GROWTH PERFORMANCE AND CARCASS QUALITY OF GRASS-FED BEEF RAISED ON TROPICAL FORAGES/LEGUMES

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BY KAYLA RYAN BUTLER

Thesis committee: Dr. Rajesh Jha, Chairperson

Dr. Yong Soo Kim

Dr. Chin Lee

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Abstract

Most beef producers in Hawaii ship cattle to the continental United States and Canada for feedlot-finishing, slaughtering and processing. Reasons for this include high transportation costs associated with shipping grain and a lack of production and slaughtering capacity in the state. There is a niche market of consumers that prefer grass-fed over grain-fed beef. However, studies have proven variation in growth performance, carcass quality and nutritional value of beef when comparing these production systems. Typically, grass-fed cattle reach slaughter weight slower, have leaner carcasses, and the meat may be healthier from a consumer standpoint. However, little research has been done to evaluate how nutrient content of different species/varieties of tropical grasses and legumes can affect these parameters in cattle grazing on Hawaii pastures.

Two studies were conducted. The objectives of study one were to determine nutrient profiles of guinea grass (GG) and the tropical legume leucaena (L), and to evaluate growth performance and carcass characteristics of steers grazed on these pastures at Ranch A on Hawaii Island. The objective of study two was to compare differences in nutrient profiles of GG and kikuyu grass (KK) due to: grass type (GG vs KK), season (summer vs winter) and ranch location (B vs C vs D vs E) on Hawaii Island.

Nutrient composition of all samples was determined by near-infrared spectroscopy. Variables determined included: percent crude protein (CP), acid detergent fiber (ADF), ash-free neutral detergent fiber (aNDFom), ash, total digestible nutrients (TDN), relative feed value (RFV), energy for maintenance (NEM, Mcal/kg), and ash-free neutral detergent fiber digestibility (NDFDom). Growth performance and carcass characteristics (hot carcass weight, backfat thickness, rib-eye area, marbling score, USDA quality grade, and Warner-Bratzler shear

force) of steers grazed on GG and GL pastures were determined. Chemical composition of rib-eye samples (moisture, fat, protein, ash, and pH) was determined as well.

Higher average daily gain (ADG) and desirable carcass characteristics were found for steers that grazed on GL pastures compared to GG pastures, which can be attributed to L having an overall higher nutrient value (CP 18.8 vs 27.3, ADF 39.2 vs 27.0, aNDFom 51.5 vs 31.5, Ash 16.3 vs 12.3, TDN 52.4 vs 59.6 % of GG vs L, respectively, $P<0.05$). Cattle grazed on GL pastures had higher average daily gains (0.46 vs 0.62 kg), shortened stay on pasture (707 vs 532 days), and carcasses with higher marbling score (8.96 vs 10.3), thicker backfat (0.38 vs 0.57 cm), and bigger rib-eye size (81.0 vs 87.9 cm^2) than GG grazed steers ($P<0.05$). Rib-eye of GG had higher intramuscular fat content than that of GL (6.77 vs 4.60%, respectively, $P<0.05$) and GL rib-eyes were found to be less tender than that of GG (4.09 vs 4.99 kg, respectively, $P<0.05$). In study two, KK grass had a higher nutrient value when comparing GG to KK (CP 12.03 vs 16.6, ADF 40.1 vs 35.4, Ash 12.7 vs 9.4, TDN 54.7 vs 58.8, RFV 90.1 vs 101.2%, NEM 0.22 vs 0.24 Mcal/kg, NDFDom 120h 67.1 vs 72.9%, respectively, $P<0.05$). Significant differences were also found due to location and season. All summer samples were found to have a higher nutritional value compared to winter samples ($P<0.05$) and samples collected from Ranch B had the highest nutritional value as well ($P<0.05$).

When comparing GG to L, L had a higher RFV (101.0 vs 200.5%, respectively, $P<0.001$) and more TDN (52.4 vs 59.6%, respectively, $P<0.001$), which resulted in GL steers having higher ADG (0.46 vs 0.62 kg), shortened stay on pasture (707 vs 532 days) and better carcass characteristics ($P<0.05$). In conclusion, GL pastures produced animals with higher ADG and more desirable carcass characteristics. Results support producers should practice more grass-fed beef production in Hawaii. It is suggested that producers allow cattle to graze mixed legume/forage pastures due to high ADG and desirable carcass characteristics found for GL cattle. Due to differences found for season and ranch location when comparing GG and KK, producers also need to consider variations in pasture nutrient profiles due to location and season in order to make decisions about when environmental conditions are appropriate to take advantage of grass-fed beef production.

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Chapter 1: Literature review

1. Introduction

Consumer perspectives and preferences have contributed to the growing popularity of grass-fed beef. Some consumers view grass-finished beef better for animal health and well-being, environmentally sustainable and healthier (USDA, 2007). However, research is lacking evaluating nutritional variation of tropical forage species and inconsistencies in beef quality are common. Previous research has shown that forage type would affect growth performance and carcass characteristics of beef cattle (Allen et al., 1996). Also, variety, season and geographic location affect forage nutrient content (Preston, 2004). Tropical beef production systems are different due to location and environment. Due to fluctuations in grain/concentrate and transportation costs, some areas such as Hawaii, would benefit from more utilization of grasses/forages as feed (Cox and Bredhoff, 2003; Campo et al., 2008).

1.1 Beef production systems

Grain and grass-finished beef production systems are both prevalent in the United States. For over 50 years United States beef producers have used grain for feedlot-finishing as a result of the oversupply of grain after World War II. Cattle graze in herds on pastures and remain with their mothers until being weaned between 227-318 kg. Most cow-calf producers sell their weaned animals to stockers and backgrounders. These animals are then placed back on pasture or in a feedlot for grain-finishing until they weigh between 363-500 kg. Currently in the United States, most producers practice grain-finishing for the last 90 days of the animal's life (McCluskey et al., 2005). However, grass-finishing has been growing in popularity. Beef is considered grass-fed when the animal consumes only grass/forage and can be labeled as organic or natural, depending on production practices. Animals are required to have continuous access to pasture during growing seasons (McCluskey et al., 2005). However, the United States Department of Agricultural Marketing Service (USDA, AMS) does not mandate a particular forage/grass nutrient profile (2007). Also, U.S. beef labeled as grass-fed without USDA certification may have consumed combinations of grass and grain feeding (McCluskey et al., 2005). Due to grain-fed/grain-finished and grass-fed/ grass-finished terms being used interchangeably, grain-finished and grass-finished terms will be used in this review of the literature. However, differences in labeling and diet regime requirements vary between countries, so these differences are noted when necessary.

Determining a superior beef production system is difficult due to limitations found in grain and grass-finished systems. Grass-finished beef is typically leaner and may have nutritional benefits from a consumer standpoint. However, an issue is determining a slaughter endpoint and how this influences beef quality (Duckett et al., 2013). Grain-finished beef production systems typically have animals reach slaughter weight faster and yield better quality grades, but have a higher fat content, thus potentially less nutritional benefits for consumers.

1.2 Tropical forage and legume species

Common tropical legume and forages species found in pastures include: leucaena (*Leucaena leucocephala*), guinea grass (*Megathyrsus maximus*), kikuyu grass (*Pennisetum clandestinum*), pangola grass (*Digitaria eriantha*), bermudagrass (*Cynodon dactylon*), and glycine (*Glycine max*). Specifically, mixed pastures containing leucaena and guinea grass have been shown to produce animals with desirable growth rates and carcass characteristics, in tropical environments such as Hawaii. The known nutritive values and successful growth of tropical pastures is useful for beef producers in tropical environments.

1.2.1 Leucaena / Haole Koa (*Leucaena leucocephala)*

Leucaena leucocephala, more commonly referred to as Haole Koa in Hawaii, is a legume tree with protein-rich leaves of extreme interest for tropical environments. Legumes were one of the first sources of human food, are 2-3 times richer in protein than cereal grains and considered high quality forages for livestock production. Nitrogen (N) that is fixed by legumes is converted into leguminous protein. Animals can meet their protein requirements by consuming legumes and what is excreted through waste is returned to soil. This is extremely beneficial because N in soil is sometimes not sufficient for efficient plant growth, especially in tropical soils that may be low in organic matter (OM), such as Hawaii (Gutteridge and Shelton, 1994).

Leucanea has been used as fodder, food, and shade for plantation crops (Brewbaker, 1987) and as a means for soil restoration in places such as Indonesia (Dijkman, 1950). Leucaena is found in Asia and the America's, but the leucocephala variety originated from the Yucatán Peninsula in Mexico. Explorers brought leucocephala to the Philippines and is now common all over the Pacific (Brewbaker, 1987). Leucaena is considered easy to grow, but takes about two years to reach maturity and maturation time is a negative aspect of this legume (Brewbaker, 1987).

Leucaena is low in sodium (Na) and iodine (I), but high in beta-carotene. Tannins found in leucaena leaves and stems reduce digestibility of dry matter (DM) and protein, but these tannins enhance the 'bypass' value of protein. Toxic non-protein amino acid mimosine is another negative aspect. Mimosine is toxic to ruminants, but *synergistes jonesii* microbes that breakdown mimosine into DHP (goitrogen) and into further non-toxic compounds, are naturally found in tropical environments, such as Indonesia and Hawaii, where cattle have historically grazed this legume (Gutteridge and Shelton, 1994).

