An investigation of the impacts of supplementing cattle finishing rations with winery by-products on feed intake, meat characteristics, fecal microbiology and pre-harvest pathogen reductions.

A Dissertation Presented

by

Paul E. Moote

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Approved as to style and content by the thesis committee:

– Jonathan D. Van Hamme, Supervisor

– John S. Church, Supervisor

– Wendy C. Gardner, Member

– Edward Topp, Member

– Doug M. Viera, External examiner

Thesis Supervisors: Jonathan Van Hamme, Ph.D and John Church, Ph.D

ABSTRACT

The production of novel beef products is crucial to ensuring the sustainability of British Columbia's beef industry as it increases product differentiation in the beef marketplace. This research has evaluated the impacts of supplementing cattle finishing rations with winery by-products (WB), mainly wine lees, at a feedlot in the South Okanagan region of British Columbia. To accomplish this, 69 Angus cross feedlot steers (live weight; 51.3 ± 27.9 kg) were randomly allocated to one of two pens, two per treatment, and fed finishing rations supplemented with either 6-7 % WB (WB; n = 18, 17) or water (control (C); n = 17, 17) for 143 d. Over the course of the study the impacts of WB-supplemented feed rations on the weight and flight speeds of cattle were evaluated, as well as the impacts of this feed on the frequency of fecal samples harbouring antimicrobial resistant *Escherichia coli* (EC) and the diversity of fecal bacterial communities. Cattle fed WB-supplemented feeds were found to require less feed than C fed steers to reach the same slaughter weight, while producing meats with no changes to the colour or composition than the control, save for the colour of ground steak meat, which was found to be darker in meats from WB-fed animals. WB-supplemented feeds did not alter cattle temperament, however cattle became habituated to handling over the feeding period. WB-fed cattle had elevated EC loads compared to C-fed animals, however no changes to the proportions of antimicrobial resistant EC were observed between treatments. Similarly, diet was not found to alter the bacterial communities of cattle feces, however these communities were found to change with time on feed. In conclusion, supplementing cattle feeds with WB can provide economic incentives to producers through reduced feed demands, without altering the loads of fecal pathogens or temperament of cattle.

Key Words: Cattle, Wine, Feed, Gains, Antimicrobial Resistance, *Escherichia coli*

TABLE OF CONTENTS

ACKNOWLEDGMENTS

DEDICATION

To my family—both blood and wed

LIST OF FIGURES

LIST OF TABLES

CHAPTER 1

1. BACKGROUND AND CONCEPTION OF "WINE FINISHED BEEF"

1.1 Project overview

This thesis provides a comprehensive analysis of the research conducted at Thompson Rivers University surrounding the production of a "wine-finished beef" product currently available through Okanagan's Finest Angus Beef in the Southern Okanagan region of British Columbia (BC). To produce "wine-finished beef" cattle are fed diets of winery by-products (WB) for a minimum of 90 d (days), primarily wine lees. Novel beef products, such as this, can positively contribute to the sustainability of the BC beef industry as they improve product differentiation in the beef marketplace. One advantage of novel food products is that their differentiation from traditional products can improve their marketability, potentially increasing consumer demands. Considering "wine-finished beef", the Toronto Sun, MacLean's, Canadian Manufacturing and others published information about this unique product when it was first being sold in BC (Findlay 2010; LeBlanc 2010; Food In Canada Staff 2010). However, this novelty comes with a cost: regulation and certification.

Novel animal feed ingredients require certification by the Canadian Food Inspection Agency (CFIA) before their use, which was not conducted for "wine-finished beef" at the commencement of this project. Without certification, the CFIA prohibited the feeding of this ration to cattle in 2010, until the safety and efficacy of this feed additive could be verified. In reply, Southern Plus Feedlots spoke with the Globe and Mail magazine to publicly voice their frustration over this decision. This worked, as the CFIA removed their restriction on the this feeding program shortly after the Globe and Mail article was released (Bailey 2010). To satisfy the requirements of the CFIA, and to verify the safety and efficacy of this product, Southern Plus Feedlots partnered with Thompson Rivers University to evaluate the impacts of this feed on animal performance, meat characteristics, as well as the changes to fecal *Escherichia coli* (EC) loads in WB-fed animals. This thesis will provide detailed accounts and discussions of the

impacts observed when WB-supplemented feeds were fed to cattle. Specifically regarding its effects on:

- – Feed efficacy and chemistry,
- – Animal gains and feed performance,
- – Cattle temperament,
- – Fecal EC loads in animals,
- – Loads of antimicrobial resistant EC in feces, as well as
- – The diversity of EC and bacteria within fecal samples.

1.2   Project rationale

To improve the sustainability of the beef industry, one strategy that Canadian cattle producers can utilize to attempt to remain economically viable is to incorporate by-products of other industries into cattle feedstuffs, thereby lowering their overall feed costs. Examples of by-products that have been used efficiently in North America include dried distillers grains, potato processing residues and citrus pulp (Stanhope et al. 1980; Leiva et al. 2000; Gibb et al. 2008). This practice can also be used to create novel beef products to differentiate the beef marketplace. As an example, feeding winery by-products to cattle enables retailers to market the resulting beef products as "wine-finished beef". This feeding practice is currently being utilized at Southern Plus Feedlots.

1.3   Study location: Southern Plus Feedlots

Southern Plus Feedlots is located in Oliver, BC and is owned and operated by Bill Freding (Figure 1.1). This feedlot has a total capacity of over 4,000 cattle, although the average capacity is often much lower, and generally consists of Angus bred cattle, harvested under the "Okanagan's Finest Angus Beef" brand of Southern Plus Feedlots, as well as custom fed cattle for other ranchers. The feedlot is located in the South Okanagan region of BC, which represents the North-most extension of the Sonoran desert (French 2009). Located around numerous wineries and restaurants, the owners have begun growing grapes and manufacturing their own wine. The close proximity of the feedlot to additional

wineries and established restaurants is ideal for the production and distribution of "wine-finished beef".
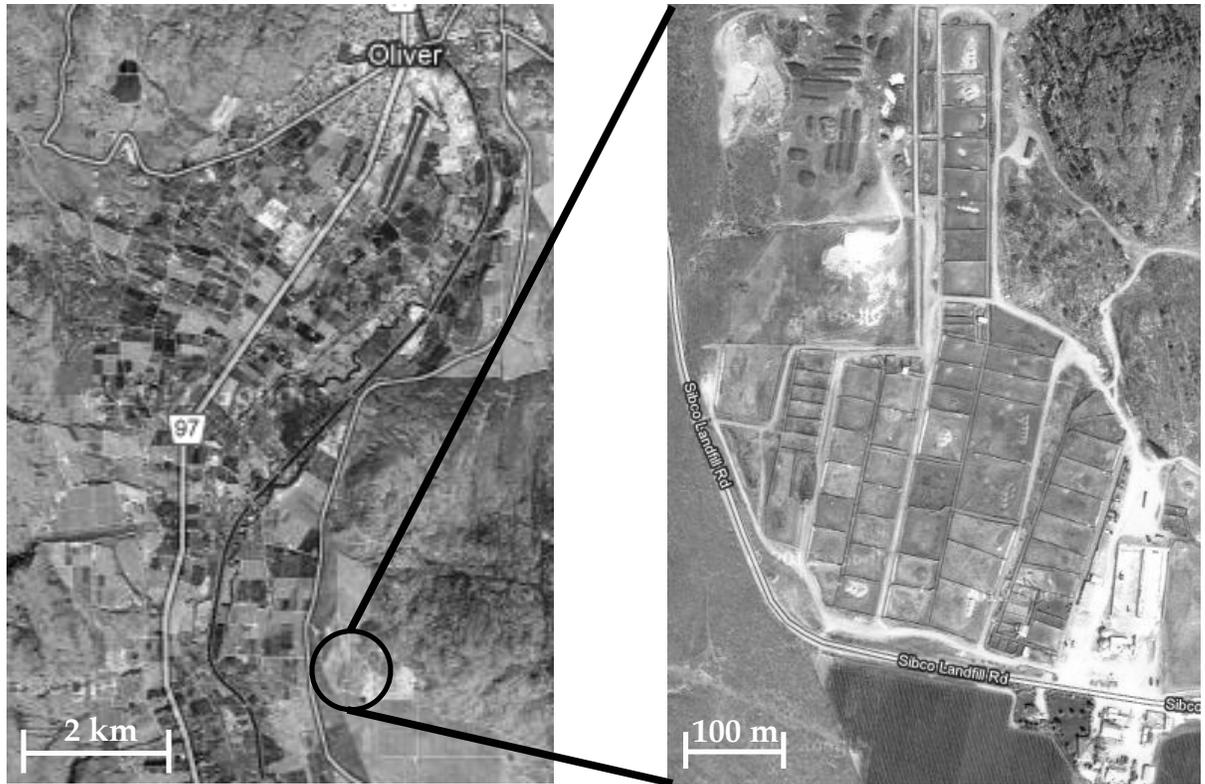
Figure 1.1 From left to right: outlined circle indicates location of Southern Plus Feedlots in relation to Oliver, BC and the image on right indicates layout of holding pens at the facility.

1.4   An introduction to the BC wine industry

The economic impact of the wine industry to BC is valued at two billion dollars, or $42 per bottle (British Columbia Wine Institute 2013a). The major regions in BC for wine production are Vancouver Island, the Fraser Valley, the Similkameen Valley and the Okanagan Valley, with the Okanagan valley being home to the majority of the provinces wineries (121 of 215) (British Columbia Wine Institute 2013b). Similarly, the majority of the province's grapes are grown in the Okanagan Valley with the city of Oliver delivering almost 50% of this (Figure 1.2; British Columbia Wine Institute 2012). With this geographically isolated wine and grape production, many companies are looking to find alternative ways to reduce or dispose of their by-products in an environmentally friendly manner, such as composting.

1.4.1   By-products of wine making and viticulture

In Greece, the by-products from wine making and viticulture have been found to account for 30% of the original grape mass, mainly in the form of pomace (also known as grape marc) (15%), stalks (2.5-7.5%), seeds of grapes (3-6%), as well as the lees produced from fermentation (3.5-8.5%) (Table 1.1) (Nerantzis & Tartaridis 2006). A majority of these wastes are valuable to the pharmaceutical industry as they contain polyphenols and anti-oxidants that can be extracted and sold as supplements. However, when these wastes cannot be sold, a consistent method for their disposal needs to be developed. Ideally, these by-products would be composted on-site and used as fertilizer for grape growing; unfortunately, this requires a large amount of organic matter and space for anaerobic composting, not readily available at most vineyards. As such, wine making wastes such as compressed pomace are commonly provided to feedlots so that they can be mixed with feces as an activator and composted to reduce the dry matter and adjust the nitrogen, phosphorus and potassium contents (Ferrer et al. 2001). However, not all products have the appropriate pH and dry matter required for anaerobic composting, such as wine lees. As an alternative form of disposal, they can be used as animal feeds (Nerantzis & Tartaridis 2006;

4

Arvanitoyannis et al. 2006b). Of note, research with goats has suggested that the condensed tannins and polyphenols in this product are of important research and practical interest for animal producers as they hold nutritive and antimicrobial properties as will be described in section 1.6.
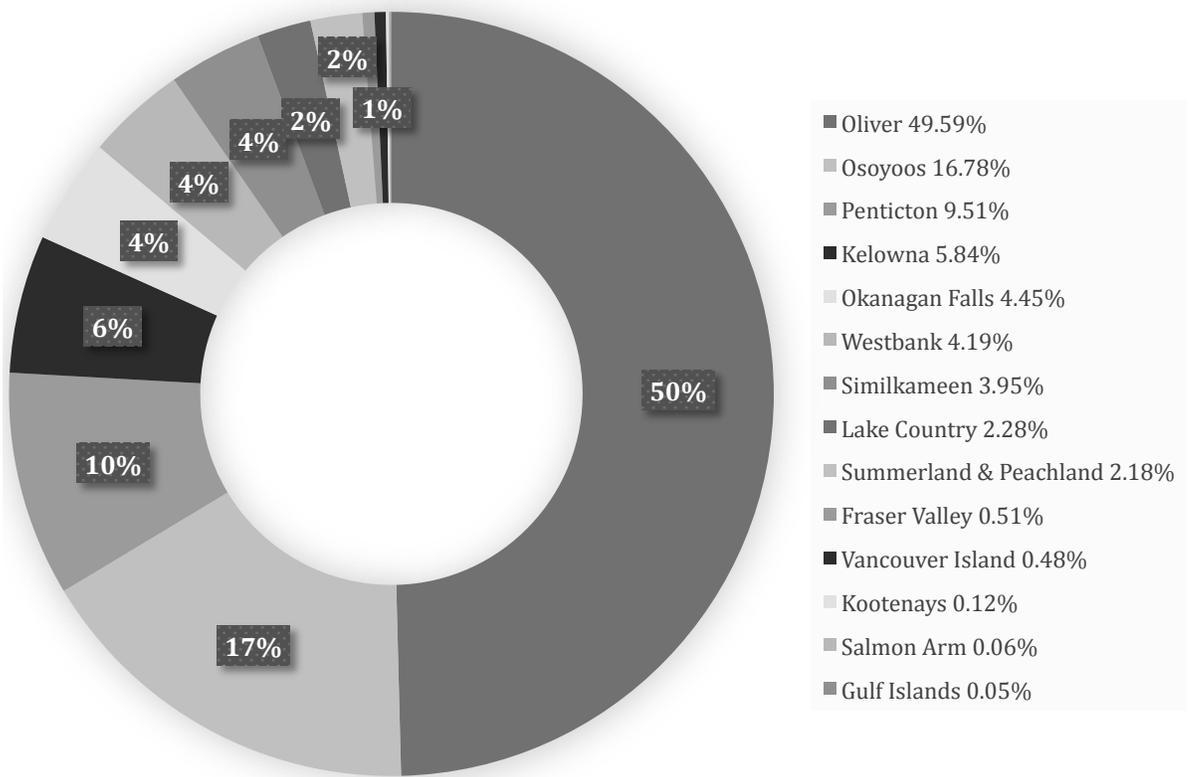
Figure 1.2 Reported tonnage of grapes produced by region; data obtained from the British Columbia Wine Institute, 2012

Table 1.1 Wastes produced from viticulture and wine making, their waste management strategies and previous uses as ruminant feeds.

	Waste				References
	Grape pomace	**Wine lees**	**Grape stalks**	**Grape seeds and stems**	
% of grapes	0.15%	3.5-8.5%	2.5-7.5%	3-6%	(Nerantzis & Tartaridis 2006)
Waste management	Composting	Solid State fermentation	Solid State Fermentation	Solid state fermentation	(Arvanitoyannis et al. 2006a)
Desirable chemical properties	Organic matter content	Polyphenolic content	Phenolic content	Flavenol, phenol, and lingo-cellulosic contents	(Arvanitoyannis et al. 2006b)
Commercial uses	Dietary supplements, animal feed supplements	Dietary supplements, animal feedstuffs	Dietary supplements	Dietary Supplements, production of laccases and phytochemicals, dark poultry meat additives	(Arvanitoyannis et al. 2006b)
Ruminant studies on waste product	Cattle, Goat (Molina-Alcaide et al. 2008), Sheep (Baumgärtel et al. 2007)	Goats (Molina-Alcaide et al. 2008),		*In vitro* (Spanghero et al. 2009)	

1.5   "Wine-finished beef": what is it?

