THESIS

THE EFFECTS OF OLIVE MEAL SUPPLEMENTATION ON FEEDLOT PERFORMANCE AND LONGISSIMUS MUSCLE FATTY ACID COMPOSITION OF WAGYU STEERS AND THE IMPACT OF CALCIUM DOSE AND OLIVE MEAL ON IN VITRO RUMEN FERMENTATION CHARACTERISTICS.

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ABSTRACT

THE EFFECTS OF OLIVE MEAL SUPPLEMENTATION ON FEEDLOT PERFORMANCE AND LONGISSIMUS MUSCLE FATTY ACID COMPOSITION OF WAGYU STEERS AND THE IMPACT OF CALCIUM DOSE AND OLIVE MEAL ON IN VITRO RUMEN FERMENTATION CHARACTERISTICS.

Two experiments were conducted to evaluate the effects of olive meal supplementation on feedlot performance of Wagyu steers and the impact of Ca dose and olive meal on in vitro rumen fermentation characteristics. Experiment 1: Eighty-three American Wagyu steers (725 ±10.7 kg) were used to evaluate the effects of olive meal supplementation on feedlot performance and carcass characteristics. The steers were blocked by BW. The heaviest 8 steers were stratified into two pens containing 3 or 4 steers per pen with similar pen BW. This was considered a paired weight block. This process was repeated until all steers were assigned to pens. Each pen contained 3 or 4 steers/pen with 11 replicates/treatment. Steers were blocked by initial body weight (BW) and randomly assigned within block to one of two treatments. Treatments consisted of: 1) Control (basal ration with no olive meal) + 1 kg of supplemental cracked corn per animal per day, or 2) Control diet + 1 kg of supplemental olive meal per animal per day. Steers were housed in feedlot pens (n=4 steers/pen; 11 replicates/treatment) and fed a traditional American Wagyu finishing diet (DM basis: 68.4% DM, 14.3% CP; 74.8% TDN, 1.16 Mcal/kg NEg, 5.3% crude fat). Diets were delivered to pens, once daily, in the morning in amounts to allow ad libitum access to feed over a 24 h period. Olive meal and cracked corn were top-dressed to the appropriate treatment pens immediately after delivery of the basal ration. Steers were individually weighed on d -1 and 0, and approximately every 28 d throughout the 177 d experiment. Equal numbers of steers per treatment were slaughtered throughout the experiment and carcass data were collected. Steers receiving olive meal had a lower final BW, ADG, DMI, and FE ($P < 0.05$) when compared to steers receiving the control diet. Longissimus muscle C18:1 tended to be greater ($P < 0.06$) in steers receiving olive meal when compared to controls. Under the conditions of this experiment, feeding olive meal at 1.0 kg/ animal /day reduced live animal performance and had minimal impacts on longissimus muscle fatty acid composition. Experiment 2: Rumen fluid from three beef steers (480 ± 10 kg) fitted with rumen canulae, was used to investigate the impact of Ca dose and olive meal on in vitro rumen fermentation characteristics. Steers were fed a high concentrate finishing diet for 21 d and rumen fluid was collected from each steer 2h post-feeding. A 2 x 4 factorial

arrangement of treatments was used for this experiment. Factors included: 1) 0 or 5% olive meal and 2) Ca dose: 0, 0.02, 0.04, and 0.08% Ca from $CaCl_2$. A McDougall's buffer-rumen fluid mixture (1:1; 30 mL 5 total volume) was added to conical tubes containing 0.5g of the ground basal diet with the appropriate treatments and incubated at 39°C for 0, 4, 8, and 12h (5 replicates per treatment per time point). After incubation, supernatant was removed for VFA analysis and the remaining digesta was dried to determine DM disappearance (DMD). There were no olive meal x Ca interactions for any response variables measured. At 4 and 8 h post incubation digestion tubes containing 0.04% Ca had greater ($P < 0.001$) DMD when compared to all other Ca doses. At 12 h post incubation, DMD was greater ($P < 0.001$) in digestion tubes containing 0.02% and 0.08% Ca compared to all other Ca doses. At 8 h post incubation, molar proportions of acetic acid were greater ($P < 0.03$) in digestion tubes containing olive meal compared to no olive meal and were greater ($P < 0.001$) in digestion tubes containing 0.08% Ca compared to all other Ca doses. At 12 h post incubation, iso-butyric acid ($P < 0.01$) and butyric acid ($P < 0.02$) were greater in digestion tubes containing 0.02% and 0.04% Ca compared to all other Ca doses. Butyric acid was lesser ($P < 0.02$) with olive meal inclusion at 12 h. Total VFA concentrations were similar across treatments. These data suggest that Ca and olive meal may impact in vitro fermentation. Dietary treatment was a significant ($P < 0.05$) source of variation for caproic (C6:0), capric (C10:0), linoleic (C18:2n-6), linolelaidic (C18:2n-6 trans), and docosahexaenoic (C22:6n-3) longissimus muscle fatty acids. Steers receiving the control diet had greater C6:0 ($P < 0.02$), C10:0 ($P < 0.02$), C18:2n-6 trans (0.02), and C22:6n-3 ($P < 0.05$) fatty acids when compared to cattle receiving olive meal. Steers receiving olive meal had greater C18:2n-6 ($P < 0.04$) when compared to controls. All other fatty acids identified were similar across treatment. Based on these data Ca addition at the concentration supplied in this experiment did not inhibit biohydrogenation of unsaturated fatty acids but did improve fermentation characteristics.

Key words: Beef cattle, carcass characteristics, volatile fatty acids, Japanese Black, Wagyu and dry matter disappearance

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Chapter 1 – Review of Literature

Introduction

The Wagyu beef breed is becoming increasingly popular worldwide. Purchasing Wagyu meat from a specialty market is no longer needed but instead it can be accessible at your local grocery store. The word Wagyu is an umbrella term and under it are multiple breed types and crossbred Japanese Black cattle produced in Australia and the United States. Once machinery was developed for agriculture the animals were no longer being used to help with farming but instead, they were used for highly marbled beef production (Gotoh et al., 2018; Minezawa, 2003).

Japanese Wagyu carcasses are graded on both yield and quality grades (JMGA, 2014). Yield grade (A, B, or C) is assigned along with four items are being assessed when assigning a meat quality grade: 1) marbling; 2) meat color and brightness; 3) meat firmness and texture; and 4) fat color, luster, and quality (JMGA, 2014). The low-fat melting point that is associated with oleic acid, 18:1, is known for making the animal's fat softer and more palatable.

Today, the renowned brand name Wagyu includes not only the cattle produced in Japan, but also cattle produced in countries such as United States and Australia. In recent years, the intramuscular fat percentage in beef (longissimus muscle) from Japanese Black cattle has increased to be greater than 30%. The Japanese Black breed is genetically predisposed to producing carcass lipids containing higher concentration of monounsaturated fatty acids than other beef breeds. This is due to a mutation in the delta 9 desaturase gene. However, there are numerous challenges with the management of this breed including high production costs, disposal of excrement, and imported feed requirements. Furthermore, due to the extensive biohydrogenation of unsaturated fatty acids that occurs in the rumen, it is difficult to alter the fatty acid composition of Wagyu muscle through dietary addition of unsaturated lipids.

Therefore, the overarching objective of this literature review is to provide background information on Wagyu breeds and to examine the literature available on modulating the fatty acid composition of Wagyu muscle.

WAGYU BREED DESCRIPTIONS

The breeds of known Wagyu cattle are Mishima cattle, Kuchinoshima feral cattle, Japanese Black, Japanese Brown, Kumamoto strain, Kochi strain, Japanese Pulled, and Japanese Shorthorn (Minezawa, 2003). Each breed has distinctive traits, including the geographic location in Japan from which they originated.