Leucaena research in Hawaii has been conducted since the 1930s (Henke, 1933; Henke et al., 1940). Cutting this crop four times a year produced a plant highest in protein and DM (Whyte, 1944) and leucaena harvested four to five times per year produced eight to ten tons of highly palatable leucaena (Kinch and Ripperton, 1962). Utilization of this legume in tropical pastures has potential to produce cattle with high ADG.

1.2.2 Guinea grass (*Megathyrsus maximus*)

Wong and Devendara (1983) found leucaena/guinea mixed pastures in Malaysia increased ADG for cattle. In Queensland, Australia leucaena/guinea pastures produced animals with 310-430 kg liveweight gain/ha (Jones, 1982; Jones and Megarrity ,1985). Mixed guinea grass/ leucaena pastures have grown successfully on Hawaii Island and currently are being evaluated to determine nutritive values, effect on growth performance, and carcass characteristics. On Hawaii Island, GG can be found in areas described as low-wet elevation (<2,000 ft. in elevation and >50 in./yr.) (Fukumoto et al., 2015) and GG has been found to grow rapidly in the rainy season and declines in nutritive value during the dry season (Aregheore, 2002).

1.2.3 Kikuyu grass (*Pennisetum clandestinum*)

Kikuyu grass is native to Kenya, but common in tropical regions such as Hawaii. This grass is considered drought-tolerant due to deep roots, but requires soil with proper water drainage. Kikuyu is palatable with a high protein content, typically low in indigestible fiber, grazing tolerant, and able to withstand soil erosion. However, kikuyu has been found to be less successful with legume species, in contrast to guinea grass. This grass can be used for dairy and beef finishing systems in high altitude tropical regions (Mears, 1970; Quinlan et al., 1975) and is commonly found in Hawaii pastures. On Hawaii Island, KK can be found in high-wet elevation (2,000-4,5000 ft. in elevation and >30 in./yr.) areas (Fukumoto et al., 2015) and grows successfully with day temperatures up to 30 C and night temperatures as low as about 15 C, which decreases the forage growth rate, but improves digestibility of KK (Ivory et al., 1974).

1.2.4 Pangola grass / digit grass (*Digitaria eriantha*)

The exact parentage of pangola is undetermined, but is considered a great pasture grass due to its high palatability and digestibility. In Western Australia, leucaena/pangola pastures produced animals of 1,420 kg liveweight gain/ha/year (Jones and Megarrity, 1985). In comparison to other grasses, such as bahia and Bermudagrass, pangola is less drought tolerant. (Burton, 1993).

1.2.5 Bermudagrass (*Cynodon dactylon*)

Bermudagrass is native to India and/or Africa, but in the 18^{th} century it was first established in the southern United States as a turf grass (Magness et al., 1971; Burton, 1993). Bermudagrass is commonly found in tropical, subtropical and mild-temperate regions worldwide, such as Hawaii. Today Bermudagrass is mainly found in pastures and lawns. In Hawaii, Bermudagrass can be grown in pastures with other forages because of its high tolerance to warm weather (Magness et al., 1971). For areas that experience frost during fall, such as high elevations on Hawaii Island and Maui, Bermudagrass can also grow successfully.

1.2.6 Glycine (*Glycine max*)

Glycine is native to Asia, but was introduced to North America and Europe as a forage crop (Caldwell, 1973). Glycine is suitable for livestock grazing, but needs to be strip-grazed to prevent wastage. This legume is considered high yielding, with a short growth period, good digestibility, high nutritive value, protein-rich and is highly accepted by livestock (Koivisto et al., 2003). Currently glycine be found in pastures on Hawaii Island.

1.3 Growth Performance of beef cattle

Animals growth rates are influenced by muscle to protein turnover (Andersen et al., 2005) and growth performance can be influenced by diet. Studies have found grass-finished beef reach slaughter endpoints slower due to lower average daily gains (ADG), compared to grain-finished cattle. Nuernberg et al. (2005) and Duckett et al. (2007) found grass-finished cattle took significantly longer to reach slaughter weight and had less ADG. Duckett et al. (2013) also found time on pasture (TOP) influenced ADG, with ADG decreasing in steers as TOP increased. Rosa et al. (2014) finished cattle on three different treatments: pasture, pasture + 4 kg supplemented corn per day and pasture + 8 kg supplemented corn per day. Pasture was mixed with Kentucky bluegrass (*Poa pratensis*), perennial ryegrass, (*Lolium perenne*), and white clover (*Trifolium repens*) and found corn supplementation significantly increased ADG. Due to corn being higher in fat, corn is expected to increase ADG and fat content.

Previous studies have proven animals obtained desirable ADG by feeding forages or grazing in pastures. In Spain, Chorfi et al. (2015) assigned crossbred beef calves to four different dietary groups: (Control) *ad libitum* access to low nutritive value timothy pasture (*Phelum pretense L.*) and hay + rolled barley grain, (Pasture) intensively managed timothy pasture, (Haylage) timothy haylage where calves were allowed free access indoors, and (Kale) 50% timothy haylage and 50% kale (*Brassica oleracea* spp. acephala*)*. Pasture grazing calves were heaviest (323.6 ± 4.2 kg body weight and 1.74 kg ADG). Chorfi et al. (2015) found kale pastures up to 50% mixed with haylage can be fed without any detrimental effects and produced animals with high ADG.

Mixed forage pastures containing high protein feed sources, such as leucaena, could help improve ADG and decrease TOP in tropical environments. Liveweight gains of 0.52 kg per day have been reported for cattle grazing leucaena only pastures in Hawaii (Henke et al., 1940). Leucaena pastures mixed with green panic, Rhodes, buffel, and signal in Queensland produced cattle of up to 1kg/head/day with a stocking rate of 3-4 animals/ha (Gutteridge and Shelton, 1994). Cardoso (1986), Wildin (1986), and Clem et al. (1986) found cattle grazing leucaena had high ADG because cattle grazed during the growing season and had no restriction to leucaena (Jones, 1979). Muir et al. (1998) found pasture-finished cattle obtained desirable growth rates on high quality, high energy spring pastures in New Zealand. Grain-finished cattle are known for reaching slaughter weight faster and having higher ADG, but properly managed pastures with high nutritive values can produce grass-finished cattle with desirable growth rates. Table 1 shows the ADG comparison of leucaena from other studies.

Table 1. Growth performance of beef raised on leucaena pastures

Publication	Location	Pasture	LWG/head/day (kg)	Duration of study (days)
Clem et al. (1993)	New Zealand	Leucaena	0.89	168
Wildin (1986)	Queensland Australia	Leucaena	1.10	90
Cardoso (1986)	Brazil	Leucaena	0.80	115
Moog (1983)	the Philippines	Leucaena x blady grass	0.36	315

1.4 Meat quality

1.4.1 Intramuscular fat (marbling)

USDA quality beef grades are evaluations of expected meat palatability (tenderness, juiciness and flavor). Grades from highest to lowest quality are prime, choice, select, standard and commercial. Quality grades are mainly determined by marbling plus maturity, and marbling (intramuscular fat) is determined by visual evaluation of intramuscular fat in the ribeye after carcasses are cut between the 12^{th} and13^{th} ribs (Hale et al., 2013). Specific grades and marbling scores will vary depending on country's specific scales. However, marbling variations in grass and grain-finished beef due to nutritional differences in feeding regimes have been found globally. Although highly marbled beef is desirable amongst consumers, and fat gives meat flavor, aroma, and texture (Nurenberg et al., 2005), shifts in consumer preferences have created a niche market for consumers who prefer grass-finished beef due to lower fat content with higher unsaturated fatty acid proportion.

Muir et al. (1998) compared liveweight gains and carcass quality of 3-year-old Angus steers finished on concentrate or pasture in New Zealand. In terms of fat depth and percentage of intramuscular far (IMF or marbling), no statistical differences were found due to diet. Muir et al. (1998) concluded pasture-finished steers could reach a similar degree of high marbling. More recently, Razminowicz et al. (2006) also found no significant differences in IMF content of Switzerland strip loins (overall <1% to about 3.5%), but conventional (concentrate-finished) steers had more IMF than pasture-finished steers.

Duckett et al. (2013) compared how diet effects (forage versus concentrate) carcass and meat quality of Angus-cross steers in a 3-year study. Duckett et al. (2013) found significantly less marbling in forage-finished steers (FOR) compared to concentrate-finished steers (CON) (marbling score: 100 point subunits where Abundant90 valued 1090 and Practically Devoid00 value 200). Percent of fat-free-lean was greater for FOR than CON steers, including longissimus muscle (LM) and other lean trim. More recently, Corbin et al. (2015) found more fat was associated with more juiciness, flavor, tenderness, liking and overall acceptability in a consumer panel study. Corbin et al. (2015) observed differences in 10 different treatment groups and fat contributed most to acceptability of beef flavor (Corbin et al., 2015), which proves importance of fat content from a general consumer standpoint.