The concept for "wine-finished beef" was originally conceived by Sezmu Meats. Initially, Southern Plus Feedlots was contracted to provide this client with a custom feeding program. The program involved feeding beef cattle 90 d+ feed rations supplemented with liquid fermented by-products of wine manufacturing, typically wine lees. This feeding program was feasible for Southern Plus Feedlots as the feedlot was already contracted to compost steady volumes of wine lees for a large local winery: Vincor Canada. After feeding, cattle were slaughtered in accordance to Sezmu's specifications and sold to local restaurants, butcher shops and individuals. On April 02, 2012, Sezmu Meats was purchased by Southern Plus Feedlots and the sale of "wine-finished beef" continues under the Okanagan's Finest Angus Beef brand.

1.6   Previous research on wine lees and other fermented winery by-products as ruminant feed supplements

For this research, wine lees were the sole experimental component within the winery by-product (WB)-supplemented feed, with the exception of one batch containing improperly fermented wines. All WB used as feedstuffs were fermented, currently the only research studies available on the impacts of feeding diets supplemented with fermented WB are with goats, no such studies exist with cattle (Molina-Alcaide et al. 2008; Wang et al. 2008). As such, this represents a novel feeding strategy. Some rationale for the limited research in this area include the lack of available products for cattle use, especially in North America, as well as the insufficient energy contents of WB, with the exception of grape seeds, limiting their use as a sole feed ingredient (Hadjipanayiotou & Louca 1976; Baumgärtel et al. 2007; Spanghero et al. 2009; Abarghuei et al. 2010). However, WB may hold promise as co-products in animal feedstuffs (Molina-Alcaide et al. 2008).

In goats, the digestion of crude protein in WB was improved when feeds were supplemented with grape shoots compared to grape pomace. It was

suggested that the elevated lignin contents of grape shoots increased the transit time of this feed, aiding digestion. When comparing the chemical profiles of various WB, wine lees were found to have elevated protein contents, however it remained low in comparison to traditional protein sources, such as legume seeds. Although high in protein, wine lees were found to not be suitable as an energy source when compared to other WB, such as grape pomace, which was found to contain similar levels of protein as olive by-products (Molina-Alcaide et al. 2008).

1.6.1   Potential benefits of condensed tannin containing feeds to ruminants

Although wine lees may have limited potential as a sole feedstuff for ruminants it has been suggested that the elevated concentrations of condensed tannins (CT) and polyphenols in WB are of special practical and research interest for animal feed science. Considering the by-products of wine making, red wine lees, vine shoots, and grape pomace, contain 98.3, 45.8 and 37.7 g(CT) kg^{-1} DM, respectively (Molina-Alcaide et al. 2008). As such, feeding WB-supplemented feeds to cattle could impart some of their health benefits onto the animal; particularly, their ability to reduce the occurrence of infections and bloat as well as increasing gains in ruminants (Min et al. 2006; Requena et al. 2010; Novobilský et al. 2011).

CT are polymers of flavenoids that have long been deemed to be anti-nutritive as they lead to weight loss through their ability to bind with dietary proteins including pectin, cellulose and hemicellulose, as well as minerals, forming CT:protein complexes. This has often been referred to as protein bypass, as these complexes remain intact in the rumen (pH 6.0 to 7.0) retarding their digestion by rumen microbes. Once proteins have passed the proteases contained in the rumen, the CT:protein bonds are cleaved in the acid environment of the lower abomassum, pH 2.5-3.5 (Aerts et al. 1999). If managed appropriately, CT containing feeds can lead to benefits in animal gains and milk production resulting from the increased availability and absorption of amino acids by the animal (McSweeney et al. 2001).

When ruminants are fed CT-containing forages, a linear correlation has been observed between the CT content in feeds and the non-ammonia-nitrogen

contents in fluids leaving the rumen (Barry & Manley 1984). When sheep are fed diets containing 2-4 % CT from *Lotus corniculatus*, improvements to animal gains and milk production are likely the result of this correlation (Waghorn et al. 1987). Additionally, the reduced exposure of proteins to proteases in the rumen can improve the quality of proteins available for animals (Aerts et al. 1999). For management purposes, the amount of CT available to animals needs to be closely monitored as over exposure to CT rich feeds can cause the rumen microbes to become starved, leading to weight loss (McSweeney et al. 2001). For research purposes, a method to evaluate the impacts of CT on animal performance is through the use of polyethylene glycol (PEG), which can be added to feeds as a control. This is useful as PEG forms PEG:CT complexes that inactivate CT throughout the digestive process (Ben Salem et al. 2000). Using this approach, researchers have been able to evaluate the impacts of CT containing forages on weight gain, meat quality, rumen and fecal microbes, methane production, bloat, as well as their ability to deter the infective larvae of nematodes from ruminants (Priolo et al. 2000; McMahon et al. 2000; Min & Hart 2003; Min et al. 2006; Chung et al. 2011; Novobilský et al. 2011). CT represent only a small sample of the polyphenols found in nature; of them, the polyphenols contained in wines have been shown to reduce the virulence of human pathogens.

1.6.2   Potential benefits of polyphenolic containing feeds to ruminants

Polyphenols are often categorized as nutraceuticals, which are compounds that can be isolated from foods, and are often sold as medicinal products (Agriculture and Agri-Food Canada 2009). In the case of wines, a nutraceutical with great commercial benefit is the phenolic *trans*-Resveratrol. This compound, along with other extractable polyphenols, has been shown to negatively impact the growth of zoonotic pathogens, such as *Campylobacter jujuni* (Gañan et al. 2009). What makes the activity of these phenolic compounds appropriate for feed supplements is that they appear to be strain specific, particularly with EC, where EC O157:H7 is more sensitive to polyphenols than non-pathogenic strains of EC (Cueva et al. 2010). Conversely, exposing *Lactobacillus* spp. to extractable polyphenols from grape pomace can increase their biomass *in vitro,* suggesting

that these polyphenols could play a role in regulating intestinal probiotics (Hervert-Hernández et al. 2009). To accompany this research, more information on the impacts of these compounds on the intestinal microflora of humans and animals is required.

Supplementing feeds with polyphenol rich grape by-products has been shown to alter the intestinal microflora of chickens. In a study by Viveros et al. (2011), broiler chickens fed grape pomace concentrate and grape seed extracts were found to increase loads of beneficial *Enterococcus* spp. while decreasing loads of *Clostridium* spp. in their feces. In addition, supplementing chicken feeds with grape by-products was found to change the biodiversity of intestinal bacteria. The feces of chicken fed grape by-products was also compared to those fed antibiotics as prophylactics to determine if these by-products could reduce the need for prophylactic antibiotics in chicken feeds. Chickens fed feeds supplemented with the grape pomace concentrate were found to have longer villi than those fed antibiotics or grape seed extracts, indicating improved gut health. As such, feeding chickens with grape by-products can be advantageous for producers (Viveros et al. 2011). Studies such as this provide insight into the possibility for diet to reduce the spread of fecal pathogens pre-harvest, by promoting a healthy gut and intestinal microflora while reducing the shedding of pathogenic bacteria.

1.7   Pre-harvest pathogen reduction

As previously described, tannins are contained in some forages, and as these forages mature the concentrations of carbohydrate lignin can increase, along with the associated carboxylic phenols including, *p*-coumaric acid and vanillin (Jung & Vogel 1992). With appropriate management, feedlot and grazing animals can be exposed to a variety of polyphenols that can reduce the spread of fecal pathogens pre-harvest. In the sole research article evaluating the impacts of WB on ruminants the authors stressed the importance of the condensed tannin and polyphenol contents of these feeds, perhaps due to their ability to reduce fecal pathogens in ruminants pre-harvest (Molina-Alcaide et al. 2008; Wang et al. 2009).

Controlling the spread of fecal pathogens pre-harvest is an important research focus as it can ensure food safety throughout all stages of meat production. Current research is aimed at evaluating the efficacy of vaccines, bacteriophages, direct fed microbials (probioitcs), and diet on the loads of various pathogens (Callaway et al. 2003; LeJeune & Wetzel 2007; Allen et al. 2011). A goal for pre-harvest treatments is to reduce the spread of zoonotic pathogens from agriculture, not only in meat products, but also in local ground water systems and in the resulting manure. Before identifying solutions, it is wise to first identify problematic feeds and management practices that can elevate fecal pathogen loads.

One feedstuff that can lead to elevated fecal pathogen loads, particularly the EC O157:H7 serotype, is wet distillers grains with solubles (40% on a dry matter basis). This finding is unfortunate as these feeds are economical for producers; as such, finding pre-harvest interventions to reduce the spread of pathogens is important (Wells et al. 2011). One pre-harvest intervention that can reduce the spread of fecal pathogens from cattle is to simply switch their diet from grain to hay based rations for one week before slaughter (Callaway et al. 2003). Identifying cattle feeds that can act to reduce the loads of fecal pathogens over the entire feeding period would be ideal, and CT and polyphenols (such as those previously found in wine lees) may hold promise in this area (Molina-Alcaide et al. 2008).

Effective supplements for pre-harvest interventions are those found in digestible forages with antimicrobial activity towards zoonotic pathogens. Examples of such extracts include phlotorotannins from seaweed as well as plant tannins; however, the antimicrobial activity of these compounds varies depending on their source (Min et al. 2007; Wang et al. 2009). Of note, providing intra-ruminal infusion of chestnut tannins has been shown to reduce the shedding of generic EC in cattle feces. Additionally, *in vitro* studies have found that incorporating these tannins into cattle diets can reduce the proliferation of EC O157:H7 in the rumen (Min et al. 2007). To reduce labour costs for producers, integrating these phenols into animal diets is beneficial. Supplementing cattle diets with the phlorotannins extracted from the seaweed *Ascophyllum nodosum* (commercially available as Tasco-14™) at 2 %dry matter for a two-week period

has been shown to reduce the loads of EC O157:H7 on cattle hides (36 %) as well as in fecal samples (11 %) before slaughter (Braden et al. 2004). This dried phlorotannin extract has also been shown to reduce the shedding of EC O157:H7 in infected animals when provided in diets at 1 % DM over a two-week period, or 2 % DM over a one-week period (Bach et al. 2008). A further aspect of pre-harvest food safety includes reducing the spread of antimicrobial resistant EC in agriculture stemming from the overuse of antibiotics as prophylactics (Alexander et al. 2009). Perhaps the CT and polyphenols in WB can improve the feed efficiency, performance and health of cattle reducing the need to use antibiotics as prophylactics in agriculture.

1.8   Antimicrobial resistance in animal agriculture

Consumers are starting to demand that meat products be produced without the use of antibiotics as growth promoters (Lusk et al. 2006). A large research project by Alexander et al. (2008) has evaluated the impacts of prophylactic antibiotics on the prevalence of antibiotic resistant pathogens, and antibiotic resistance genes, in the feces of beef cattle. These researchers found that the administration of prophylactic antibiotics leads to elevated resistance to tetracycline and ampicillin. Additionally, when animals are fed prophylactics, antibiotic resistance can increase within non-type specific and O157 serotype EC (Rao et al. 2010). Further, the storage of manure from cattle fed antibiotics as prophylactics can act as reservoirs for these antimicrobial resistance genes (Whitehead & Cotta 2013). As such, finding ways to reduce the need for antimicrobials as prophylactics is a priority for animal agriculture. Research aimed at identifying diets that can improve animal performance is required to convince producers to reduce the use prophylactics in agriculture.

CT, as mentioned previously, can improve gains in animals as they can bind to proteins, reducing wasted metabolism by rumen microbes and increasing the amino acids available for absorption by the animal. In chicks, the gains associated with diets supplemented with proanthocyanidin extracts from grape seeds were compared to those fed salinomyacin or maduramicin antibiotics as prophylactics. Here, it was found that the performance of chicks fed this extract

13

compared with those fed antibiotic prophylactics. Additionally, when chicks were infected with the avian parasite *Eimeria tella* those fed the extract had improved gains and recovery compared to birds fed salinomyacin , but not those given maduramicin. The improved resilience of these chicks to infection may have resulted from the extracts restoring the balance between oxidants and antioxidants post-infection (Wang et al. 2008). The authors did note that the anti-inflammation effects of grape extracts may come with a risk, as increasing the dosage of grape seed extracts to infected birds increased their mortality rates. Although similar research has not been conducted in ruminants, this research provides a basis of how grape, and potentially wine extracts, can play a role in reducing the dependency of prophylactic antibiotics in animal agriculture. From here it is important to ensure that the meat products generated from these novel production strategies are similar to conventional meats to ensure consumer satisfaction.

1.9   Resulting meat products

Concerning the CT content of WB, a wealth of information is available on the impacts of CT to the meats of ruminants, particularly with sheep. Meat products from sheep fed diets supplemented with the pulp from the CT rich fruits of *Ceratonia siliqua* (carob pulp) were shown to be more tender, with increased pH, than sheep fed a maize diet; however, carcass yields decreased when sheep were fed the carob pulp diet (Priolo et al. 2005). Further, the meat from sheep fed a concentrate diet supplemented with quebracho CT showed improve colour stability, likely as a result of haem pigment and metmyoblobin formation during the 14-d refrigeration (Luciano et al. 2009). In cattle, a great deal of research has been conducted on the impacts of supplementing feeds with phlorotannin extracts from the brown seaweed *A. nodosum*, commercially available as the CT supplement Tasco-14™.

When fed to cattle, Tasco-14™ has been shown to increase the marbling scores of carcasses, as well as increasing the occurrence of choice graded carcasses by 39.6% (Anderson et al. 2006). Further, meats from cattle fed this supplement are more tender, with less off-flavor, than those fed a control diet.

Additionally, these meats contained greater concentrations of extractable fats, with less protein than meats from control fed animals. Of note, supplementing cattle feeds with seaweed extracts can improve the retail shelf-life of strip loin samples by reducing discolouration scores compared to the meats from animals not fed this extract (Braden et al. 2007). Although WB contain CT and polyphenols, it is important to understand the impacts of feeds from animals fed grape by-products or extracts, as a combination of chemical components may be acting to cause the observed changes in meats from animals fed supplemented diets.

When comparing the antioxidant potential and shelf life of lamb meats from diets supplemented with grape seed extract or essential oils it was found adding essential oils into lamb diets can increase the antioxidant potential of meats, determined through a ferrous/hydrogen peroxide system, however, the shelf life of meats from animals fed the grape seed extracts was superior to those fed the essential oils (Jerónimo et al. 2012). Additionally, meats from lambs fed the grape seed extract-supplemented diet did not differ from the control fed sheep in terms of colour, volatile compounds or fatty acid composition (Vasta et al. 2010a; Jerónimo et al. 2010; Jerónimo et al. 2012). In lambs, similar research has been conducted on diets supplemented with extracts from red wine.

Rivas-Cañedo et al. (2013) evaluated the antioxidant potential and shelf life of omega-3 enriched lambs meats from sheep fed a red wine extract-supplemented diet and compared these meats to those from animal fed vitamin E-supplemented feeds as well as a control feed (no supplement). Of note, the lipids of meat from animals fed the red wine-supplemented diet were more protected during storage than the control; however, greater protection was observed from the animals fed the vitamin E-supplemented diet. The WB samples fed to cattle at Southern Plus Feedlots are mainly wine lees, and although they contain a large amount of wine and related polyphenols, they are not extracts. As such, it is unlikely that WB could improve the shelf life of meats, however they may alter the behaviour of cattle leading to changes in meat colour.