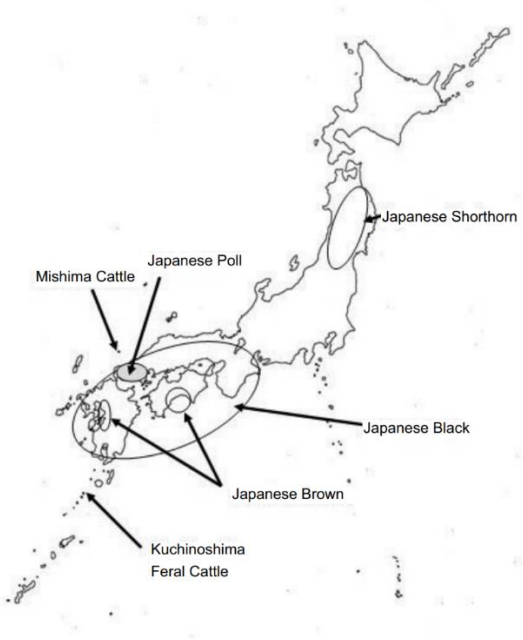

Figure 1. Native breed and population location adapted from Cattle Genetic Resources in Japan: One Successful Crossbreeding story and genetic diversity Erosion (Minezawa, 2003).

MISHIMA CATTLE

Mishima cattle come from a small island in the Sea of Japan called Mishima Island (Figure 1; Minezawa, 2003). These animals are small in stature and have a good disposition, making them the perfect fit for farmers with small amounts of land. These cattle are typically thought of as the "original' black cattle (Minezawa, 2003). These cattle are late maturing cattle with a dark brown coat color, small horns, and a narrow body (Harada et al., 1996). The average female Mishima is 60 months old, with a height of 112.8 cm, a chest circumference of 152.1 cm and 261.1 kg body weight (Harada et al., 1996).

Figure 2. Adapted from Mishima Cattle (Bull) taken from Cattle Genetic Resources in Japan: One Successful Crossbreeding story and genetic diversity Erosion (Minezawa, 2003)

KUCHINOSHUMA FERAL CATTLE

Kuchinoshima feral cattle are from the northern portion of the Tokara Isles, approximately 200 km south of Kyushu (Minezawa, 2003). There is evidence these cattle were in existence in 1727, but according to Hayashida and Nozawa (1964) it is thought that Kuchinoshima cattle are descendants of Kagoshima cattle that escaped from pasture in 1918 and 1919 (Tominta, 1996). The Kuchinoshima cattle are typically smaller than the Mishima cattle with an average height at 110 cm and length at 120 cm for mature female (Minezawa, 2003). The mature female also has a mainly black coat but can also have a white spot on the belly and/or on the limbs with a small possibility of being brown in color (Minezawa, 2003).

Figure 3. Adapted from Kuchinoshima Feral Cattle taken from Cattle Genetic Resources in Japan: One Successful Crossbreeding story and genetic diversity Erosion (Minezawa, 2003).

JAPANESE BLACK CATTLE

The Japanese Black cattle were crossbred making the modern type of this breed known for the high amounts of intramuscular fat. The main purpose of this breed was to be used as a draft animal to pull firewood for steel production (Minezawa, 2003). In 1867 the government told farmers that crossbreeding the native cattle would increase the body size and milk production of the Japanese Black breed (Minezawa, 2003). When agricultural machinery was developed in the mid 1950's this breed was no longer used as work animals but shifted to beef production (Minezawa, 2003). Wagyu cattle are known for their ability to "marble" (intramuscular fat; IMF) when compared to breeds of beef cattle such as Angus. This breed can now be found in all regions of Japan. The Japanese Black breed is characterized as a breed with a dull black coat and skin, horns, small to medium body frame with the height being 124 cm and the average weight being 700 kg in both a cow and bull (Minezawa, 2003).

Figure 4. Adapted from Japanese Black taken from Cattle Genetic Resources in Japan: One Successful Crossbreeding story and genetic diversity Erosion (Minezawa, 2003).

JAPANESE BROWN

The Japanese brown breed comes from two breeds of cattle: the Kumamoto and Kochi breeds. The Kumamoto cattle have a red colored coat developed from Korean cattle being crossed with Simmental and Devon breeds. The unique features of this breed are the large body size with a mature female and male body weights ranging from 600 kg and 950 kg (Minezawa, 2003). The Kochi breed of cattle was developed from crossing Simmental cattle with cattle from Kyushu Island. The unique characteristic of this breed is the yellow-brown coat, lighter than the Kumamoto breed but the performance is similar to the Kumamoto breed with body weights of the mature female and male ranging from 600 kg and 950 kg (Minezawa, 2003).

Figure 5. Japanese Brown, Kumamoto strain (Bull) adapted from Cattle Genetic Resources in Japan: One Successful Crossbreeding story and genetic diversity Erosion (Minezawa, 2003).

JAPANESE POLLED

The Japanese Polled breed has been around since 1916, originating from the cross of Japanese indigenous cattle and the Aberdeen Angus imported from England (Minezawa, 2003). In order to increase the meat quality of the Japanese Polled breed they were crossed with the Japanese Black Breed in 1975 (Minezawa, 2003). Characteristics of the Japanese Polled breed are hornless cattle with a black coat, with a height averaging 122cm and body weight averaging 450 kg for mature cows.

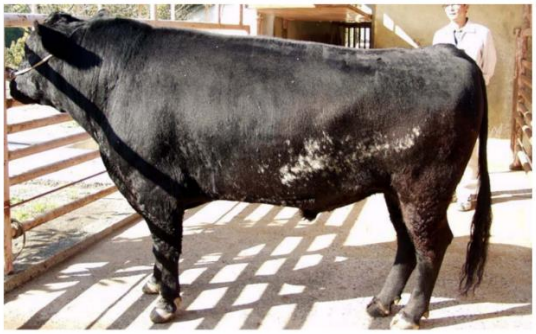

Figure 6. Japanese Poll (Bull) adapted from Cattle Genetic Resources in Japan: One Successful Crossbreeding story and genetic diversity Erosion (Minezawa, 2003).

JAPANESE SHORTHORN

The Japanese Shorthorn breed was developed from the crossbreeding of imported dairy Shorthorn cattle and indigenous cattle from the Tohoku region. This breed is known for being able to graze in the mountain pastures better than other breeds and they are mainly found in the Tohoku and Hokkaido regions of Japan (Minezawa, 2003).

The coat color of this breed is a reddish-brown color that is darker when compared to the Japanese Brown. The height of an average mature female is 128 cm and 500 kg and a mature bull of 140 cm and 800 kg.

Figure 7. Japanese Shorthorn (Cow) adapted from Cattle Genetic Resources in Japan: One Successful Crossbreeding story and genetic diversity Erosion (Minezawa, 2003).

In the early 1900's Japan was crossbreeding the native cattle to Braunvieh and Simmental cattle from Switzerland; Ayrshire, Devin and Shorthorn cattle from the United Kingdom; and Holstein cattle from Germany and the Netherlands. It wasn't until Japan stopped crossbreeding in 1944 that the Japanese Black Breed was started (Morita et al., 2000).

WAGYU IN THE UNITED STATES

Just like in the United States and other countries, Wagyu production is separated into different categories or phases. The two main categories are calf-production and fattening production (Motoyama et al., 2016). Calf-production farmers' goal is to produce calves and sell feeder cattle. Once the calves are between the ages of 2 to 4 months, the animals are taken to "small markets" for auction while calves between the ages of 6 to 12 months are taken to "feeder cattle markets" (Motoyama et al., 2016). Today these farmers breed by using artificial insemination (Morita et al., 2000). The challenges that one might face when raising Wagyu are feed cost and animal prices. Producers raising the Japanese cattle breeds must take into consideration factors such as growth rate, feed efficiency, health, animal welfare, disease tolerance and intramuscular fat (Gotoh et al., 2018). With the finishing period being most important for the Japanese Black cattle, feed usually consist of a high concentrate diet to help accumulate the intramuscular fat. From 11 to 18 months of age, the diet consists of a large majority concentrate with a small amount of roughage such as Jamboree, hay, or rice hay (Gotoh et al., 2018). During the final stage, the diet is typically 60-

85% concentrate and 13 to 15 % roughage (Gotoh et al., 2018). During the whole life span the cattle will have access to water and minerals. A large majority of the feed being given during the fattening process is imported.

