1		0.043	0.04	0.044	0.003	0.62
C14:0	Myristic Acid	2.12	2.28	2.29	0.11	0.78
C14:1	Myristicoleic Acid	1.54	1.57	1.6	0.13	0.51
C16:0	Palmitic Acid	20.67	20.79	21.24	0.32	0.71
C16:1	Palmitoleic Acid	6.25	6.29	6.45	0.22	0.62
C17:0	Heptadecanoic Acid	1.15	1.14	1.19	0.11	0.82
C17:1	Heptadecenoic Acid	1.11	1.09	1.13	0.06	0.67
C18:0	Stearic Acid	8.81	7.78	7.76	0.62	0.14
C18:1 t-9	Elaidate Acid	0.122	0.116	0.109	0.008	0.48
C18:1 t-10		0.418	0.415	0.428	0.09	0.48
C18:1 t11	Vaccenic Acid	2.31	2.27	2.4	0.16	0.58
C18:1 t-12		3.24	3.63	3.25	0.21	0.57
C18:1c9	Oleic Acid	47.74	48.2	47.57	0.93	0.74
C18:1c11	<i>Cis</i> -Vaccenic Acid	1.91	1.93	1.93	0.02	0.81
C18:2	Linoleic Acid	1.14	1.09	1.19	0.07	0.63
C18:3	Linolenic Acid	0.157	0.146	0.144	0.004	0.08
C20:0	Arachidic Acid	0.046	0.045	0.047	0.002	0.64
C18:2c9t11	CLA4- Rumenic Acid	0.478	0.471	0.494	0.012	0.68
C18:2t10c12	CLA4	0.064	0.062	0.068	0.001	0.38
C20:1	Eicosenoic Acid	0.239	0.236	0.247	0.04	0.72
C20:2	Eicosadienoic Acid	0.01	0.011	0.011	0.001	0.91
C20:4	Eicosatetraenoic Acid	0.109	0.108	0.112	0.001	0.87
C20:5	Eicosapentaenoic Acid	ND ^a	ND	ND	---	---
C24:0	Lignoceric Acid	0.036	0.034	0.04	0.008	0.68
C22:6	Docosahexaenoic Acid	ND	ND	ND	---	---

¹ 10%= roughage inclusion of 10% on dry matter basis; 20%= roughage inclusion of 20% on dry matter basis; 30%= roughage inclusion of 30% on dry matter basis.

² Adjusted Diet Fatty Acid by weight percentage or g/100g.

³ Probability that treatment means are not different.

⁴ Conjugated Linoleic Acid

^aND= not detected.

Table 3.7 Influence of 10, 20, and 30% roughage inclusion diets fed during finishing phase on fatty acid composition by treatment and tissue type in Wagyu cattle

Item	Treatment			SEM(trt)	<i>P</i> <		
	10%	20%	30%		Trt	Tissue	Trt x Tissue
C10:0					0.26	0.14	0.76
IMF	0.05	0.05	0.05	0.009			
SQ	0.04	0.04	0.04	0.011			
C12:0					0.64	0.04	0.25
IMF	0.10	0.10	0.09	0.002		*	
SQ	0.08	0.08	0.08	0.001		**	
C12:1					0.73	0.05	0.38
IMF	0.04	0.03	0.03	0.003		*	
SQ	0.04	0.04	0.04	0.003		**	
C14:0					0.26	0.01	0.11
IMF	2.53	2.12	2.29	0.150		*	
SQ	2.12	2.28	2.29	0.110		**	
C14:1					0.12	0.001	0.78
IMF	1.23	1.33	1.30	0.110		*	
SQ	1.54	1.57	1.60	0.130		**	
C16:0					0.46	0.63	0.44
IMF	20.62	20.45	20.71	0.340			
SQ	20.67	20.79	21.24	0.320			
C16:1					0.84	0.38	0.61
IMF	5.95	6.17	6.07	0.180			
SQ	6.25	6.29	6.45	0.220			
C17:0					0.84	0.0001	0.76
IMF	1.44	1.38	1.45	0.120		*	
SQ	1.15	1.14	1.19	0.110		**	
C17:1					0.64	0.45	0.76
IMF	1.11	1.09	1.10	0.040			
SQ	1.11	1.09	1.13	0.060			
C18:0					0.50	0.29	0.09
IMF	7.90	7.81	7.89	0.580			
SQ	8.81	7.78	7.76	0.620			
C18:1 t-6,8					0.45	0.35	0.24
IMF	0.11	0.10	0.11	0.008			
SQ	0.12	0.12	0.11	0.008			
C18:1 t-9					0.24	0.04	0.37
IMF	0.39	0.37	0.41	0.020		*	
SQ	0.42	0.42	0.43	0.090		**	
C18:1 t11					0.17	0.03	0.47
IMF	1.76	1.72	1.75	0.140		*	
SQ	2.31	2.27	2.40	0.160		**	
C18:1 t-10					0.46	0.02	0.3
IMF	2.79	2.50	2.67	0.210		*	
SQ	3.24	3.63	3.25	0.210		**	
C18:1c9					0.59	0.05	0.84
IMF	48.88	49.65	49.30	0.880		*	
SQ	47.74	48.20	47.57	0.930		**	
C18:1c11					0.72	0.04	0.87
IMF	2.09	2.13	2.05	0.060		*	
SQ	1.91	1.93	1.93	0.020		**	
C18:2					0.46	0.05	0.77
IMF	1.97	1.82	1.70	0.015		*	
SQ	1.14	1.09	1.19	0.070		**	
C18:3					0.07	0.06	0.31
IMF	0.12	0.16	0.16	0.020		*	
SQ	0.16	0.15	0.14	0.004		**	
C20:0					0.59	0.28	0.79

IMF	0.05	0.05	0.06	0.008			
SQ	0.05	0.05	0.05	0.002			
C18:2c9t11					0.19	0.02	0.28
IMF	0.36	0.37	0.34	0.018		*	
SQ	0.48	0.47	0.49	0.012		**	
C18:2t10c12					0.81	0.001	0.58
IMF	0.04	0.04	0.03	0.001		*	
SQ	0.06	0.06	0.07	0.001		**	
C20:1					0.68	0.04	0.14
IMF	0.18	0.19	0.17	0.020		*	
SQ	0.24	0.24	0.25	0.040		**	
C20:2					0.73	0.16	0.28
IMF	0.01	0.01	0.01	0.001			
SQ	0.01	0.01	0.01	0.001			
C20:4					0.85	0.72	0.81
IMF	0.15	0.11	0.10	0.009			
SQ	0.11	0.11	0.11	0.001			
C20:5					0.83	---	---
IMF	0.02	0.01	0.02	0.001			
SQ	ND ^a	ND	ND	---			
C24:0					0.48	0.05	0.63
IMF	0.02	0.02	0.02	0.001		*	
SQ	0.04	0.03	0.04	0.008		**	
C22:6					0.79	---	---
IMF	0.01	0.01	0.01	0.001			
SQ	ND	ND	ND	---			

¹ Adjusted Diet Fatty Acid by weight percentage or g/100g

*,** Overall means within a row with different symbols differ ($P < 0.05$)

ND = Not Detected

Table 3.8. Mean concentration of saturated, unsaturated, *trans*, n-3, and n-6 fatty acids in Wagyu cattle fed 10%, 20%, and 30% roughage inclusion diets during finishing phase

Fatty Acid	Treatment			SEM (trt)	P-Value	
	10%	20%	30%		TRT	TISSUE
SFA				0.480	0.84	0.75
IMF	32.71	32.41	32.55			
SQ	32.95	32.14	32.69			
UNSAT				0.540	0.42	0.49
IMF	67.19	67.79	67.32			
SQ	66.88	67.14	67.17			
MUFA				0.460	0.51	0.68
IMF	64.53	65.27	64.96			
SQ	64.92	65.78	65.15			
PUFA				0.120	0.42	0.05
IMF	2.68	2.53	2.37			*
SQ	2.31	1.92	2.02			**
n-3				0.110	0.15	0.82
IMF	0.14	0.18	0.18			
SQ	0.16	0.15	0.14			
n-6				0.130	0.43	0.05
IMF	2.13	1.94	1.81			*
SQ	1.26	1.21	1.31			**
Total trans				0.240	0.42	0.03
IMF	5.05	4.69	4.94			*
SQ	6.09	6.43	6.19			**
c9, t11 CLA				0.150	0.57	0.05
IMF	0.36	0.37	0.34			*
SQ	0.48	0.47	0.49			**
Total CLA				0.140	0.35	0.02
IMF	0.40	0.41	0.37			*
SQ	0.54	0.53	0.56			**
MUFA:SFA				0.350	0.49	0.39
IMF	1.97	2.01	2.00			
SQ	1.97	2.04	1.99			
PUFA:SFA				0.003	0.41	0.12
IMF	0.08	0.08	0.07			
SQ	0.07	0.06	0.06			
n-6:n-3				2.000	0.16	0.04
IMF	15.20	11.08	10.16			*
SQ	8.02	8.28	9.12			**

¹ 10%= roughage inclusion of 10% on dry matter basis; 20%= roughage inclusion of 20% on dry matter basis; 30%= roughage inclusion of 30% on dry matter basis.

² Adjusted Diet Fatty Acid by weight percentage or g/100g.

³ Total SFA=Σ 10:0, 12:0, 14:0, 16:0, 18:0, 20:0, and 24:0.

⁴ Total Unsaturated = Σ 12:1,14:1, 16:1, 17:1, 18:1t9, 18:1t10, 18:1t11, 18:1t12, 18:1c9, 18:1c11, 18:2, 18:3, 18:2c9t11,18:2t10,c12, 20:1, 20:2, 20:4, 20:5, and 22:6.

⁵ Total MUFA = Σ 12:1,14:1, 16:1, 17:1, 18:1t9, 18:1t10, 18:1t11, 18:1t12, 18:1c9, 18:1c11, and 20:1.

⁶ Total PUFA = Σ 18:2, 18:3, 18:2c9t11,18:2t10,c12, 20:2, 20:4, 20:5, and 22:6.

⁷ Total n-3 = Σ 8:3, 20:5, and 22:6.

⁸ Total n-6 = Σ 18:2, 20:2, and 20:4.

⁹ Total Trans = Σ 18:1t9, 18:1t10, 18:1t11, and 18:1t12.

¹⁰ Total CLA = Σ 18:2c9t11, and 18:2t10,c12.

** Overall means within a row with different symbols differ ($P < 0.05$).

Table 3.9. Least square means of carcass imaging characteristics of Wagyu cattle fed diets with 10, 20, and 30% roughage inclusion during the finishing phase

Item	Treatment ¹			SEM	P-value ²
	10%	20%	30%		
No. of Pens	6	7	6		
No. of steers	8	10	8		
No. of heifers	4	2	4		
Ribeye area, cm ²	87.85	100.99	94.83	3.726	0.051
Ribeye length, cm ²	15.72	15.80	16.02	0.369	0.824
Ribeye width, cm ²	8.17	8.96	8.73	0.303	0.164
Visible lean area, mm ²	112.82	122.93	118.55	3.541	0.126
Visible fat area, mm ²	120.18	112.32	116.71	3.672	0.283
Marbling degree	794.93	809.70	838.33	44.978	0.766
Total percent fat, %	10.84	9.95	12.77	1.034	0.119
Mean marbling size, mm ²	2.77	2.57	2.97	0.173	0.206
Max marbling size, mm ²	153.53	181.90	231.62	27.336	0.130
Mean distance between marbling, mm	4.56	4.57	4.06	0.347	0.455
Max distance between marbling, mm	30.00	27.66	27.22	2.660	0.718

¹ 10%= roughage inclusion of 10% on dry matter basis; 20%= roughage inclusion of 20% on dry matter basis; 30%= roughage inclusion of 30% on dry matter basis

² Probability that treatment means are not different.

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CHAPTER IV

Evaluation of roughage inclusion rate within a total mixed ration effects on feedlot performance, carcass characteristics, and fatty acid composition of subcutaneous adipose, intramuscular adipose, and muscle tissue of Wagyu Cattle

Introduction

Understanding feedlot performance, carcass quality, and fatty acid composition in Wagyu cattle is key to making production decisions to optimize all three factors. To make improvements it is necessary to understand how Wagyu cattle compare to conventional feedlot breeds in and the effect of diet variation on feedlot performance, carcass quality and fatty acid composition for Wagyu steers and heifers through the measurement of body weight, dry matter intake, average daily gain, gain to feed conversion, ribeye area, back fat, fatty acid composition, and marbling distribution.