1.10   Effects of diet on cattle behaviour

Before the commencement of this research, un-validated claims were made by the marketers of the "wine-finished beef" product, suggesting that feeding wine lees to beef cattle altered their behaviour (Findlay 2010). To evaluate these claims a field study was designed to objectively measure the impacts of feeding WB on beef cattle behaviour, via flight speed (FS). FS is a quantitative behavioural assessment tool that measures animal temperament (Petherick et al. 2009; Schwartzkopf-Genswein et al. 2012; Stockman et al. 2012). Understanding ways to reduce anxiety in cattle is important both for animal welfare and financial reasons, as calm animals have been found to gain weight at faster rates, yielding more tender meats than more flighty animals, however the mechanisms behind this are not clear (Voisinet et al. 1997a; King et al. 2006; Ferguson & Warner 2008). A potential exists for WB-supplemented diets to alter the temperament of cattle by altering the energy, carbohydrate and chemical components of feeds.

Of the dietary supplements evaluated in cattle, supplementing feeds with the amino acid tryptophan has been shown to increase lying and eating behaviours in dairy cattle (Nakanishi et al. 1998). By increasing the serum concentration of tryptophan a potential exists for stress responses in cattle to decrease when tryptophan is converted to the neurotransmitter serotonin in the brain (Gregorini et al. 2006). Further, feeds containing high levels of carbohydrates can stimulate insulin secretion leading to increased levels of tryptophan in the blood, and in turn, elevating serotonin levels in the brain (Nelson 1995). In addition to tryptophan, diets supplemented with electrolytes or glycerol also have potential to reduce stress in cattle.

One supplement commercially available to reduce stress in cattle during transportation is the electrolyte Nutricharge®, which has been shown to increase carcass yields and reduce the occurrence of dark cutting carcasses caused during transport (Schaefer et al. 1997; Parker et al. 2007). Individual electrolytes, including chromium and magnesium, have been evaluated for their ability to reduce stress and improve immune responses of animals. Of note, supplementing chromium into diets deficient in this element, such as corn silage,

can reduce the concentration of serum cortisol while improving the immune system of cattle by increasing the concentration of serum immunoglobulins (Chang & Mowat 1992). On the other hand, magnesium supplementation acts to relax skeletal muscles by antagonizing calcium, which in turn reduces the secretion of neurotransmitters from membrane currents required for neuromuscular stimulation, i.e. muscle contractions (Hubbard 1973; Hagiwara et al. 1974). In pigs, magnesium supplementation has been shown to reduce stress, as indicated by plasma cortisol concentrations, however similar results have not been found with cattle fed magnesium-supplemented feeds (Niemack et al. 1979; Kietzmann & Jablonski 1985; Bass et al. 2010). If supplementing WB into cattle feeds yields chemical changes, a potential exists for the temperament of cattle to be altered over the experiment, potentially leading to increased gains and more tender meat products in animals as observed by Voisinet et al. (1997a, 1997b).

1.11   Summary of research objectives

This thesis contains the results of the research on "wine-finished beef" and a detailed discussion on the impacts of this supplementation on cattle performance, temperament and meat quality as well as the loads of EC and bacteria in the feces of cattle fed these diets. The goal of this work is to improve the scientific understanding of the role that WB can play on animal performance and welfare, as well as the environmental impacts of the associated manure when supplemented into cattle feeds. The goal of this information is to provide Southern Plus Feedlots, as well as other beef producers and marketers, with an understanding of the potential that exists for by-product synergy between the beef and wineries of BC.

CHAPTER 2

2. EFFECT OF FERMENTED WINERY BY-PRODUCT SUPPLEMENTED RATIONS ON BEEF CATTLE TEMPERAMENT, FEED INTAKE, GROWTH PERFORMANCE AND MEAT QUALITY

2.1 Introduction

Feeding animals with novel diets, particularly ones that provide potential benefits to both the animal and consumer, is one way to create product differentiation in the meat marketplace. Additionally, consumer trends surrounding beef purchases are influenced by factors including animal production practices (i.e. feeding management), as well as welfare information provided with the product (Napolitano et al. 2010). Co-marketing the production of novel beef products with sustainability and animal welfare attributes could help to facilitate positive consumer responses to a meat product. One such beef product has recently emerged, known as "wine-finished beef" by producers, and is currently marketed in the wine country of the Okanagan Valley of British Columbia, Canada, by feeding feedlot cattle finishing rations containing winery by-products.

Due to escalating feed grain costs experienced in the feedlot industry there exists a need to reduce dependency on grain-based rations (Molina-Alcaide et al. 2008; Hersom et al. 2010). At the same time, costs associated with the disposal of by-products of the wine-making industry are also increasing as current environmental initiatives require more stringent technologies for disposal, such as solid state fermentation or composting (Arvanitoyannis et al. 2006a). Therefore, the use of winery by-products (WB) as an alternative feed and energy source in feedlot diets could provide an economic benefit to both industries (Arvanitoyannis et al. 2006b). The major by-products from wine production include grape stalks, pomace, seeds and stems, as well as wine lees; in addition, these wastes can represent 2.5 to 7.5 %, 15 %, 3 to 6 % and 3.5 to 8.5 % of the original grape mass, respectively (Nerantzis & Tartaridis 2006). Many of these

wastes have pharmacological value and can be sold to the pharmaceutical industry as a source of phenol, flavenol and lignocellulosic compounds (Arvanitoyannis et al. 2006a, 2006b). Winery by-products have been used as dietary supplements, feed stuffs, as well as for the production of laccases and phytochemicals (Arvanitoyannis et al. 2006b). Of these products, grape pomace has been used most widely in animal agriculture, including studies with dairy and feedlot cattle, sheep, and goats (Hadjipanayiotou & Louca 1976; Nielsen & Hansen 2004; Baumgärtel et al. 2007; Molina-Alcaide et al. 2008; Alipour & Rouzbehan 2010).

Although grape pomace has been widely used, grape seed extracts have been shown to present the best source of energy for high producing ruminants (Baumgärtel et al. 2007). However, the value of seeds to the pharmacological industry is reducing the availability of this product in pomace and as an extract (Nerantzis & Tartaridis 2006). In the absence of seeds, WB such as grape pomace, have been shown to be limited in energy and not sufficient to support animal growth or milk production as a sole animal feed (Hadjipanayiotou & Louca 1976; Baumgärtel et al. 2007; Spanghero et al. 2009; Abarghuei et al. 2010). Grape pomace has been successfully used as a coproduct with high energy forages in animal feeds, and has been shown to reduce methane emissions from dairy cattle (Tsiplakou & Zervas 2008; Molina-Alcaide et al. 2008; Hersom et al. 2010). Fermented WB, such as wine lees, are wastes produced during the decanting or raking process of wine and have been shown to be a source of protein and tannins suitable as feed supplements for ruminants (Molina-Alcaide et al. 2008). As the feeding of winery by-products, such as wine lees, to animals is a relatively new practice, few studies have assessed its potential as a supplemental feed for beef cattle.

During the marketing of "wine-finished" beef in Canada, un-validated claims that feeding wine lees to beef cattle altered their behaviour were widely reported in the media (Findlay 2010). To evaluate the potential impact of this novel feed ingredient on the behaviour of beef cattle, at the request of the producers and marketers of this new niche beef product, this investigation was initiated. A field study was designed to attempt to validate the marketing claims made by the proponents of the product by measuring the impacts of feeding

winery by-products on beef cattle flight speed (FS), a quantitative behavioural assessment tool which has been widely used as a measure of beef cattle temperament and has been correlated with weight gain in cattle (Petherick et al. 2009; Schwartzkopf-Genswein et al. 2012; Stockman et al. 2012). Temperament in cattle has been defined and measured in numerous ways, the most common of which involves an animal's response to handling (Schwartzkopf-Genswein et al. 2012). Past research has indicated that animals that differed in their FS also exhibited differences in their personality traits, which has often resulted in differences in their average daily gain (Muller & von Keyserlingk 2006). It is possible that substituting WB for water in the cattle feeds could alter animal behaviour through changes made to the energy and carbohydrate contents of the diet, as the residual levels of alcohol present in the cattle rations seems unlikely to be sufficient to alter cattle behaviour.

Diet has been shown to alter cattle behaviour in previous studies; for instance, Gregorini et al. (2006) found that shifts to the carbohydrate concentration of forage corresponded to changes in biting behaviour, which was attributed to tryptophan. The addition of tryptophan to cattle feed has also been shown to increase lying and eating behaviours in dairy cattle (Nakanishi et al. 1998). Tryptophan circulates in the blood at low levels and is later converted to serotonin in the brain (Gregorini et al. 2006). Diet can affect this conversion process because carbohydrates stimulate pancreatic ß-cells to secrete insulin, which affects the uptake of sugars and non-tryptophan amino acids into peripheral cells (Nelson 1995). This in turn results in a relatively high ratio of tryptophan to other amino acids in the blood, which then outcompetes other amino acids for access to the central nervous system, by selectively crossing the blood brain barrier and producing higher levels of serotonin. Beef cattle diets supplemented with electrolytes or glycerol have also been shown to reduce stress and agitation during transport, likely through this mechanism (Schaefer et al. 1997; Parker et al. 2007). If the inclusion of WB into cattle feeds results in substantial differences in dietary constituents, it may affect cattle behaviour as indicated by FS, and lead to increased gains in the animals as well as yielding more tender meat products (Voisinet et al. 1997a, 1997b).

It is well established that the addition of tryptophan to pig rations results in calmer behaviour as well as lower plasma cortisol concentrations (Peeters et al. 2004; Li et al. 2006; Koopmans et al. 2006; Guzik et al. 2006). Further, meat quality in tryptophan fed pigs was improved compared to control fed pigs with respect to meat colours (lightness (L*)) and 45-minute pH (Guzik et al. 2006). Marketers of "wine-finished" beef are also claiming that the practice of feeding WB has altered the colour of the final beef product. If supplementing finishing rations with WB can alter cattle behaviour as indicated by FS, it is reasonable to suggest that it might also cause a colour change in the final beef product as well.

The primary objective of the study was to establish if substantial nutritional differences in rations supplemented with winery by-products exist; and to determine the effects of feeding custom feedlot finishing rations supplemented with winery by-products on feed intake, performance, and meat quality in beef cattle.

The secondary objective of this study was to determine if feeding winery by-products has an impact on FS, which is an indicator of temperament, which has been associated with stress in beef cattle.

2.2   Materials and methods

2.2.1   Experimental design

2.2.1.1   Animals

Before commencing this project, animal use research protocols were reviewed and approved by the Thompson Rivers University animal care committee. The study followed the Canadian Council of Animal Care guidelines for Farm Animals (Canadian Council on Animal Care 2009) and the Canadian Beef Cattle Code of Practice guidelines (Agriculture Canada 1991). Upon commencing the research project, a total of 69 Angus-cross steers (351.3 ± 27.9 kg) were purchased from the Stirrup Ranch near Kirsley, BC and transported 678 km to a small custom feedlot near Oliver, BC (Southern Plus Feedlots). Once at the feedlot, cattle were acclimated to finishing rations by stepping up the grain ration content from 0 to 50 % in the diets over a 30 d period. Once acclimated,

animals were randomly separated into four freshly cleaned pens, two pens per treatment (n = 18, 17, 17, and 17).

2.2.1.2 Housing

Treatment pens were each 1600 m^2 (40 m × 40 m) and pen walls were made of 2-m tall wood panels along three sides. The remaining side contained a feed bunk with metal railings above a concrete bunk to prevent cattle from exiting the pen through this area. For this experiment, pens shared water bowls with an adjacent pen (one bowl per two pens) that contained animals fed the same diets. Pens were cleaned on a monthly basis, or as needed, using front-end loaders, and bedding of either gypsum or wood chips were added after cleaning.

2.2.1.3 Dietary treatments

Animals were fed finishing diets containing either 6-7 % winery by-products (WB; 18, 17) or 6-7 % water (C; 17, 17) for a 143 d period (Table 2.1). Wine lees were the sole source of the winery by-products for this experiment, except for one batch, which contained improperly fermented wine. For this reason we refer to the wine supplement as WB, as opposed to wine lees. The oats and barley used in the feeds were at least 489 and 618 kg m^{-3} respectively and rolled on site. Further, malt contents were fermented cereal grains provided from local breweries and the liquid supplement contained minerals and protein designated for beef cattle. Variation in the feed contents occurred as a result of a dietary change from corn silage to chopped hay when silage supplies diminished (d105) two thirds of the way through the feeding trial.

Animals were limit fed freshly prepared feeds twice daily, at 0800 and 1500, using industrial feeders equipped with scales (± 4.55 kg) that mixed the ingredients via five large rotors. Weighing back the feed daily to determine feed intake was logistically impractical in this custom feedlot setting. During the experiment the feedlot manager monitored the daily feed weight delivered to each pen through slick bunk demand management, which is routinely utilized at the feedlot.

Table 2.1 Wet matter composition of experimental diets used during the 143-d feeding program; control diets and experimental diets differed from one another only by their contents of water and winery by-products.

Item	% Diet (d1-d105)		% Diet (d106-d148)	
	WB	C	WB	C
Corn Silage	30	30	0	0
Chopped Hay	0	0	12	12
Fermented cereal grains (Malt)	10	10	31	31
Oats	20	20	20	20
Barley	30	30	28	28
Protein and mineral feed supplement	3	3	3	3
Winery by-products	7	0	6	0
Water	0	7	0	6
Percent Dry Matter (%) $\pm$ SD	64.3 $\pm$ 2.3	58.5 $\pm$ 8.1	63.3 $\pm$ 0.8	84.6 $\pm$ 32.4
Energy content (Kcal gm^{-1} DM $\pm$ SD)	4.9 $\pm$ 0.4	4.19 $\pm$ 0.7	4.21 $\pm$ 0.2	4.7 $\pm$ 0.5

2.2.2　Data collection

2.2.2.1　Feed analysis and intake

WB and C rations were compared using NIRS (Near Infrared Spectrophotometry) and bomb calorimetry to determine chemical and energy contents, respectively. To evaluate changes between batches of WB, three samples of total mixed rations were collected at each of the five sampling events (d1, 36, 63, 98 and 143) during the experiment for NIRS; and a total of nine samples of each diet, representing three sampling events, were used for bomb calorimetry analyses. Ethanol contents in WB products were evaluated by capillary electrophoresis, and although the total alcohol concentration in mixed feeds was not measured, their contributing energy levels should have been observed through bomb calorimetry as seen with studies on grape pomace (Baumgärtel et al. 2007). In order to evaluate the different feeds, samples were processed within 8 h of collection to determine dry matter (DM) composition by placing the samples in a drying oven at 60ºC for 48 h. Samples were then ground using a sample mill (FOSS CyclotecTM 1093, Foss, Hillerød, Denmark) and the feeds were analyzed via NIRS using the Total Mixed Rations parameters of the Ruminant Feed Package following the manufactures instructions (FOSS InfraXact^{TM}, FOSS Hillerød, Denmark); information supporting this NIRS calibration is listed in Table 2.2 (FOSS 2005, n.d.). The dietary components selected for evaluation were determined from CFIA guidelines for feed requirements (Government of Canada 2012). After NIRS analysis, samples were subjected to oxygen bomb calorimetry to determine the kcal g^{-1} DM, using a calibrated Parr oxygen bomb calorimeter (Parr model 1108 combustible bomb and calorimeter) as outlined in (Galyean & May 1989).