Marbling will influence flavor, juiciness, tenderness and overall palatability (Realini et al., 2009) and as fat content increases, palatability increases with an acceptable fat level of fat being between 3% and 7.3% (Miller, 2004). The propensity to deposit fat can be influenced by breed (Orellana et al., 2009). Also is has been found animals fed a diet higher in fat (concentrate) deposit more fat, which supports Duckett et al. (2013) finding CON finished animals containing more fat than FOR. Variation in IMF (marbling) content is common depending on diet, but other factors can influence marbling. Differences are expected due to the nutritional value of grass compared to grain, but differences cannot be significantly dependent on pasture quality.

1.4.2 Muscle and fat color

Consumer perceptions drive the beef industry and consumers are drawn to physically appealing products. Myoglobin is responsible for the red pigment of meat and many extrinsic factors can affect the myoglobin concentration, thus meat color. At the same time, some extrinsic factors can affect the color perception. Extrinsic factors affecting meat color found in the literature include species, genetics, nutrition and postmortem conditions. Due to color being hard to universally interpret by the human eye, different tools have been developed to interpret expression of color. The basic principle of color determination is light hits a surface and is reflected back to the detector, then the processor (human eye or instrument) interprets color (Hunt and King, 2012). Research has shown diet (grain versus grass) impacts meat color, which influences the economic value of products. Typically, grain-finished beef is lighter and grass-finished beef is darker with higher incidence of yellow fat.

Razminowicz et al. (2006) compared beef cattle produced according to specific Switzerland standards. Groups included: pasture sucklers (PS), pasture-finished steers/heifers supplemented with low levels of concentrate for finishing (PF), intensively fattened young bulls following specific husbandry conditions (LB), concentrate-finished or conventional heifers (CH) and conventional young bulls (CB). Color of longissimus dorsi (LD) was determined on three areas on the surface bloomed for 1 h at 4 C with a Chroma Meter (model 300-CR, Minolta, Dietikon, Switzerland) using the L*a*b* system and D65 as the light source. Razminowicz et al. (2006) found LB were lightest in color, followed by PS (L*= 41.0 and 39.8) and CH were darkest (L*=37.5). PF were most red and PS were least red (a*= 23.6 and 20.3). However, the small amount of concentrate supplemented to PF beef for finishing may be why LD were most red. Studies comparing concentrate and pasture diets have found concentrate-finished steers lighter and forage-finished steers darker in color, such as Realini et al. (2009), Leheska et al. (2014) and Nuernberg et al. (2005). Realini et al. (2004) also found longissimus muscle (LM) of concentrate-finished steers was lighter in comparison to pasture-finished steers (using a Minolta chromameter, CR-210, Minolta Inc., Osaka, Japan).

Yellow fat common in pasture-finished steers can be attributed to high levels of beta-carotene in forages (Dunne et al., 2009), but variation has been found. Razminowicz et al. (2006) found no significant differences in fat color (b*) between different diets or seasons of forages and Duckett et al. (2013) also found no significant differences in color due to beta-carotene content of forages. Realini et al. (2004) tested vitamin E supplementation in concentrate-finished beef to enhance shelf life, which Daniel M. Schaefer discussed in, Fresh beef marketing opportunities due to dietary vitamin E (2007). However, vitamin E supplemented to concentrate-finished beef had no significant effect when compared to pasture-finished beef with no supplementation. Some literature has shown fat from pasture-finished beef is significantly more yellow (Realini et al., 2004; Muir et al, 1992). Realini et al. (2004) determined color of subcutaneous fat at the $12/13^{th}$ rib 24 h post-mortem. Findings from Realini et al. (2004) agree with previously mentioned studies because fat from pasture-finished beef was significantly more yellow (higher b* values) compared to concentrate-finished (b*: yellowness/blueness; positive values: yellow, negative values: blue).

Countries have laws to ensure health of consumers is not being compromised when processing, manufacturing or selling food products. Although darker meat can be undergo faster spoilage, darker meat is not necessarily harmful to consumers because color can be impacted by reasons previously mentioned. Grass-finished animals contain more myoglobin in their muscle due to more physical activity than grain-finished animals before slaughter, thus more activity leads to darker meat color (Varnam and Sutherland, 1995). Prior to this Reagan et al. (1977) found grass-finished animals muscle was darker in color in comparison to grain-finished animals, but this was attributed to animals requiring more time to reach slaughter weight. Previous work has shown variation in meat color can be impacted by various factors. Comparisons of animals fed concentrate and pasture diets support that diet impacts color, thus contributes to consumer perceptions.

1.4.3 pH of muscle

During postmortem, muscle pH declines from 7.0 to 5.6 in normal muscle due to accumulation of lactic acid produced by postmortem glycolysis. Decline in pH will vary, but for beef ultimate pH is usually achieved 24 h postmortem. However, dark-cutting is the quality problem associated with pH and occurs due to limited glycogen stores in living animals causing inadequate lactic acid buildup postmortem, thus resulting in a higher than normal pH (pH≥ 6.0). When determining pH in a laboratory, iodoacetate is added to inhibit glycolysis (prevents further production of lactate), and measurements are taken with a pH meter (Hale et al., 2013). Variations in pH comparing grass and grain-finished beef have been found.

The LM pH was lower for CON finished steers than FOR finished steers (pH= 5.49 and 5.64) (Duckett et al., 2013). Muir et al. (1998) measured ultimate pH at the 12th rib following chilling for 18 h at 10 C. Mean ultimate pH (pH 24 h after slaughter) values were acceptable overall (pH < 5.8). However, grain-finished beef had overall lower pH, which was attributed to treatment. Nuernberg et al. (2005) found pH after 24 h varied due to breed and diet in German Holstein (GH) and German Simental Bulls (GS) fed concentrate or pasture diets. This contrasts French et al. (2000) and Razminowicz et al. (2006) because no significant differences in pH were found due to diet in these studies. Realini et al. (2004) also found dietary treatment had no significant effect on pH.

Meat producers aim to produce consistent products at an optimal cost. The pH of pasture-finished animals has been higher due to meat being darker, which can be an indicator of dark firm dry beef (DFD). Dark meat is an undesirable characteristic for consumers, but darker meat poses no threat, unless meat is spoiled. Microbial growth can be determined by pH and the industry maintains regulatory requirements to reduce risks of disease outbreaks (Queeney, 2007).

Differences found for pH mentioned are due to meat being darker in color and this can be attributed to feeding regime or diet.

1.4.4 Meat tenderness

Tenderness is considered the most important consumer satisfaction parameter in the beef industry (Dransfield, 1998) and differences in tenderness have been found for different beef production systems (grass versus grain-finished). Tenderness is commonly determined following the Warner-Bratzler shear force test protocol developed by Savell et al. (1994). Dietary fatty acids have been suggested to influence tenderness and juiciness, but differences are more likely due to overall fatty acid content rather than one single fatty acid (Wood et al., 2012). Orenalla et al. (2009) concluded genetics influence fat deposition due to different breeds having variation in propensity to deposit fat. Specifically marbling can influence tenderness (Razminowicz et al., 2006). Typically, researchers have found grain-finished beef will be tenderer due to animals being higher in fat and younger at slaughter. However, studies have found variation for tenderness.

Tenderness is influenced by diet, age, pre-slaughter growth rate, and length of finishing period (Campo et al., 2008) and post-mortem ageing may improve tenderness. Duckett et al. (2013) reported no significant differences in Warner-Bratzler shear force (WBSF) values 14 days and 28 days post-mortem for the LM (10^{th} rib) when comparing CON and FOR diets. Type of finishing system had no significant effect on LM tenderness when steers were slaughtered at similar endpoints (Duckett et al., 2013), which supports Kim et al. (2012). Findings from Kim et al. (2012) found no significant differences in tenderness when comparing grass to grain-finished beef. Duckett et al. (2013) also found no differences in sensory panel juiciness, initial tenderness, and overall tenderness of the LM.

Realini et al. (2004) found no variation in initial tenderness of LM (WBSF values) comparing treatments (pasture versus concentrate), however 7 and 14 days postmortem pasture-finished were significantly more tender than concentrate-finished (2.91 kg vs. 3.84 and 2.83 vs. 3.45). Realini et al. (2004) concluded post-mortem aging could improve overall tenderness, but more for pasture-finished steers. French et al. (2000) also found post-mortem aging increased LM tenderness 2 days post-mortem in pasture grazed steers with low levels of concentrate.

No significant statistical differences in tenderness were found for Nuernberg et al. (2005) and Razminowicz et al. (2006) due to diet. The LM of pasture-finished samples had lower Warner-Bratzler values compared to concentrate-finished (Razminowicz et al., 2006). However, concentrate supplementation was provided to pasture-finished steers for finishing, which may have increased tenderness. Nuernberg et al. (2005) compared fatty acid composition and beef quality of concentrate or grass-finished German Holstein (GH) and German Simental Bulls (GS). Significant differences were reported for tenderness, with concentrate steers being more tender. Cox et al. (2006) also found pasture-finished beef not as tender as feedlot-finished beef due to pasture-finished steers containing less fat. Overall, variation in tenderness is common for beef and post-mortem ageing may improve tenderness, but differences are not always significant.

1.5 Fatty acid profile of beef

Variations in fatty acid content of beef due to diet, breed, and type or variety of forage/grass is common (Elswyk and McNeill, 2014) and grass-finished beef fatty acid content may be healthier for consumers. Ruminants naturally contain conjugated linoleic acids (CLAs) (Leheska et al., 2014) and grass-finished beef contains more CLAs due to grass nutrient composition. By definition, CLA refers to different isomers of linoleic acid and cis9-trans11 CLA isomers are beneficial to human health and are commonly found in higher ratios in grass-finished beef. These CLAs can reduce the risk of cancer, heart disease, hypertension and lower cholesterol (Chin et al., 1992).