The taste that is consistent with Wagyu cattle comes from the mono-unsaturated fatty acid (MUFA) mostly from the oleic acid (18:1). Oleic acid 18:1 is known for causing the animal's fat to be softer and more palatable, which allows for a lower melting point (Motoyama et al., 2016). A lower melting point allows the fat to be able to melt in the consumer's mouth (Motoyama et al., 2016). However, 18:0, stearic acid is responsible for increasing fat hardness which reduces eating quality (Motoyama et al., 2016).

Yield grade and quality grade in Japan are based on the $6^{th}/7^{th}$ rib cross section (Motoyama et al., 2016). Quality grade in Japan is comprised of color and brightness, marbling, firmness and texture, fat color, luster, and quality. In Japanese marbling is called "Shimo-furi" which translates to "frosting" (Motoyama et al., 2016). The Japanese rely heavily on imported feed estimating around 58.9 million tonnes (Sithyphone et al., 2011). According to the United States Department of Agriculture in 2009 over 24,911,960 million tons of grain was imported to Japan and of that 4,564,228 million tons was for the beef cattle industry alone (USDA, 2009). Both the feed and labor cost are high to support Wagyu cattle. The feed increased due to the importation of feedstuffs to Japan. Furthermore, the animals are individually penned and fed which increases the amount of labor needed.

MEAT GRADING & QUALITY

The Japan Meat Grading Association (JMGA) is the accredited group of graders from which most of the Japanese breeds are graded. The grading system used today was established in 1988. It includes a yield grade (A, B, or C) and a meat quality grade (JMGA, 2014). The four items being assessed when assigning a meat quality grade: 1) marbling; 2) meat color and brightness; 3) meat firmness and texture; and 4) fat color, luster, and quality (JMGA, 2014). The grade is assigned based on the lowest of the four items (Horii et al., 2009; Albrecht et al., 2011).

Muscle from Wagyu is made up of multiple myofiber types with type 1, type IIA and type IIB being the most common. The type and size of muscle fibers has an impact on meat quality (Klont et al., 1998). Iwamoto et al. (1991) investigated the differences between the muscle fiber composition of Japanese Black, Japanese Brown, and Holstein cattle (Iwamoto et al., 1991). The authors reported that a higher percentage of type IIB muscle fiber existed in Japanese Brown and Holstein breeds while a higher number of type I and type IIA muscle fibers existed in the

Japanese Black cattle (Iwamoto et al., 1991). This can have a direct impact on the shear force values. Warner-Bratzler shear force is a technique used to determine meat tenderness (AMSA, 1995). In Japanese Black steers the longissimus muscle was shown to decrease after 20 months of age and the intramuscular fat percentage has been greater than 30% in recent years (Nishimura et al., 1999; Gotoh et al., 2014). This shows that the higher level of marbling is closely related to meat tenderness.

FATTY ACIDS

Fatty acids are a subcategory of a larger group called lipids. Lipids are insoluble in water but are soluble in organic solvents (O'Fallon et al., 2015). Examples of lipids compounds containing lipids are steroids, terpenes, carotenoids, and bile acids which are soluble in solvents like ether, hexane, benzene, chloroform, or methanol.

Unsaturated fatty acids contain double bonds while monounsaturated fatty acids (MUFA) are single bonds. Fatty acids can range from 2 carbons to more than 30 carbons but the most common have 12-22 carbons (O'Fallon et al., 2015). Oleic acid, C18:1 omega-9, accounts for 41% of the fatty acid in a Wagyu Ribeye steak containing 18 carbons and 1 double bond in the omega-9 position (O'Fallon et al., 2015). Linoleic acid, C18:2 omega 6, contains two double bonds making it a polyunsaturated fatty acid (PUFA).

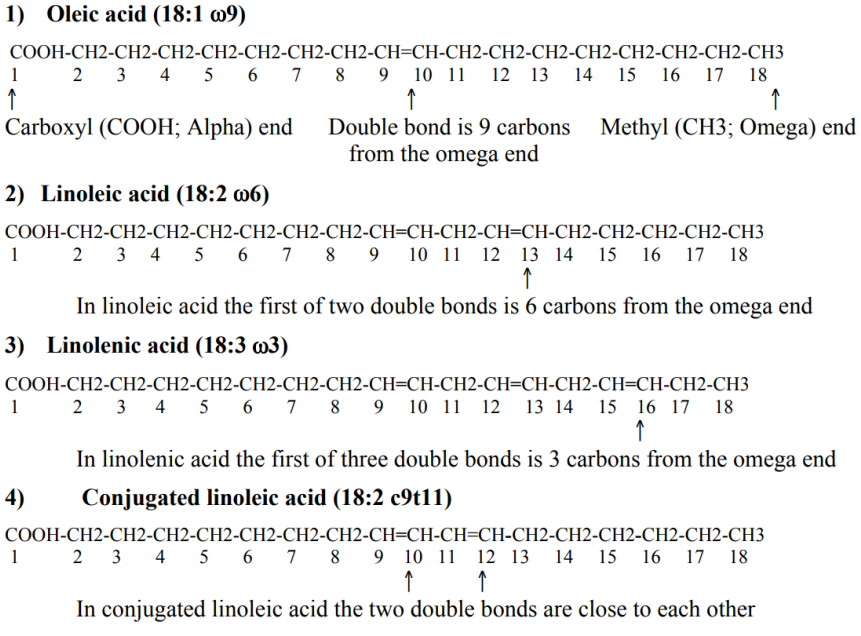

Figure 8. Structure of selected fatty acids adapted from Fatty Acids and Wagyu Beef (O'Fallon et al., 2015).

The number of carbons, type and location of the bonds affect how the fatty acid function in food with the larger number of carbons, the higher melting point, and the more double bonds the lower the melting point (O'Fallon et al., 2015). Stearic acid, C18:0, does not have any double bonds so its melting point is approximately 70 ℃ which makes it solid at room temperature. Oleic acid, C18:1, has one double bond and a melting point at 17 ℃ making it a liquid at room temperature.

Three major factors that influence fatty acid composition of beef are age of animal, diet, and breed type. The age and breed factors impact the MUFA of beef by affecting the stearoyl-CoA desaturase (SCD) gene. Pasture and hay feeding decreases the SCD, therefore increasing the SFA (Chung et al., 2007; Duckett et al., 2009) composition of muscle.

Rumen microorganisms are responsible for saturating unsaturated fatty acids consumed by the animals. This process is known as biohydrogenation (Ekeren et al., 1992). In a study conducted by Kucek et al. (2001), increasing the forage in the diet increased the duodenal flow of stearic acid (C18:0) and α-linolenic acid but at the same time decreased the flow of oleic and linoleic acid. By feeding a high-corn diet, the rumen pH is lowered which can slow the rumen biohydrogenation process (Devillard et al., 2006; Wallace et al., 2006).