Feed intake (FI) was measured by determining the mass delivered to feed bunks divided by the number of animals in the pen, the frequency of feed refusals (orts) were not measured during this experiment. Although orts were not gathered to measure feed intake, feed bunks were monitored on a daily basis to govern the feed required and to ensure adequate feeding levels to maximize

gains in cattle. This was accomplished using slick (clean) bunk management, which is a form of regulated feed delivery commonly used in feedlots where feed intakes are regulated, but not necessarily reduced, to ensure animal gains (Alberta Agriculture and Rural Development 2012). Slick bunk management is an effective way to meet the nutritional needs of cattle, while limiting refusals and feed spoilage, and has been found to not alter the ADG of cattle when compared with *ad libitum* feeding strategies (Mader & Davis 2004). Feed was not removed, which in this case helped ensure intake measurement accuracy as this management strategy ensured that all feed offered to cattle was eaten. Cattle were fed the diets until harvest.

Table 2.2 Number of samples (#), mean values, standard error of the calibration (SEC), R^2 (RSQ), and the standard error in cross validation (SECV) used to prepared the calibration of the NIR hardware and software used for determining the % ash, fat, protein, acid detergent fibre (ADF), neutral detergent fibre (NDF), and starch of cattle feeds containing 6-7 % winery by-products or 6-7 % water as a control.

Variable	#	Mean	SEC	RSQ	SECV
Ash	22	3.53	0.11	0.99	0.16
Fat	23	4.55	0.09	0.95	0.2
Protein	291	12.77	0.39	0.99	0.47
ADF	288	20.69	1.12	0.99	1.28
NDF	172	36.29	2.35	0.98	2.76
Starch	22	57.63	0.88	0.99	1.14

Table 2.3 Near infrared analysis of ash, fat, protein, acid detergent fibre (ADF), neutral detergent fibre (NDF), and starch, as well as the amounts of dry matter and calories within the finishing rations of cattle feeds containing 6-7 % winery by-products (WB) or 6-7 % water (C).

Diet	Measurement	Units	n	Mean	STDEV	t	P
WB	Ash	%	15	4.10	0.33	-1.1900	0.2449
C			13	4.69	0.36		
WB	Fat	%	15	1.03	0.33	-1.3440	0.1905
C			13	1.65	1.75		
WB	Protein	%	15	14.07	1.00	-1.1960	0.2424
C			13	15.55	4.70		
WB	ADF	%	15	14.17	2.03	-0.6550	0.5185
C			13	15.24	6.00		
WB	NDF	%	15	21.61	2.34	-1.4790	0.1512
C			13	25.18	9.04		
WB	Starch	%	15	50.51	3.21	0.7620	0.4530
C			13	48.86	7.66		
WB	TDN-TMR	%	15	78.9420	2.0981	0.2780	0.7841
C			13	78.6346	3.4771		
WB	Dry Matter	%	15	64.32	3.53	0.3200	0.7516
C			13	65.84	18.12		
WB	Energy	kcal g^{-1} DM	9	4.44	0.47	-0.7070	0.4896
C			9	4.61	0.55		

2.3.2 Growth and performance

At d1, 36, 64, 98, and 143, two handlers and a dog directed cattle from their home pens to holding pens near the processing area. Cattle were then directed towards a handling facility that enabled cattle to be pushed towards a chute. This chute curved 180º to direct cattle towards a squeeze and scale. Here, the ID of cattle was determined by radio-frequency ID readers of government ID tags, and their weights were determined using scales underneath the squeeze that were tested before each use by ensuring accurate measurement of a handler. Cattle weights were compared over time to determine their average daily gains (ADG – kg gain per day) and feed conversion (FC – kg of Gain kg^{-1} of FI) over the course of the experiment.

2.3.2 Flight speed

After weights and cattle IDs were determined, the flight speed of cattle was evaluated. Flight speed is a standard method of quantifying an aspect of cattle temperament as described previously (Muller & von Keyserlingk 2006; Petherick et al. 2009; Schwartzkopf-Genswein et al. 2012). Modifications to these methods were that the first set of mirrors and reflectors was placed at 150 cm from the squeeze exit, and the second set was placed at 245 cm from the previous set, for a total distance of 395 cm. The apparatus used in this research was provided by Alberta Agriculture Food and Rural Development (Lethbridge, AB) and consisted of four steel posts, two housing infrared sensors and two housing mirrors. In this way, cattle exiting the squeeze would trip the first beam, starting a timer, and stop the timer upon breaking the second beam, providing the time between sensors from which velocity was calculated.

2.3.3 Meat quality

Cattle were weighed and transported 573 km (approximately a six hour drive from the feedlot) to an abattoir in two groups, the first groups on d143 (n = 10, 5 per treatment) and the second on d147 (n = 10, 5 per treatment). After

slaughter, carcasses were aged for 14 d in a cooler with temperatures between 0 - 1.1℃ and a humidity of 68 %. The weights of the rights side of carcasses were compared to determine if changes in diet impacted carcasses weight. Dressing percentage and carcass yield were not measured. Two rib eye steaks (2.5 cm thick, *longissimus dorsi*) were obtained between the 11th and 12th rib from each carcass; one steak was used for tenderness evaluation, and the other for meat colour and meat chemistry measures. To ensure meat samples were objectively evaluated, steaks were provided to the analyst without specifying the ID of the animal they were removed from. After samples were analyzed, the information linking the carcass to the animal was provided preventing operational biases. Steaks were analyzed 6 h after sampling.

Dietary impacts on the colour of red steak meat, the surface of trim fat, as well as the surface of ground steak samples were determined using a colourimeter (Hunter Lab ColorFlex® EZ, Reston, VA). Any debris created during the removal of the steak from its carcass was removed from the surfaces of steaks prior to measurement. Eight readings from each sample were averaged and the results were reported according to the International Commission on Illumination (CIE) profile as Lightness (L*), redness (a*) and yellowness (b*) (CIE 1978). Additional bloom time was not provided, as steaks had been removed from the carcasses at least 6 h prior and stored in food safe bags, and meat samples were not dried after they were ground.

Protein, fat, moisture and collagen contents were determined with an NIRS designed for meat samples (FOSS FoodScan™ Meat analyzer, Hillerød, Denmark). Rib eye steak samples were trimmed of excess fat and then ground in a commercial grinder (Hobart model FP41, Hobart Food Equipment, Toronto Ontario).

Meat tenderness was evaluated by removing the bone of rib eye meat samples and cooking the meats in a heated clamshell cooker until an internal temperature of 71℃ was reached; samples were flipped at 40℃ to ensure consistency in cooking. All temperature measures were recorded using a meat thermometer (VWR International LLC, Vandor PA). Once cooled to room temperature, eight 1-cm diameter cores were removed from each steak and a shear force (SF) test was conducted using a Lloyd Instruments Texture Analyzer

with a 50-kN load cell (C.S.C. Force Measurement Inc., Agawam MA). Cores were sheared using a flat V-shaped blade directed towards the core at a speed of 20 cm min^{-1}. The instrument then recorded the force (kgf) required to shear each core.

2.4   Statistical analysis

Statistical analyses were performed using JMP Software V8 (SAS, Carey, NC). Repeated measures multivariate analyses of variance (MANOVA) were used to identify the impacts of WB on FI, weight gain, ADG, FC, and FS. Further, for statistical analyses the pen served as the experimental unit over the five sample periods with the pens being represented in the statistical equation through the use of an ID function. To accomplish this, data tables were organized by sorting determinate values (FI, ADG, FS, etc.) into one column and then mixed model MANOVAs were carried out to determine the changes to the main factors of time and diet between results, as well as the significance of results towards the time×diet interactions, using the univariate approach (S.A.S. Institute Inc 2012). The normality of collected data was determined using the Shapiro-Wilk test in JMP, with results greater than 0.05 being deemed as coming from normally distributed samples. Of the data collected, all were from normally distributed samples with the exception of the feed intake and feed chemistry (NIR) values. As such, all changes in meat variation were determined using Student's *t*-tests assuming equal variances, and a *post hoc* power analyses was conducted to validate results. To address the smaller sample size, *post hoc* power analyses were completed by providing the software program with the standard deviation, sample size and mean difference between values for each result. These power tests yielded values above 80% in all cases with the exception of observed changes to ground steak colour and feed intake, which had power values of 45 and 27% respectively. These *post hoc* results indicate that despite the small sample size used in this study, in the majority of cases there was sufficient statistical power in our tests to detect differences between the two groups at the 0.05 significance level. For all the statistical analyses, significance was declared at $P \leq 0.05$ and trends at $P > 0.05 < 0.10$.

2.3   Results

2.3.1   Feed analysis

In order to evaluate the impacts of a WB-supplemented feedlot finishing ration, changes to the chemical and energy composition of the feed was evaluated by NIRS and bomb calorimetry, respectively. Feed analysis showed no differences ($P > 0.10$) between the two diets with respect to percentages of ash, crude fibre, fat, moisture, protein, and total dietary nitrogen for each of the total mixed rations. Further, no changes between the WB-supplemented and control rations were observed in the percent dry matter or energy contents of the feeds, which were compared on a dry matter basis ($P = 0.4897$ and 0.7516, respectively; Table 2.3). The ethanol contents of four separate WB batches were found to be 10.6 % (n=4).

2.3.2   Feed intake

Changes to the FI of cattle fed WB and C fed cattle are important determinants of the overall cost to producers. When comparing the mass of feeds delivered to pens between d1 and d 36, 64, 98 and 143, differences were observed between the main and interactive effects of time and diet ($P = 0.0130$; Table 2.4). Means for FI were found to increase over time ($P < 0.001$); in addition, FI for cattle fed WB-supplemented feeds was less then cattle fed C-supplemented feeds at each sampling event ($P \leq 0.05$). .

Table 2.4 Impacts of a 143d diet containing 6-7 % winery by-products (WB) or 6-7 % water (C) on the average daily gains (ADG), wet matter feed intake (FI), feed conversion (FC) and flight speeds (FS) of cattle; similar letters within each measurement indicate statistically similar numbers; standard error of measurements are indicated after each value.

Diet	Value	Units	Time on Feed (d) 1	36	64	98	143	P(diet)	P(time)	P(time ×diet)
WB	n		35	35	35	35	34	NA	NA	NA
C			33	33	33	31	30			
WB	FI	Kg	NA	213.2^{e} ±4.3	185.4^{f} ±3.7	232.6^{d} ±12.7	337.1^{b} ±34.4	0.013	<0.0001	<0.0001
C			NA	234.0^{d} ±2.3	210.5^{e} ±3.8	276.2^{c} ±19.8	380.7^{a} ±25.5			
WB	Gain	Kg	NA	119^{e} ±4.0	218^{d} ±4.5	319^{c} ±6.7	442^{a} ±8.6	0.1388	<0.0001	0.8069
C			NA	130^{e} ±5.2	229^{d} ±6.1	337^{b} ±7.7	447^{a} ±9.9			
WB	ADG	$Kg\ d^{-1}$	NA	1.5^{ab} ±0.31	1.5^{bc} ±0.23	1.4^{cd} ±0.35	^{d} ±0.29	0.675	<0.0001	0.0864
C			NA	1.7^{a} ±0.39	1.5^{bc} ±0.27	1.4^{bc} ±0.34	1.1^{e} ±0.47			
WB	FC	---	NA	1.88 ± 0.4	0.87^{bc} ±0.1	0.74^{d} ±0.1	0.77^{cd} ±0.1	0.2825	<0.0001	0.6605
C			NA	1.88^{a} ± 0.4	0.94^{b} ±0.1	0.83^{bcd} ±0.1	0.86^{bc} ±0.1			
WB	FS	$m\ s^{-1}$	±0.11	1.82^{bc} ±0.09	1.84^{bc} ±0.07	1.67^{cd} ±0.09	1.45^{d} ±0.08	0.9849	<0.0001	0.8619
C			±0.14	1.82^{bc} ±0.11	1.77^{bc} ±0.10	1.56^{cd} ±0.10	1.61^{cd} ±0.14			

2.3.3   Growth and performance

The ADG and FC results are presented in Table 2.4. No differences ($P =$ 0.1379) were observed between the diet treatments. Overall, the ADG of animals decreased over time, with the ADG of animals fed the WB ration decreasing from 1.54 to 1.25 kg d^{-1} while the ADG of animals fed the C ration decreased from 1.69 to 1.10 kg d^{-1} (P=0.0864). Changes in ADG between d1, d36, 64, 98 and 143 illustrated economic advantages between the C and WB feeds. No changes to the FC of treatment cattle were observed by diet ($P = 0.2825$); however, FC was found to change over time ($P < 0.0001$). In a *post hoc* analysis of power, the power with which we were able to detect changes in ADG and FC among 67 samples was determined to be 0.94 and 0.99 respectively.

2.3.4   Animal mortality

Surveying the impacts of a WB-supplemented feed on animal health was not an objective of this research. During the experiment, four cattle were removed from the study, three fed C rations (d95, 97, 112) and one fed WB rations (d106). Of these animals, one fatality was observed, and catalogued, from the animals fed the C ration. The cause of death was thought to be bloat by the onsite animal health technician. These samples sizes are too small to draw any valid conclusions.

2.3.5   Flight speed

Cattle FS was found to decrease over the course of the experiment ($P < $ 0.0001), however, when comparing flight speeds between cattle fed WB and C diets, no differences were observed ($P = 0.9849$), and similarly no interactions were observed over time with respect to diet ($P = 0.8619$; Table 2.4). In our *post hoc* power analysis, the observed power of our experiment detect changes in flight speed was 0.95 for 66 samples.

2.3.6 Carcass characteristics and meat quality

Carcass data are presented in Table 2.5. No treatment differences (P = 0.4399) were observed in the carcass half weights, indicating that all carcasses chilled similarly and that any changes to meat properties would likely be the result of the feed differences, and not simply as a result of the energy contents of the finishing rations. Tables 5, 6, and 7 summarize the results of changes to chemical, tenderness and colour characteristics of 14 d aged rib eye steaks measured using near infrared spectrophotometric, shear force and calorimetric tests, respectively. Results shown in Table 2.6 illustrate that no differences (P > 0.34) were observed in the protein, fat, moisture or collagen content of WB and C steak samples. Similarly, the tenderness of steaks was not impacted by diet (P = 0.2343) (Table 2.5). When comparing steak meat colour, no differences (P > 0.17; Table 2.7) were observed between the WB or C treatments. However, the colour of ground steak samples was darker (P = 0.0477; Table 2.7) in WB than C finished cattle; the incidence of dark, firm and dry (DFD) meat or pH of meat samples was not evaluated in this study, but no obvious cases of DFD were detected by personnel at slaughter. Further, trends towards significance were observed in the fat colours between meats from treatment diets. Specifically, the fats of steak meats were found to be redder in samples from cattle fed the C feeds (P = 0.0517; Table 2.7). Other than these findings, there were no colour or tenderness changes observed. In *post hoc* analyses of power, the power with which we were able to detect differences within half carcass weights, as well as steak and trim meat colours was found to be > 0.8825; however, the observed power within our experiment to discern changes to ground meat colour was found to be 0.4514, which was a result of reduced sample size to accommodate tenderness evaluations.

Table 2.5 Half weights of carcasses (kg) and the observed tenderness of resulting steaks (load at maximum; kgf) determined using the shear force test between cattle fed either 6-7 % winery by-products (WB) or 6-7 % water as control (C).