Isomers of cis9-trans11have been discovered to form by two main ways. One is from ruminal biohydrogenation of linoleic acid to stearic acid in the rumen due to *butyrinvibrio fibrisolvens* bacteria (Kepler et al., 1966; Jenkins, 1993). Second is conversion of trans-11 C18:1 by D9-desaturatase in ruminant adipose tissue and dairy cow mammary glands (Griinari et al., 2000; Kay et al., 2004). Nurenberg et al. (2005) found CLA content in LM of grass-finished beef significantly higher due to diet (mg/100 g muscle) and significantly higher CLA content in GS fed a concentrate diet, which was due to breed.

Linolenic acid (C18: 3) and linoleic acid (C18: 2 cis9-cis12) are commonly found in high ratios in grass-finished beef and can lower cholesterol and reduce the risk of heart disease (Acetoze and Rossow, 2014). Ruminant animals can have beneficial (low) ratios of omega-6: omega-3 (n-6: n-3) PUFA and are common in animals grazing pastures high in C18: 3 (alpha linolenic acid). Specifically, grass-finished beef is high in alpha-linolenic acid (ALA) due to forages being high in ALA (Wood et al., 2003) and animals that grazed pasture finished on a diet with linseed had two to three fold higher concentrations of n-3 fatty acids (Nurenberg et al., 2005). Weill et al. (2002) found linseed supplementation, which is high in n-3 fatty acids, increased n-3 fatty acids found in human blood. Seafood is high in omega-3 LC-PUFA (long chain polyunsaturated fatty acids), but pasture grazing can contribute to this requirement for consumers that do not consume seafood (Meyer et al., 2003).

A proper n-6: n-3 fatty acid ratio is important for a well-balanced diet. Usually, humans consume diets too high in n-6, lacking n-3 fatty acids. Pasture contains more linolenic acid and is higher in n-3 fatty acids (Leheska et al., 2014). 100 g of grass-finished beef provided an average of 40-47 mg of n-3 LC-PUFA ratios and grain-finished beef provided 16-19 mg (Nurnberg et al., 2002). Similarly, n-3 LC-PUFA ratios were higher in grass versus grain-finished beef (38mg/100g to 18mg/100g) (Raes et al., 2003). Increasing levels of n-3 fatty acids in livestock animal diets by pasture-finishing, such as beef, may help improve Western beef from a dietary standpoint without forcing consumers into dietary changes (Razminowicz et al., 2006).

Studies have found increased ratios of n-6: n-3 fatty acids in grain-finished compared to grass-finished beef (French et al., 2000; Realini et al., 2004). French et al. (2000), shown in table 2, found treatments: 6 kg grazed grass (DM basis) + 5 kg concentrate (CG), 12 kg grazed grass (DM basis) + 2.5 kg concentrate (GC) and 22 kg grazed grass (DM basis) (GO) all had the highest ratios of n-6 and n-3 fatty acids in LM IMF. These three treatment groups also had the healthiest ratios of n-6: n-3 fatty acids with GO cattle being healthiest. Overall, differences in fatty acid content of pasture and feedlot beef are present with pasture beef appearing to be healthier for consumers.

Table 2. Comparison of omega-3, omega-6, and omega-6: omega-3 ratios

Publication	Location	n-3	n-6	n-6: n-3 ratio
Duckett et al. (2013)	Intramuscular fat of longissimus muscle: total fatty acid composition (%)	GF: 0.56 GS: 2.67	GF: 3.18 GS: 3.51	GF: 6.01 GS: 1.33
Nuernberg et al. (2005)	Longissimus muscle fatty acid composition (%)	German Holstein: GF: 0.96 GS: 3.25 German Simmental: GF:0.90 GS: 4.70	German Holstein: GF: 6.14 GS: 6.30 German Simmental: GF: 7.73 GS: 9.80	German Holstein: GF: 6.49 GS: 1.94 German Simmental: GF: 8.34 GS: 2.04
French et al. (2000)	Intramuscular fatty acid composition in longissimus muscle (g/100 g fatty acid methyl esters)	SC: 0.91 CO: 0.84 CG: 1.13 GC: 1.25 GO: 1.36	SC: 2.96 CO: 3.21 CG: 3.12 GC:3.04 GO: 3.14	SC: 3.61 CO: 4.15 CG: 2.86 GC: 2.47 GO: 2.33

*GF- grain-finished, *GS-grass-finished
*SC- grass silage ad libitum + 4 kg concentrate, *CO- 8 kg concentrate+ 1 kg hay, *CG- 6 kg grazed grass (DM) + 5 kg concentrate, *GC 12 kg grazed grass (DM) + 2.5 kg concentrate, *GO- 22 kg grazed grass (DM)

Increasing knowledge about benefits of grass-finished beef has caused a lot of interest in this product amongst consumers. In 2000, the USDA published information recommending consumers consume lean steaks with low marbling. Following this, the USDA (2005) stated conventionally produced beef (grain-finished) is higher in saturated fatty acids (SFA) and the USDA recommends consuming a diet less than 10% SFA. Overall, research has proven the importance of a healthy fatty acid composition from a consumer nutritional standpoint and fatty acid composition can influence meat quality (Oliver et al., 2006).

1.6 Conclusions and implications for this study

Literature published proves variation in diet (grass versus grain) can influence beef quality parameters including: growth performance, marbling, muscle and fat color, pH, tenderness and fatty acid profile. Grass-finished beef tends to reach slaughter endpoint slower and animals tend to be leaner, darker, and have a healthier fatty acid composition from a consumer standpoint. However, studies have proven not all differences significant. Also grass-finished beef is better in terms of animal welfare (Morrow-Tesch, 2000) and has less of an environmental impact than grain-finished beef (Horrigan et al., 1999; Kim et al., 2012).

Duckett et al. (2007 and 2009) and Neel et al. (2007) found forage-finished beef leaner with higher concentrations of n-3 fatty acids and CLAs. However, it was concluded nutritional information available on specific forage species was lacking. Drewhurst et al. (2001) and Clapham et al. (2005) also noted fatty acid content of forages varies due to differences in species, variety, harvest time and growing season. For tropical environments research on the nutritional value of various forage pasture species would benefit the beef industry. Research needs to continue on nutritional value of specific varieties and species of tropical pastures. Improving inconsistencies in beef quality, increasing ADG, and proper tropical pasture management is necessary for the grass-fed beef industry to expand and better serve consumers seeking healthy and environmentally sustainable products.

1.7 Objectives for this study

For this thesis project, two independent studies were conducted. The objective of study one was to determine nutrient profiles of guinea grass (GG, *Megathyrsus maximus*) and leucaena (L, *Leucaena leucocephala*) collected in the AM and PM to compare how animals that grazed either 100 % GG or 60% GG + 40% L pastures on Hawaii Island (Ranch A) influenced growth performance, carcass characteristics, chemical composition of rib-eye samples. The objective of study two was to determine nutrient profiles of guinea grass and kikuyu grass (KK, *Pennisetum clandestinum*) to compare differences due to grass type (GG vs KK), season (summer vs winter), and ranch location on Hawaii Island (B vs C vs D vs E).

Chapter 2: Materials and Methods

Study 1: Nutrient profiles of guinea grass and leucaena and growth performance and carcass quality of beef cattle grazed on these pastures in Hawaii

2.1 Study location and sample collection

Guinea grass (GG) and leucaena (L) samples were collected along the Hamakua Coast from Ranch A on Hawaii Island. 50 Angus-cross weaned steers were divided into two groups (n=25 per group) and placed on 48.5 acres of 100% GG pasture or 48.5 acres of 60% GG + 40% L (GL) with 10 paddocks following a 70-day rotation. Animals were on pasture for 365-611 days and started on pasture at about 294 kg and were slaughtered when they all reached a similar body conformation at about 630 kg. Animals were slaughtered following United States Department of Agriculture (USDA) Food Safety and Inspection Service regulations (FSIS) at a slaughterhouse on Hawaii Island, but not all rib-eye samples were available for analysis. Due to this limitation, only 23 GG and 10 GL rib-eye samples were available for chemical composition analysis.

The GG and L samples were randomly hand collected to determine the nutritional value of pastures. Samples were collected in the AM and PM to determine if time of day influenced nutritional value; a total of 16 replicates of GG and L were collected from the two pastures. Pasture sampling took place close to the time of grazing to give a better representation of nutritive values of intake. Samples were hand collected using sheers and clippings were taken at a height at which the forage would be grazed by the animal or harvested. Samples were placed in paper bags and labeled. Once samples arrived at the University of Hawaii-Manoa Agricultural Sciences building, samples were oven dried at 65 °C (Fisher Scientific, Isotemp Oven Model 750G) overnight to determine dry-matter (DM). Samples were ground in a 1-mm screen using a

Wiley mill (Thomas Model 2®- Thomas scientific) to get samples to a uniform particle size and were stored until proximate analysis.