Lunt et al. (1993) compared Angus and Wagyu calves fed a corn and barley based diet for 522 days. The Wagyu calves consumed 12.3 kg of feed for each kg of weight gained while Angus calves consumed 11.1 kg of feed for each kg of weight gained resulting in a difference in feed to gain ratio of 1.2 kg of feed consumed for each kg of weight gained between the breeds (Lunt, 1993). In addition to feed to gain, Lunt et al. (1993) reported values for average daily gain of 0.9 kg/head/day for Angus steers and 0.7 kg/head/day for Wagyu steers. Harvest weights were significantly different between the breeds. Angus steers weighed 713.4 kg at harvest compared to Wagyu steers weighing 648.6 kg at harvest.

Average daily gain varied by 0.07 kg/head/day between Piedmontese gaining 1.70kg/head/day and Wagyu gaining 1.62 kg/head/day. Wagyu steers averaged 678 kg at harvest and Piedmontese steers were 14 kg heavier or 692 kg at harvest (Café et al., 2009).

Mir et al. (1998) compared conversion capabilities of cross bred steers with increased influence of Wagyu genetics fed over a two year period with increasing concentrate inclusion. Feed to gain ratios increased and dry matter intake decreased with increasing Wagyu influence, but there was no difference between 75% Wagyu steers and cross-bred steers with no Wagyu influence (Mir et al., 1998). Differences were seen between crossbred steers and Wagyu cross steers for average daily gain, but there were no differences were observed with increasing Wagyu influence (Mir et al., 1998).

Lunt et al. (2005) and Gotoh et al. (2009) compared Wagyu cattle and other breeds of cattle over extended feeding period. Body weight and hot carcass weight increased linearly from 6 to 24 months regardless of breed when fed decreasing percentage of roughage over a continuous feeding period (Gotoh et al., 2009). Angus steers had greater final weight than Wagyu steers regardless of forage or concentrate diet fed and Japanese or U.S. endpoint (Lunt et al. 2005). Wagyu fed decreasing roughage described by Gotoh et al. (2009) showed steady growth of Longissimus dorsi area up to 26 months but other muscles showed decreased growth after 14 months. For Wagyu and Angus steers fed to both Japanese and U.S. endpoints on either a high roughage or high concentrate diet described by Lunt et al. (2005). Wagyu steers had 20 % extractable lipid at the 20-month endpoint when hay fed while Angus steers had 12% extractable lipid. For the Angus steers intramuscular fat increased up to 16 months on feed while Wagyu steers continued to show increased intramuscular fat with increased time on feed past 16 months (Lunt et al., 2005).

Wagyu and Angus steers fed a corn and barley based diet for 522 days Lunt et al. (1993) reported significant decrease of 41.10 kg in hot carcass weight for Wagyu steers which was a direct reflection in the difference in final weight between Angus and Wagyu steers. No differences in other yield grade factors including adjusted fat thickness, rib eye area and kidney, pelvic and heart fat between the breeds were seen. Japanese grading standards include cold left side weight and a yield estimate. Angus steers produced larger carcasses than Wagyu, but yield estimate was lower than Wagyu (Lunt et al., 1993). Overall yield grade favored Wagyu steers based on the Japanese grading scale (Lunt et al., 1993). Barker et al., (1995) reported an increase in the dressing percentage for concentrate fed F1 Wagyu and Angus crossbred steers compared to a roughage fed group. Live weight did not differ for the two feeding styles. The variation in dressing percentage attributed to accelerated gastrointestinal growth (Barker et al., 1995). There were no differences in Japanese yield estimates between the two groups and rib eye area was only numerically greater for the linear fed group (Barker et al., 1995). Lunt et al. (2005) compared the impact of breed as well as diet and endpoint on carcass characteristics. Rib eye area was significantly increased for both Wagyu and Angus steers fed either a hay based or concentrate diet to an extended endpoint of 16 or 20 months. Additionally, yield grade was increased for Angus steers fed to the extended endpoints of 16 and 20 months on feed compared to the shorter endpoints of 8 and 12 months on feed (Lunt et al., 2005).

Quality grade was also discussed by Lunt et al. (1993), Barker et al. (1995), and Lunt et al. (2005). For Wagyu and Angus steers fed to both US and Japanese endpoint a numerical increase in quality grade was seen for Angus steers at the shorter US endpoints (Lunt et al., 2005). This was reversed for the extended Japanese endpoint; Wagyu steers had numerically higher quality grades (Lunt et al., 2005). These results were in agreement with the previous study

conducted by Lunt et al., (1993). Marbling score was numerically a full degree higher for Wagyu steers. The lack of significance was attributed to the high variation within the breed (Lunt et al., 1993). On the Japanese grading scale significant differences were seen for beef marbling score, texture score and firmness score. Wagyu expressed favorable values for all three factors compared to their Angus counterparts, resulting in better quality grades for the Wagyu steers (Lunt et al., 1993). When comparing feeding regimes Barker et al. (1995) presented significant increases in beef marbling score for linear fed steers than deferred fed steers of 4.74 and 3.30 respectively. Additionally, the linear fed group presented significantly increased values for all quality grade values on the Japanese grading system resulting in improved quality grades for linear fed Wagyu steers (Barker et al., 1995).

Lunt et al. (1993) reported that Wagyu were more likely to qualify for the highest marbling grade compared to Angus steers, but variation within the breed provides opportunities to select sire lines with increased propensity to deposit intramuscular adipose (Lunt et al., 1993). Wagyu continue to increase intramuscular adipose with continued time on feed compared to Angus (Gotoh et al., 2009 and Lunt et al., 2005). Wagyu steers showed a 20% increase in IMF percentage within the longissimus dorsi over German Angus steers fed by Japanese standards for 26 months (Gotoh et al., 2009). Wagyu continued to increase intramuscular adipose past 16 months on feed while Angus steers no longer increased intramuscular adipose after 16 months on feed (Lunt et al., 2005). Marbling scores were not significantly different regardless of breed, diet or endpoint, but extractable lipid provided better illustration of the differences in intramuscular fat content between Angus and Wagyu steers. Hay fed Wagyu had 20% extractable lipid and hay fed Angus had 12 % extractable lipid when fed to the extended 20 months on feed (Lunt et al., 2005). In addition to the increased extractable lipid, Angus cattle deposited more subcutaneous

adipose regardless of diet or endpoint when compared to Wagyu (Lunt et al., 2005). Barker et al. (1995) reported an increase in 6th rib fat thickness for concentrate fed Wagyu compared to grass fed Wagyu of 3.14 cm and 3.09 cm respectively.

Wagyu are known for significantly higher ratios of monounsaturated fatty acids (MUFA) to saturated fatty acids (SFA) than domestic breeds. This is valuable because increased consumption of MUFA paired with decreased consumption of SFA is believed to prevent increases and possibly decrease blood cholesterol (Boylston et al., 1995). Wagyu have higher lipid content than other breeds with consistently higher content of unsaturated fatty acid (Boylston et al., 1995). This is in agreement with Sturdivant et al. (1992) and May et al. (1993) who reported significant decreases in 16:0 and 18:0 fatty acids for Wagyu compared to Angus steers. The percentage of unsaturated fatty acids 14:1, 16:1, 18:1, and 18:2 were significantly higher compared to Angus steers (Boylston et al., 1995). May et al. (1993) compared fatty acid composition within tissue type. Wagyu consistently produced more 16:1 and 18:1 fatty acid for subcutaneous and intramuscular tissues compared to Angus and produced significantly less 18:0 and 16:0 fatty acids regardless of tissue type compared to Angus (May et al., 1993). For all tissue types the differences in fatty acids resulted in significant differences in MUFA:SFA ratio with Wagyu having consistently higher ratios (May et al., 1993). Although increased MUFA:SFA ratio is desirable for human nutrition, Wagyu have higher propensity to lay down adipose than other breeds (Boylston et al., 1995) and may create an issue with total fat as dietary recommendations suggest that less than 30%, but not less than 20%, of the dietary energy is derived from fat (Lee et al., 2001).

The majority of the research completed at this time has utilized crossbred Wagyu genetics ranging from F1 to $\frac{3}{4}$ and $\frac{7}{8}$ bloodlines. Research conducted with full blood Wagyu

will be valuable to clarify variation presented in the literature for carcass characteristics. Little is known about the dietary effect of adipose deposit size. Gotoh et al. (2009) compared fleck size of marbling deposit between Wagyu and German Angus. This study reported 37.0 cm² size flecks for Wagyu and 6.7cm² size flecks for German Angus. There were no differences in the number of flecks between the breeds and distribution was not different for the breeds (Gotoh et al., 2009). Additionally Kuchida et al. (1999) compared image analysis to grader assigned beef marbling scores. Beef marbling standards assigned by examiners differed by -1 to +2 from the beef marbling score based on the marbling percentage estimated by image analysis (Kuchida et al., 1999). Image analysis provides an objective measurement to understand variation in adipose distribution as well as reduce grading error.

Therefore, the objectives of this experiment were to determine the effect of roughage inclusion of 10, 20 and 30% on body weight, average daily gain, dry matter intake and gain to feed conversion in full blood Wagyu steers and heifers, as well as carcass quality characteristics and fatty acid composition.

Material And Methods

Animals

Experimental procedures conducted with animals were approved by the Colorado State University Animal Care and Use Committee prior to initiation of the experiment. The experiment utilized 42, 24±3 m Wagyu calves from a single supplier. There were 12 heifers and 30 steers. Sexes were unequal due to limited availability of feeder heifers. All animals were provided by Emma Farms Wagyu (Colorado Wagyu Emma Farms Cattle Company, Walden CO), due to limited availability of feeder heifers animal numbers are unbalanced. Upon arrival all calves were housed in a standard feedlot pen and fed a high forage starter ration to acclimate to bunk

feeding. Calves were transitioned to treatment rations over 14 d. Calves were randomly distributed into three treatment groups, blocking by sex and BW and then randomly assigned to one of 7 treatment allotted 5x33 m feedlot pen in groups of 2 animals. The resulting design consisted of 3 treatment groups, and 21 pens, with each treatment consisting of 7 pens of 2 animals apiece. Calves were transitioned to treatment rations over a 14 d period.

Diets

Three total mixed rations (TMR) were formulated to contain 10, 20 and 30 percent roughage. The rations consisted of corn silage, alfalfa hay or grass hay, cracked corn, dry distillers grains, soybean meal, limestone, soybean oil, and molasses based liquid mineral supplement (Archer Daniels Midland Co., Chicago, IL). All rations were formulated to be isocaloric, isoamylolytic and isonitrogenous, and to meet or exceed NRC (2000) requirements for growing beef animals (NRC, 2000). Diet composition including ingredient and chemical composition is summarized in Table 4.1. Fatty acid composition of diets is summarized in Table 4.2. Total lipid were extracted according to the procedures of Folch et al. (1957) for composite samples of treatment rations collected weekly throughout entire feeding trial. Folch-extracted samples were transmethylated according to the method of Park and Goins (1994) and evaporated on a rotary evaporator under a gentle stream of nitrogen at 25° C. Dried fatty acid methyl esters were reconstituted with 2µL of hexane and analyzed by an Agilent 6890 series gas chromatograph (Agilent Technologies, Santa Clara, CA) equipped with flame ionization detector and a 100 m x 0.25mm (i.d.), fused silica capillary column (SP-2560, 0.2µm film thickness, Supelco, Bellefonte, PA). A triacylglycerol of tridecanoic acid (13:0, 1.0mg) was used as the internal standard. Oven temperature was maintained at 175° C for 40 min, and then increased to

240° C at 10° C/min. Injector and flame-ionization detector temperatures were 245° C/min. Helium was the carrier gas at a split ratio of 50:1 and a constant flow rate of 0.8 mL/min. Fatty acid peaks were recorded and integrated using GC ChemStation software (version A.09.03, Agilent Technologies). Retention times were compared with known FAME standards to identify individual FA (Nu-Check Prep, Inc., Elysian, MN, and Matreya Inc., Pleasant Gap, PA). Trial was initiated on May 1, 2014 calves were fed once daily at 0800 with ad libitum access to water. Feed was initially offered at the daily rate of 15kg (AF basis) per animal and quantity was adjusted daily until trace amounts of uneaten feed remained at time of subsequent feeding. Calves were weighed at 7d intervals and weighing was initiated at 0100. Feed refusals were measured at 7d intervals prior to feeding and weight collection.