BIOHYDROGENATION

Hartfoot (1978) found that two microbial transformations happen in the rumen (Engle, 1999). The important transformations are lipolysis and biohydrogenation. The lipases convert acylglycerols to make fatty acids and glycerol (Hawke and Wilcock, 1970). The glycerol is then fermented creating propionic acid (Garton et al., 1961). Much is still needed to be learned about biohydrogenation, but Hartfoot (1978) has claimed that biohydrogenation in the rumen helps with detoxification. The first step in to biohydrogenation is the conversion of an unsaturated fatty acid cis-12 double bond to a trans-11 isomer (Engle, 1999). After the trans-11 bond is formed, hydrogenation of cis-9 bond in C18:2 forms because of microbial reductase (Engle, 1999). The biohydrogenation of unsaturated fatty acids heavily relies on the conditions of the rumen. Biohydrogenation is low when high grain diets are fed which helps explain the low numbers of lipolytic bacteria in grain diets since a free carboxyl group is a requirement for the first step in biohydrogenation (Kemp et al., 1981; Palmquist and Schanbacher, 1991; Jenkins 1994; Engle, 1999).

Several traditional Wagyu feeding operation is Japan include olive meal byproduct in rations. Olive meal is high in oleic acid (C18:1; approximately 70%). The intent of feeding olive meal is to further increase the C18:1 fatty acid in the muscle of Wagyu beef. Inherent physical and chemical properties in olive meal have been suggested to inhibit ruminal biohydrogenation of C18:1 in olive meal. Therefore, allowing more C18:1 Fatty acid to be absorbed from the small intestine and incorporated into muscle.

Based on the information presented in this literature review, the following experiments described in Chapter 2 were designed to: 1) examine the impact of olive meal supplementation on live animal performance and fatty acid composition in Wagyu steers; and 2) determine if calcium supplementation can inhibit rumen biohydrogenation of unsaturated fatty acids in vitro.

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CHAPTER 2 - EXPERIMENT

THE EFFECTS OF OLIVE MEAL SUPPLEMENTATION ON FEEDLOT PERFORMANCE AND LONGISSIMUS MUSCLE FATTY ACID COMPOSITION OF WAGYU STEERS AND THE IMPACT OF CALCIUM DOSE AND OLIVE MEAL ON IN VITRO RUMEN FERMENTATION CHARACTERISTICS.

SUMMARY

Two experiments were conducted to evaluate the effects of olive meal supplementation on feedlot performance of Wagyu steers and the impact of Ca dose and olive meal on in vitro rumen fermentation characteristics. Experiment 1: Eighty-three American Wagyu steers (725 ±10.7 kg) were used to evaluate the effects of olive meal supplementation on feedlot performance and carcass characteristics. The steers were blocked by BW. The heaviest 8 steers were stratified into two pens containing 3 or 4 steers per pen with similar pen BW. This was considered a paired weight block. This process was repeated until all steers were assigned to pens. Each pen contained 3 or 4 steers/pen with 11 replicates/treatment. Steers were blocked by initial body weight (BW) and randomly assigned within block to one of two treatments. Treatments consisted of: 1) Control (basal ration with no olive meal) + 1 kg of supplemental cracked corn per animal per day, or 2) Control diet + 1 kg of supplemental olive meal per animal per day. Steers were housed in feedlot pens (n=4 steers/pen; 11 replicates/treatment) and fed a traditional American Wagyu finishing diet (DM basis: 68.4% DM, 14.3% CP; 74.8% TDN, 1.16 Mcal/kg NEg, 5.3% crude fat). Diets were delivered to pens, once daily, in the morning in amounts to allow ad libitum access to feed over a 24 h period. Olive meal and cracked corn were top-dressed to the appropriate treatment pens immediately after delivery of the basal ration. Steers were individually weighed on d -1 and 0, and approximately every 28 d throughout the 177 d experiment. Equal numbers of steers per treatment were slaughtered throughout the experiment and carcass data were collected. Steers receiving olive meal had a lower final BW, ADG, DMI, and FE ($P < 0.05$) when compared to steers receiving the control diet. Longissimus muscle C18:1 tended to be greater ($P < 0.06$) in steers receiving olive meal when compared to controls. Under the conditions of this experiment, feeding olive meal at 1.0 kg/ animal /day reduced live animal performance and had minimal impacts on longissimus muscle fatty acid composition. Experiment 2: Rumen fluid from three beef steers (480 ± 10 kg) fitted with rumen canulae, was used to investigate

the impact of Ca dose and olive meal on in vitro rumen fermentation characteristics. Steers were fed a high concentrate finishing diet for 21 d and rumen fluid was collected from each steer 2h post-feeding. A 2 x 4 factorial arrangement of treatments was used for this experiment. Factors included: 1) 0 or 5% olive meal and 2) Ca dose: 0, 0.02, 0.04, and 0.08% Ca from $CaCl_2$. A McDougall's buffer-rumen fluid mixture (1:1; 30 mL 5 total volume) was added to conical tubes containing 0.5g of the ground basal diet with the appropriate treatments and incubated at 39°C for 0, 4, 8, and 12 h (5 replicates per treatment per time point). After incubation, supernatant was removed for VFA analysis and the remaining digesta was dried to determine DM disappearance (DMD). There were no olive meal x Ca interactions for any response variables measured. At 4 and 8 h post incubation digestion tubes containing 0.04% Ca had greater ($P < 0.001$) DMD when compared to all other Ca doses. At 12 h post incubation, DMD was greater ($P < 0.001$) in digestion tubes containing 0.02% and 0.08% Ca compared to all other Ca doses. At 8 h post incubation, molar proportions of acetic acid were greater ($P < 0.03$) in digestion tubes containing olive meal compared to no olive meal and were greater ($P < 0.001$) in digestion tubes containing 0.08% Ca compared to all other Ca doses. At 12 h post incubation, iso-butyric acid ($P < 0.01$) and butyric acid ($P < 0.02$) were greater in digestion tubes containing 0.02% and 0.04% Ca compared to all other Ca doses. Butyric acid was lesser ($P < 0.02$) with olive meal inclusion at 12 h. Total VFA concentrations were similar across treatments. These data suggest that Ca and olive meal may impact in vitro fermentation. Dietary treatment was a significant ($P < 0.05$) source of variation for caproic (C6:0), capric (C10:0), linoleic (C18:2n-6), linolelaidic (C18:2n-6 trans), and docosahexaenoic (C22:6n-3) longissimus muscle fatty acids. Steers receiving the control diet had greater C6:0 ($P < 0.02$), C10:0 ($P < 0.02$), C18:2n-6 trans (0.02), and C22:6n-3 ($P < 0.05$) fatty acids when compared to cattle receiving olive meal. Steers receiving olive meal had greater C18:2n-6 ($P < 0.04$) when compared to controls. All other fatty acids identified were similar across treatment. Based on these data Ca addition at the concentration supplied in this experiment did not inhibit biohydrogenation of unsaturated fatty acids but did improve fermentation characteristics.

INTRODUCTION

Today, the brand name Wagyu includes not only the cattle produced in Japan, but also cattle produced in countries such as United States and Australia. In recent years, the intramuscular fat percentage in beef (longissimus muscle) from Japanese Black cattle has increased to be greater than 30%. The Japanese Black breed is genetically predisposed to producing carcass lipids containing higher concentration of monounsaturated fatty acids (oleic acid; C18:1) than other beef breeds. This is due to a mutation in the delta 9 desaturase gene. However, there are numerous challenges with the management of this breed including high production costs, disposal of excrement, and imported feed requirements.

Furthermore, due to the extensive biohydrogenation of unsaturated fatty acids that occurs in the rumen, it is difficult to alter the fatty acid composition of Wagyu muscle through dietary addition of unsaturated lipids. Several traditional Wagyu feeding operation is Japan include olive meal byproduct in rations. Olive meal is high in oleic acid (C18:1; approximately 70%). The intent of feeding olive meal is to further increase the C18:1 fatty acid in the muscle of Wagyu beef. Inherent physical and chemical properties in olive meal have been suggested to inhibit ruminal biohydrogenation of C18:1 in olive meal. Therefore, allowing more C18:1 Fatty acid to be absorbed from the small intestine and incorporated into muscle.