2.1.1 Nutrient profile of forages

Nutrient composition of forage samples was determined using near-infrared reflectance spectroscopy (NIR), which is recognized by the Association of Analytical Chemists (AOAC) at a certified commercial laboratory (Dairy One Forage Laboratory, Ithaca, NY). This is an analytical technique to rapidly determine the spectral properties of a feed. Molecular bonds between hydrogen, nitrogen, oxygen, carbon, sulfur and phosphorus exist in feeds and are able to absorb light in or beyond the region of near-infrared. NIR can be explained by absorbance/reflection effects, surface effects, and interface effects. This instrument is able to measure the difference of light emitted and the light that is absorbed or reflected.

In order to analyze samples calibration of several samples from that feed needs to be analyzed, which can be seen as a limitation for this method. However, this method is less expensive compared to wet chemistry. The cost of running a single sample using wet chemistry is less or equal to the cost of running multiple samples using NIR. Also NIR requires no sample preparation, requires less amount of sample and no chemicals are used for this method. Multiple nutrients were determined by utilization of spectral properties of feeds using the LOCAL calibration software and samples were analyzed for overall nutrient composition.

2.2 Chemical composition of rib-eye

Nutritional composition of rib-eye samples was determined for GG (n=23) and GL (n=10) rib-eye samples according AOAC methods (2006). Samples taken out of a freezer at -20 C thawed for at least 30 min. Meat was ground two or three times using a Sportsman Clamp-On

Meat Grinder with a 1/8 plate (3 mm). Samples were immediately placed on ice and analyzed for moisture, fat, ash, protein and pH. External (subcutaneous) fat was removed and stored in a freezer at -20 °C for future analysis.

2.2.1 Moisture

Moisture of the rib-eye samples was determined by oven drying. Duplicates of 5-7 g of sample were dried in an oven at 125 C for 2-4 h in aluminum dishes. After drying samples were placed in a desiccator for at least 30 min, then weights were taken. Samples were saved for Soxhlet extraction of fat.

2.2.2 Fat

Moisture of the rib-eye samples was determined by ether extraction. Samples (from moisture determination) were wrapped in Whatmann filter paper. 6-8 samples were placed in a Soxhlet extraction apparatus for 4-5 h with petroleum ether (boiling point: 40-50 C). After samples were removed and placed in a fume hood until no petroleum odor remained. Samples were then oven dried for 1 h at 100 C and then placed in a desiccator. Filter paper was unwrapped carefully and weight was recorded to determine fat.

2.2.3 Protein

Protein was calculated on a percentage basis by calculating the difference:

Protein = l00% – moisture% – fat% – ash%

2.2.4 Ash

Ash was determined in duplicate by weighing porcelain crucibles first alone, then with 2 g of sample. Samples were placed in a furnace at 600 C for 4-6 h. After samples were cooled and placed in a desiccator and weighed to determine organic matter.

2.2.5 pH value

To determine pH iodoacetate solution was prepared and added to samples to inhibit glycolysis. 2 g of sample was homogenized with 10 ml of solution using a Brinkmann Homogenizer. The pH was measured using a Metter Toledo seven easy pH meter.

Solution preparation involved 1 liter of 0.005M Na-iodoacetate dissolved in 0.15M KCl

Grams= 0.005M Na-iodoacetate * 207.93= 1.03965 grams/ liter

Grams= 0.15M KCl * 74.5513= 11.182694 grams/ liter

1.0486 grams of Na-iodoacetate and 11.2049 grams of KCl for a total of 12.2234 grams was added to make one liter of solution.

2.3 Carcass characteristics and growth performance

Carcass characteristics were determined following United States Department of Agriculture (USDA) beef grading standards performed by University of Hawaii Research Extension Agent Glen Fukumoto and Mealani Research Station staff on Hawaii Island. Carcass characteristics determined included: hot carcass weight (HCW, kg), backfat thickness (cm), rib-eye area (cm^2), USDA marbling score, USDA quality grade and Warner-Bratzler shear force values (tenderness, kg). Growth performance data collected included initial body weight to final body weight. A total of 7 growth periods were determined for a total of 1.5 years. Weights were taken for 7 periods which included: 1/13/2013 (1), 4/8/2013 (2), 7/15/2013 (3), 9/23/2013 (4), 12/2/2013 (5), 2/10/2014 (6), and 4/21/2014 (7). Average daily gain (ADG) was determined for periods 1, 3, 6 and overall ADG (kg).

ADG= finish weight – start weight / age (days)

2.3.1 Hot carcass weight

The hot carcass weight is the weight of the carcass after slaughter. The head, hide, intestinal tract and internal organs were removed (Knight, 2013).

2.3.2 Backfat thickness

Backfat thickness is a measure of the external fat thickness over the ribeye. Fat thickness was measured three-fourths of the distance of the length of the ribeye (Knight, 2013).

2.3.3 Rib-eye area

The method for determining the rib-eye area involves using a grid to measure the ribeye. The grid is placed on the surface of the cut and squares are counted. The number of squares are divided by 10 and this is the ribeye area in square inches. For this study, data was presented in cm^2 (Knight, 2013).

2.3.4 USDA marbling score

Marbling is the amount of intramuscular fat within the ribeye and is used for determining USDA quality grade. Evaluations were done visually at the 12^{th} rib cross-section on the ribeye (Knight, 2013).

2.3.5 USDA quality grade

The USDA quality grade is determined using maturity and marbling factors. Maturity is determined by evaluating the size, shape and ossification of bone and cartilage on the carcass. Maturity is also determined by the color and texture of the ribeye muscle. These two factors can be found on the USDA beef grading chart. A younger carcass with more marbling is desirable and will increase the quality of the carcass (Knight, 2013).

2.3.6 Tenderness determination using Warner-Bratzler instrument

Vacuumed packed steak samples were thawed overnight, then heated in a 70 C water bath for 1 h followed by cooling to room temperature for 1 h. 6 core samples were taken from each sample by drilling in the direction of muscle fibers. Cores were cut using a Warner-Bratzler blade with a TA.XT2 Texture Analyzer (Texture Technologies Group, Scarsdale, NY). Shear force values were determined by the amount of force needed to cut through each core sample and averages were calculated.

Study 2: Seasonal and locational variation of nutrient profile and in vitro digestion kinetics of guinea grass and kikuyu grass on Hawaii Island

2.4 Study location and sample collection

A total of 38 forage samples were randomly hand collected at a time right before grazing from pastures of four different ranches on Hawaii Island in winter (12/18/2014-12/20/2014) and summer (8/17/2015/-8/19/2015). Guinea grass (GG) samples were collected from: Ranch B (4 winter, 6 summer), Ranch C (4 winter, 2 summer), Ranch D (4 winter, 4 summer), and Ranch E (4 summer). Kikuyu grass (KK) samples were collected from Ranch B (4 winter, 2 summer) and Ranch D (4 winter). Once samples arrived at the University of Hawaii-Manoa Agricultural Sciences building, samples were dried at 65 C (Fisher Scientific, Isotemp Oven Model 750G) overnight to determine dry-matter (DM). Samples were ground in a 1-mm screen using a Wiley mill (Thomas Model 2®- Thomas scientific) to get samples to a uniform particle size and were stored until proximate analysis.

2.4.1 Nutrient profile of forages

Nutrient composition of forage samples was determined using near-infrared reflectance spectroscopy (NIR), which is recognized by the Association of Analytical Chemists (AOAC), at a certified commercial laboratory (Dairy One Forage Laboratory, Ithaca, NY). Multiple nutrients were determined by utilization of spectral properties of feeds using the LOCAL calibration software and samples were analyzed for overall nutrient composition

2.5 Statistical Analyses

In mixed effect models, it is assumed: observation = fixed effects + random effects + error

PROC MIXED was used in both studies using the *restricted maximum likelihood* (REML) procedure. REML is more beneficial for field-correlated work compared to ANOVA because it allows for changing variances and is good for studies where treatments have a changing variance structure (Oehlert, 2012; O'Neill, 2010).

The data is considered normally distributed and independent of each other. For this reason, all data was analyzed by the MIXED procedure using SAS software v9.2 (SAS institute Inc., Cary, NC). Means were compared using Tukey's method and all differences were considered significant at $P<0.05$. PROC MIXED, followed by data= specifies the data set. CLASS is entered because it indicates qualitative variables and MODEL specifies dependent variables and fixed effects.

Nutrient profiles of GG and L were compared to see differences due to type (GG vs L) and time of collection (AM vs PM). In this analysis, nutrient variables were treated as the response (dependent) variables or the fixed factor. Type and time were the explanatory (independent) variables or the random factors. Testing for an interaction between type X time was of interest because there may be differences due to both type and time simultaneously. An interaction has occurred when the effect of an independent variable depends on the effect of another independent variable.

For growth performance, chemical composition, and carcass characteristics data, type (GG vs L) was treated as the independent variable or the random factor. Body weight, chemical composition, and carcass characteristics were the dependent variables or the fixed factors for these analyses.

Nutrient profiles of GG and KK were also analyzed by the MIXED procedure using SAS software v9.2 (SAS institute Inc., Cary, NC). Means were compared using Tukey's method and all differences were considered significant at $P<0.05$. GG and KK nutrient profiles were compared by type (GG vs KK), season (summer vs winter) and location (Ranch B vs Ranch C vs Ranch D vs Ranch E). Nutrient variables were the dependent variables or the fixed factor. Type, season and ranch were treated as independent variables or random factors. Type X season and type X ranch were also considered to determine if there was an interaction in this study.