Endpoint Selection

Projected finish weights were set based on literature review to 590 kg for heifers and 680 kg for steers. The first six calves were harvested and animal owners were not satisfied with marbling levels. The weight endpoint was eliminated and endpoint was determined to be a visual fat covering evaluation. Endpoint selection as based on visual evaluation of frame size, evenness of fat cover and weight by an owner representative. The change in endpoint caused an unforeseen extension to the feeding component of the trial. Calves were consolidated due to lack of pen space into fewer pens on October 31, 2014. Final weights for the initial feeding phase were collected over a two-day period. Weight was first collected at d 6 of the weight interval and again at d 7 of the weight interval as normal. Final weights were determined by the average of these two weights. Calves remained within original treatment groups and sexes were not combined. Pens had no more than 4 head per pen after consolidation. Initial weights were collected for the second phase of feeding and 7 d interval weights continued to be collected along

with feed refusals as conducted in phase 1. Calves remained on feed until January 30, 2015 to conclude the feeding trial. Weights were collected consecutively over two days and averaged to determine final weight. One heifer was removed from the study when she was discovered to be pregnant.

Tissue Collection and Processing

Calves were transported 55 km to a commercial packing company (Innovative Foods, LLC., Evans, CO) where they were humanely harvested. Adipose and skeletal muscle tissue samples were collected between ribs 12 and 13 immediately after the carcass was separated. Tissue samples were immediately dissected into subcutaneous adipose, intramuscular adipose, and intact skeletal muscle including intramuscular adipose and adjacent subcutaneous adipose. Samples were snap frozen in liquid nitrogen. Hot carcass weight was determined prior to cooler storage. After 21 d at 0°C carcasses were cross-sectioned between ribs 12 and 13; circumferences of LM were traced and BF measurements were made at the lateral three-quarter point over the LM. During fabrication process a 1.27cm cross section from the anterior face of the short loin was collected for imaging. Steaks were frozen on a sheet tray at -20°C for 18±6 h prior to vacuum packaging and stored at -20°C. All samples collected on the harvest floor were stored at -80°C.

Samples of adipose and muscle tissue were homogenized for nutrient analysis. Subcutaneous and composition samples were ground using a robot coupe blender. Sample was cut into 2x2 cm cubes and submerged in liquid nitrogen. During blending liquid nitrogen is added to blender at 45 s intervals to keep samples frozen. Intramuscular adipose samples were diced finely with scalpels to prevent loss of product. 1 g samples of subcutaneous and composition samples were oven dried at 100° C for 24 h. Moisture content of dried samples was

determined by weight loss after cooling in a desiccation chamber at 25° C for 1 to 2 h. To determine ash content, 2 g samples of subcutaneous and composite samples were dried in muffle furnace at 600° C for 18h. After cooling for 30 to 45 min samples were transferred to desiccation chamber for 1 to 2 h. Percent ash was determined by weight loss after cooling as a percentage of initial weight. Total lipid were extracted with duplicate samples run every ten from subcutaneous adipose, intramuscular adipose, and composite samples according to the procedures of Folch et al (1957). Folch-extracted samples were transmethylated according to the method of Park and Goins (1994) and evaporated on a rotary evaporator under a gentle stream of nitrogen at 25° C. Dried fatty acid methyl esters were reconstituted with 2µL of hexane and analyzed by an Agilent 6890 series gas chromatograph (Agilent Technologies, Santa Clara, CA) equipped with flame ionization detector and a 100 m x 0.25mm (i.d.), fused silica capillary column (SP-2560, 0.2µm film thickness, Supelco, Bellefonte, PA). A triacylglycerol of tridecanoic acid (13:0, 1.0mg) was used as the internal standard. Oven temperature was maintained at 175° C for 40 min, and then increased to 240° C at 10° C/min. Injector and flame-ionization detector temperatures were 245° C/min. Helium was the carrier gas at a split ratio of 50:1 and a constant flow rate of 0.8 mL/min. Fatty acid peaks were recorded and integrated using GC ChemStation software (version A.09.03, Agilent Technologies). Retention times were compared with known FAME standards to identify individual FA (Nu-Check Prep, Inc., Elysian, MN, and Matreya Inc., Pleasant Gap, PA).

Frozen steaks were stored at -20°C until all samples were collected for imaging. Prior to imaging, steaks were thawed at 0°C for 3 d within vacuum packaging. All steaks were removed from packaging 30 min before imaging to allow for color to bloom. Images were taken using Computer Vision System Rib Eye Camera (Research Management Systems, Fort Collins, CO)

to objectively measure rib eye area, shape, marbling percentage, fat thickness, and lean and fat color.

Statistical Analysis

Differences in feedlot performance were analyzed using the MIXED procedure in SAS (SAS institute Inc., Cary, NC) which fits generalized mixed models. Backward model selection was utilized to include all significant factors ($\alpha=0.05$) were used in the final statistical model to analyze feedlot performance. Factors that were available to the model included treatment, sex, initial weight, initial age, and days on feed. Differences in body weight were analyzed using the REG procedure to calculate a predicted 168-day weight. This was conducted to account for variation in days on feed due to initial endpoint selection based on weight prior to modification of design. Values for average daily gain and gain to feed were calculated using results of regression analysis and further analyzed using the MIXED procedure. Feedlot performance data collected up to pen consolidation or 168 days on feed was utilized for analysis. Data collected for feedlot performance following pen consolidation was not analyzed for presentation due to collapse of experimental unit and variation of harvest date within the consolidated pens.

Differences in carcass characteristics were analyzed using the MIXED procedure in SAS (SAS institute Inc., Cary, NC) which fits generalized mixed models. Backward selection was utilized to include all significant factors ($\alpha=0.05$) in the final statistical model to analyze carcass performance. Factors that were available to the model included treatment, sex, initial weight, initial age, and days on feed. Fatty acid analysis was performed with the MIXED procedure. Backward model selection was also conducted. Tissue type was also included as a factor for the final model. The final model consisted of treatment, tissue type, and the treatment \times tissue type interaction.

Results And Discussion

Feedlot Performance

This experiment was designed to explore the effect on feedlot performance in Wagyu cattle due to the alteration of roughage inclusion rate within a total mixed ration to 10%, 20% or 30%. Treatments were designed to maintain isonitrogenous and isoamylolytic nature of the diet and allow for variation of roughage. All feedlot data corresponds to the 168 days on feed prior to pen consolidation.

The experimental data indicate that Wagyu feedlot performance including ADG, total BW, DMI and G:F conversion is not significantly affected by roughage inclusion rate.

Information regarding treatment means and variation are presented in Table 1.3 as an overview of the study. Feedlot performance statistics are presented in Table 1.4. Predicted initial weight and predicted 168d weight represent values calculated by regression of weekly weight collection during feeding trial. Actual initial weight varied between the treatments by 8.12 kg ($P=0.95$) and did not influence other weight factors. There was no significant difference in harvest weight ($P=0.27$). Predicted initial weight and predicted 168d weight represent estimated values of growth based on a regression of all weekly collected weights for 168 days on feed. Cattle continued to be on feed past 168 days, but consolidation of pens as well as variation in harvest date within consolidated pen confounded the analysis of weight gain. Predicted initial weight values are greater than actual initial weight but there were no significant differences between treatment groups ($P=0.66$). Treatment group did not result in significant variation for predicted 168 d weights ($P=0.46$). Numerically, ADG was lowest for 20% roughage inclusion rate at $0.92 \text{ kg*hd}^{-1}\text{*d}^{-1}$ and highest for 30% roughage inclusion rate of $1.45 \text{ kg*hd}^{-1}\text{*d}^{-1}$. Treatment groups did not significantly differ for ADG or DMI ($P=0.53, 0.35$ respectively). Gain

to feed ratio followed the same numeric pattern as ADG. The 20% roughage inclusion rate produced the lowest conversion ratio while 30% roughage inclusion rate produced the highest conversion ratio, although not significantly different ($P=0.50$). Total DMI for 168 days on feed was also not affected by treatment group ($P=0.27$).

Initial weights for the cattle on this study were heavier than initial weights reported by Barker et al. (1995), Lunt et al. (1993& 2005), and Mir et al. (1999). The listed studies utilized weaned calves of approximately 8-9 months of age. Initial age was much older 24 ± 1 m at the initiation of the feeding trial. Harvest weights for this study were comparable to the results presented by Ozawa et al. (2000) of 654 ± 6 kg for steers fed 75% concentrate and 25% roughage and Barker et al. (1995) of 671.18 ± 13 kg for steers continuously fed grain based diets. Conversely Mir et al. (1999) reported lesser harvest weights of 415kg for steers with increasing Wagyu influence fed 35% barley and 65% barley silage. This reduced final weight is a result of a selection for harvest at 460kg. When compared to Angus cattle, Wagyu were lighter weight at harvest. Lunt et al., (2005) compared Angus and Wagyu steers at various days on feed as well as grain or forage based diets. Angus steers were consistently heavier (Lunt et al., 2005). Harvest weights are comparable to steers fed for 20 months on a forage based diet of 603.4 kg and 663.1 kg for Wagyu and Angus steers respectively (Lunt et al., 2005). Few comparisons have been made with Angus cattle that focus on forage inclusion rate without other treatment factors to make comparisons to this study.

During the 168 d feeding trial treatment groups 10%, 20%, and 30% roughage gained 1.27, 0.92, and 1.45 $\text{kg}\cdot\text{hd}^{-1}\cdot\text{d}^{-1}$ ($P=0.53$) respectively. These results are in agreement with Mir et al. (1998) who reported 0.9-1.2 $\text{kg}\cdot\text{hd}^{-1}\cdot\text{d}^{-1}$ ADG values for steers with increasing Wagyu influence. Conversely ADG values from this study were greater than values reported by Barker

et al. (1995), Lunt et al. (2005), and Ozawa et al. (2000). Barker et al. (1995) reported ADG of $0.79 \text{ kg*hd}^{-1}\text{*d}^{-1}$ and Ozawa et al. (2000) reported ADG as low as 0.57 kg/day and an overall ADG of $0.71 \text{ kg*hd}^{-1}\text{*d}^{-1}$. ADG is only comparable to steers fed 8 months on a corn-based diet as reported by Lunt et al. (2005) of $1.03 \text{ kg*hd}^{-1}\text{*d}^{-1}$. There is also a similarity between Angus steers within this same treatment group of $1.25 \text{ kg*hd}^{-1}\text{*d}^{-1}$ (Lunt et al., 2005). Average daily gains were reduced with increased time on feed regardless of diet (Lunt et al., 2005). In comparison to this current study, long fed Wagyu and Angus had reduced ADG values that presented in the current study.

Treatment did not have an effect on DMI ($P=0.35$). The 10% roughage inclusion group consumed $9.98 \text{ kg*hd}^{-1}\text{*d}^{-1}$, the 20% roughage inclusion group consumed the least per day at $9.38 \text{ kg*hd}^{-1}\text{*d}^{-1}$ and the 30% roughage inclusion group consumed $9.92 \text{ kg*hd}^{-1}\text{*d}^{-1}$. Dry matter intake values were greater than values presented by Yamada et al. (2009) and Mir et al. (1999). Dry matter intake for steers of increasing Wagyu influence consumed $7.5 \pm 2 \text{ kg*hd}^{-1}\text{*d}^{-1}$ (Mir et al., 1999). Dry matter intake varied from $7.74 \text{ kg*hd}^{-1}\text{*d}^{-1}$, $4.73 \text{ kg*hd}^{-1}\text{*d}^{-1}$, and $1.93 \text{ kg*hd}^{-1}\text{*d}^{-1}$ of silage and $4.97 \text{ kg*hd}^{-1}\text{*d}^{-1}$, $5.86 \text{ kg*hd}^{-1}\text{*d}^{-1}$, and $6.68 \text{ kg*hd}^{-1}\text{*d}^{-1}$ of concentrate for the high medium and low roughage diets respectively (Yamada et al., 2009). Combining both concentrate and silage, steers consumed $12.71 \text{ kg*hd}^{-1}\text{*d}^{-1}$, $10.56 \text{ kg*hd}^{-1}\text{*d}^{-1}$, and $8.61 \text{ kg*hd}^{-1}\text{*d}^{-1}$ on the high medium and low roughage rations. Both the high and medium rations contain more forage than the current treatment formulations. The low roughage diet corresponds to the current study the most. Comparing the DMI for the low roughage group of $8.61 \text{ kg*hd}^{-1}\text{*d}^{-1}$ to the DMI of the current study of $9.98 \text{ kg*hd}^{-1}\text{*d}^{-1}$, $9.38 \text{ kg*hd}^{-1}\text{*d}^{-1}$, and $9.92 \text{ kg*hd}^{-1}\text{*d}^{-1}$ shows a difference of $1.2 \pm .5 \text{ kg*hd}^{-1}\text{*d}^{-1}$ between the two studies (Yamada et al., 2009). Moore et al. (1990) compared crossbred steers fed 35% roughage of various sources. For steers fed alfalfa,

the most comparable to the current study, DMI of $5.9 \text{ kg} \cdot \text{hd}^{-1} \cdot \text{d}^{-1}$ was observed. The large difference in DMI for Moore et al. (1990) and the current study can be attributed to the difference of 300 kg in initial body weight.