Diet	Value	Units	n	Mean	STDEV	t	P
WB	Weights	kg	10	146.0	7.18	-0.790	0.4399
C			10	148.9	9.12		
WB	Tenderness	kgf	32	2.92	0.77	1.203	0.2343
C			24	2.71	0.51		

Table 2.6 Near infrared analysis of % protein, fat, moisture and collagen contained in steak samples from cattle fed either 6-7 % winery by-products (WB) or 6-7 % water as control (C).

Diet	Item	n	Mean	STDEV	t	P
WB	Protein	5	21.93	0.91	-0.325	0.7562
C		3	22.11	0.24		
WB	Fat	5	13.17	4.30	-1.030	0.3429
C		3	16.22	3.51		
WB	Moisture	5	64.90	3.24	0.864	0.4210
C		3	62.91	2.95		
WB	Collagen	5	1.96	0.45	0.044	0.9667
C		3	1.95	0.43		

Table 2.7 Comparison of lightness (L*), red (a*) and yellow (b*) colours in rib eye meats (a), trim fats (b), and ground rib eye steaks from 14-d aged meat samples of cattle fed 143-d diets of either 6-7 % winery by-products (WB) or 6-7 % water as control (C).

(a) Meat	Diet	Light	n	Mean	STDEV	t	P
	WB	L*	9	39.86	3.42	-0.361	0.7231
	C		8	40.34	1.51		
	WB	a*	9	19.75	2.33	-0.881	0.3920
	C		8	20.58	1.32		
	WB	b*	9	17.40	1.86	-0.673	0.5109
	C		8	17.89	0.90		
(b) Fat	WB	L*	10	62.70	5.72	0.487	0.6340
	C		6	61.46	3.20		
	WB	a*	10	6.77	1.33	-2.126	0.0517
	C		6	8.44	1.80		
	WB	b*	10	16.39	1.83	-1.416	0.1786
	C		6	17.82	2.17		
(c) Ground	WB	L*	4	49.02	2.35	-2.609	0.0477
	C		3	54.59	3.36		
	WB	a*	4	23.14	1.20	1.405	0.2189
	C		3	2.58	2.58		
	WB	b*	4	22.04	0.22	-1.158	0.2992
	C		3	0.94	0.94		

2.4 Discussion

2.4.1 Feed analysis

The addition of WB into finishing rations was found to not alter the chemical and energy contents of the feed. These results are in agreement with previous studies that show grape pomace is an insufficient sole source of energy (Baumgärtel et al. 2007; Spanghero et al. 2009; Abarghuei et al. 2010). As the diets contained less than 7% WB or water, these results were expected. Additionally, as changes to the % DM of feeds were not found between samples, it was assumed that changes to cattle performance characteristics could be extrapolated between wet and dry matter feed references.

2.4.2 Feed intake

Before considering the impacts of WB and C feed on cattle feed intake (FI) it is important to discuss the limitations of this study. As the cattle diet changed from a silage to a chopped hay based diet, the rumen microbes would require a period of time to adapt (Fernando et al. 2010). However, both WB and C diet groups maintained consistent contents of either WB or water throughout the course of the study.

The changes to FI in cattle between the main and interactive effects of diet and time are thought to be the result of either the alcohol or the condensed tannins of the WB fraction of feeds because no changes were determined in either the chemical or energy contents of the feeds. In a study evaluating the impacts of alcohol on cattle feed intake and behaviour, Asato et al. (2003) showed that increases to intra-ruminal alcohol concentrations can increase bolus formation, mastication and rumination over time as well as lowering FI for up to 6 h after alcohol infusion. The reasoning for this is that the alcohol in the rumen acts to signal neural receptors stimulating mastication through increases in plasma isopropanol levels (Asato et al. 2003). This may partially explain our modest findings that feed intake decreased through the interaction of time×diet between the two groups.

Another possibility could be that the diminished FI in WB fed steers was caused by the condensed tannin (CT) fraction of these by-products. The impacts of CT on feed intake and palatability are well understood. When supplemented into animal feeds; condensed tannins have been shown to reduce FI and lower animal performance by forming insoluble protein complexes inaccessible to rumen microbes (Reed 1995). To accommodate feeding of high CT containing forages, such as *Acacia cyanophylla*, to ruminants, Ben Salem et al. (1999) found Polyethylene Glycol (PEG) could deactivate the CT preventing protein bypass. As a research tool, this method provides a way to discriminate the impacts of CT within individual feeds. In lambs, the feeding of *Acacia cyanophylla* without PEG has been shown to increase weight gains while not impacting FI (Ben Salem et al. 1999, 2000; Priolo et al. 2000). In cattle, it has been reported that when animals are fed high CT containing diets, quebracho and *Acacia mearnsii* for example, depressions in FI are observed (Landau et al. 2000; Grainger et al. 2009). Further, Landau *et al*. (2000) found cattle fed quebracho tannins to have more frequent eating bouts over a longer feeding period than animals fed diets not supplemented with quebracho tannins (Landau et al. 2000). The concentration of CT contained in WB feeds was not evaluated as it was beyond the scope of our initial research design; however, their concentration in red wine lees has been reported to be 180.3 g kg^{-1} DM (Molina-Alcaide et al. 2008). These studies do lend support to the results obtained from this current study, which show that feeding WB-supplemented feeds can alter the FI of cattle.

2.4.3 Growth and performance

Supplementing finishing rations with WB was not found to alter the average daily gains in cattle. These results are in agreement with previous studies that show grape pomace is an insufficient sole source of energy (Baumgärtel et al. 2007; Spanghero et al. 2009; Abarghuei et al. 2010). Information on the use of wine lees as a feed for cattle was not previously available, and as such, studies utilizing grape pomace remain the closest comparable feed (Molina-Alcaide et al. 2008). Similar to the resulting gains observed, no changes were observed when comparing the feed conversion rates by diet ($P = 0.2825$).

These results suggest that the WB volumes provided to cattle may lack the required levels of condensed tannins or alcohol to elicit a change in animal performance.

Other benefits of CT-supplemented feeds, not evaluated in this study, include reduced methane production, reduced occurrence of parasites including nematodes, as well as reductions to bloat in cattle (McMahon et al. 2000; Grainger et al. 2009; Novobilský et al. 2011). As the observations of bloat in this study were small it is not possible to state that WB reduced the occurrence of bloat in cattle. Other studies have shown that feeds containing elevated levels of CT can reduce the occurrence of bloat by suppressing the activity of rumen microbes. Specifically, *in vivo* studies of lamb micro flora found that the inclusion of tannins into feeds increased the numbers of the bacteria *Butyrivibrio fibrisolvens*, while decreasing the abundance of *Butyrivibrio proteoclasticus* bacteria (Vasta et al. 2010b). Further, Min et al. (2005) found that supplementing the diets of cattle grazing winter wheat with quebracho CT reduced bloat scores as well as increasing weight gains. Finding diets that can act to reduce bloat in feedlot rations provides an ideal way to reduce the need for bloat preventing antimicrobials, such as monensin.

2.4.4   Flight speed

To evaluate claims surrounding changes to animal behaviour made by the marketers of WB finished cattle, the objective and quantifiable evaluation of flight speed was chosen over other more subjective measurements. Although flight speed is not a thorough evaluation of animal behaviour, this method has been found to correlate with other subjective measurements of behaviour including crush scores, exit scores and pen behaviour (Muller & von Keyserlingk 2006; Petherick et al. 2009; Cafe et al. 2011). Further advantages of flight speed include its repeatability and correlation with serum concentrations of cortisol (Curley et al. 2006).

This study identified that cattle became habituated to handing during the course of the study, as indicated by increasingly slower cattle flight speeds as the study progressed. This relationship is supported by other studies which found

cattle habituated to handling over time (Petherick et al. 2009; Schwartzkopf-Genswein et al. 2012). Overall, diet was not found to alter cattle behaviour in this study and measured by flight speed. Currently, no research has been conducted on the impacts of WB on ruminant behaviour. Previous research on the role of diet on cattle behaviour has found that providing oral supplements of tryptophan to beef cattle produced sedative effects as well as increased lying and eating behaviours (Nakanishi et al. 1998). Supplementing cattle diets with electrolytes, such as Nutricharge®, has also been found to reduce the stress of transport in animals, improving carcass gains and yield grades during transport (Schaefer et al. 1997). As no change to the chemical and energy contents of experimental diets were observed, it is somewhat expected that the diets tested would not alter the flight speeds of cattle.

2.4.5   Carcass characteristics and meat quality

The treatment diets in this study did not alter the hot carcass weights. Further, as carcass weights did not differ, changes to meat quality are most likely the result of the micronutrients in the treatment diet and not the chilling properties of the individual carcasses. Our results, observing darker colours in ground steak meats (L*) between WB and C treatments, are consistent with other studies. Guzik *et al.* (2006) found darker colouration in pigs fed L-tryptophan-supplemented feeds. Additionally, Luciano *et al.* (2009) found similar colour patterns when supplementing lamb diets with quebracho tannins. The colour changes observed in the latter study were found to be caused by increased metmyoglobin formation in animals fed tannin-supplemented feeds. Similar research has also been conducted on the impacts of citrus pulp inclusive diets on lamb meat. Here, researchers found that these diets can lead to slightly less tender meats, as the animals fed the treatment diets produced meats with shear force values that were slightly more than animals fed the control feed (Scerra et al. 2001). Additionally, it has also been demonstrated that citrus pulp inclusive diets can reduce the redness of lamb meats, which was observed in the fats of the WB steaks, albeit at non-significant levels when compared to C animals (Caparra

et al. 2007). Overall, the lack of changes to cattle FS and the chemical properties of treatment feeds indicate that changes to meat colour were likely caused by components not measured in this study, such as CT, and not animal behaviour.

2.5   Conclusion

Winery by-products (WB) are not an effective sole energy source for cattle finishing rations as they do not increase the energy or chemical contents of feeds. However, as a co-product WB can potentially compliment high-energy rations through additions of nutritive factors not yet identified, such as condensed tannins. As a co-product, winery by-products were found to reduce the feed intake of cattle and improve their feed efficiency without altering the weights of carcasses at slaughter. Further, ground muscle meats were found to be darker in colour from animals fed this co-product. No differences were observed in the FS between cattle fed the different diets, suggesting that the behaviour of cattle is likely unaffected by the inclusion of WB. Further research in this area should be conducted to repeat and validate this work on a dry matter basis and verify if unidentified nutritive factors exist in the WB-supplemented feed. Understanding the biochemical processes that impacted the colour changes observed in this study is required to ensure consistent production, especially if the overall goal is to improve meat quality.

3. EFFECT OF FERMENTED WINERY WASTE SUPPLEMENTED RATIONS ON THE DIVERSITY AND ANTIMICROBIAL RESISTANCE PROFILES OF FECAL *E. COLI* LOADS IN BEEF CATTLE

3.1   Introduction

As novel diets are introduced in animal production their impacts on animal welfare, food safety and environmental issues must be evaluated. Of specific interest in animal agriculture is identifying diets that have the ability to reduce fecal pathogens and antimicrobial resistance (AMR) in manure while maintaining growth yields (Nakanishi et al. 1998; Callaway et al. 2003, 2003, 2009, 2010; Gilbert et al. 2005; Ferguson & Warner 2008). The focus of this work was to evaluate the impacts of a grain-based feed supplemented with fermented winery by-products (WB), mainly wine lees, on the proliferation of fecal *Escherichia coli* (EC), their antimicrobial resistance phenotypes, as well as the bacterial diversity of feces from cattle fed WB-supplemented feeds. This diet is currently being fed to cattle in the South Okanagan Valley of British Columbia, Canada.

Understanding the impacts of diet on the spread of antimicrobial resistant organisms from animal agriculture is essential as the use of antibiotics as growth promoters has increased the loads of drug resistant pathogens in fecal wastes, which can be spread through manure to drinking water sources (Duriez & Topp 2007; Alexander et al. 2008; Jokinen et al. 2011). In healthcare, tetracycline and ampicillin are often used as first line drugs to treat infections, as well as being used in agriculture as prophylactics (Hamilton-Miller 1984). The subtherapeutic administration of antimicrobials as prophylactics promotes the prevalence of resistant EC in cattle feces. Additionally, the role that diet plays in promoting this resistance is not understood (Alexander et al. 2008; Sharma et al. 2008). Dibner & Richards (2005) have suggested that consumer trends will force producers to reduce the use of antimicrobials as prophylactics; further they suggest that dietary supplements able to increase animal performance need to be

identified to replace antibiotics. In Europe, the use of antimicrobial prophylactics is restricted, yet producers have found ways to avoid losses to animal performance; however, the EU still allows the administration of ionophore antibiotics, such as salinomycin in poultry and monensin in cattle (Dibner & Richards 2005). While ionophores are used to manage rumen microbes during dietary changes, they are still antimicrobial in nature, however they have been shown to not increase the prevalence of specific serotypes or antimicrobial resistance in fecal pathogens when added to cattle diets (McAllister et al. 2006; Nisbet et al. 2008).

Previous research on the use of WB in animal feeds has focused on the addition of grape pomace, also known as grape marc, with limited studies on wine lees, likely due to the availability of by-products (Bravo & Saura-Calixto 1998; Baumgärtel et al. 2007; Alipour & Rouzbehan 2010; Abarghuei et al. 2010). Of note, by-products from wine making are good sources of condensed tannins (CT) and polyphenols, which have been found to reduce fecal pathogen loads and methane production in beef and dairy cattle (Min et al. 2007; Molina-Alcaide et al. 2008; Grainger et al. 2009). Although CT can restrict the growth of bacteria, such as EC and *Salmonella* spp., and nematodes in cattle intestinal systems, some species have developed resistance to CT through the degradation of the CT molecules (Goel et al. 2005; Min et al. 2007; Wang et al. 2009; Berard et al. 2009; Novobilský et al. 2011). Molina-Alcaide et al. (2008) found higher levels of free CT in feeds supplemented with red wine lees compared to those supplemented with grape pomace, and similar ratios of protein- and fiber-bound CT. Both wine lees- and pomace-supplemented feeds were found to be good sources of protein and energy for ruminants. Further, Bahrami et al. (2010) found that supplementing lamb rations with dried grape pomace increased the crude protein and dry matter contents of feeds while also improving animal gains and feed conversion rates. Unfortunately, grape pomace alone lacks sufficient energy to maintain animal growth and milk production and, as such, it can be only used as a feed supplement; however, it holds promise as a co-product (Hadjipanayiotou & Louca 1976; Baumgärtel et al. 2007; Tsiplakou & Zervas 2008; Molina-Alcaide et al. 2008; Spanghero et al. 2009; Hersom et al. 2010; Abarghuei et al. 2010).

Considering the impacts of diet on fecal pathogen loads, Diez-Gonzalez et al. (1998) found that switching cattle from grain based to forage based rations five days before harvest reduced EC loads 1,000 fold while also reducing their virulence. Wang et al. (2009) evaluated the impacts of a phlorotannin-supplemented feed and found them capable of reducing the viability of EC O157:H7 *in vitro*. Further, Min et al. (2007) found that chestnut tannins reduced fecal EC loads in cattle feces when supplied directly into the rumen. However, *in vivo* trials by Berard et al. (2009) have found tannin-supplemented feeds to have limited impacts on reducing the loads of EC O157:H7 in cattle feces.