Chapter 3: Results

Study 1: Nutrient profiles of guinea grass and leucaena and growth performance and carcass quality of beef cattle grazed on these pastures in Hawaii

3.1 Nutrient profiles of guinea grass and leucaena

There was variation in nutrient profiles of guinea grass and (GG) and leucaena (L) for samples collected Winter 2014 from Ranch A. In this study, L was found to have a higher nutritional value than GG. When comparing GG to L, ash was higher for GG compared to L (16.3 vs 12.3%, respectively; $P<0.0001$), which indicated less organic matter. Comparing GG to L, acid detergent fiber (ADF) and ash-free neutral detergent fiber (aNDF) were both lower for L, indicating less indigestible fiber (39.2 vs 27.0 and 51.5 vs 31.5%, respectively, $P<0.0001$). Total digestible nutrients (TDN) and crude protein were higher for L (52.4 vs 59.6 and 18.8 vs 27.3%, respectively, $P<0.0001$). Overall type (GG vs L) had a significant effect on all variables except lignin content. All variables contributed to L having an overall higher relative feed value (RFV) (101.0 vs 200.5%, respectively, $P<0.0001$).

Time of day was found to significantly affect net energy for maintenance (NEM), ash, TDN, and crude fat. Samples collected in the AM were found to provide more NEM (0.24 vs 0.21 Mcal/kg, respectively, $P<0.05$) more organic matter (13.0 vs 15.6%, respectively, $P<0.0001$), more TDN (58.0 vs 54.0%, respectively, $P<0.000l$) and more crude fat (2.4 vs 1.5%, respectively, $P<0.05$). There was a two-way interaction for type of grass and time for ash. Type of grass and time of collection influenced each other when comparing ash for GG and L samples. Organic matter was significantly higher in L samples (13.3 vs 12.3%, respectively, $P<0.0001$) and AM had more organic matter (13.0 vs 15.6%, respectively, $P<0.001$) which can be attributed to time of collection (AM vs PM). Table 3 summarizes the nutritional differences of GG and L.

3.1.1 Growth performance and carcass characteristics

When comparing GG to GL pastures, L being incorporated into pasture improved ($P<0.05$) average daily gain (ADG) of steers (0.46 vs 0.62 kg) resulting in shortened ($P<0.05$) stay in pasture (707 vs 532 days), shown in table 4. Chemical composition of GG and GL rib-eye samples determined significant differences in fat and protein. Rib-eye samples from GG treatment had more fat (6.77 vs 4.60%, respectively, $P<0.05$). However, GL had more protein based on difference (22.5 vs 23.5%, respectively, $P<0.05$), shown in table 5. The GL carcasses had thicker backfat (0.38 vs 0.57 cm, respectively, $P<0.05$), bigger rib-eye areas (81.0 vs 87.9 cm^2, respectively, $P<0.001$) better marbling scores (8.96 vs 10.3, respectively, $P<0.01$) and better USDA quality grades (3.16 vs 6.84, respectively, $P<0.001$), shown in table 6. However, shear force values of GL rib-eye samples were greater ($P<0.05$) than that of GG, indicating less tenderness, (4.09 vs 4.99 kg, respectively, $P<0.001$).

Table 3. Nutrient profile of guinea grass and leucaena

	Guinea grass		Leucaena		Main effects				SEM[1]	*P*-value		
					Type		Time					
	AM	PM	AM	PM	Guinea grass	Leucaena	AM	PM		Type	Time	Type x Time
DM	20.9	15.5	27.9	24.4	18.2[b]	26.1[a]	24.4	19.9	2.75	0.013	0.136	0.736
RFV[2]	99.0	103.0	209.8	191.3	101.0[b]	200.5[a]	154.4	147.1	6.46	<0.001	0.284	0.107
CP	19.3	18.3	27.8	26.9	18.8[b]	27.3[a]	23.5	22.6	0.67	<0.001	0.208	0.913
NEM[3]	0.22	0.18	0.27	0.25	0.20[b]	0.26[a]	0.24	0.21[b]	0.01	<0.001	0.002	0.103
Ash	14.0[b]	18.6[a]	12.0[b]	12.6[b]	16.3[a]	12.3[b]	13.0	15.6[b]	6.48	<0.001	0.002	0.001
ADF	38.2	40.2	25.7	28.3	39.2[a]	27.0[b]	31.9	34.3	1.10	<0.001	0.064	0.807
aNDFom	49.3	53.8	30.3	32.7	51.5[a]	31.5[b]	39.8	43.2	3.00	<0.001	0.272	0.750
TDN[4]	55.0	49.8	61.0	58.3	52.4[b]	59.6[a]	58.0	54.0[b]	0.72	<0.001	0.001	0.112
Crude fat	2.3	1.3	2.4	1.7	1.8	2.1	2.4[a]	1.5[b]	0.29	0.324	0.011	0.568
Lignin	4.8	5.1	7.3	7.8	4.9	7.5	6.0	6.4	0.28	0.375	0.169	0.896

[a–b] Within a row, means without a common superscript differ ($P < 0.05$).
[1] Pooled SEM (4 replicates per treatment).
[2] Relative feed value
[3] Net energy for maintenance,
[4] Total digestible nutrients

Table 4. Average daily gain (kg)

	Type			
	GG	GL	SEM	*P*-value
Body weight				
Initial	294.6	293.5	5.36	0.746
Final	508.4[b]	582.9[a]	10.08	<0.001
Overall gain	213.8[b]	289.4 [a]	7.55	<0.001
Period 1	0.64[b]	0.84[a]	0.04	0.003
Period 3	0.49[b]	0.90[a]	0.03	<0.001
Period 6	0.56[b]	0.98[a]	0.03	<0.001
Overall period	0.46[b]	0.62[a]	0.01	<0.001

a–b Within a row, means without a common superscript differ (P < 0.05).

Table 5. Chemical composition of rib-eye

	Type		SEM	*P*-value
	GG	GL		
Parameters				
Moisture content, %	69.7	70.7	0.868	0.277
Fat, %	6.77[a]	4.60[b]	0.998	0.042
Ash, %	1.02	1.15	0.790	0.065
Protein, %	22.5[b]	23.5[a]	0.401	0.016
pH	5.57	5.54	0.031	0.418

a–b Within a row, means without a common superscript differ ($P < 0.05$).

Table 6. Carcass characteristics

	Type			
	GG	GL	SEM	*P*-value
Variable				
HCW, kg	322.0[b]	336.5[a]	5.05	0.048
Backfat thickness, cm	0.38[b]	0.57[a]	0.04	0.004
Rib-eye area, cm^2	81.0[b]	87.9 [a]	1.46	<0.001
Marbling score[a]	8.96[b] (slight)	10.3[a] (small)	0.36	0.013
USDA quality grade[b]	3.16[b] (select+)	6.84[a] (choice -)	0.25	<0.001
Warner-Bratzler shear force, kg	4.09[b]	4.99[a]	0.58	<0.001

a–b Within a row, means without a common superscript differ ($P < 0.05$).

[a] Practically devoid (-, 0, and +)– 1, 2, and 3; trace (-, 0, and +)– 4, 5 and 6; slight (-, 0, and +)– 7, 8, and 9; small (-, 0, and +)– 10, 11, and 12; modest (-, 0, and +)– 13, 14, and 15; moderate (-, 0, and +)– 16, 17, and 18; slightly abundant– 19; moderately abundant–20; abundant– 21

[b] Standard (-, 0, and +)– 1, 2, and 3; Select (-, 0, and +)– 4, 5, and 6; Choice (-, 0, and +)– 7, 8, and 9; Prime (-, 0, and +)– 10, 11, and 12

Study 2: Seasonal and locational variation of nutrient profile and in vitro digestion kinetics of guinea grass and kikuyu grass on Hawaii Island

3.2 Nutrient profiles of guinea grass and kikuyu grass

Nutrient profiles of guinea grass (GG) and kikuyu grass (KK) were compared to determine differences due to grass type (GG vs KK), season (summer vs winter) and ranch location (B vs C vs D vs E). When comparing GG to KK, KK had a significantly higher nutritional value (CP 12.0 vs 16.6, ADF 40.1 vs 35.4, Ash 12.7 vs 9.4, TDN 54.7 vs 58.8, NEM 0.49 vs 0.53 Mcal/kg, and RFV 90.1 vs 101.2%, respectively, $P<0.05$). However, GG had a higher NDFDom (neutral detergent fiber digestibility) at 120h, compared to KK (72.9 vs 67.1%, respectively, $P<0.05$) shown in table 7. Significant differences were found due to season when GG and KK were analyzed together, with summer yielding higher nutritive values for certain traits (ADF 35.87 vs 39.78, WSC 6.23 vs 3.75, Ash 9.79 vs 12.34, TDN 58.2 vs 55.3, NDFDom 30h 79.8 vs 60.2%, respectively, $P<0.05$) shown in table 8.