Gain to feed ratio was the lowest for the 20% roughage group. Although not significantly different from the other treatment groups, the numerical decrease in conversion reflects the decreased ADG exhibited by the 20% roughage treatment. Gain to feed values were similar to values presented by Wertz et al. (2002) of 0.138 for two year old Wagyu heifers and 0.157 for Wagyu heifer calves. Angus conversion ratios are also similar at 0.130 for two-year-old Angus heifers and 0.173 for Angus heifer calves (Wertz et al., 2002).

Carcass Characteristics

Experimental data indicates that carcass characteristics for Wagyu cattle are not significantly affected by roughage inclusion rate.

A summary of treatment means is presented in Table 1.3 as an overview of carcass characteristics as well as feedlot performance. Carcass characteristic statistics are presented in Table 1.5. Harvest weight was collected 12 ± 3 h prior to transport to harvest. No differences were seen between the treatment groups ($P=0.27$). Numerically, the 10% roughage inclusion treatment were the heaviest at 691.05 kg and 20% roughage inclusion treatment were the lightest to harvest at 673.95 kg. Hot carcass weight was not significantly different ($P=0.58$) between the treatment groups. This similarity is observed in dressing percentage as well. All treatment groups were in the low 60% for dressing percentage and no differences were seen ($P=0.77$). Back fat thickness as well as rib eye area was collected immediately following ribbing of the carcass and all measurements were collected from the right side. No differences were observed for back fat or rib eye area ($P=0.22, 0.14$, respectively). The 20% roughage inclusion treatment had the

largest rib eye area of 107.15 cm. for back fat the 10% roughage treatment had the greatest fat coverage of 1.68cm.

Harvest weights for this study were similar to weights reported by Barker et al. (1995) and Yamada et al. (2009). In comparison to harvest weights presented by Lunt et al. (2005) there were only similarities between steers fed hay based ration for 20 months. Harvest weights were lighter for both Angus and Wagyu steers fed either corn or hay at shorter feeding lengths (Lunt et al., 2005). Mir et al., (1999) reported harvest weights of 450 kg for steers on feed 175 days. The feeding period is similar to the current study, but initial weights were also much lesser than the current study. In comparison to Angus steers, harvest weights were lighter for the Wagyu steers of the current study (Gorocica-Buenfil and Loerch 2005; Quinn et al., 2011). This is in agreement with Lunt et al. (2005) who reported increased harvest weights for Angus steers compared to Wagyu steers regardless of diet type or time on feed.

Hot carcass weights varied from 416.32 kg to 424.94 kg for the current study. Hoque et al., (2006), Ozawa et al., (2000), and Xie et al., (1996) reported carcass weights ranging from 409 kg to 431.63kg for Wagyu steers similar to values observed on the current study. In contrast, Lunt et al., (2005), Mir et al., (1999), and Wertz et al., (2002) reported hot carcass weights ranging from 250 kg to 350 kg for Wagyu steers. Dressing percentages values were not reported by Lunt et al. (2005), but Mir et al., (1999) reported dressing percentages of 58.3% for 75% Wagyu steers. The 10% difference in dressing percentage between values reported by Mir et al. (1999) and the current study are a direct reflection of the differences in hot carcass weights reported by the two studies. Hot carcass weights for Angus steers reported by Gorocica-Buenfil and Loerch (2005) and Quinn et al. (2011) were less than the weights of Wagyu steers on the current study. This is not in agreement with the data presented by Lunt et al. (2005). Angus

steers consistently produced heavier carcasses than their Wagyu counterparts regardless of diet or time on feed (Lunt et al., 2005). Hot carcass weights were heavier for Wagyu heifer calves than their Angus counterparts, but were lighter for two-year-old Wagyu heifers and two-year-old Angus heifers of same breeding style (Wertz et al., 2002).

No differences in dressing percentage were observed between treatment groups ($P=0.77$). Values are less than values reported by Barker et al. (1995) of 65.05% for Wagyu and Angus cross steers. In comparison, steers with increasing Wagyu genetics had dressing percentage values of 57.6% for steers with 75% Wagyu influence (Mir et al., 1999). Dressing percentage values for Angus steers were similar (Gorocica-Buenfil and Loerch, 2005; Quinn et al., 2011; Sexten et al., 2012).

Twelfth rib back fat was not significantly different between treatment groups ($P=0.22$). Values ranged from 1.28 cm to 1.68 cm, with 10% roughage inclusion rate having the most and then back fat decreasing with increasing roughage inclusion rate. Similar values for back fat were presented by Xie et al. (1996a), Lunt et al. (2005), Oka et al. (2002), Mir et al. (1999), Xie et al. (1996b), and Ozawa et al. (2000). Lunt et al. (2005) and Ohsaki et al. (2009) reported back fat values of 2.71 cm and 2.97 cm respectively. Description of the measurement location is unclear. The elevated measurements may be due to variation in grading practices of Japanese grading systems and American grading systems as Japanese measurements are collected at the 6th rib rather than the 12th rib. Back fat for Wagyu cattle on the current study are comparable to Angus back fat measurements of 1.5 cm, 1.38 cm, and 1.11 cm reported by Quinn et al. (2011), Gorocica-Buenfil and Loerch, (2005), and Sexten et al., (2012). Lunt et al. (2005), Wertz et al. (2002) and Xie et al. (1996b) made direct comparisons between Wagyu and Angus carcass performance. Angus steers produced carcasses with greater back fat than Wagyu steers. Wertz et

al. (2002) reported Angus heifers with 2.71 cm of back fat and Wagyu heifers with 1.90 cm of back fat. Similarly, Xie et al. (1996b) reported 1.96 cm back fat for Angus heifers and 1.56 cm back fat for Wagyu steers. Lunt et al. (2005) reported the greatest quantity of back fat for Angus steers fed corn-based diet for 16 months of 2.51 cm and 1.53 cm for Wagyu steers within the same group.

Rib eye area varied greatly within the literature for Wagyu cattle. Oka et al. (2002) and Ozawa et al. (2000) reported values of 42.1 cm² and 48.7 cm² for rib eye area respectively. Xie et al. (1996b) reported rib eye area of 101.4 cm². For the current study rib eye area ranged between 98.67 cm² and 107.15 cm². These values are similar to values presented by Wertz et al. (2002), Xie et al. (1996a), and Xie et al. (1996b) who reported rib eye area of 93.0 cm², 101.4 cm², and 95.5 cm² respectively. In comparison to Angus cattle rib eye area was larger for Wagyu cattle on the current study. Sexten et al. (2012) reported rib eye area of 79.74 cm² and Quinn et al. (2002) and Gorocica-Buenfil and Loerch (2005) reported similar values of 62cm² rib eye area. Rib eye area was numerically greater for Wagyu compared to Angus (Wertz et al., 2002). For both Lunt et al. (1993) and Lunt et al. (2005) Angus had numerically greater rib eye area compared to their Wagyu counterparts. The data from this study suggests that Wagyu have greater rib eye area than Angus cattle, but this is not supported by other literature comparisons of Wagyu and Angus cattle (Lunt et al., 1993; Lunt et al., 2005).

Fatty Acid Composition

The percent inclusion of C10-C24 fatty acids (FA) in i.m. adipose (IMF) and s.c. adipose (SQ) are quantified and compared (Table 4.6, and Table 4.7, respectively). Intramuscular FA composition did not differ between treatments. There is a tendency ($P=0.07$) for linolenic acid (C18:3) concentration to increase with increasing percentage of forage inclusion rate. Conjugated

linoleic acid isomer C18:2t10c12 tends to increase with decreasing forage inclusion rate ($P=0.09$). No differences were detected in FA composition of SQ tissue. There was a tendency for linolenic acid (C18:3) concentration to be increased with decreasing forage inclusion ($P=0.08$).

Comparison of FA by treatment across tissue types is presented in Table 1.8. Intramuscular adipose contained the greatest concentration of Lauric acid (C12:0) ($P=0.05$). Subcutaneous adipose contained the greatest concentration of Lauroleic acid (C12:1) and the least Myristic acid (C14:0) ($P=0.05$ and 0.01 , respectively). Differences were seen between tissue types for Myristicoleic acid (C14:1) ($P=0.001$). Subcutaneous adipose contained the greater concentration than IMF. No differences were detected between SQ and IMF for Palmitic Acid (C16:0), Palmitoleic acid (C16:1), or $P=0.63$ and 0.38 , respectively). Intramuscular adipose contained a greater concentration of Heptadecanoic Acid (C17:0) than SQ ($P=0.001$). No differences were seen for Heptadecenoic Acid (C17:1), Stearic Acid (C18:0), or C18:1 t-6,8 ($P=0.45$, 0.05 , and 0.04 , respectively). Subcutaneous adipose contained the greatest concentration of Elaidate Acid (C18:1, t-9) ($P=0.04$), C18:1t-10 ($P=0.02$), and Vaccenic Acid (C18:1 t11) ($P=0.03$). Intramuscular adipose contained the greatest concentration of Oleic Acid (C18:1c9) as well as *Cis*-Vaccenic Acid (C18:1c11) ($P=0.05$, 0.01 , respectively). The greatest concentration of Linoleic Acid (C18:2) was found in IMF compared to SQ ($P=0.01$). No difference was detected between treatment groups for Linolenic acid (C18:3) ($P=0.06$). Subcutaneous adipose contained the greatest concentration of both Conjugated Linoleic Isomers, C18:2c9t11 and C18:2t10c12, Eicosenoic Acid (C20:1), and Lignoceric Acid (C24:0) ($P=0.02$, 0.001 , 0.04 , and 0.05 , respectively). No difference was detected in concentration of (C20:2),

Lignoceric acid (C20:4), Eicosapentaenoic Acid (C20:5) and Docosahexaenoic Acid (C22:6) ($P>0.05$).

Mean concentrations of saturated FA (SFA), unsaturated FA (UFA), trans FA, as well as n-3 and n-6 FA are presented in Table 1.9. No differences were observed for SFA, UFA, MUFA, and Omega 3 fatty acids ($P>0.05$). Intramuscular adipose had greater concentration of PUFA and Omega 6 fatty acids ($P=0.05$). Subcutaneous adipose contained greater concentrations of trans fatty acids as well as conjugated linoleic acids ($P=0.03$ and 0.02 , respectively). No differences were seen for ratios of MUFA and PUFA to SFA ($P>0.05$). Omega 6 to Omega 3 ratio was more favorable for SQ compared to IMF as the ratio was lower ($P=0.04$).

No significant treatment effects of fatty acid composition were seen for SQ and IMF. Treatment showed a tendency for Linolenic Acid C18:3. Few studies report concentration of C18:3 within skeletal muscle for Wagyu cattle. French et al. (2000) reported values of 0.71 g/100g C18:3 for grass fed crossbred steers. Leheska et al. (2008) also reported an increase in C18:3 concentration with grass fed beef. Concentration of C18:3 increased with increasing rate of roughage inclusion. Values of C18:3 are less than those reported by French et al. (2000), Laheska et al. (2008) and Nuernberg et al. (2005). Limited comparison is available for C18:3 within skeletal muscle. Oka et al. (2002) and Chung et al. (2006) compare C18:3 within SQ tissues and concentrations are similar. Concentrations of C14:0 and C18:0 are comparable to values presented by May et al. (1993), Elias Calles et al. (2002) and Xie et al. (1996a) for skeletal muscle. Concentration of C16:0 reported in the literature are elevated compared to concentrations within SM (Elias Calles et al., 2000; May et al., 1993; Xie et al., 1996a and 1996b). This pattern remains true for SQ and IMF concentrations of C14:0, C18:0, and C16:0. Oleic acid concentration is correlated to positive beef palatability and increased consumption of