Based on the information presented in this literature review, the following experiments described in Chapter 2 were designed to: 1) examine the impact of olive meal supplementation on live animal performance and fatty acid composition in Wagyu steers; and 2) determine if calcium supplementation can inhibit rumen biohydrogenation of unsaturated fatty acids in vitro. We hypothesized that supplementation of olive meal to Wagyu steers would improve feedlot performance and longissimus muscle intramuscular fat composition due to the increased energy content from fat and that Ca addition to an in vitro rumen system, would inhibit rumen biohydrogenation of unsaturated fatty acids due to Ca soap formation.

MATERIAL AND METHODS

Prior to the initiation of this experiment all animal care, handling, and procedures described herein were approved by the Colorado State University Animal Care and Use Committee (Institutional Animal Care and Use # 667).

EXPERIMENT 1:

CATTLE

Ninety-six American Wagyu steers (725 ±10.7 kg) were used to evaluate the effects of olive meal supplementation on feedlot performance and carcass characteristics. Steers were housed at the Agricultural, Research, Development, and Education Center (ARDEC) in Fort Collins, CO. Prior to starting the experiment, all steers were vaccinated at their place of purchase. Upon arrival to the ARDEC facility, steers were individually weighed and identified with a unique electronic ear tag.

Steers were then assigned to treatments based on BW. Briefly, steers that were beyond ± 2 SD from the overall mean BW were eliminated from further consideration for enrolment into the experiment. The 83 eligible steers were blocked by BW. The heaviest 8 steers were stratified into two pens containing 3 or 4 steers per pen with similar pen BW. This was considered a paired weight block. This process was repeated until all steers were assigned to pens. Each pen contained 3 or 4 steers/pen with 11 replicates/treatment.

DIET AND ANIMAL CARE

Animals were housed in feedlot pens (7 m x 40 m) equipped with a concrete feed bunk, a 3 m x 7 m concrete bunk pad and automatic waterers that were shared between two pens. Once steers were sorted into their appropriate pens, pens within a paired weight block were randomly assigned to treatments. Treatments consisted of: 1) Control (basal ration with no olive meal) + 1 kg of supplemental cracked corn per animal per day, or 2) Control diet + 1 kg of supplemental olive meal per animal per day. All animals received a basal American Wagyu finishing diet that contained: DM basis: 68.4% DM, 14.3% CP; 74.8% TDN, 1.16 Mcal/kg NEg, 5.3% crude fat; Table 1). Feed was delivered to all pens, once daily, in amounts to allow ad libitum access to feed for a 24 h period. When excess feed accumulated in the feed bunks, feed was removed, weighed, subsampled for DM determination, and then discarded. Diet samples were collected weekly. Weekly diet samples were composited by month and analyzed for nutrient composition.

Steers were monitored daily by trained animal care personnel for health and mobility problems and to ensure the cleanliness of the feed bunks and water troughs. Steers showing health and mobility problems were assessed by a licensed veterinarian from the Colorado State University Veterinary Teaching Hospital and treated per

the recommendation of the attending veterinarian. The animal was returned to its home pen and allowed to recover. If an animal did not recover, the animal was removed, weighed, transported to the veterinary teaching hospital. Feed in the feed bunk was weighed and feed delivery to that pen was adjusted for the next day.

WEIGHTING, SAMPLING, AND CARCASSES DATA COLLECTION

Steers were individually weighed on 2 consecutive days at the beginning and end of the experiment. Interim BW were also obtained every 28 d throughout the experiment. Steers were harvested when they reached a finished live BW of approximately 900 kg. On the day of slaughter, the steers were transported to a commercial abattoir and presented for slaughter using standard U.S. beef industry practices and USDA/Food Safety Inspection Service criteria. At this time individual hot carcass data weight collected. Carcasses were allowed to chill for approximately 14 d then a 25.4 mm ribeye cut was obtained from the upper portion of the ribbing cut. Standard carcass data were collected by Center for Meat Safety and Quality personnel at Colorado State University and included marbling score, quality grade, and yield grade.

After carcasses were chilled for 14 days, a longissimus muscle sample was obtained from the 12^{th} and 13^{th} ribs. Samples were placed in Ziploc bags and frozen at -80°C degrees until analyzed for fatty acid composition as described by AOAC (2006), Bligh et al. (1959) and Folch et al. (1957).

Feed samples were taken using a Hay Core Sampler (AgraTronix) on both round and square bales. The samples were then combined based on geographical location on the farm to be sent out for processing. The water samples were collected on site in a Thermo Scientific Nalgene 250mL bottle. Samples were collected from the river and on-site water troughs in the pasture and processed using the suitability option (Dairy One Forage Laboratory, Ithaca, New York). Their process looks at the water quality and nutrition physiochemical properties for most classes of livestock (Dairy One Forage Laboratory, Ithaca, New York). Feed and water samples were sent to an established laboratory (Dairy One Forage Laboratory, Ithaca, New York) for routine nutrient, mineral, and water quality analysis. The forage samples were processed using the ration balancer wet chemistry analysis which looked at dry matter, crude protein, ADF. and, NFC, RFV, TDN, NEI, NEm, Neg, ME, DE, CA, P, Mg K, Na, Fe, Zn, Cu, Mn, Mo, and S (DairyOne).

EXPERIMENT 2:

ANALYTICAL PROCEDURES:

Rumen fluid from three beef steers (480 ± 10 kg) fitted with rumen canulae, was used to investigate the impact of Ca dose and olive meal on in vitro rumen fermentation characteristics. Steers were fed a high concentrate finishing diet for 21 d and rumen fluid was collected from each steer 2h post-feeding. A 2 x 4 factorial arrangement of treatments was used for this experiment. Factors included: 1) 0 or 5% olive meal and 2) Ca dose: 0, 0.02, 0.04, and 0.08% Ca from $CaCl_2$. A McDougall's buffer-rumen fluid mixture (1:1; 30 mL) was added to conical tubes containing 0.5g of the ground basal diet with the appropriate treatments and incubated at 39°C for 0, 4, 8, and 12h (5 replicates per treatment per time point). Following fermentation, tubes were centrifuged at 2,000 x g for 25 minutes at room temperature. A sub sample of supernatant was collected from all tubes, stored in a 25% metaphosphoric acid solution, and frozen at -20 °C until analyzed for VFA composition via gas chromatography as described by Ropotă et al. (2016). Another sub sample of supernatant were frozen until analysed for fatty acid composition as described by by AOAC (2006), Bligh et al. (1959) and Folch et al. (1957). The remaining digesta was dried to determine DM disappearance (DMD).

STATISTICAL ANALYSIS:

Feedlot performance data, longissimus muscle fatty acid composition, and in vitro rumen fermentation characteristics were analyzed as a randomized block design using PROC MIXED of SAS (SAS Institute Inc., Cary, NC). Treatment was included in the model as a fixed classification effect. Covariates of pen initial BW, number of animals used in the pen average, and days on feed were used in the analysis of all performance, lipid, and carcass response variables. There were two missing live animals pen observations. Outlier tests were performed on all data, and no outliers were removed from the data set. A type three ANOVA table was constructed using the Kenward-Roger method of computing denominator degrees of freedom. Backwards elimination with AIC criteria was used to remove nonsignificant ($P \geq 0.10$) covariates from the model. When a significant treatment effect was detected, treatment means were separated using the PDIFF option of the LSMEANS statement of SAS. (SAS Inst. Inc., Cary, NC). Pen was considered the experimental unit for all live animal data and in vitro tube was considered the experimental unit for all in vitro data. Categorical carcass, data, including USDA quality grade and yield grade, were analyzed using PROC GLIMMIX of SAS using the same model as listed above. A binomial was assumed for the categorical data.