In the case of supplementing cattle rations with WB, we have previously evaluated their impacts on the weight gains, temperament, and feed conversion rates of cattle (Moote et al. 2012). The addition of WB to feeds did not alter their caloric or chemical composition, however it was found to decrease the feed intake and increase feed conversion ratios of cattle, without impacting cattle temperament. In this report, we evaluate the impacts of WB-supplemented feeds on the loads of fecal EC, their antimicrobial resistances, and the bulk changes in the diversity of fecal bacterial communities.

3.2   Materials and methods

3.2.1   Experimental design

3.2.1.1   Animals

Before commencing this project, animal use research protocols were reviewed and approved by the Thompson Rivers University Animal Care Committee. The study followed the Canadian Council of Animal Care guidelines for Farm Animals (Canadian Council on Animal Care 2009) and the Canadian Beef Cattle Code of Practice guidelines (Agriculture Canada 1991). The experimental design used for this project followed that of Moote et al. (2012). Specifically, 69 single-sourced Angus-cross steers (351.3 ± 27.9 kg) were transported 678 km to a small custom feedlot near Oliver, BC (Southern Plus Feedlots). On arrival, cattle were acclimated to finishing rations by stepping up the grain ration content from 0 to 50% in the diets over a 30 d period. Once

acclimated, animals were randomly separated into four freshly cleaned pens, two pens per treatment (n = 18, 17, 17, and 17).

3.2.1.2 Housing

Cattle were held in pens of 1,600 m² (40 m × 40 m) and pen walls were made of 2-m wood panels along three sides. The remaining side contained a feed bunk with metal railings above a concrete bunk to prevent cattle from exiting the pen through this area. To minimize the spread of bacteria from animals of varying diets, pens shared water bowls, with an adjacent pen (one bowl per two pens), that contained animals fed the same diets. Pens were cleaned on a monthly basis, or as needed, using front-end loaders, and bedding of either gypsum or wood chips were added after cleaning.

3.2.1.3 Dietary treatments

Animals were fed finishing diets, twice daily at 0800 and 1500, containing either 6-7 % (w w^{-1}) winery by-products (WB; 18, 17) or 6-7 % water (C; 17, 17) for a 143 d period (Table 3.1) using industrial feeders equipped with scales (± 4.55 kg) that mixed feeds before delivery. Wine lees were the sole source of the winery by-products for this experiment, except for one batch, which contained improperly fermented wine. For this reason we refer to the wine supplement as WB, as opposed to wine lees. The oats and barley used in the feeds were at least 489 and 618 kg m^{-3} respectively and rolled on-site. Further, malt contents were fermented cereal grains provided from local breweries and the liquid supplement contained minerals and protein designated for beef cattle. Variation in the feed contents occurred as a result of a dietary change from corn silage to chopped hay when silage supplies diminished (d105) two thirds of the way through the feeding trial. During the experiment the feedlot manager monitored the daily feed weight delivered to each pen through slick bunk demand management, which is routinely utilized at the feedlot.

Table 3.1 Wet matter composition of experimental diets used during the 143d feeding program; control diets and experimental diets differed from one another only by their contents of winery by-products and water respectively.

Item	% Diet (d1-d105)		% Diet (d106-d148)	
	WB	C	WB	C
Corn Silage	30	30	0	0
Chopped Hay	0	0	12	12
Fermented cereal grains (Malt)	10	10	31	31
Oats	20	20	20	20
Barley	30	30	28	28
Protein and mineral feed supplement	3	3	3	3
Winery by-products	7	0	6	0
Water	0	7	0	6
Percent Dry Matter (%) $\pm$ SD	64.3 $\pm$ 2.3	58.5 $\pm$ 8.1	63.3 $\pm$ 0.8	84.6 $\pm$ 32.4
Energy content (Kcal gm^{-1} DM $\pm$ SD)	4.9 $\pm$ 0.4	4.19 $\pm$ 0.7	4.21 $\pm$ 0.2	4.7 $\pm$ 0.5

3.2.2   Data collection

3.2.2.1   Fecal sampling

Fecal samples were gathered from the cattle six times during the experiment using sterile double-pronged swabs (StarSwab SO9D; Starplex Scientific Inc., Etobicoke On, Can). Swabs were inserted ~4 cm into the rectum and rotated around the mucosal membrane to ensure an even distribution of feces, a technique referred to as a Rectal Anal Mucosal Swab (RAMS). At each sampling event, whole fecal samples (n = 6 per pen) were gathered from the first six animals to enter the squeeze providing the animal had not recently had a prolapsed rectum. Grab samples were extracted by hand using a fresh sterile nitrile examination glove (High Five Products Inc., Chicago IL) for each sample, and transferred directly into a sterile urine analysis cup (VWR International Inc., Randor PA). All samples were stored on ice for no more than four hours before being processed at the on-site laboratory operating under the Biohazard regulations of Thompson Rivers University (TRU) and approval of Southern Plus Feedlots. RAMS samples were prepared for EC isolation by re-weighing the sampled StarSwab container to determine the mass of fecal sample gathered. Grab samples were processed by adding ~15% sterile glycerol (w w^{-1}) into the urine analysis tubes followed by freezing at -20°C until being transferred back to the main lab where they were stored at -80°C.

3.2.2.2   *Escherichia coli* (EC) isolation

RAMS samples were diluted in 4.5 mL Brain Heart Infusion Broth (BHI) (EMD Chemical Inc., Darnstadt, GER) and vortexed until homogenous slurries were produced. The resulting slurries were then serially diluted up to 10,000 times in BHI broth and duplicate 100 μL samples were spread onto Membrane Fecal Coliform Agar supplemented with 100 mg L^{-1} 5-Bromo-4-chloro-3-indolyl-ß-D-glucuronic acid (BCIG) (mFc agar) (Oxoid Ltd., Basingstoke, Hants, ENG) that contained no antibiotics, 4 μg mL^{-1} of tetracycline hydrochloride or 4 μg mL^{-1} ampicillin sodium salt to produce mFc, mFc^T and mFc^A plates, respectively. The 1

× 10^4, 10^5, 10^6 dilutions were spread onto mFc plates to enumerate the EC load of selected cattle (n = 6 per pen). All mFc, mFc^T and mFc^A plates were incubated at 44.5ºC overnight, and presumptive EC were identified by the production of a blue pigment indicating the presence of ß-D-glucuronidase. Loads were only determined from the mFc plates if between 30-300 cultures were present. An error was made when starting this experiment as the concentration in mFc^A plates should have been 32 μg mL^{-1} or 50 μg mL^{-1} as done previously (Alexander et al. 2008). As such, only the changes to the susceptibility of EC in samples to ampicillin can be monitored, where the changes to resistance patterns to tetracycline can be determined through the mFc^T plates.

From a single mFc, mFc^A or mFc^T plate, four presumptive isolates were selected and streaked onto fresh Luria Bertani (LB) agar (5 g L^{-1} tryptone, 10 g L^{-1} yeast extract, 10 g L^{-1} NaCl, 15 g L^{-1} agar) and incubated at 37ºC overnight; after, single colonies were plate purified on LB agar. Isolates were then transferred into 96-well microtiter plates (Grenier Bio-One North America, Inc., Monroe, NC) using sterile toothpicks and grown at 37ºC overnight; after incubation, 15% (v v^{-1}) glycerol (Sigma-Aldrich, St. Louis MO) was added and the plates were frozen at -20ºC for transport to TRU.

Presumptive isolates were confirmed as EC by their growth at 44.5ºC, their production of ß-glucuronidase, as well as their ability to metabolize lactose as a sole carbon source using Lactose broth (EMD Chemicals Inc., Darnstadt, GER), and produce indole by adding 10 μl of Kovacs' Indole reagent (EMD Chemicals Inc., Darnstadt, GER) following growth in Peptone broth (Oxoid Ltd., Basingstoke, Hants, ENG). Confirmed EC isolates were transferred into microtiter plates along with the control strain EC K12 MG1655 (provided by Dr. Julian Davies, UBC) being added to one well per plate.

3.2.2.3   Antimicrobial susceptibility of *E. coli* isolates

The minimum inhibitory concentration (MIC) for EC isolates was estimated using 25-cm^2 bioassay plates (Corning Inc., Corning NY, USA) with 250 mL Mueller Hinton agar (BD, Sparks, MD, USA) supplemented with either 0, 1, 2, 4, 8, 16, 32, 64, 128 μg mL^{-1} ampicillin or 0, 0.25, 0.50, 1, 2, 4, 8, 16, 32, 64, 128

μg mL^{-1} tetracycline. Freshly cultured EC isolates were replicated onto these plates and incubated at 37°C for 16 h. The MIC for each isolate was determined by the absence of growth occurring at the lowest concentration of each antibiotic. MIC values were grouped by diet and compared using a Student's t-tests, assuming un-equal variances, to determine if changes occurred between diet and over time via JMP statistical software (SAS Institute Inc., Cary NC).

3.2.2.4   DNA extraction for 16S rRNA gene sequencing

DNA was extracted from frozen fecal grab samples taken at d1, 64, and 143 using MoBio Ultra Clean Soil Isolation Kits (Carlsbad CA, USA lots SD11E5 and SD11G11) as described by the supplier; deviations to the supplier protocol were that the frozen samples were partially thawed at 37°C before beginning the isolation. DNA concentrations were determined fluorometrically using a Qubit (Invitrogen Inc., Grand Island, NY) as well as visually following gel electrophoresis. Fecal DNA extractions from two cattle per pen were pooled and adjusted to 20 ng μl^{-1}. A total of 12 DNA samples were sent to Research and Testing Laboratories (RTL) (Lubbock, TX) for pyrosequencing.

3.2.2.5   Massively parallel bTEFAP

Bacterial tag-encoded FLX amplicon pyrosequencing (bTEFAP) was performed by Research and Testing Laboratory (Lubbock, TX) using the Gray28F (5′TTTGATCNTGGCTCAG) and Gray519r primers (5′ GTNTTACNGCGGCKGCTG) (Suchodolski et al. 2009, 2012; Handl et al. 2011). The bacterial DNA tagging and sequencing was performed as described by Dowd *et al*. (Dowd et al. 2008). A Roche 454 FLX sequencer with Titanium reagents was used to sequence DNA from within the 28F and 519R regions of the 16S rRNA gene.

3.2.2.6   Pyrosequencing and data analyses

Data analysis was performed using the macQiime V1.6.0 Software package downloaded through the Werner Lab website, SUNY Cortland

University (Caporaso et al. 2010b). In total, 81,214 raw sequences were filtered based on length, eliminating those <200bp, and quality. From this, a total of 49,578 sequences averaging 313.7 bp were extracted. The resulting sequences were aligned with the UCLUST function in Qiime, to generate an OTU table outlining sequence similarity. The UCLUST function has been shown as an effective way to align generated sequences in Qiime (Edgar 2010; Jami & Mizrahi 2012). In parallel, the taxonomy of generated OTUs was assigned using the Ribosomal Database Project (RDP) to generate alignment seeds against the Greengenes reference database available at blog.qiime.org as "Most recent Greengenes OTUs" ("QIIME News and Announcements" n.d.; Wang et al. 2007). A 97% alignment between OTUs and BLAST results was set to predict the identity to the species level for each sequence. In total, 11,433 OTUs were generated from the 81,214 sequence reads for analysis; further, to reduce biases 2,028 OTUs were evaluated from each sample yielding a total of 7,882 OTUs for analyses. During the same workflow script, the Pynast algorithm was used to calculate sample phylogeny, enabling the generation of a tree through the FastTree algorithm to determine the similarities between the bacterial communities within samples (Price et al. 2009; Caporaso et al. 2010a). To identify similarities between the bacterial communities within samples by time and diet, Principal Component Analyses were generated using the unweighted Unifrac discrete parameters. Species richness and bacterial diversity was evaluated using the Chao1, Shannon's index and observed species functions in Qiime. Changes between specific Genera and Phyla over time were evaluated using the Student t-test package available in JMP Software V8 (SAS, Carey, NC), incorporating un-equal sample variances.

3.2.2.7   BOX-PCR (Rep-PCR)

Rep-PCR was performed on confirmed EC isolates using the BOX A1R primer (5'-CTACGGC AAGGCGACGCTGACG -3') (Alpha DNA, Montreal QC, CAN Lots 399,824 and 444,206) in 25-μl PCR reactions that contained 3 mM MgCl$_2$ 1X EconoTaq PCR buffer, 2 μM BOX A1R primer, 600 μM dNTPs and 1U of 5U μL^{-1} Taq polymerase (EconoTaq, Lucigen Corp., Lot 3672). PCR

amplification was performed using a MyCycler thermal cycler (BioRad Laboratories Inc, Segrate, Italy) as follows: after an initial denaturation step of 94°C for 10 minutes, reactions underwent 34 cycles of denaturation (94°C for 3 seconds, 92°C for 30 seconds), annealing (50°C for 1 minute) and extension (65°C for 8 minutes), followed by a final extension (65°C for 8 minutes). PCR products (10 μl) were then separated on 1.5% agarose gels using 50-cm OWL A3-1 or 30-cm A5 gel electrophoresis boxes (Owl Separation Systems Inc., Plymouth NH) at 20 V cm^{-1} for 16 h using BioRad PowerPack Basic power supplies (BioRad Laboratories Inc, Segrate, Italy). For each gel, 5 μl of VWR 1Kb ladder with fragments ranging from 0.3 to 10 Kb in size (VWR International Inc., Randor PA lot MD03100301) was added in every eighth lane; in addition, 5μl of PCR reactions from positive and negative controls (EC K12MG1655 and water, respectively) were added to each gel. Gels were stained using GelRed (Biotium Hayward, CA) and visualized using a BioRad GelDoc XR imager (BioRad Laboratories Inc, Segrate, Italy).

3.2.2.8   Computer assisted fingerprinting

Normalization of gel images and assignment of fingerprints to isolates were done with BioNumerics (version 6.5; Applied Maths, Kortrijk, Belgium). Positions of fingerprints on gels were normalized using the MassRuler DNA ladder as the external standard in the range of 300 bp to 3,000 bp. Similarity coefficients were generated using the curve-based cosine correlation coefficient. Similarity trees were generated using the unweighted-pair group method using average linkage, and a similarity cutoff of 80 % was used in order to determine related fingerprint types. Comparisons of 355 isolates from the d143 sampling event were made using the Multi-Dimensional Scaling (MDS) package available in BioNumerics in order to determine the change in bacterial diversity by diet Figure 3.5 and by isolation method Figure 3.6.

3.2.3   Statistical analyses

Statistical analyses were performed using JMP Software V8 (SAS, Carey, NC). Repeated measures mixed model MANOVAs were used to identify the

impacts of WB on changes to fecal EC loads over time. The number of cultures isolates able to grow on antibiotic containing media was evaluated by providing values for samples with resistant cultures as 1 and those without as 0. In this way all samples could be evaluated and compared through the repeated measures and Student's *t*-test functions available in JMP. Further, for statistical analyses the pen served as the experimental unit over the five sample periods with the pens being represented in the statistical equation through the use of an ID function. To accomplish this, data tables were organized by sorting determinate values (ex: loads) into one column, and then mixed model MANOVAs were carried out to determine changes to the main factors of time and diet between results, as well as time×diet interactions using the univariate approach outlined by the software manufacturer (S.A.S. Institute Inc 2012). The normality of collected data was determined using the Shapiro-Wilk test in JMP, with results greater than 0.05 being deemed as coming from normally distributed samples. Of the data collected, all were from normally distributed samples with the exception of the analysis of samples containing tetracycline and ampicillin resistant EC as the results were limited to presence/ absence, as well changes to Genera and Phyla diversity. As such, all changes to the minimum inhibition concentrations, as well as Genera and Phyla diversity, were determined using Student *t*-test functions incorporating un-equal sample variations. For all the statistical analyses, significance was declared at $P \leq 0.05$ and trends at $0.05 < P < 0.10$.