oleic acid can reduce risk factors for metabolic diseases in humans (Smith et al., 2006). Wagyu express elevated levels of Oleic acid compared to conventional feedlot breeds (May et al., 1993; Oka et al., 1992; Sturdivant et al., 1992). French et al. (2002) reported C18:1 concentrations of 39.74 g/100g for concentrate fed and 40.58 g/100g for grass fed crossbred steers. While May et al. (1993) reported values of 45.22%, 50.25% and 50.25% for SM, IMF, and SQ concentration of C18:1 for Wagyu cattle. Oleic acid concentrations presented are similar to reported Wagyu values and support the statement that Wagyu produce more oleic acid than conventional beef breeds. No differences were detected for Conjugated linoleic acid between treatment groups. De La Torre et al. (2006) reported CLA *c9,t11* concentrations of 0.77% for Charolais bulls fed 30% roughage. Alfaia et al. (2009) reported 81.34 mg/g for CLA *c9,t11* in Alentejano bulls. Grass fed crossbred steers expressed increased levels of CLA *c9,t11* compared to concentrate fed steers (French et al., 2000). CLA isomers are not delineated apart from C18:2 by Chung et al. (2006), Oka et al. (2002), and Ohaski et al. (2009). Concentrations of CLA *c9,t11* are comparable to values presented by French et al. (2000), Alfaia et al. (2009), and De La Torre et al. (2006). Linoleic acid concentrations are comparable to values presented for C18:2 undifferentiated by Chung et al. (2006), May et al. (1993), Ohaski et al. (2009), and Oka et al. (2002). Differences in FA composition were significant by tissue type. Skeletal muscle contained the greatest concentration of SFA. This is in agreement with values presented by Ellias Calles et al. (2000) who reported SFA concentrations of 45.7% for longissimus dorsi muscle and 41.1% for SQ. Xie et al. (1996b) reported elevated levels of SFA within the longissimus dorsi muscle than SQ. Oka et al. (2002) reported values of 46.07% and 38.02% SFA within IMF and SQ respectively. Intramuscular fat was not significantly different from SQ and does not support findings of Oka et al. (2002). Monounsaturated ratios are greater for IMF and SQ than SM. This is supported by

results presented by Ellias Calles et al. (2000) and Xie et al. (1996a). Oka et al. (2002) reported significantly greater concentrations of MUFA in SQ than IMF. Tissue type was statistically significant for total PUFA. Skeletal muscle contained greater concentration of PUFA than IMF and SQ. This is supported by results presented by Ellias Calles et al. (2000) and Xie et al. (1996a and 1996b). Oka et al. (2002) reported greater concentrations of PUFA in SQ compared to IMF and does not support findings of the current study that IMF contains greater PUFA. No differences were observed for MUFA: SFA ratio. Skeletal muscle expressed greater levels of MUFA and SFA than SQ and IMF. Proportions were not different for tissue types. Xie et al. (1996a) reported 1.40 and Xie et al. (1996b) reported 1.21 MUFA:SFA ratio for longissimus dorsi tissue. Chung et al. (2006) reported MUFA:SFA ratio of 1.41 and 1.18 of SQ for Wagyu and Angus steers respectively. MUFA:SFA ratios are greater than values reported in the literature. The ratio of PUFA: SFA is greatest for SM and not different for SQ and IMF. Xie et al. (1996a) reported PUFA:SFA ratio of 0.10 and 0.06 for longissimus dorsi muscle and SQ. Elias Calles also reported greater PUFA:SFA ratios for longissimus dorsi than SQ tissues.

Carcass Imaging

Comparisons of carcass characteristics obtained using Computer Vision System Cold Camera (CVS; Research Management Systems, USA, Inc., Fort Collins, CO) are presented in Table 4.10. Treatment did not account for significant variation in carcass characteristics except for ribeye area. The 20% roughage inclusion treatment group produced the largest rib eye area of 100.99 cm² followed by 30% roughage inclusion treatment at 94.83 cm² and 87.85 cm² for 10% roughage inclusion treatment ($P=0.05$). No differences were observed for ribeye length of rib eye width between the treatment groups ($P=0.824$, 0.164 respectively). Numerically, Marbling degree increased with roughage inclusion rate although not significantly ($P=0.76$). Distribution

and size of marbling was not effected by treatment. The 10% roughage inclusion treatment had mean marbling size of 2.77 mm² and a maximum marbling size of 153.33 mm². The mean marbling size for 20% roughage inclusion was 2.57 mm² and maximum marbling size of 181.90 mm². Both mean marbling size and maximum marbling size was numerically greater for the 30% roughage inclusion treatment of 2.97 mm² and 231.62 mm² respectively. No differences were detected for values of distance between marbling deposits. The 10% treatment group had the numerically greatest distance between marbling while the 20% and 30% treatment groups were similar at 27mm. Mean distance between marbling was greatest for 20% treatment group at 4.57 mm ($P=0.45$). The 30% roughage inclusion treatment had the numerically lowest mean distance between marbling deposits of 4.06mm.

Limited information is available utilizing imaging technology to analyze carcass characteristics of Wagyu cattle. Comparisons with the Computer Vision System Cold Camera utilized for this study such as data presented by Cannell et al. (2002) and Vote et al. (2003) have been limited to validation of quality grade factors as well as comparison of image values to eating experience such as tenderness. Gotoh et al. (2009) utilized a computerized image analysis system working with oil red stained samples of longissimus dorsi muscle. Digital images of carcass cross sections were taken between the 6th and 7th rib by Ozawa et al. (2008) of Japanese black steers. Both studies compared marbling fleck size, the number of marbling flecks as well as distribution patterns within the muscle tissue. Ozawa et al. (2008) describe distribution in terms of overall coarseness of marbling fleck and coarseness of maximum marbling fleck. Conclusions regarding the number of adipose cells as well as the total amount of adipose within each deposit can be made by comparing marbling fleck number and size. Greater fat content is

reflected by greater marbling fleck area as well as the proportion of marbling area within the ribeye area, but does not directly correlate to number of marbling flecks (Gotoh et al., 2009).

Consumers utilize marbling as an indicator of quality and nutritional value (Pena et al., 2013). Intramuscular fat content is moderately correlated with number of marbling flecks, but the proportion of the largest flecks doesn't correlate to IMF content allowing for greater IMF contents to be reached without being negatively perceived by consumers (Pena et al., 2013).

Values of ribeye area varied greatly within the literature for Wagyu cattle depending on the system of measurement used. Oka et al. (2000) reported values of 42.1 cm² and 48.7 cm² for rib eye area respectively. Xie et al. (1996b) reported ribeye area of 101.4 cm². Rib eye area acquired from image analysis for the current study range between 87.85 cm² and 100.99 cm². These values presented by Wertz et al. (2002), Xie et al. (1996a) and Xie et al. (1996b) who reported ribeye area of 93.0 cm², 101.4 cm², and 95.5 cm² respectively. In comparison to Angus, cattle rib eye measurements are greater for Wagyu cattle. This is in agreement with data presented by Wertz et al. (2002), Lunt et al. (1993) and Lunt et al. (2005). Length and width measurements for ribeye area are not commonly discussed when describing ribeye area or dimensions. Discussion of image analysis of Wagyu cattle is limited within the literature. Factors such as visible lean area and visible fat area are not discussed. Marbling degree is often assessed through the assignment of USDA quality grades by Japanese quality grades or by Japanese quality grade standards. Xie et al. (1996a) reported marbling score of 538.2 for Wagyu compared to 478.2 for Angus steers. In comparison, days on feed caused an increase in marbling score for Wagyu steers from 450.7 to 458.5 as reported by Xie et al. (1996b). Wagyu carcasses are also graded based on Japanese grading standards. Limited comparisons can be made for marbling score between the U.S. system and Japanese system. Mir et al. (1999) reported increased

marbling score with increased Wagyu genetics. Similar results were presented by Ozawa et al., (2000) who reported beef marbling score values ranging from 5.4 to 10.2 on the Japanese scale. Wagyu cattle produced greater marbling scores than their Angus counterparts. Lunt et al (2005) reported marbling scores of 612.5 to 897.5 for Wagyu steers. This is in agreement with values observed with the current study.

Imaging allows for in depth description of marbling distribution including size of marbling flecks, as well as distance between flecks. No differences were observed for distribution characteristics. Numerically maximum marbling fleck size increased with the increase of roughage inclusion and maximum distance between flecks decreased with roughage inclusion. Data presented by Gotoh et al. (2009) discussed marbling fleck area, proportion of area accounted for by marbling and the total number of flecks. Wagyu steers had the greatest marbling fleck area of 37.0 cm² compared to Belgian Blue at 1.9 cm² and German Angus at 6.7 cm². The marbling present accounted for 35.8% of ribeye area for Wagyu cattle and 6.1% in Angus cattle (Gotoh et al., 2009). Although Wagyu had the greatest intramuscular fat content over Angus, Belgian Blue, and Holsteins, they had fewer numbers of marbling flecks (Gotoh et al., 2009). The difference in fat content was accounted for by larger fleck area rather than a greater number of flecks and may be a consequence of fusion of marbling flecks as fat deposition increased (Gotoh et al., 2009). Osawa et al. (2008) discussed marbling distribution in terms of overall coarseness of marbling particles as well as fat area ratios. Muscle area of longissimus muscle was much smaller at 52.3 cm² than values reported within the current study. Fat area ratio was reported at 39.3% (Osawa et al., 2008). Overall coarseness of marbling particles index was 19.6 and coarseness of maximum marbling particle index was 4.8 (Osawa et al., 2008). In comparison to other breeds marbling fleck size was greater for the present study. Albrecht et al.

(2006) reported marbling fleck size of 1.59 mm² for Angus, 1.31 mm² for Galloway, and 1.32 mm² for Holstein-Friesian at 24 months of age. Marbling fleck size increased with age for all breeds (Albecht et al., 2006). The Wagyu steers were 24 months of age at initiation of the feeding trial and may explain the increase in marbling fleck size. Number of marbling flecks for Angus, Belgian Blue, and Holstein are similar between Gotoh et al. (2009) and Albrecht et al. (2006), but proportion of marbling fleck area percentage values were lower for all breeds than values presented by Gotoh et al., (2009).

Implications

No significant differences were observed in feedlot performance for Wagyu cattle fed 10%, 20%, or 30% roughage within a total mixed ration. Cattle on this trial were older and all experienced similar background feeding prior to feedlot entry. More variation due to treatment may be apparent when fed to cattle started on feed at a younger age. Although not significant, average daily gain was numerically highest for 30% roughage inclusion rate at 1.45 kg/day. There was not a benefit of feeding any of the three treatment diets over the other. This can result in economic implications of feeding Wagyu cattle. Feed costs can be reduced by feeding the higher roughage diets without compromising feedlot performance.

Minor differences were observed in carcass characteristics, fatty acid composition or skeletal muscle, subcutaneous adipose and intramuscular adipose, or image analysis of carcass characteristics. No differences were observed in carcass weight backfat thickness, dressing percentage or yield grade. Linoleic acid concentration was decreased in 10% roughage inclusion rate treatment, but no difference was observed between the 20% and 30% roughage treatments. The diet analysis shows a stepwise increase in linoleic acid of 4.619 g/100g for 10% roughage, 5.611 g/100g for 20% roughage and 9.355 g/100g for 30% roughage concentrations within the

feed. The variation in availability of the Linoleic fatty acid follows the same pattern observed within the tissues of increasing linoleic acid concentration with increasing roughage inclusion. The elevated levels of linoleic acid within the diet allow for increased integration within the tissue. Concentrations of reported fatty acids were comparable to reported figures and support the claim that Wagyu produce greater concentrations of oleic acid which can have positive effect on reducing metabolic disease risk factors in humans (Smith et al., 2006). Ratios of saturation of fatty acid are in agreement with values reported by Elias Calles et al. (2000), Xie et al. (1996a), Oka et al. (2002), and Chung et al. (2006). The limited effect of treatment on fatty acid composition does not highlight benefits or disadvantages of roughage inclusion rate within the diet for modifying fatty acid composition. Roughage inclusion rate resulted in no treatment effects for carcass imaging characteristics involving marbling amount, distribution or size of marbling flecks. Rib eye area measured via carcass imaging was affected by treatment, but did not follow a pattern regarding roughage rate inclusion. Ribeye values obtained from carcass imaging do agree with values presented by Xie et al. (1996a and 1996b), Lunt et al. (1993 and 2005), and Wertz et al. (2002) and supports the statement that rib eye area is greater for Wagyu cattle than Angus cattle when fed for Extended periods of time (Lunt et al., 1993). No discernable treatment effects were observed for marbling amount, distribution, and fleck size. Consumers infer taste, tenderness, juiciness, and nutrition from intrinsic and extrinsic factors of color, size, visible leanness, and price and labeling (McIlveen and Buchanan, 2001). Grunert et al. (1997) concluded that fat content and color are most important to quality evaluation by customers, but consumers avoid obvious fat due to perceptions of reduced nutritional value (Chambers and Bowers, 1993). Number of marbling flecks is moderately correlated to IMF content, but large particles do not serve as a good representation of IMF content (Pena et al.,

2013). Finer marbling fleck size allows for increased marbling content within the muscle tissue without being identified by consumers as a non-desirable product (Chambers and Bowers, 1993). Although no treatment differences were observed, marbling degree values reached prime quality grades and support that Wagyu are known for marbling ability and are more likely to reach highest quality than Angus cattle (Lunt et al., 1993). Marbling fleck size, although not effected by treatment was greater than values reported by Albecht et al. (2006) and Osawa et al. (2008). The increased coarseness may be due to fusion of marbling flecks (Gotoh et al., 2009) and may be perceived negatively by consumers (McIlveen and Buchanan, 2001). Treatment groups did not identify a superior roughage inclusion rate for carcass characteristics, fatty acid composition or marbling distribution.