RESULTS & DISCUSSION

Table 1 shows the ingredient and nutrient composition of the basal experimental diet. The diet contained 23% roughage and 77% concentrate. In a study conducted by Oka et al. the diet consisted of primarily of concentrate diet, timothy hay with a small amount of chopped rice straw (Oka et al., 2002). According to a study conducted by Sithyphone et al., the typical cattle feeding program in Japan consists of the early fattening stage, the second stage and the third stage. In the first stage the cattle are around 11 to 15 months of age and being fed a 73-50% roughages like hay, rice straw, alfalfa cube and beer lees and 27-50% concentrate (Sithyphone et al., 2011). In the second stage of cattle from 16-19 months of age are being fed a 40-30% roughage and 60-70% concentrate diet (Sithyphone et al., 2011). Finally in the third stage the 20-28 months animals are being fed a diet of 16% roughages of rice straw and 84% concentrate (Sithyphone et al., 2011). The project's objective was to compare feed cost, palatability, and environmental impacts in three feeding systems. They used 20 Japanese Black male Wagyu calves and divided them into three groups a high concentrate fattening group, a hay fattening group that was fed hay after high concentrate feeding until 10 months of age, and a consistent grass-only feeding group that was only fed grass throughout full lives. They found that reducing the feeding period had lower environmental impacts by 5.7-5.8% (Sithyphone et al., 2011). The feed cost were significantly different among the three groups. The cost were highest high concentrate feeding group compared to the grass-only feeding group and the hay fattening group (Sithyphone et al., 2011). Data in Table 1 indicates that the diet used in the current experiment was between stage 2 and stage 3 and that the steers used in the current experiment were older than the steers used by Sithyphone et al. (2011).

The impact of olive meal supplementation on live animal performance is shown in Table 2. Initial BW were similar across treatments. Steers receiving olive meal supplementation had a lighter ($P < 0.01$) final BW and tended ($P < 0.07$) to have a lower overall ADG when compared to the controls. Average dry matter intake was higher in the control group (9.49 kg/animal/d) compared to the supplemented olive group (8.46 kg/animal/d) with a significant $P < 0.01$. Feed efficiency was also greater in the control group compared to the supplemental olive group. In a study conducted by McGee et al. in 2013, 92 yearling Wagyu bulls were used to test the performance and residual feed intake during a 70 d period. These researchers reported that the mean dry matter intake was 10.35 kg/d,

the minimum was 7.36 kg/d and the maximum was 15.87 kg/d (McGee et al., 2013). These results are similar to the dry matter intakes reported in the current experiment. Average daily gain of the cattle in the McGee et al. experiment showed a mean of 1.39 kg/d, a minimum of 0.79 kg/d and a maximum of 2.30 kg/d. The average daily gains reported in the current experiment were lower overall when compared to those reported by McGee et al. (2013). This could be due to cattle age differences between the two experiments. Cattle in the McGee et al. (2013) experiment started at an average body weight of 415 ± 55 kg while our animals in the current experiment had initial body weights averaging 724 kg. It is known that younger animals can gain weight faster than older animals.

Table 3 shows the effects of olive supplementation on carcass characteristics of American Wagyu cattle. Treatment impacted ($P<0.04$) on dressing percentage. Cattle receiving the olive meal had a greater dressing percentage when compared to the controls. All others carcass characteristics were similar across treatments. In a study conducted by Wertz et al. (2002), twelve Angus and 12 Wagyu-cross heifers were chosen to evaluate the performance of Angus and Wagyu heifers from weaning to slaughter in order to define the relationship of marbling score, 12^{th}-rib fat thickness and feed efficiency using ultrasound. Two angus and two Wagyu heifers approximately the same age, were assigned to 12 pens and individually fed. They were weighed every 21 days and dry matter intake was recorded daily. The Wagyu heifers reportedly consumed less daily than Angus. This supports the data collected as it shows the ADG of the American Wagyu feedlot steers were less than expected from a feedlot animal.

Table 4 shows the effects of olive meal on long chain fatty acid composition of American Wagyu. Dietary treatment was a significant ($P < 0.05$) source of variation for caproic (C6:0), capric (C10:0), linoleic (C18:2n-6), linolelaidic (C18:2n-6 trans), and docosahexaenoic (C22:6n-3) longissimus muscle fatty acids. Steers receiving the control diet had greater C6:0 ($P < 0.02$), C10:0 ($P < 0.02$), C18:2n-6 trans (0.02), and C22:6n-3 ($P < 0.05$) fatty acids when compared to cattle receiving olive meal. Steers receiving olive meal had greater C18:2n-6 ($P < 0.04$) when compared to controls. All other fatty acids identified were similar across treatment. Three studies were conducted by Sturdivant et al. (1992) to determine fatty acid composition differences between the Japanese grades, the fatty acid composition between crossbred Wagyu and lastly to investigate the genetic differences among breeds of cattle. In the first experiment, twenty-three tissue samples were taken from a packing plant in Japan and sent to Texas A&M for analysis. There were no relationships between crude fat percentage and quality grades scores. Myristate (C14:0) tended to be of significance ($P < 0.07$) as it decreased as the quality grade increased. It was also found that grade 5

was significantly higher ($P < 0.05$) compared to grade 3 for the monounsaturated fatty acids and saturated fatty acid ratio.

The second experiment had one-half to 7/8 Wagyu crossbred steers fed out in a feedlot for 174 days then switched to a corn-based diet until harvest. Cattle were determined to be ready for harvest when back fat reached 1-8 cm and the animals were approximately twenty- seven months of age. Subcutaneous adipose tissue, longissimus dorsi muscle, and intermuscular adipose tissue were collected for fatty acid analysis. The results of this second experiment showed there was significantly more myristoleate and palmitoleate in the subcutaneous tissue and significantly less stearate and linoleate compared to muscle. There was no significant difference between the monounsaturated fatty acid and saturated fatty acid ratio.

Experiment three utilized eleven strip steaks from Japanese Black Wagyu Steers from three different regions of Japan. Subcutaneous adipose tissue, musculus longissimus dorsi and intermuscular adipose tissue were taken for analysis of region differences. There was no interaction between regions and tissue site. When comparing the current results to the results reported by Sturdivant et al. (1992), there was no significant difference between myristoleate and palmitoleate but instead there was a difference in caproic, capric, linolenic and linolelaidic acids.

In the study, Oka et al. utilized 293 Japanese Black Wagyu Steers to determine if there were genetic effects on fatty acid composition of carcass fat. Fatty acid composition can be affected by characteristics such as age and time on feed (Waldman et al. 1968; Leat, 1975; Rule et al., 1997). In this study the steers were fed the same diet for 365 d, slaughtered at similar ages, body weight was taken at the beginning and end of the fattening period and withers height was taken into account. The carcasses were chilled and evaluated at the sixth and seventh ribs by official graders according to the Japan Meat Grading Standards (JMGA, 1988). The carcasses were evaluated for meat quality, marbling, color, longissimus muscle area and subcutaneous fat thickness. The samples were taken after 24 hr of chilling from the longissimus muscle, intermuscular adipose tissue and subcutaneous tissue at the six-seventh rib and perinephric adipose tissue with a glass slide and used for fatty acid analysis. Characteristics of the steers varied on withers height, BW, carcass weight, and carcass characteristics and all steers were found to be clinically normal. Results were as follows: Subcutaneous adipose tissue lipid had higher percentages in myristoleic (14:1), palmitoleic (16:1) and oleic (18:1) acids. Monounsaturated fatty acids were higher, the C18:1/C18:0 ratio was lower and while it was lower percentages of 18:0 and saturated fatty acids than lipid from other sites. These

finding were consistent with previous studies reporting that the higher percentages of monounsaturated fatty acids bovine tissue near the body surface than internal tissues (Waldman et al., 1968; Ozutsumi et al., 1983; Sturdivant et al., 1992). The animals with a higher percentage of monounsaturated fatty acids had better flavor. The fatty acid C18:1 and monounsaturated fatty acids positively correlated to a positive flavor experience (Dryden and Marchello, 1970; Mandell et al., 1998) while meat with a elevated percentages of 18:0 and 18:3 were negatively correlated to eating experience (Melton et al., 1982).