Differences were found for Ranches B and D when comparing overall GG and KK nutrient profiles with KK also having a higher nutritional value at these ranches when compared together (CP 11.9 vs 17.7, ADF 40.0 vs 36.7, Ash 12.3 vs 9.92, TDN 55.2 vs 58.5, P 0.30 vs 0.41, K 2.60 vs 3.26%, respectively, $P<0.05$). GG samples were also found to have the highest NDFDom at 120h at these ranches (73.3 vs 67.5%, respectively, $P<0.05$), shown in table 10. Ranch B was found to have better nutritional (when analyzing GG and KK forages together) compared to Ranch D (water soluble carbohydrates, 3.96 vs 5.59, crude fat 1.90 vs 2.55%, Mg 0.26 vs 0.29, S 0.19 vs 0.25%, respectively, $P<0.05$) shown in table 11. There were no interactions found for type × season and type × ranch location in this study.

Table 7. Overall nutrient analysis, %DM basis

Traits	Type		SEM	*P*-value
	GG	KK		
DM	26.8	20.0	5.24	0.214
CP	12.0[b]	16.6[a]	1.70	0.010
ADF	40.1[a]	35.4[b]	1.26	0.007
Lignin	4.46[b]	5.06[a]	0.50	0.239
WSC	4.82[b]	5.16[a]	0.66	0.613
CF	2.07	2.45	0.22	0.106
Ash	12.7[a]	9.4[b]	0.65	<.0001
TDN	54.6[b]	58.8[a]	1.28	0.002
NEM, Mcal/kg	0.22	0.24	0.01	0.054
RFV	90.1	101.2	5.46	0.051
aNDFom	60.1	56.5	2.45	0.157
NDFDom 30h	55.1	56.7	2.63	0.546
NDFDom 120h	72.9[a]	67.1[b]	2.15	0.010
NDFDom 240h	77.7	72.9	2.71	0.081
Ca	0.50	0.47	0.07	0.711
P	0.32	0.37	0.04	0.237
Mg	0.27	0.26	0.01	0.575
K	2.65	3.08	0.27	0.129
S	0.20	0.23	0.02	0.235
Cl	0.93	1.02	0.12	0.489

a–b Within a row, means without a common superscript differ ($P < 0.05$).
*DM= Dry matter, *CP= Crude protein, *ADF=Acid detergent fiber, *WSC=Water soluble carbohydrates, *CF= Crude fat, *TDN= Total digestible nutrients, *NEM= Net energy for maintenance, *RFV= Relative feed value, *aNDFom= Ash free neutral detergent fiber, *NDFDom= Neutral detergent fiber digestibility-organic matter basis

Table 8. Summer and winter overall forage nutrient analysis, %DM basis

Traits	Season		SEM	*P*-value
	Summer	Winter		
DM	22.5	24.3	5.24	0.727
CP	13.0	15.7	1.70	0.118
ADF	35.9^{b}	39.8^{a}	1.26	0.004
Lignin	4.69	4.84	0.50	0.762
WSC	6.23^{a}	3.75^{b}	0.66	0.006
CF	2.45	2.07	0.22	0.100
Ash	9.79^{b}	12.3^{a}	0.65	0.004
TDN	58.2^{a}	55.3^{b}	1.28	0.031
NEM, Mcal/kg	0.24	0.23	0.01	0.360
RFV	96.5	94.8	5.46	0.765
aNDFom	59.2	57.4	2.45	0.470
NDFDom 30h	59.3^{a}	52.5^{b}	2.63	0.014
NDFDom 120h	70.8	69.2	2.15	0.466
NDFDom 240h	77.7	72.9	2.71	0.086
Ca	0.46	0.51	0.07	0.430
P	0.34	0.36	0.04	0.663
Mg	0.24^{b}	0.30^{a}	0.01	0.003
K	2.64	3.09	0.27	0.106
S	0.21	0.23	0.02	0.486
Cl	0.75^{b}	1.20^{a}	0.12	0.001

a–b Within a row, means without a common superscript differ ($P < 0.05$).
*DM= Dry matter, *CP= Crude protein, *ADF=Acid detergent fiber, *WSC=Water soluble carbohydrates, *CF= Crude fat, *TDN= Total digestible nutrients, *NEM= Net energy for maintenance, *RFV= Relative feed value, *aNDFom= Ash free neutral detergent fiber, *NDFDom= Neutral detergent fiber digestibility-organic matter basis

Table 9. GG and KK nutrient analysis of Ranch B and Ranch D, %DM basis

	Type			
Traits	GG	KK	SEM	*P*-value
DM	27.2	20.0	5.35	0.187
CP	11.8[b]	17.6[a]	1.63	0.001
ADF	40.0[a]	36.7[b]	1.47	0.032
Lignin	4.48	4.79	0.42	0.475
WSC	5.01	4.53	0.64	0.454
CF	2.18	2.27	0.18	0.610
Ash	12.3[a]	9.92[b]	0.77	0.005
TDN	55.2[b]	58.5[a]	1.27	0.016
NEM, Mcal/kg	0.22	0.24	0.01	0.110
RFV	90.8	99.7	5.08	0.093
aNDFom	59.9	56.6	2.25	0.156
NDFDom 30h	55.5	56.1	2.84	0.858
NDFDom 120h	73.3[a]	67.5[b]	1.93	0.006
NDFDom 240h	77.7	72.7	2.56	0.059
Ca	0.53	0.43	0.07	0.142
P	0.30[b]	0.41[a]	0.03	0.009
Mg	0.27	0.28	0.01	0.935
K	2.60[b]	3.26[a]	0.25	0.016
S	0.20	0.24	0.20	0.140
Cl	0.88	1.08	0.13	0.141

a–b Within a row, means without a common superscript differ ($P < 0.05$).
*DM= Dry matter, *CP= Crude protein, *ADF=Acid detergent fiber, *WSC=Water soluble carbohydrates, *CF= Crude fat, *TDN= Total digestible nutrients, *NEM= Net energy for maintenance, *RFV= Relative feed value, *aNDFom= Ash free neutral detergent fiber, *NDFDom= Neutral detergent fiber digestibility-organic matter basis

Table 10. Overall nutrient analysis of Ranch B and Ranch D, %DM basis

	Ranch			
Traits	B	D	SEM	*P*-value
DM	24.5	22.7	5.35	0.745
CP	13.5	15.9	1.63	0.148
ADF	39.5	37.2	1.47	0.151
Lignin	4.22	5.05	0.42	0.062
WSC	3.96[b]	5.59[a]	0.64	0.018
CF	1.90[b]	2.55[a]	0.18	0.001
Ash	11.2	11.0	0.77	0.769
TDN	56.2	57.4	1.27	0.355
NEM, Mcal/kg	0.22	0.24	0.01	0.131
RFV	89.9[b]	100.5[a]	5.08	0.047
aNDFom	60.3	56.2	2.25	0.078
NDFDom 30h	53.9	57.7	2.84	0.195
NDFDom 120h	71.0	69.8	1.93	0.529
NDFDom 240h	74.9	75.5	2.56	0.817
Ca	0.42	0.54	0.07	0.114
P	0.36	0.35	0.03	0.730
Mg	0.26[b]	0.29[a]	0.01	0.042
K	2.79	3.06	0.25	0.296
S	0.19[b]	0.25[a]	0.02	0.013
Cl	0.90	1.06	0.13	0.252

a–b Within a row, means without a common superscript differ ($P < 0.05$).
*DM= Dry matter, *CP= Crude protein, *ADF=Acid detergent fiber, *WSC=Water soluble carbohydrates, *CF= Crude fat, *TDN= Total digestible nutrients, *NEM= Net energy for maintenance, *RFV= Relative feed value, *aNDFom= Ash free neutral detergent fiber, *NDFDom= Neutral detergent fiber digestibility-organic matter basis

Chapter 4: Discussion

4.1 Nutrient profile of guinea grass and leucaena

Proper pasture maintenance is important in order to achieve optimal average daily gains (ADG) and carcass characteristics. In study one, nutrient profiles of guinea grass (GG) and leucaena (L) were determined to evaluate GG and GL pastures effects on growth performance and carcass characteristics of steers. Morning and evening samples were collected because variation in nutrient content has been found due to time of day.

The GG and L were found to vary in their nutrient profiles with L having an overall higher nutritional value compared to GG. When comparing GG to L, dry matter (DM) content of L was higher than GG (18.2 vs 26.1%, respectively) and producers want less moisture in order for pastures to yield higher nutritive values. Also L has protein-rich leaves which make it suitable for livestock grazing and L was found to be higher in crude protein (CP) (18.8 vs 27.3%, respectively) comparing GG to L. Overall, L was found to have a higher RFV (101.0 vs 200.5%, respectively) and more total digestible nutrients (TDN) (52.4 vs 59.6 %, respectively).