Table 4.1. Ingredient and nutrient composition of treatment diets of 10, 20, and 30% roughage inclusion on dry matter basis fed to Wagyu cattle during finishing phase

Item	Treatment ¹		
	10%	20%	30%
Alfalfa hay, kg	-	15.00	25.00
Grass hay, kg	5.00	-	-
Corn silage, kg	10.00	9.80	10.00
Cracked corn, kg	49.80	46.62	43.77
Dry distillers grains, kg	27.20	17.29	-
Soy bean meal, kg	-	-	9.05
Soy bean oil, kg	2.00	4.60	5.50
Ground limestone, kg	1.00	1.00	1.00
Urea, kg	-	0.69	0.68
Mineral premix ² , kg	5.00	5.00	5.00
Theoretical Composition			
EE, mg/kg	0.069	0.069	0.069
CP, %	14.04	14.00	14.00
ME, Mcal/kg	3.12	3.07	2.99
NEm, Mcal/kg	2.13	2.06	1.97
NEg, Mcal/kg	1.43	1.42	1.35
TDN, %	86.50	83.71	79.92
Chemical composition, DM basis			
CF, %	5.25	6.03	6.31
CP, %	14.88	14.80	15.89
ASH, %	2.77	4.25	4.12
DM, %	20.32	20.02	25.02

¹ 10%= roughage inclusion of 10% on dry matter basis; 20%= roughage inclusion of 20% on dry matter basis; 30%= roughage inclusion of 30% on dry matter basis

² 30% CP Hay Treat R400 (ADM Alliance Nutrition, Quincy, IL)

Table 4.2. Fatty acid composition of treatment diets of 10, 20, and 30% roughage inclusion fed to Wagyu Cattle during finishing period

Fatty Acid ^{1,3}	Common Name	Treatment ²		
		10%	20%	30%
C12:0	Lauric Acid	ND	ND	ND
C14:0	Myristic Acid	ND	ND	0.018
C16:0	Palmitic Acid	10.67	14.05	16.73
C16:1	Palmitoleic Acid	0.36	0.74	0.99
C18:0	Stearic Acid	1.79	2.55	3.20
C18:1	Oleic Acid	45.61	42.08	37.42
C18:2	Linoleic Acid	36.38	34.50	31.91
C18:3	Alpha-linoleic Acid	4.62	5.61	9.36
C20:0	Arachidic Acid	0.19	0.15	0.10
C20:1	Eicosenoic Acid	0.14	0.12	0.08
C22:0	Behenic Acid	0.06	0.04	0.05
C22:1	Erucic Acid	0.06	0.07	0.07
C24:0	Lignoceric Acid	0.14	0.11	0.09
C24:1	Nervonic Acid	ND	ND	ND

¹ Adjusted diet fatty acid by weight percentage or g/100g.

² 10%= roughage inclusion of 10% on dry matter basis; 20%= roughage inclusion of 20% on dry matter basis; 30%= roughage inclusion of 30% on dry matter basis.

³ Analysis performed on composite sample of mixed feed samples collected throughout the feeding trial.

ND= Not detected

Table 4.3. Summary statistics of Wagyu cattle fed diets with 10, 20, and 30% roughage inclusion during finishing phase

Item	Mean	SD	Min	Max
Initial d0 BW, kg	547.6	62.06	431.82	634.09
Harvest BW, kg	675.2	53.27	584.09	804.55
Predicted initial BW, kg ²	544.5	58.48	446.62	620.50
Predicted final BW, kg ²	753.0	169.15	596.30	954.79
Age at d0	720.8	76.96	594.00	794.00
Age at harvest, d	873.4	83.46	720.50	1036.00
Days on feed, d	130.4	54.64	33.00	168.00
Total DMI, kg ¹	1208.6	479.74	407.54	1797.04
ADG, kg/d ¹	1.2	0.86	0.43	4.66
G:F ¹	0.12	0.077	0.05	0.44
HCW, kg	425.6	32.90	371.36	487.95
Dressing percentage, %	62.2	1.40	59.38	65.34
Rib eye area, cm ²	102.1	8.93	77.40	114.49
Back fat thickness, cm	1.4	0.44	0.76	2.28
Calculated yield grade	3.5	0.61	2.72	4.71

¹ Values measured over first 168 d of feeding period

² Values calculated from regression analysis of weekly body weights

Table 4.4. Least square means of feedlot performance in Wagyu cattle during 168d feeding trial fed diets with 10, 20, and 30% roughage inclusion

Item	Treatment ¹			SEM	P-value ²
	10%	20%	30%		
No. of Pens	7	7	7		
No. of steers	10	10	10		
No. of heifers	4	3	4		
Days on feed	126.0	120.9	144.3	21.38	0.72
BW, kg					
Initial	523.4	531.5	528.2	18.99	0.95
Harvest	691.1	674.0	689.4	8.04	0.28
Predicted, d0 ³	546.1	547.3	540.0	5.96	0.66
Predicted, d168 ³	767.5	700.5	791.0	52.41	0.46
ADG, kg/d ³	1.27	0.93	1.46	0.332	0.53
DMI, kg/d ³	9.98	9.38	9.93	0.319	0.35
G:F ³	0.12	0.10	0.15	0.030	0.50

¹ 10%= roughage inclusion of 10% on dry matter basis; 20%= roughage inclusion of 20% on dry matter basis; 30%= roughage inclusion of 30% on dry matter basis

² Probability that treatment means are not different.

³ Values calculated from regression analysis of weekly body weights

Table 4.5. Least square means of carcass characteristics of Wagyu cattle fed diets with 10, 20, and 30% roughage inclusion during finishing phase

Item	Treatment ¹			SEM	P-value ²
	10%	20%	30%		
No. of Pens	6	7	6		
No. of steers	8	10	8		
No. of heifers	4	2	4		
Days on feed	117.80	170.14	154.0	38.40	0.58
Initial Wt., kg	523.3	531.5	528.2	18.99	0.95
Harvest Wt., kg	691.1	674.0	689.4	8.04	0.28
HCW, kg	424.9	416.3	417.1	7.23	0.58
Dressing %	61.91	61.82	61.43	0.580	0.77
Back fat thickness, cm	1.69	1.52	1.28	0.174	0.22
Ribeye area, cm ²	98.67	107.15	99.13	3.265	0.11
Calculated yield grade	3.98	3.27	3.45	0.239	0.11

¹ 10%= roughage inclusion of 10% on dry matter basis; 20%= roughage inclusion of 20% on dry matter basis; 30%= roughage inclusion of 30% on dry matter basis

² Probability that treatment means are not different.

Table 4.6 Influence of 10, 20, and 30% roughage inclusion diets fed during finishing phase on intramuscular adipose tissue fatty acid composition of Wagyu cattle

Item ²	Common Name	Treatment ¹			SEM	P-Value ³
		10%	20%	30%		
C10:0	Capric Acid	0.05	0.05	0.05	0.009	0.62
C12:0	Lauric Acid	0.10	0.10	0.09	0.002	0.32
C12:1		0.04	0.03	0.03	0.003	0.11
C14:0	Myristic Acid	2.53	2.12	2.29	0.150	0.1
C14:1	Myristicoleic Acid	1.23	1.33	1.30	0.110	0.21
C16:0	Palmitic Acid	20.60	20.45	20.71	0.340	0.87
C16:1	Palmitoleic Acid	5.95	6.17	6.07	0.180	0.41
C17:0	Heptadecanoic Acid	1.44	1.38	1.45	0.120	0.76
C17:1	Heptadecenoic Acid	1.11	1.09	1.10	0.040	0.82
C18:0	Stearic Acid	7.90	7.80	7.89	0.580	0.74
C18:1 t-9	Elaidate Acid	0.11	0.10	0.11	0.008	0.39
C18:1 t-10		0.39	0.37	0.41	0.020	0.29
C18:1 t11	Vaccenic Acid	1.76	1.72	1.75	0.140	0.48
C18:1 t-12		2.79	2.50	2.67	0.210	0.68
C18:1c9	Oleic Acid	48.88	49.65	49.30	0.880	0.87
C18:1c11	<i>Cis</i> -Vaccenic Acid	2.09	2.13	2.05	0.060	0.64
C18:2	Linoleic Acid	1.97	1.82	1.70	0.015	0.84
C18:3	Linolenic Acid	0.12	0.16	0.16	0.020	0.07
C20:0	Arachidic Acid	0.05	0.05	0.06	0.008	0.36
C18:2c9t11	CLA ⁴ - Rumenic Acid	0.36	0.37	0.34	0.018	0.42
C18:2t10c12	CLA ⁴	0.04	0.04	0.03	0.001	0.09
C20:1	Eicosenoic Acid	0.18	0.19	0.17	0.020	0.62
C20:2	Eicosadienoic Acid	0.01	0.01	0.01	0.001	0.76
C20:4	Eicosatetraenoic Acid	0.15	0.11	0.10	0.009	0.56
C20:5	Eicosapentaenoic Acid	0.02	0.01	0.02	0.001	0.42
C24:0	Lignoceric Acid	0.02	0.02	0.02	0.001	0.48
C22:6	Docosahexaenoic Acid	0.01	0.01	0.01	0.001	0.73

¹ 10%= roughage inclusion of 10% on dry matter basis; 20%= roughage inclusion of 20% on dry matter basis; 30%= roughage inclusion of 30% on dry matter basis.

² Adjusted Diet Fatty Acid by weight percentage or g/100g.

³ Probability that treatment means are not different.

⁴ Conjugated Linoleic Acid

Table 4.7 Influence of 10, 20, and 30% roughage inclusion diets fed during finishing phase on subcutaneous adipose tissue fatty acid composition of Wagyu cattle

Item2	Common Name	Treatment1			SEM	P-value ³
		10%	20%	30%		
C10:0	Capric Acid	0.042	0.041	0.042	0.011	0.54
C12:0	Lauric Acid	0.078	0.075	0.081	0.001	0.12
C12:1		0.043	0.04	0.044	0.003	0.62
C14:0	Myristic Acid	2.12	2.28	2.29	0.11	0.78
C14:1	Myristicolenic Acid	1.54	1.57	1.6	0.13	0.51
C16:0	Palmitic Acid	20.67	20.79	21.24	0.32	0.71
C16:1	Palmitoleic Acid	6.25	6.29	6.45	0.22	0.62
C17:0	Heptadecanoic Acid	1.15	1.14	1.19	0.11	0.82
C17:1	Heptadecenoic Acid	1.11	1.09	1.13	0.06	0.67
C18:0	Stearic Acid	8.81	7.78	7.76	0.62	0.14
C18:1 t-9	Elaidate Acid	0.122	0.116	0.109	0.008	0.48
C18:1 t-10		0.418	0.415	0.428	0.09	0.48
C18:1 t11	Vaccenic Acid	2.31	2.27	2.4	0.16	0.58
C18:1 t-12		3.24	3.63	3.25	0.21	0.57
C18:1c9	Oleic Acid	47.74	48.2	47.57	0.93	0.74
C18:1c11	<i>Cis</i> -Vaccenic Acid	1.91	1.93	1.93	0.02	0.81
C18:2	Linoleic Acid	1.14	1.09	1.19	0.07	0.63
C18:3	Linolenic Acid	0.157	0.146	0.144	0.004	0.08
C20:0	Arachidic Acid	0.046	0.045	0.047	0.002	0.64
C18:2c9t11	CLA4- Rumenic Acid	0.478	0.471	0.494	0.012	0.68
C18:2t10c12	CLA4	0.064	0.062	0.068	0.001	0.38
C20:1	Eicosenoic Acid	0.239	0.236	0.247	0.04	0.72
C20:2	Eicosadienoic Acid	0.01	0.011	0.011	0.001	0.91
C20:4	Eicosatetraenoic Acid	0.109	0.108	0.112	0.001	0.87
C20:5	Eicosapentaenoic Acid	ND ^a	ND	ND	---	---
C24:0	Lignoceric Acid	0.036	0.034	0.04	0.008	0.68
C22:6	Docosahexaenoic Acid	ND	ND	ND	---	---

¹ 10%= roughage inclusion of 10% on dry matter basis; 20%= roughage inclusion of 20% on dry matter basis; 30%= roughage inclusion of 30% on dry matter basis.