Tables 5, 6 and 7 shows the effects of Ca dose and olive meal on in vitro rumen fatty acid composition, dry matter disappearance and rumen fermentation characteristics, respectively. There were no olive meal x Ca interactions for any response variables measured. At 4 and 8 h post incubation digestion tubes containing 0.04% Ca had greater ($P < 0.001$) DMD when compared to all other Ca doses. At 12 h post incubation, DMD was greater ($P < 0.001$) in digestion tubes containing 0.02% and 0.08% Ca compared to all other Ca doses. At 8 h post incubation, molar proportions of acetic acid were greater ($P < 0.03$) in digestion tubes containing olive meal compared to no olive meal and were greater ($P < 0.001$) in digestion tubes containing 0.08% Ca compared to all other Ca doses. At 12 h post incubation, iso-butyric acid ($P < 0.01$) and butyric acid ($P < 0.02$) were greater in digestion tubes containing 0.02% and 0.04% Ca compared to all other Ca doses. Butyric acid was lesser ($P < 0.02$) with olive meal inclusion at 12 h. Total VFA concentrations were similar across treatments. These data suggest that Ca and olive meal may impact in vitro fermentation. Dietary treatment was a significant ($P < 0.05$) source of variation for caproic (C6:0), capric (C10:0), linoleic (C18:2n-6), linolelaidic (C18:2n-6 trans), and docosahexaenoic (C22:6n-3) longissimus muscle fatty acids. Steers receiving the control diet had greater C6:0 ($P < 0.02$), C10:0 ($P < 0.02$), C18:2n-6 trans (0.02), and C22:6n-3 ($P < 0.05$) fatty acids when compared to cattle receiving olive meal. Steers receiving olive meal had greater C18:2n-6 ($P < 0.04$) when compared to controls. All other fatty acids identified were similar across treatment.

Oils such as whole cottonseed, soybean and palm oil are added to cattle diets (Rabiee et al., 2012). There is limited information on Ca soaps of fatty acids (CSFA). A study was conducted by Cappellozza et al. (2020) to understand the effectiveness of oil-based sources co Ca soaps on rumen pH in vitro. There were six treatment groups: oil, soybean oil, palm + soybean oil, palm + cottonseed oil, and palm + cottonseed + soybean oil assigned to four rumen pH (5.5, 6.0, 6.5, and 7.0) in a 6×4 factorial design (Cappellozza et al., 2020). Three rumen cannulated

Angus steers were used and fed a free-choice access to stargrass hay and 2 kg d of 50:50 soybean meal and cottonseed meal supplement along with access to water and mineral supplement (Cappellozza et al., 2020). Rumen samples were taken daily and were analyzed for Ca and total fatty acid concentrations, and fatty acid profiles (Cappellozza et al., 2020). Oleic acid decreased ($P \leq 0.03$) for soybean oil vs palm oil 1 at all pH levels. Oleic acid did not differ ($P \geq 0.07$) in palm + cottonseed + soybean oil verses palm oil 1 at a pH of 6.5 and 7.0 while palm + cottonseed + soybean oil verses palm oil 2 at a pH level of 6.0 and 6.5.

The mineral that is most abundant in the body is calcium absorbed through the duodenum and jejunum (McDowell, 1992). Calcium helps make up 98 percent of bones and teeth. Calcium requirements are influenced by age, weight, and stage of cattle production (NRC, 2000). Calcium can be found in forages grains and oilseeds. Oilseed meal is an excellent source of calcium and has a higher percentage the grains (NRC, 2000). When adding more than 0.3 percent calcium to finishing cattle diets, gain was improved in one of two trials done by Huntington (1983) but it did not affect the calcium in bone calcium, bone ash, and plasma ionizable calcium concentrations.

In a study conducted by Sklan et al., they tested the effects and interactions of fatty acids, cottonseed, and ruminal inert fat in diets for high yielding dairy cows fed low forage rations (1992). 252 cows were used between 4 experiments and the duration lasted from 90 d to 140 d depending on the experiment. These experiments took different sources of fatty acids to see the effects. In one experiment Sklan et al. added 2% of calcium soaps fatty acid (CSFA) to the ration with the treatments being no added fat or 0.43 kg/d of fatty acids or 0.5 kg/d of CSFA. In this experiment the results showed milk yield increased in both groups after 150 DIM and milk fat percentages and yield also increased in the animal fed CSFA before 150 DIM. Milk protein was not increased nor decreased by the diets before 150 DIM (Sklan et al., 1992) indicating that by adding fatty acids to the diet of animals it can increase the performance of that animal.

Overall, under the conditions of experiment 1 there data suggest that olive byproduct supplementation for 177 d to American Wagyu cattle reduced overall live animal performance and the longissimus muscle fatty acid composition was similar across treatment. Data from experiment 2 suggests that Ca addition may improve DM digestibility in the rumen. The inclusion of olive byproduct did not impact DM digestibility but did influence VFA molar proportions in vitro. At the concentration of the calcium used in this experiment we are unable to conclude if

calcium prevents biohydrogenation of unsaturated fatty acids. Further research is warranted investigating if calcium prevents biohydrogenation of unsaturated fatty acids.

Table 1. Ingredient and nutrient composition of the basal experimental diet containing olive meal.

Item[a]	%DM
Ingredient	
Grass hay	13
Corn silage	20
Cracked corn	38.8
Soybean meal	18.2
Olive meal	8.1
Limestone	1.3
White salt	0.5
Calculated nutrient composition	
Dry matter, %[b]	63.9
Concentrate dry matter, %	67
Crude protein, %	15.8
NDF, %[c]	28.8
fNDF, %[d]	12.07
NEm, Mcal/kg	1.74
NEg, Mcal/kg	1.14
Ether extract, %	3.5
Calcium, %	0.7
Phosphorus, %	0.34
Potassium, %	1.14
Magnesium, %	0.24
Sulfur, %	0.22
Analyzed nutrient composition	
Dry matter, %	68.42
Crude protein, %	14.33
Crude Fiber, %	11.14
NEg,Mcal/kg	1.16
TDN, %	74.76
Ether extract, %	5.25

[a]Percentage, unless otherwise stated.

[b]Percentage of as-fed.

[c]Neutral detergent fiber.
[d]NDF from the forage component of the diet.

Table 2. The effects of olive meal on live animal performance of American Wagyu steers.

	Treatment			
Item	Control	Olive meal	SEM	P<
Body weight, kg				
Initial	724.82	725.75	15.17	0.96
Final	804.24	771.25	20.23	0.01
Average daily gain, kg/animal/d	0.6226	0.4372	0.06215	0.07
Dry matter intake kg/d	9.49	8.46	0.53	0.01
Feed efficiency	0.06250	0.05050	0.008	0.30

Table 3. Effects of olive meal supplementation on carcass characteristics of American Wagyu steers

Item	Treatment		SEM	P<
	Control	Olive meal		
N=	16	16	---	---
Hot carcass weight, kg	530.3	524.9	22.4	0.71
Live weight, kg	861.2	832.1	30.4	0.15
Dressing percent	61.6	63.0	0.47	0.04
Adjusted preliminary yield grade	4.3	4.2	0.16	0.65
Adjusted fat thickness, cm	4.4	4.3	0.16	0.71
longissimus muscle area, cm^2	121.9	118.7	4.64	0.61
Kidney pelvic heart fat, %	3.1	3.1	0.23	0.89
Yield grade	4.0	4.6	0.40	0.27
Marbling score[a]	1023.5	1042.0	17.3	0.46
USDA quality grade	PR	PR	-	-

[a] Marbling score; 300 = $Slight^0$, 400 = $Small^0$, 500 = $Modest^0$, etc.