Samples of GG and L were collected in the AM and PM because previous studies found differences in nutritive value of forages due to time of day. A diurnal cycling of sugars occurs from accumulation of sugars during the day due to photosynthesis. These accumulated sugars are then used up at night which causes PM forage to be higher in sugar, thus having a higher RFV. Due to the increased sugar content of PM forages, animals prefer grazing in the afternoon compared to morning (Maryland and Idaho, 2004). Some advantages for feeding PM versus AM forages include increased: rate of passage, dry matter intake and digestibility, efficiency of forage and protein utilization, and energy intake for production (Maryland et al., 2005). In this present study energy for maintenance (NEM), ash, total digestible nutrients (TDN), and crude fat were found to be significantly higher for AM samples, which disagrees with the idea of PM forages containing less sugar than AM forages (Maryland and Idaho, 2004). This difference could be due to differences in sunlight at specific hours of the day. Also, Hawaii can change from being sunny to cloudy very rapidly, which may influence the ability of plants to photosynthesize. Further research could help to explain these resultsuy

4.1.1 Nutrient profile of guinea grass and kikuyu grass

Tropical soils have been described as being infertile and unproductive, but research at Hawaii Agricultural Experiment Station has proven soils in Hawaii can yield high crop production under proper conditions and management. It has also been noted soils can vary in short distances in Hawaii due to soils forming rapidly because of high temperatures and rainfall. Soil along the Hamakua Coast is the Maile series, which is considered suitable as pasture land and is well-drained, silty clay loam that developed in volcanic ash. Rainfall is 60 to 90 inches annually and soil temperature is 58 F (Ikawa et al. 1985; Hawaii Soil Atlas, 2014).

In study two, guinea grass (GG) and kikuyu grass (KK) nutrient profiles were determined and compared to determine differences due to grass type (GG vs KK), season (summer vs winter) and ranch location (B vs C vs D vs E) on Hawaii Island. An estimated 570, 662 acres of pasture and rangeland are available on Hawaii Island and zones considered suitable for grass-finished beef production have been determined. For GG and KK, 210,368 acres of pastureland have been determined as usable and suitable for high-quality grass-finished beef production (Fukumoto et al., 2015).

When comparing nutrient profiles of GG to KK, KK was found to have a higher nutritional value compared to GG. Kikuyu has been found to grow successfully when fertilized with nitrogen (N), but the accumulation of N is too high for animal requirements, thus negatively effecting digestion and utilization for animals. Also KK has been described as lacking readily digestible non-structural carbohydrates, thus resulting in low digestibility of structural components (Marais, 2001). Low digestibility of kikuyu can be attributed to lignification of xylem cells, which are responsible for transferring water and nutrients in plants. Also it can be attributed to high concentrations of sclerenchyma cells, which are woody cells that support the plant and as maturity increases the lignin content increases (Akin et al., 1990). This explains why GG was found to have a higher neutral detergent fiber digestibility on an organic matter basis compared to KK in this present study (NDFDom 120h 72.9 vs 67.1%, respectively). Oxalic acid found in KK has also been found to bind to calcium (Ca) making it unavailable for the grazing animal, thus Ca needs to be considered while developing grazing programs (Marais, 2001). Also it has been noted that the mineral requirement of grazing animals is sufficient for fertilized KK, however Ca is the exception for beef cattle in Hawaii (Younge and Otagaki, 1985).

Nutritional value of pastures can vary due to season and differences were found in this study. Summer forages had a higher nutritive value compared to winter forages. However, it was expected for winter forages to yield higher nutritive values than summer due to weather conditions for different seasons. Hawaii experiences mild seasonal variation and typically winter is the wet season (November to April) and summer is the dry season (May to October). The weather on each island is also influenced by trade winds with the east and north side typically experiencing more wind and rain than the south and west side. In this present study, summer forages yielded a higher nutritive value, which can be attributed to the 2015 El Niño. This results in warmer than normal waters, thus increasing the likelihood for storms to hit Hawaii. Meteorologists observed changes beginning in summer 2015 with increased tropical storms striking in the Pacific. During August and September 2015, Hawaii experienced abnormal amounts of rainfall, thus influencing pasture growth and quality. Due to El Niño, meteorologists are predicting lower than average rainfall for the whole state and drought conditions are expected until April 2016 (Erdman and Dolce, 2015).

4.1.2 Growth performance

One objective of study one was to evaluate growth performance of animals that grazed GG or GL pastures. Steers that grazed GL pastures were found to have higher ADG (0.46 vs 0.62 kg, respectively) compared to GG steers and a shortened stay on pasture (707 vs 532 days). Previous studies have concluded when animals are allowed free access to pasture and grain or concentrate, animals that consumed concentrate had higher ADG (French et al., 2000). However, previous studies have also found successful ADG for animals that grazed mixed forage and legume pastures (Gutteridge and Shelton, 1994) and specifically L has produced animals with liveweight gains of 0.90 kg/head/day (Clem et al., 1993) in New Zealand and 1.10 kg/head/day in Queensland, Australia (Wildin, 1986). It has been determined that L can be 30% of a diet and fulfil the role as a supplemental protein, roughage and mineral source (Brewbaker, 1987). Results from this study suggest grass-fed beef production can produce animals with desirable ADG and it is possible for tropical environments such as Hawaii.

4.1.3 Chemical composition of rib-eye

In study one, chemical composition variables determined included: moisture, fat, ash, protein and pH. Fat was found to be significantly higher in GG compared to GL rib-eyes (6.77 vs 4.60%, respectively) and overall fat was higher compared to results from previous studies. Protein was determined by difference and was found to be higher for GL rib-eyes (22.5 vs

23.5%, respectively). Interestingly fat was higher in GG steers, however GL yielded carcasses with better marbling scores and thicker backfat thickness. When determining marbling scores measurements are determined objectively, which may explain the variations found.

Chemical composition has been found to not be significantly affected when comparing forage to concentrate ratio of diets (French et al., 2000). However, Marino et al. (2006) did find significant differences in protein, fat and ash when comparing concentrate and forage fed steers, with concentrate-fed steers having been better in all variables. Fatty acid content can vary when comparing diet (grass versus grain) and type of fat is more important than the amount of fat. Fatty acid profiles can vary due to forage variety, species, harvest time and growing season (Drewhurst et al., 2001; Clapham et al., 2005). For future work, it would be interesting to determine the fatty acid profiles of GG and GL carcasses, which may help to better understand differences found for chemical composition in this study.

4.1.4 Carcass characteristics

Determination of slaughter endpoints for grass-fed beef and effects on meat quality are seen as the biggest weaknesses of grass-fed beef production systems (Duckett et al., 2013). Overall, GL carcass traits were better than GG carcass traits in study one. Backfat thickness for GL carcasses was greater and rib-eye area for GG carcasses was less than that of GL carcasses. The GL yielded better carcasses overall when comparing marbling scores and USDA quality grades according to USDA grading standards. These results were expected due to the higher nutritive value of L and higher ADG of steers that grazed GL pastures.

In the present study, rib-eyes from GG steers were more tender compared GL rib-eyes (4.09 vs 4.99 kg, respectively). This result was somewhat unexpected considering that GL carcasses had higher marbling scores and USDA quality grades. However, proximate analysis showed GG rib-eyes contained significantly more fat than GL rib-eyes. Therefore, it is not currently possible to explain what factor(s) are potentially associated with the difference in meat tenderness between these groups, and future studies need to examine more closely how leucaena incorporation in pasture affect meat tenderness and carcass quality.

Producers have explored different ways to improve tenderness due to consumers desiring more lean, but tender meat. Post-mortem ageing (French et al., 2000) and electrical stimulation have been found to improve tenderness of beef cattle (Kim et al., 2007). Previous work has shown Hawaii originated pasture-finished cattle (mainly kikuyu grass pasture) and Hawaii originated feedlot-finished cattle were not significantly different in tenderness. However, in this present study GG rib-eyes were more tender than previous Hawaii studies (4.09 kg, respectively). The USDA (2013) determined beef products as "USDA Certified Tender" at 4.4 kg and "USDA Certified Very Tender" at 3.9 kg (American Society for Testing and Materials International Publication F2925-11). This supports the idea that improvements have occurred, which may be attributed to pasture quality. Variation is common and it can be concluded shear force values will vary due to slaughter age, diet, pH and fatness (Fiems et al., 2000). For future work, a larger sample size, post-mortem ageing and electrical stimulation may help address differences found for carcass quality in this present study.

4.3 Conclusions and recommendations

The common practice for Hawaii beef producers is to ship weaned calves to the continental United States and Canada. Grain feed is no longer shipped because of high transportation costs and the state lacks feedlot and slaughter capacity. Mixed guinea grass and leucaena pastures were found to be successful for cattle grazing on the Island of Hawaii. When comparing GG to L, L was found to have a higher nutritive value, which contributed to the positive impact GL pastures had on factors such as growth performance, marbling score, backfat thickness, and rib-eye area. Nutrient profile differences were also found due to season (summer vs winter) and ranch location (B vs C vs D vs E) on Hawaii Island for guinea grass and kikuyu grass (KK). When comparing GG to KK, KK was found to have a higher nutritive value and forages collected in the summer and from Ranch B had a better nutritive value when comparing all ranches. This proves season can influence pasture quality, even in tropical environments such as Hawaii. Also differences due to location were found, which proves soil conditions can vary for ranches short distances from each other.

The state of Hawaii relies heavily on imported resources due to the state's increasing population and lack of space for production. This is true for the beef industry and why local grass-fed beef cannot supply the demand alone. However, due to more information becoming available on differences in species/varieties of forages and legumes, season and location, grass-fed production could be practiced more by local producers. Also a niche market for grass-fed beef has been established in the state and grass-fed beef is growing in popularity. Overall, results from these studies show more producers in Hawaii could practice grass-fed beef production by utilization of local forage and legume species if proper pasture management and paddock rotation are considered.

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