² Adjusted Diet Fatty Acid by weight percentage or g/100g.

³ Probability that treatment means are not different.

⁴ Conjugated Linoleic Acid

^aND= not detected.

Table 4.8 Influence of 10, 20, and 30% roughage inclusion diets fed during finishing phase on fatty acid composition by treatment and tissue type in Wagyu cattle

Item	Treatment			SEM(trt)	<i>P</i> <		
	10%	20%	30%		Trt	Tissue	Trt x Tissue
C10:0					0.26	0.14	0.76
IMF	0.05	0.05	0.05	0.009			
SQ	0.04	0.04	0.04	0.011			
C12:0					0.64	0.04	0.25
IMF	0.10	0.10	0.09	0.002		*	
SQ	0.08	0.08	0.08	0.001		**	
C12:1					0.73	0.05	0.38
IMF	0.04	0.03	0.03	0.003		*	
SQ	0.04	0.04	0.04	0.003		**	
C14:0					0.26	0.01	0.11
IMF	2.53	2.12	2.29	0.150		*	
SQ	2.12	2.28	2.29	0.110		**	
C14:1					0.12	0.001	0.78
IMF	1.23	1.33	1.30	0.110		*	
SQ	1.54	1.57	1.60	0.130		**	
C16:0					0.46	0.63	0.44
IMF	20.62	20.45	20.71	0.340			
SQ	20.67	20.79	21.24	0.320			
C16:1					0.84	0.38	0.61
IMF	5.95	6.17	6.07	0.180			
SQ	6.25	6.29	6.45	0.220			
C17:0					0.84	0.0001	0.76
IMF	1.44	1.38	1.45	0.120		*	
SQ	1.15	1.14	1.19	0.110		**	
C17:1					0.64	0.45	0.76
IMF	1.11	1.09	1.10	0.040			
SQ	1.11	1.09	1.13	0.060			
C18:0					0.50	0.29	0.09
IMF	7.90	7.81	7.89	0.580			
SQ	8.81	7.78	7.76	0.620			
C18:1 t-6,8					0.45	0.35	0.24
IMF	0.11	0.10	0.11	0.008			
SQ	0.12	0.12	0.11	0.008			
C18:1 t-9					0.24	0.04	0.37
IMF	0.39	0.37	0.41	0.020		*	
SQ	0.42	0.42	0.43	0.090		**	
C18:1 t11					0.17	0.03	0.47
IMF	1.76	1.72	1.75	0.140		*	
SQ	2.31	2.27	2.40	0.160		**	
C18:1 t-10					0.46	0.02	0.3
IMF	2.79	2.50	2.67	0.210		*	
SQ	3.24	3.63	3.25	0.210		**	
C18:1c9					0.59	0.05	0.84
IMF	48.88	49.65	49.30	0.880		*	
SQ	47.74	48.20	47.57	0.930		**	
C18:1c11					0.72	0.04	0.87
IMF	2.09	2.13	2.05	0.060		*	
SQ	1.91	1.93	1.93	0.020		**	
C18:2					0.46	0.05	0.77
IMF	1.97	1.82	1.70	0.015		*	
SQ	1.14	1.09	1.19	0.070		**	
C18:3					0.07	0.06	0.31
IMF	0.12	0.16	0.16	0.020		*	
SQ	0.16	0.15	0.14	0.004		**	
C20:0					0.59	0.28	0.79
IMF	0.05	0.05	0.06	0.008			
SQ	0.05	0.05	0.05	0.002			
C18:2c9t11					0.19	0.02	0.28

IMF	0.36	0.37	0.34	0.018		*	
SQ	0.48	0.47	0.49	0.012		**	
C18:2t10c12					0.81	0.001	0.58
IMF	0.04	0.04	0.03	0.001		*	
SQ	0.06	0.06	0.07	0.001		**	
C20:1					0.68	0.04	0.14
IMF	0.18	0.19	0.17	0.020		*	
SQ	0.24	0.24	0.25	0.040		**	
C20:2					0.73	0.16	0.28
IMF	0.01	0.01	0.01	0.001			
SQ	0.01	0.01	0.01	0.001			
C20:4					0.85	0.72	0.81
IMF	0.15	0.11	0.10	0.009			
SQ	0.11	0.11	0.11	0.001			
C20:5					0.83	---	---
IMF	0.02	0.01	0.02	0.001			
SQ	ND ^a	ND	ND	---			
C24:0					0.48	0.05	0.63
IMF	0.02	0.02	0.02	0.001		*	
SQ	0.04	0.03	0.04	0.008		**	
C22:6					0.79	---	---
IMF	0.01	0.01	0.01	0.001			
SQ	ND	ND	ND	---			

[†] Adjusted Diet Fatty Acid by weight percentage or g/100g

*** Overall means within a row with different symbols differ ($P < 0.05$)

ND = Not Detected

Table 4.9. Mean concentration of saturated, unsaturated, *trans*, n-3, and n-6 fatty acids in Wagyu cattle fed 10%, 20%, and 30% roughage inclusion diets during finishing phase

Fatty Acid	Treatment			SEM (trt)	P-Value	
	10%	20%	30%		TRT	TISSUE
SFA				0.480	0.84	0.75
IMF	32.71	32.41	32.55			
SQ	32.95	32.14	32.69			
UNSAT				0.540	0.42	0.49
IMF	67.19	67.79	67.32			
SQ	66.88	67.14	67.17			
MUFA				0.460	0.51	0.68
IMF	64.53	65.27	64.96			
SQ	64.92	65.78	65.15			
PUFA				0.120	0.42	0.05
IMF	2.68	2.53	2.37			*
SQ	2.31	1.92	2.02			**
n-3				0.110	0.15	0.82
IMF	0.14	0.18	0.18			
SQ	0.16	0.15	0.14			
n-6				0.130	0.43	0.05
IMF	2.13	1.94	1.81			*
SQ	1.26	1.21	1.31			**
Total trans				0.240	0.42	0.03
IMF	5.05	4.69	4.94			*
SQ	6.09	6.43	6.19			**
c9, t11 CLA				0.150	0.57	0.05
IMF	0.36	0.37	0.34			*
SQ	0.48	0.47	0.49			**
Total CLA				0.140	0.35	0.02
IMF	0.40	0.41	0.37			*
SQ	0.54	0.53	0.56			**
MUFA:SFA				0.350	0.49	0.39
IMF	1.97	2.01	2.00			
SQ	1.97	2.04	1.99			
PUFA:SFA				0.003	0.41	0.12
IMF	0.08	0.08	0.07			
SQ	0.07	0.06	0.06			
n-6:n-3				2.000	0.16	0.04
IMF	15.20	11.08	10.16			*
SQ	8.02	8.28	9.12			**

¹ 10%= roughage inclusion of 10% on dry matter basis; 20%= roughage inclusion of 20% on dry matter basis; 30%= roughage inclusion of 30% on dry matter basis.

² Adjusted Diet Fatty Acid by weight percentage or g/100g.

³ Total SFA=Σ 10:0, 12:0, 14:0, 16:0, 18:0, 20:0, and 24:0.

⁴ Total Unsaturated = Σ 12:1,14:1, 16:1, 17:1, 18:1t9, 18:1t10, 18:1t11, 18:1t12, 18:1c9, 18:1c11, 18:2, 18:3, 18:2c9t11,18:2t10,c12, 20:1, 20:2, 20:4, 20:5, and 22:6.

⁵ Total MUFA = Σ 12:1,14:1, 16:1, 17:1, 18:1t9, 18:1t10, 18:1t11, 18:1t12, 18:1c9, 18:1c11, and 20:1.

⁶ Total PUFA = Σ 18:2, 18:3, 18:2c9t11,18:2t10,c12, 20:2, 20:4, 20:5, and 22:6.

⁷ Total n-3 = Σ 8:3, 20:5, and 22:6.

⁸ Total n-6 = Σ 18:2, 20:2, and 20:4.

⁹ Total Trans = Σ 18:1t9, 18:1t10, 18:1t11, and 18:1t12.

¹⁰ Total CLA = Σ 18:2c9t11, and 18:2t10,c12.

** Overall means within a row with different symbols differ ($P < 0.05$).

Table 4.10. Least square means of carcass imaging characteristics of Wagyu cattle fed diets with 10, 20, and 30% roughage inclusion during the finishing phase

Item	Treatment ¹			SEM	P-value ²
	10%	20%	30%		
No. of Pens	6	7	6		
No. of steers	8	10	8		
No. of heifers	4	2	4		
Ribeye area, cm ²	87.85	100.99	94.83	3.726	0.051
Ribeye length, cm ²	15.72	15.80	16.02	0.369	0.824
Ribeye width, cm ²	8.17	8.96	8.73	0.303	0.164
Visible lean area, mm ²	112.82	122.93	118.55	3.541	0.126
Visible fat area, mm ²	120.18	112.32	116.71	3.672	0.283
Marbling degree	794.93	809.70	838.33	44.978	0.766
Total percent fat, %	10.84	9.95	12.77	1.034	0.119
Mean marbling size, mm ²	2.77	2.57	2.97	0.173	0.206
Max marbling size, mm ²	153.53	181.90	231.62	27.336	0.130
Mean distance between marbling, mm	4.56	4.57	4.06	0.347	0.455
Max distance between marbling, mm	30.00	27.66	27.22	2.660	0.718

¹ 10%= roughage inclusion of 10% on dry matter basis; 20%= roughage inclusion of 20% on dry matter basis; 30%= roughage inclusion of 30% on dry matter basis

² Probability that treatment means are not different.

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APPENDIX I

SAS Code for Chapter II

Code for analysis of summary statistics:

```
proc sort data=summarystats;  
by trt;  
run;
```

```
proc means data=summarystats;  
by trt;  
run;
```

Code for analysis of feedlot performance:

Regression Analysis of Weight

```
proc sort;  
by pen trt sex;  
run;
```

```
proc reg data=weight;  
by pen trt;  
model wt=week2 week;  
run;
```

```
proc sort data=wagyu;  
by trt;  
proc means data=wagyu;  
by trt;
```

```
title "Dry Matter Intake";  
proc mixed data=wagyu;  
class trt;  
model dmi=trt initialage donfeed;  
lsmeans trt /pdiff cl;
```

```
title "Gain to Feed";  
proc mixed data=wagyu;  
class trt;  
model gf=trt;  
lsmeans trt /pdiff cl;  
run;
```

```
title "Average Daily Gain";  
proc mixed data=wagyu;  
class trt;
```

```
model adg=trt;
lsmeans trt /pdiff cl;
run;
```

```
title "intercept";
proc mixed data=wagyu;
class trt;
model intercept=trt initialwt;
lsmeans trt /pdiff cl;
run;
```

```
title "slope of week";
proc mixed data=wagyu;
class trt;
model slopew=trt;
lsmeans trt /pdiff cl;
run;
```

```
title "slope week2";
proc mixed data=wagyu;
class trt;
model slopew2=trt;
lsmeans trt /pdiff cl;
run;
```

```
title "Predicted Final Weight";
proc mixed data=wagyu;
class trt;
model predfwt=trt initialwt;
lsmeans trt /pdiff cl;
run;
```

```
title "harvest weight";
proc mixed data=wagyu;
class trt;
model harvestwt=trt;
lsmeans trt /pdiff cl;
run;
```

```
title "Total DMI";
proc mixed data=wagyu;
class trt;
model totdmi=trt;
lsmeans trt /pdiff cl;
run;
```

SAS Code for Chapter III

Code for analyzing summary statistics:

```
proc sort data=summarystats;  
by trt;  
run;
```

```
proc means data=summarystats;  
by trt;  
run;
```

Code for analyzing carcass characteristics:

```
title "harvest weight";  
proc mixed data=wagyu;  
class trt;  
model harvestwt=trt;  
lsmeans trt /pdiff cl;  
run;
```

```
title "Yield Grade";  
proc mixed data=wagyu;  
class trt;  
model YG=trt;  
lsmeans trt /pdiff cl;  
run;
```

```
title "hcw";  
proc mixed data=wagyu;  
class trt;  
model hcw=trt;  
lsmeans trt /pdiff cl;  
run;
```

```
title "Back Fat cm";  
proc mixed data=wagyu;  
class trt;  
model bfc=trt;  
lsmeans trt /pdiff cl;  
run;
```

```
title "Rib Eye Area";  
proc mixed data=wagyu;  
class trt;  
model reacm=trt;
```

```
lsmeans trt /pdiff cl;  
run;
```

```
title "Dressing Percentage";  
proc mixed data=wagyu;  
class trt;  
model DP=trt;  
lsmeans trt /pdiff cl;  
run;
```

```
Title "Days on Feed";  
proc mixed data=wagyu;  
class trt;  
model donfeed=trt;  
lsmeans trt /pdiff cl;  
run;
```