3.3   Results

3.3.1   Fecal EC loads

To evaluate the impacts of WB-supplemented cattle rations on fecal *Escherichia coli* (EC) loads, the numbers of EC were evaluated on a Log Colony Forming Unit per gram (LogCFU g^{-1}) basis after incubation on selective media. Over the course of experiment, the EC loads per sample averaged 7.01 ± 0.96 LogCFU g^{-1} (Figure 3.1). Fecal EC loads gradually declined from d1 to d64. From here, a spike in numbers was observed at d98, and these numbers remained consistent until harvest at d143. Repeated measures MANOVAs revealed

changes occurring to the EC loads when monitoring the interactions of time×diet ($P = 0.0498$); however, no changes were observed to the fecal EC loads at any sampling event (Table A.1). This indicates that over the feeding period cattle fed WB were found to have elevated EC loads over time than cattle fed C diets.
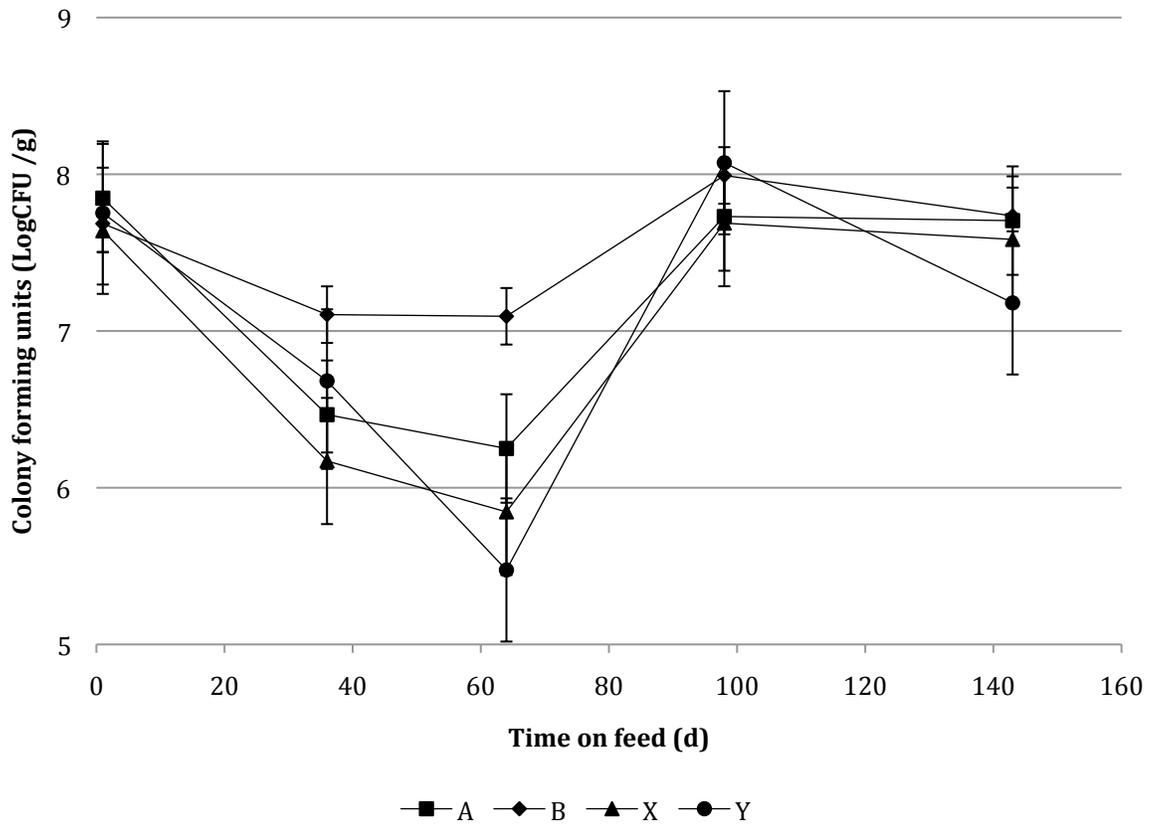
Figure 3.1 Shifts to the loads of fecal *E. coli* in cattle fed a 143-day diet supplemented with either 6-7% winery by-products (A and B; ■ and ◆) or 6-7% water (X and Y; ▲ and ●).

3.3.2 Tetracycline and ampicillin resistance

To understand the impacts of WB on EC resistance to tetracycline and ampicillin, the number of fecal samples harbouring EC able to grow in 4 μg mL^{-1} of these antibiotics was determined at each sampling event. Resistance to ampicillin was found to be greatest at d1, 98 and 143, with greater than 95.5% of samples containing resistant organisms, while EC were found to be most susceptible to ampicillin at d64 with 81.8 % of samples containing resistant organisms (Figure 3.2). Diet, and the interactions of time×diet, were not found impact the number of samples containing ampicillin resistant EC over time ($P >$ 0.05; Table A.1).
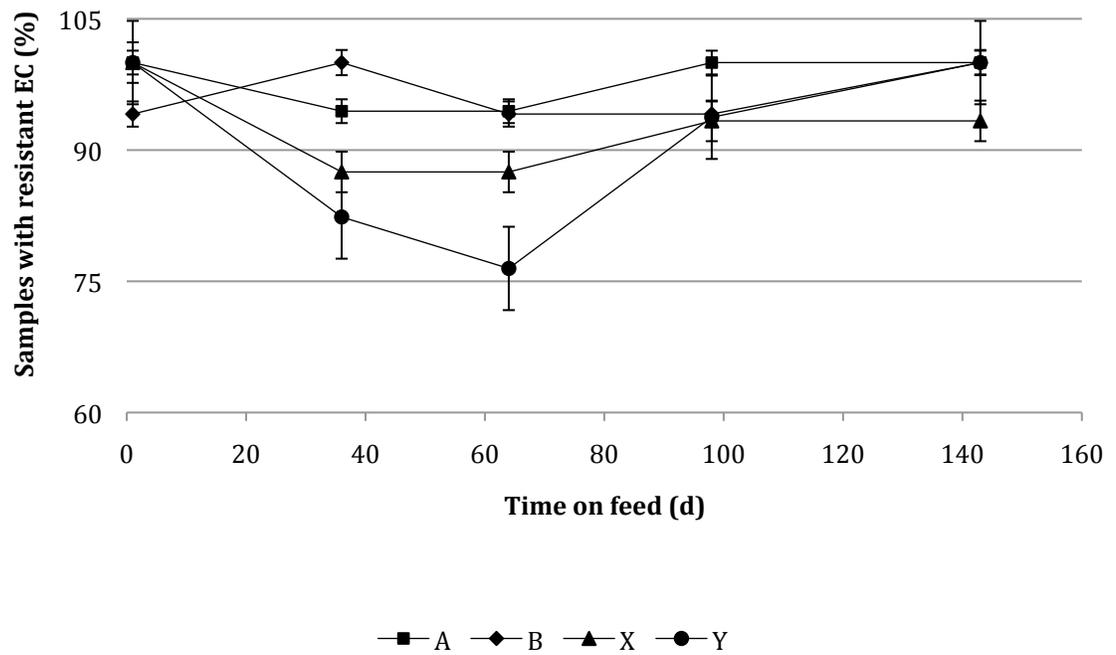
Figure 3.2 Proportion of fecal samples containing *E. coli* capable of growth at 4 *μ*g mL^{-1} ampicillin from cattle fed a 143-day diets containing either 6-7 % wine (A abd B; ■ and ◆) or 6-7% water (X and Y; ▲ and ●).

Resistance to tetracycline among samples was similarly found to be the greatest at d1, 98 and 143, with 95.5% of samples containing EC resistant to tetracycline. The greatest proportion of samples containing resistant EC were found at d63, with 84.3 % of samples found to be harbouring resistant EC (Figure 3.3). The number of samples containing resistant EC changed over time (repeated measure mixed model MANOVA, $P = 0.0062$); however, diet was not found to have an impact ($P = 0.1008$; Table A.1). The proportion of samples harbouring tetracycline resistant EC were different between diets at d64, where 72.7% of samples from C cattle were found to harbour resistant EC compared to 97.0% of samples from cattle fed WB rations. From here, it is important to further evaluate the antimicrobial resistance patters of EC to understand if diet altered the resistance profile of these organisms.
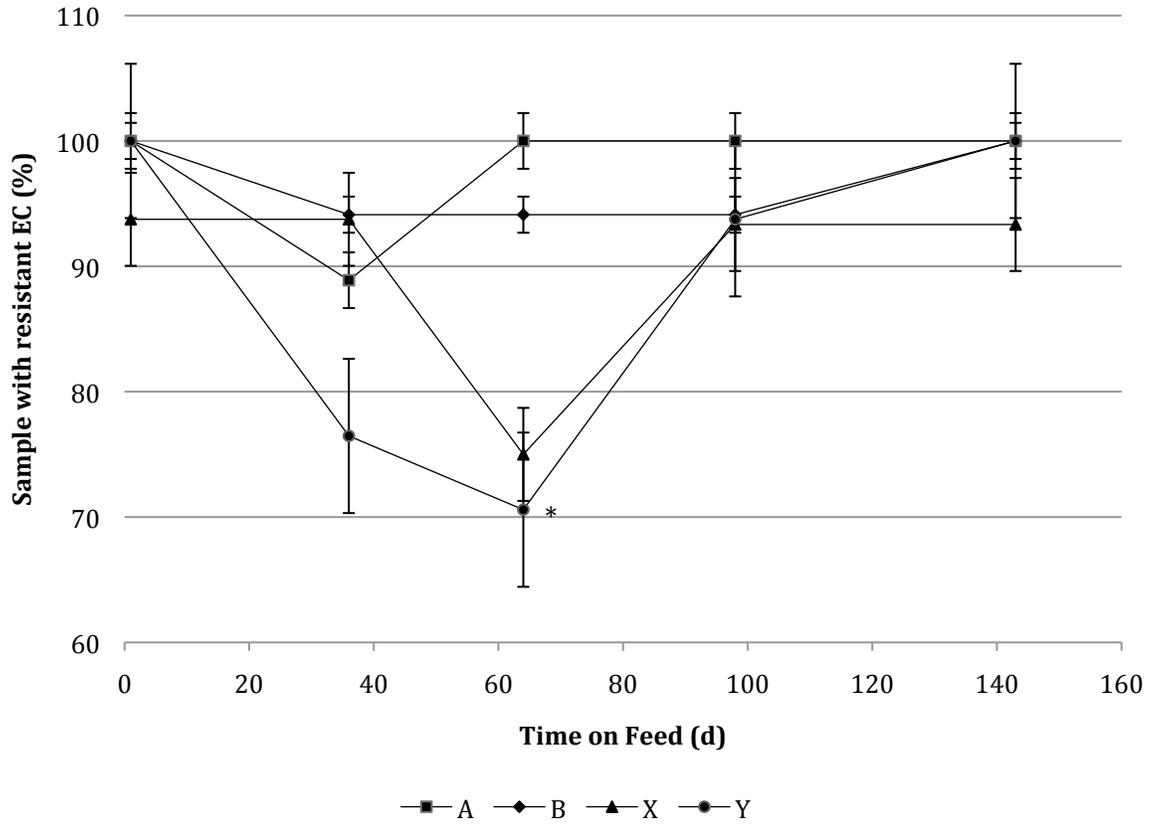
Figure 3.3 Proportion of fecal samples containing *E. coli* capable of growth at 4 *μ*g mL^{-1} tetracycline from cattle fed a 143-day diets containing either 6-7% wine (A and B; ■ and ◆) or 7% water (X and Y; ▲ and ●). * Indicates findings between diets ($P < 0.5$, n=4).

3.3.3 Minimum inhibition concentrations (MIC)

The MIC of EC was determined on all isolates through agar-based techniques. Resistance to ampicillin antibiotics was found to be highest in EC isolates from d1 with an average MIC of 202.3 ± 73.3 μg mL^{-1} (Table 3.2). The lowest MIC values recorded were from d64 with an average of 51.8 ± 11.5 μg mL^{-1}. At d143, differences between diets ($P < 0.05$) were observed within the MIC values of EC isolated from all thee selection methods (mFc, mFc^A and mFc^T). Diet was also found to impact the MIC values of EC isolated on d1 from mFc plates and on d36 from mFc^A and mFc^T plates. No trends were observed between EC isolated on mFc, mFc^A or mFc^T plates between diets. In general, the MIC of all isolates remained above the resistance threshold of 32 μg mL^{-1} throughout the experiment.

Table 3.2 Minimum inhibitory concentrations of ampicillin antibiotics for *Escherichia coli* **isolates obtained from fecal samples of cattle fed 143-day diets supplemented with 6-7% winery by-products (WB) or water as a control (C) on membrane fecal coliform agar containing BCIG and supplemented with no antibiotics (mFc) 4 μg mL^{-1} tetracycline (mFc^T) or 4 μg mL^{-1} Ampicillin Sodium Salt (mFc^A); letters indicate statistically similar numbers ($P = 0.05$), not compared between isolation methods.**

											Day	
			1		36		64		98		143	
Diet	Media	n	mean	n	mean	n	mean	n	mean	n	mean
WB	mFc	28	231^a	83	30^{bfgh}	84	62^{cefh}	78	74^{cfh}	103	122^d
C	mFc	12	55^{cdfh}	15	54^{bcefgh}	14	32^{bfgh}	25	42^{bcegh}	101	51^{cefh}
WB	mFc^T	40	240^a	51	151^{bf}	90	49^{cdf}	76	56^{cdf}	114	57^e
C	mFc^T	27	211^a	85	40de	70	48^{cdcf}	65	48^{bcdcf}	82	90^{cdcf}
WB	mFc^A	28	231^a	83	30^b	84	62^{cdef}	78	74^{cdefg}	103	122^{cdefg}
C	mFc^A	21	247^{ab}	50	121^{cdef}	65	58^{cdef}	53	49^{cdef}	89	53^{def}
Total			202.3		71.0		51.8		57.3		82.6
STDEV			73.3		52.1		11.5		13.9		33.6

The MIC values for tetracycline were found to be the greatest at d1, with an average MIC of 162.9 ± 85.2 μg mL^{-1} (Table 3.3). The lowest average MIC was recorded on d143 at 25.1 ± 20.0 μg mL^{-1}, the MIC values of EC were observed to differ between diets when isolated on various media at each sampling event. Of note, WB fed cattle consistently yielded EC with elevated MIC values compared to EC from C fed cattle when EC were selected on mFc and mFc^A plates. Conversely, EC from WB fed cattle were found to have lower MIC values compared to EC isolated from cattle fed the C diet when isolated on mFc^T plates.