Table 4. The effects of olive meal supplementation on fatty acid composition (weight percentage) of longissimus muscle from American Wagyu steers.

	Treatment			
Long Chain Fatty Acid	Control	Olive meal	SEM	P<
C12:0	0.27	0.13	0.08	0.19
C13:0	0.11	0.03	0.04	0.09
C14:0	6.66	5.2	0.53	0.06
C14:1	4.67	4.58	0.39	0.87
C15:0	1.3	0.64	0.4	0.25
C15:1	0.13	0.05	0.04	0.15
C16:0	28.71	29.79	1.79	0.68
C16:1	15.48	13.41	0.95	0.13
C17:0	1.15	0.79	0.29	0.39
C17:1	3.35	2.05	0.84	0.28
C18:0	5.86	5.99	0.46	0.84
C18:1	26.42	32.07	2.07	0.06
C18:2 n-6, *cis*	1.17	1.94	0.26	0.04
C18:2 n-6 *trans*	2.56	1.17	0.38	0.02
C18:3 n-6	0.08	0.08	0.03	0.78
C18:3 n-6	0.23	0.21	0.03	0.69
C20:0	0.06	0.08	0.01	0.14
C20:1 n-9	0.66	0.84	0.1	0.18
C20:2	0.07	0.07	0.01	0.58
C20:3 n-6	0.03	0.02	0.007	0.28
C21:0	0.03	0.03	0.006	0.46
C20:3 n-3	0.02	0.02	0.003	0.86
C20:4 n-6	0.007	0.02	0.008	0.4
C20:5 n-3	0.12	0.16	0.03	0.25
C22:0	0.09	0.1	0.02	0.73
C22:1 n-9	0.04	0.07	0.02	0.1
C22:2	0.2	0.18	0.04	0.79
C23:0	0.13	0.14	0.03	0.69
C24:0	0.07	0.05	0.02	0.45
C22:6 n-3	0.05	0.008	0.02	0.05
C24:1 n-9	0	0.02	0.006	0.2

Table 5: Effects of Ca dose and olive meal inclusion on in vitro rumen supernatant fatty acid composition (weight percentage).

Ca%	0		0.02		0.04		0.08		SEM	Olive	Ca	Olive x Ca
Olive	+	-	+	-	+	-	+	-				
Fatty Acid												
C14:0	6.51	19.18	8.61	9.45	13.50	9.66	10.12	6.20	3.54	0.57	0.51	0.07
C14:1	0.79	2.98	0.68	2.28	1.55	1.40	1.54	4.24	0.93	0.02	0.47	0.47
C15:0	1.84	3.91	1.98	2.41	3.23	2.63	3.09	1.28	0.98	0.98	0.78	0.22
C15:1	1.04	2.90	4.18	1.50	1.90	2.35	5.14	1.62	1.41	0.33	0.73	0.18
C16:0	13.29	13.28	16.57	17.07	13.03	13.72	13.02	10.20	2.33	0.81	0.15	0.87
C16:1	0.64	1.05	2.08	1.08	0.95	0.49	2.15	—	0.09	0.53	0.35	0.57
C17:0	2.23	1.76	1.60	1.66	1.32	1.56	1.54	0.92	0.34	0.42	0.16	0.53
C17:1	0.44	0.72	0.49	0.11	0.19	0.13	0.61	—	0.35	0.84	0.52	0.43
C18:0	27.48	26.49	31.63	30.20	34.44	36.88	34.80	40.45	3.94	0.62	0.04	0.79
C18:1	9.94	11.58	12.31	11.79	8.29	9.86	9.16	9.75	2.05	0.58	0.46	0.95
C18:2 N6C	11.08	5.85	9.51	0.41	0.87	—	—	11.08	9.46	0.71	0.58	0.97
C18:2N6T	0.27	—	0.63	0.56	0.40	0.33	1.04	—	0.23	0.40	0.16	0.73
C18:3 N6	3.26	1.10	3.01	0.84	3.39	2.56	1.68	0.25	1.78	0.20	0.78	0.98
C18:3 N3	1.70	1.53	1.38	1.67	0.79	4.07	1.60	2.66	1.15	0.18	0.85	0.47
C20:0	0.63	1.45	0.62	1.06	0.85	0.67	1.54	3.24	0.60	0.10	0.02	0.44
C20:1 N9	1.79	2.01	1.12	1.38	1.02	2.34	0.35	2.14	0.50	0.02	0.46	0.31
C20:2	1.31	1.80	1.21	1.40	1.57	1.77	1.22	1.70	0.40	0.22	0.82	0.97
C20:3 N6	1.00	1.88	0.14	1.13	2.88	0.95	0.62	0.80	0.82	1.00	0.40	0.27
C21:0	2.16	2.43	1.51	1.26	1.42	1.06	2.83	2.27	0.59	0.59	0.07	0.90
C20:3 N3	0.12	0.28	0.10	0.23	0.39	0.41	0.45	0.25	0.13	0.77	0.20	0.47
C20:4 N6	0.78	1.00	0.57	0.52	2.46	6.26	2.40	2.51	1.33	0.30	0.03	0.44
C20:5 N3	0.98	0.80	0.36	0.39	0.43	0.45	0.22	0.52	0.21	0.77	0.06	0.71
C22:0	1.86	1.70	1.15	2.15	1.32	2.48	1.66	3.39	0.73	0.08	0.62	0.55
C22:1 N9	0.33	1.13	0.18	0.28	0.43	0.45	0.06	0.61	0.31	0.10	0.41	0.54
C22:2	26.20	7.33	9.77	12.24	10.24	8.99	10.94	17.84	3.93	0.34	0.26	0.01
C23:0	4.72	2.56	2.34	2.32	1.91	0.81	8.75	1.54	2.31	0.12	0.38	0.41
C24:0	0.64	2.12	0.45	1.91	3.10	0.79	1.33	1.36	0.75	0.76	0.82	0.09
C22:6N3	0.22	1.89	0.38	0.50	7.21	1.26	2.40	8.65	1.39	0.58	0.02	0.01

Table 6. The effects of calcium dose on in vitro DM disappearance.

Hour	Calcium Concentration				SEM	*P*<
	0	0.02	0.04	0.08		
4	23.61[a]	24.18[a]	29.31[b]	27.92[b]	1.12	<.0001
8	32.88[a]	36.54[ab]	38.77[b]	39.80[b]	1.84	0.0031
12	43.26[a]	53.44[b]	46.47[c]	54.22[d]	1.22	<.0001

Table 7. The effects of calcium dose on in vitro molar proportions of VFA.

	0	0.02	0.04	0.08	SEM	*P*<
Acetic						
0h	76.50	76.50	76.50	76.50	---	---
4h	72.82	72.09	72.52	72.50	0.34	0.19
8h	69.64	69.51	69.82	70.31	0.15	0.0001
12h	67.05	66.17	65.97	66.51	0.79	0.54
Propionic						
0	15.76	15.76	15.76	15.76	---	---
4	18.90	19.41	18.76	18.86	0.17	0.005
8	21.46	21.57	20.67	20.59	0.16	0.0001
12	22.51	23.47	22.69	22.77	0.51	0.25
Isobutyric						
0	0.69	0.69	0.69	0.69	---	---
4	0.63	0.68	0.71	0.66	0.04	0.24
8	0.64	0.63	0.66	0.66	0.17	0.26
12	0.59	0.57	0.67	0.61	0.02	0.001
Butyric						
0h	7.04	7.04	7.04	7.04	---	---
4h	7.62	7.80	8.03	8.00	0.17	0.17
8h	8.24	8.27	8.87	8.46	0.10	0.001
12h	9.85	9.79	10.66	10.11	0.29	0.05

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