Code for analyzing fatty acid composition:

```
proc mixed method = reml data=perf covtest cl;  
class trt tissue;  
model bb= trt|tissue/ddfm=satterth;  
random trt (tissue);  
lsmeans trt|tissue/pdiff;  
run;
```

Code for analysis of carcass imaging characteristics:

```
title "RMS_Marbling_Degree";  
proc mixed data=images;  
class trt sex;  
model RMS_Marbling_Degree=trt;  
lsmeans trt /pdiff cl;  
run;
```

```
title "Ribeye Area";  
proc mixed data=images;  
class trt sex;  
model Ribeye_Area=trt;  
lsmeans trt /pdiff cl;  
run;
```

```
title "Ribeye length";  
proc mixed data=images;  
class trt sex;  
model Ribeye_length=trt;
```

```
lsmeans trt /pdiff cl;  
run;
```

```
title "Ribeye Width";  
proc mixed data=images;  
class trt sex;  
model Ribeye_Width=trt;  
lsmeans trt /pdiff cl;  
run;
```

```
title "Grade Percent Fat";  
proc mixed data=images;  
class trt sex;  
model Grade_Percent_Fat=trt;  
lsmeans trt /pdiff cl;  
run;
```

```
title "Total Percent Fat";  
proc mixed data=images;  
class trt sex;  
model Total_Percent_Fat=trt finalwt initialwt dfeed;  
lsmeans trt /pdiff cl;  
run;
```

```
title "Ribeye R";  
proc mixed data=images;  
class trt sex;  
model Ribeye_R=trt;  
lsmeans trt /pdiff cl;  
run;
```

```
title "Ribeye G";  
proc mixed data=images;  
class trt sex;  
model Ribeye_G=trt;  
lsmeans trt /pdiff cl;  
run;
```

```
title "Ribeye B";  
proc mixed data=images;  
class trt sex;  
model Ribeye_B=trt;  
lsmeans trt /pdiff cl;  
run;
```



```
title "Ribeye Ls";
proc mixed data=images;
class trt sex;
model Ribeye_Ls=trt;
lsmeans trt /pdiff cl;
run;
```

```
title "Ribeye as";
proc mixed data=images;
class trt sex;
model Ribeye_as=trt;
lsmeans trt /pdiff cl;
run;
```

```
title "Ribeye bs";
proc mixed data=images;
class trt sex;
model Ribeye_bs=trt;
lsmeans trt /pdiff cl;
run;
```

```
title "Fat R";
proc mixed data=images;
class trt sex;
model Fat_R=trt;
lsmeans trt /pdiff cl;
run;
```

```
title " Fat G";
proc mixed data=images;
class trt sex;
model Fat_G=trt;
lsmeans trt /pdiff cl;
run;
```

```
title "Fat B";
proc mixed data=images;
class trt sex;
model Fat_B=trt;
lsmeans trt /pdiff cl;
run;
```

```
title "Fat Ls";
proc mixed data=images;
class trt sex;
model Fat_Ls=trt;
```

```
lsmeans trt /pdiff cl;  
run;
```

```
title "Fat as";  
proc mixed data=images;  
class trt sex;  
model Fat_as=trt;  
lsmeans trt /pdiff cl;  
run;
```

```
title "Fat bs";  
proc mixed data=images;  
class trt sex;  
model Fat_bs=trt;  
lsmeans trt /pdiff cl;  
run;
```

```
title "Visible Fat Area";  
proc mixed data=images;  
class trt sex;  
model Visible_Fat_Area=trt ;  
lsmeans trt /pdiff cl;  
run;
```

```
title "Visible Lean Area";  
proc mixed data=images;  
class trt sex;  
model Visible_Lean_Area=trt;  
lsmeans trt /pdiff cl;  
run;
```

```
title "Visible Fat Percent";  
proc mixed data=images;  
class trt sex;  
model Visible_Fat_Percent=trt;  
lsmeans trt /pdiff cl;  
run;
```

```
title "Mean Marb Size";  
proc mixed data=images;  
class trt sex;  
model MeanMarbSize=trt finalwt initialwt dfeed;  
lsmeans trt /pdiff cl;  
run;
```

```
title "Max Marb Size";
proc mixed data=images;
class trt sex;
model MaxMarbSize=trt;
lsmeans trt /pdiff cl;
run;
```

```
title "Mean Dist to Marb";
proc mixed data=images;
class trt sex;
model MeanDistToMarb=trt;
lsmeans trt /pdiff cl;
run;
```

```
title "Max Dist to Marb";
proc mixed data=images;
class trt sex;
model MaxDistToMarb=trt;
lsmeans trt /pdiff cl;
run;
```

```
title "raw percent fat";
proc mixed data=images;
class trt sex;
model raw_percent_fat=trt finalwt initialwt dfeed;
lsmeans trt /pdiff cl;
run;
```

SAS Code for Chapter IV

Code for analysis of summary statistics:

```
proc sort data=summarystats;
by trt;
run;
```

```
proc means data=summarystats;
by trt;
run;
```

Code for analysis of feedlot performance:

Regression Analysis of Weight

```
proc sort;
by pen trt sex;
```

```

run;

proc reg data=weight;
by pen trt;
model wt=week2 week;
run;

proc sort data=wagyu;
by trt;
proc means data=wagyu;
by trt;

title "Dry Matter Intake";
proc mixed data=wagyu;
class trt;
model dmi=trt initialage donfeed;
lsmeans trt /pdiff cl;

title "Gain to Feed";
proc mixed data=wagyu;
class trt;
model gf=trt;
lsmeans trt /pdiff cl;
run;

title "Average Daily Gain";
proc mixed data=wagyu;
class trt;
model adg=trt;
lsmeans trt /pdiff cl;
run;

title "intercept";
proc mixed data=wagyu;
class trt;
model intercept=trt initialwt;
lsmeans trt /pdiff cl;
run;

title "slope of week";
proc mixed data=wagyu;
class trt;
model slopew=trt;
lsmeans trt /pdiff cl;
run;

```

```
title "slope week2";
proc mixed data=wagyu;
class trt;
model slopew2=trt;
lsmeans trt /pdiff cl;
run;
```

```
title "Predicted Final Weight";
proc mixed data=wagyu;
class trt;
model predfwt=trt initialwt;
lsmeans trt /pdiff cl;
run;
```

```
title "harvest weight";
proc mixed data=wagyu;
class trt;
model harvestwt=trt;
lsmeans trt /pdiff cl;
run;
```

```
title "Total DMI";
proc mixed data=wagyu;
class trt;
model totdmi=trt;
lsmeans trt /pdiff cl;
run;
```

Code for analyzing carcass characteristics:

```
title "harvest weight";
proc mixed data=wagyu;
class trt;
model harvestwt=trt;
lsmeans trt /pdiff cl;
run;
```

```
title "Yield Grade";
proc mixed data=wagyu;
class trt;
model YG=trt;
lsmeans trt /pdiff cl;
run;
```

```
title "hcw";
proc mixed data=wagyu;
```

```
class trt;
model hcw=trt;
lsmeans trt /pdiff cl;
run;
```

```
title "Back Fat cm";
proc mixed data=wagyu;
class trt;
model bfc=trt;
lsmeans trt /pdiff cl;
run;
```

```
title "Rib Eye Area";
proc mixed data=wagyu;
class trt;
model reacm=trt;
lsmeans trt /pdiff cl;
run;
```

```
title "Dressing Percentage";
proc mixed data=wagyu;
class trt;
model DP=trt;
lsmeans trt /pdiff cl;
run;
```

```
Title "Days on Feed";
proc mixed data=wagyu;
class trt;
model donfeed=trt;
lsmeans trt /pdiff cl;
run;
```

Code for analyzing fatty acid composition:

```
proc mixed method = reml data=perf covtest cl;
class trt tissue;
model bb= trt|tissue/ddfm=satterth;
random trt (tissue);
lsmeans trt|tissue/pdiff;
run;
```

Code for analysis of carcass imaging characteristics:

```
title "RMS_Marbling_Degree";
proc mixed data=images;
```

```
class trt sex;
model RMS_Marbling_Degree=trt;
lsmeans trt /pdiff cl;
run;
```

```
title "Ribeye Area";
proc mixed data=images;
class trt sex;
model Ribeye_Area=trt;
lsmeans trt /pdiff cl;
run;
```

```
title "Ribeye length";
proc mixed data=images;
class trt sex;
model Ribeye_length=trt;
lsmeans trt /pdiff cl;
run;
```

```
title "Ribeye Width";
proc mixed data=images;
class trt sex;
model Ribeye_Width=trt;
lsmeans trt /pdiff cl;
run;
```

```
title "Grade Percent Fat";
proc mixed data=images;
class trt sex;
model Grade_Percent_Fat=trt;
lsmeans trt /pdiff cl;
run;
```

```
title "Total Percent Fat";
proc mixed data=images;
class trt sex;
model Total_Percent_Fat=trt finalwt initialwt dfeed;
lsmeans trt /pdiff cl;
run;
```

```
title "Ribeye R";
proc mixed data=images;
class trt sex;
model Ribeye_R=trt;
lsmeans trt /pdiff cl;
run;
```

```
title "Ribeye G";  
proc mixed data=images;  
class trt sex;  
model Ribeye_G=trt;  
lsmeans trt /pdiff cl;  
run;
```

```
title "Ribeye B";  
proc mixed data=images;  
class trt sex;  
model Ribeye_B=trt;  
lsmeans trt /pdiff cl;  
run;
```

```
title "Ribeye Ls";  
proc mixed data=images;  
class trt sex;  
model Ribeye_Ls=trt;  
lsmeans trt /pdiff cl;  
run;
```

```
title "Ribeye as";  
proc mixed data=images;  
class trt sex;  
model Ribeye_as=trt;  
lsmeans trt /pdiff cl;  
run;
```

```
title "Ribeye bs";  
proc mixed data=images;  
class trt sex;  
model Ribeye_bs=trt;  
lsmeans trt /pdiff cl;  
run;
```

```
title "Fat R";  
proc mixed data=images;  
class trt sex;  
model Fat_R=trt;  
lsmeans trt /pdiff cl;  
run;
```

```
title " Fat G";  
proc mixed data=images;  
class trt sex;  
model Fat_G=trt;
```



```
lsmeans trt /pdiff cl;  
run;
```

```
title "Fat B";  
proc mixed data=images;  
class trt sex;  
model Fat_B=trt;  
lsmeans trt /pdiff cl;  
run;
```

```
title "Fat Ls";  
proc mixed data=images;  
class trt sex;  
model Fat_Ls=trt;  
lsmeans trt /pdiff cl;  
run;
```

```
title "Fat as";  
proc mixed data=images;  
class trt sex;  
model Fat_as=trt;  
lsmeans trt /pdiff cl;  
run;
```

```
title "Fat bs";  
proc mixed data=images;  
class trt sex;  
model Fat_bs=trt;  
lsmeans trt /pdiff cl;  
run;
```

```
title "Visible Fat Area";  
proc mixed data=images;  
class trt sex;  
model Visible_Fat_Area=trt ;  
lsmeans trt /pdiff cl;  
run;
```

```
title "Visible Lean Area";  
proc mixed data=images;  
class trt sex;  
model Visible_Lean_Area=trt;  
lsmeans trt /pdiff cl;  
run;
```

```
title "Visible Fat Percent";
proc mixed data=images;
class trt sex;
model Visible_Fat_Percent=trt;
lsmeans trt /pdiff cl;
run;
```

```
title "Mean Marb Size";
proc mixed data=images;
class trt sex;
model MeanMarbSize=trt finalwt initialwt dfeed;
lsmeans trt /pdiff cl;
run;
```

```
title "Max Marb Size";
proc mixed data=images;
class trt sex;
model MaxMarbSize=trt;
lsmeans trt /pdiff cl;
run;
```

```
title "Mean Dist to Marb";
proc mixed data=images;
class trt sex;
model MeanDistToMarb=trt;
lsmeans trt /pdiff cl;
run;
```

```
title "Max Dist to Marb";
proc mixed data=images;
class trt sex;
model MaxDistToMarb=trt;
lsmeans trt /pdiff cl;
run;
```

```
title "raw percent fat";
proc mixed data=images;
class trt sex;
model raw_percent_fat=trt finalwt initialwt dfeed;
lsmeans trt /pdiff cl;
run;
```