Table 3.3 Minimum inhibitory concentrations of tetracycline antibiotics for *E. coli* isolates obtained from fecal samples of cattle fed 143-day diets supplemented with 6-7% winery by-products (WB) or water as a control (C) on membrane fecal coliform agar containing BCIG and supplemented with no antibiotics (mFc) 4 μg mL^{-1} tetracycline (mFc^T) or 4 μg mL^{-1} Ampicillin Sodium Salt (mFc^A); letters indicate the presence of similar numbers ($P = 0.05$), not compared between isolation methods.

| | | Day | | | | | | | | |
Diet	Media	n	mean	n	mean	n	mean	n	mean	n	mean
		1		36		64		98		143	
WB	mFc	29	222^a	92	52^{bch}	86	38^{bcegh}	84	65^{bcdeh}	104	49^{bcdeh}
C	mFc	12	2^f	15	16^{cghi}	14	2^f	24	$31^{bcdeghi}$	97	8^i
WB	mFc^T	36	141^a	55	38bc	80	14^{bc}	75	16^b	116	8^{bc}
C	mFc^T	25	218^a	85	8d	67	65^d	65	75^d	86	30^d
WB	mFc^A	29	222^a	92	52bcdfgi	86	38^{bcdh}	84	65^{bbcdhj}	104	49^{ch}
C	mFc^A	20	173^e	55	13bcij	61	8^{bfgi}	53	20^{bfgi}	89	7^{bdfj}
AVG			162.9		36.1		27.5		45.1		25.1
STDEV			85.2		17.5		23.9		25.9		20.0

3.3.4 Fecal bacterial diversity

Sequencing of 16S rRNA gene fragments from fecal DNA, using Gray28F and 519R primers, generated 49,578 sequences with an average length of 313.7 bp after being filtered for length (<200 bp) and quality. On average, $4,131.5 \pm 637.5$ sequences were observed per sample, generating a total of 11,733 OTUs for community comparisons (Supplemental information, Table A.2). Table 3.4 outlines how species richness and bacterial diversity increased between d1 and 143 through shifts in the Observed Species and Shannon's indices, respectively (P < 0.05, n=4 per sampling date). No changes to species richness or bacterial diversity were observed between diets, and dominant phyla were not found to change over the feeding period or between diets, however, changes were observed between the less dominant *Cyanobacteria* and *Lentisphaerae* phyla over time (P <0.05, n=4, Table A.3). Numbers of *Ruminococcus* were found to increase over time (n=4, P < 0.05), however not between diets (Table 3.4). The changes are in agreement with the principal component analyses, which showed isolation of the d1 sample by component and conversely, an isolation of the sample from d143 through the analysis of principal component two (Table 3.4).
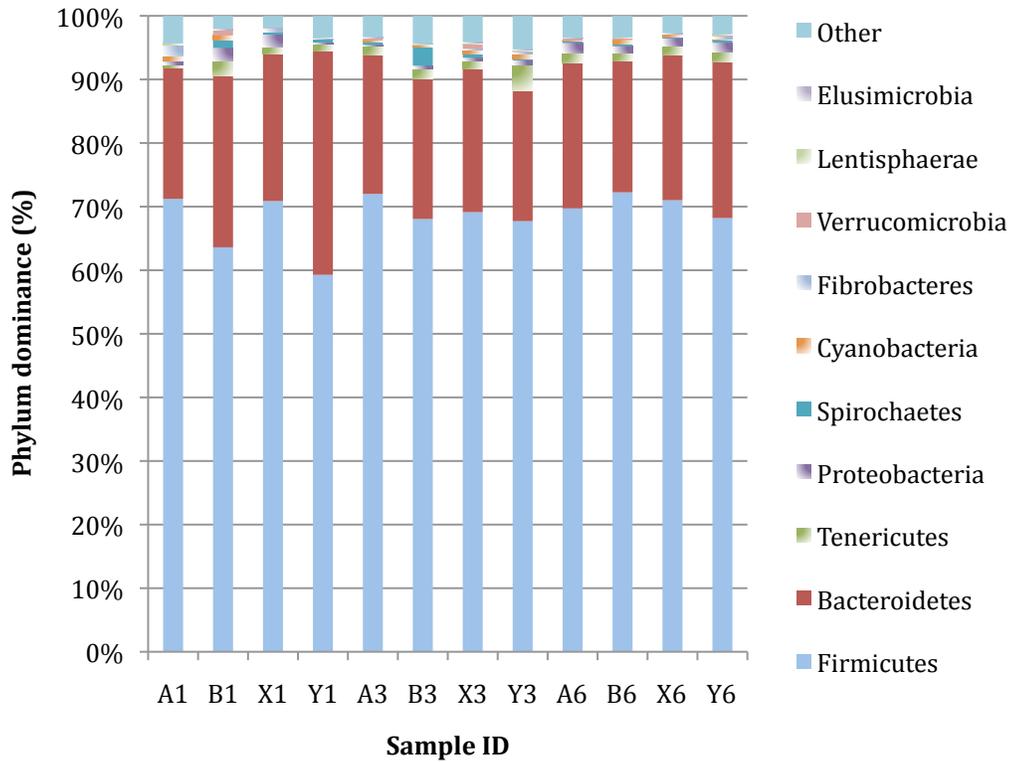
Figure 3.4 Bacterial Phyla diversity between samples. Letters of sample ID indicate pens and diets that feces originated from: A and B from cattle fed a 143-day diet supplemented with 6-7 % winery by-products (WB) and X and Y from cattle fed diets supplemented with 6-7 % water as the control (C). The numbers in sample ID indicates sampling event, 1 at d1, 3 at d63 and 6 at d143.

Table 3.4 Bacterial diversity and species richness of fecal samples obtained from cattle fed 143-day diets of 6-7 % WB or 6-7 % water as the control and sequenced using bTEAFAP pyrosequencing as outlined through Chao1, Shannon's diversity (Shann.) as well as the observed species (Obs. Spec.) indices at 2,518 sequence reads and Principal Component analyses of sequence reads (PCoA) and the change in Phylum and Genera between samples over time (P1 = *Firmicutes*; P2 = *Bacteroidetes*; P3 = *Tenericutes*; P4 = *Proteobacteria*; P5 = *Spirochaetes*; P6 = *Cyanobacteria*; P7 = all other observed Phyla; G1 = *Ruminococcus*); Letters indicate statistically significant similarities between numbers in columns.

Sample Description ID	Date	Diet	Phylum identification (%)				Genera (%)	PCoA		
			P4	P5	P6	P7	G1	PC1	PC2	PC3
WB1	1	WB	0.5	0.1	0.7	6.4	26.9[ab]	0.195[a]	-0.121[bc]	0.054
C1	1	C	2.1	1.2	0.7	3.1	18.8[b]	0.258[a]	0.008[bc]	-0.098
WB64	64	WB	1.9	0.4	0.1	2.5	17.0[a]	-0.136[b]	-0.010[bc]	-0.06
C64	64	C	0.3	0.5	0	3.7	22.4[ab]	-0.143[b]	-0.204[c]	0.101
WB143	143	WB	0.2	0.4	0.3	3.9	29.9[a]	-0.050[b]	0.261[a]	-0.032
C143	143	C	0.4	2.9	0.3	4.6	29.6[a]	-0.125[b]	0.067[ab]	0.034

ID	Sample Description		Richness	Bacteria Diversity	Phylum identification (%)		
	Date	Diet	Chao1	Shann.	P1	P2	P3
WB1	1	WB	9.6[b]	1244[b]	71.2	20.6	0.6
C1	1	C	9.2[b]	1166[b]	63.6	27	2.2
WB64	64	WB	10.0[ab]	1492[a]	70.9	23.1	1.1
C64	64	C	10.1[a]	1531[a]	59.3	35.2	1.1
WB143	143	WB	10.0[ab]	1483[a]	72	21.8	1.4
C143	143	C	10.0[ab]	1482[a]	68.1	22.1	1.6

3.3.5 *E. coli* isolate fingerprinting

To determine if diet impacted the diversity of EC isolated immediately prior to animal harvest (d143), BOX-PCR generated fingerprints were analyzed using Multi-Dimensional Scaling Plots. Fingerprint types from cattle fed WB and C diets were found to cluster together and not in distinct groups (Figure 3.5). Similarly, no distinct clustering was observed between EC isolated from various selection media (Figure 3.6).
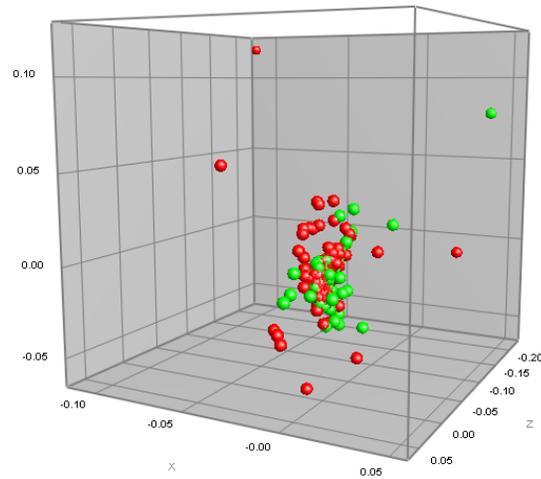
Figure 3.5 Multi-dimensional scaling plots of Rep-PCR patterns created from EC obtained from cattle fed 143-day diets of 6-7 % winery by-products (Green), or water as the control (Red) at harvest.
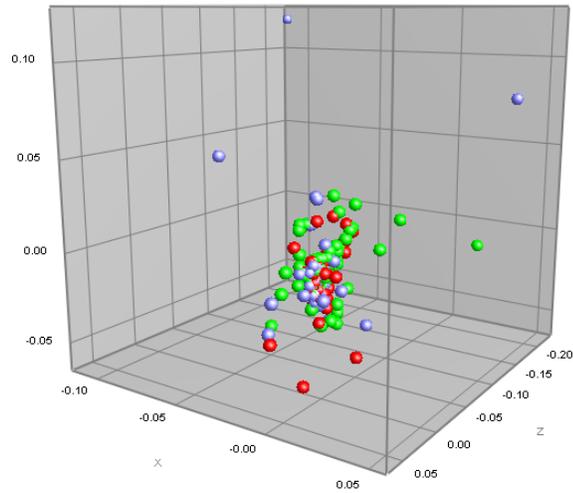
Figure 3.6 Multi-dimensional scaling plots of Rep-PCR patterns obtained from *Escherichia coli* **isolates obtained from the feces of cattle fed 143-day diets of 6-7 % winery by-product or 6-7 % water as a control by membrane fecal coliform plates containing no antibiotics (Red), 4 μg mL^{-1} Tetracycline (Green), or 4 μg mL^{-1} Ampicillin (Blue).**

3.4 Discussion

The impact of diet on the loads of fecal pathogens has been a topic of research for decades (Brownlie & Grau 1967; Callaway et al. 2009). Diez-Gonzalez et al. (1998) found shifting grain finished animals to hay rations before harvest to be an effective strategy for reducing the loads of EC at harvest. Other approaches that have been evaluated for reducing fecal microbial loads are the inclusion of Sainfoin tannins and direct-fed microbials (probiotics) to feeds, and the use of phages to reduce the loads of specific pathogens in cattle feces (Callaway et al. 2003; Loneragan & Brashears 2005; Sheng et al. 2006; Casey et al. 2007; Mirzaagha et al. 2011). The goal for this research was to evaluate the impacts of supplementing cattle finishing rations with WB on the loads of fecal EC, the resistance of these isolates toward ampicillin and tetracycline antibiotics, and overall fecal microbial diversity.

Switching cattle diets from silage (d64) to hay based rations (d98) increased EC loads in feces, and is in agreement with the observations of Brownlie and Grau (1967) (Brownlie & Grau 1967). Conversely, over the feeding period cattle fed the WB feeds were found to have higher fecal EC loads when comparing the interactions of time×diet ($P = 0.0498$). This is contrary to our original hypothesis as it was thought that the antimicrobial and polyphenol contents of WB could reduce the EC loads in cattle feces.

The elevated EC loads observed between diets were not found to increase the number of samples harbouring EC resistant to ampicillin or tetracycline antibiotics ($P = 0.4247$ and 0.0806 respectively); however, the number of samples harbouring EC resistant to tetracycline increased with time on feed ($P=0.0062$). Unfortunately, the concentration of ampicillin in plates used in this experiment was inappropriately set to 4 μg mL^{-1} instead of the breakpoint concentration of 36 μg mL^{-1} or 50 μg mL$^{-1}$ as done previously (Mirzaagha et al. 2011). To accommodate this, the numbers of samples containing EC capable of growth in 32 μg mL^{-1} ampicillin was evaluated at harvest (Results not shown). Diet had no effect on the number of samples harbouring resistant EC when plated on media containing 32 μg mL^{-1} ampicillin ($P = 0.3268$). The decision to not correct this mistake was decided upon to maintain consistency throughout the experiment. If

the limit was set to 50 μg mL^{-1} it is expected that the number of samples harbouring resistant EC would have increased over time, but not between diets as seen in the tetracycline study.

The MIC study identified greater average MIC values in EC when compared to other studies. These changes are likely due to the differing methods, where we evaluated resistance patterns up to 128 μg mL^{-1} and other studies stopped evaluating for resistance past the 32 μg mL^{-1} resistance threshold (Alexander et al. 2008, 2011; Mirzaagha et al. 2011). The average MIC values of EC varied greatly over time. In general, the values for both ampicillin and tetracycline decreased with time on feed regardless of the antibiotic used for isolation. This in agreement with Alexander et al. (2009) where the average MIC of EC from animals fed no antibiotics was greatest at d0 and decreased there after. MIC values from EC isolated were heavily impacted by selection method. This was observed as EC from cattle fed WB feeds had elevated MIC values when isolated on mFc^T plates, and reduced MIC values when isolated on mFc^A plates compared to EC from C fed cattle. Additional trials identifying specific genes present in isolated EC would assist in understanding this polarization.

When comparing the changes to the diversity of EC isolates at harvest, the diversity among isolates within both diets and isolation methods was too great to identify any degree of dissimilarity in BOX-PCR patterns. This lack of change is unexpected as other studies utilizing Pulsed Field Gel Electrophoresis have were able to differentiate from cattle fed differing back grounding diets (Su et al. 2011).

Many studies have evaluated the bacterial communities in soils, animals and humans using deep sequencing technologies and this present study adds more knowledge to this area of research (Finegold et al. 2010; Gaidos et al. 2011; Nacke et al. 2011; Handl et al. 2011; Goddard et al. 2012; Suchodolski et al. 2012). The Phyla observed in our study are similar to those observed by Durso et al. (2010), as we found that *Firmicutes*, *Bacteroidetes*, and *Tenericutes*, dominated the fecal Phyla at 68.5, 23.6, and 1.6 %, respectively, where Durso et al. (2010) found *Firmicutes*, *Bacteroidetes* and *Proteobacteria* to represent 62.8, 29.5, and 4.4 %, respectively. The similarities between these two studies is expected as overlap occurred with sequencing 16S regions; i.e., Durso et al. (2010) sequenced within the 27 and 1392 bp and we sequenced within the 27 and 519 bp region of the 16S

gene. When comparing identified Genera with the literature, Rice et al. (2012) similarly found *Ruminococcus* spp. to dominate cattle feces, similarly their study found that the members of this Genus were impacted by the concentrations of Dried Distillers Grain in diets, which is similar to our results. These reports illustrate how this dominant Genus is highly impacted by cattle diet and stresses further understanding of its impact on cattle health.

3.5   Conclusion

Supplementing cattle feeds with winery by-products (WB) was not found to alter the bacterial populations within cattle feces. However supplementing cattle rations with fermented winery by-products (WB) was found to increase the EC loads over time ($P = 0.0498$). Although greater levels of EC were found, the proportion of samples containing resistant EC was not altered ($P > 0.0874$). The isolation methods used were found to alter the resistances of EC towards tetracycline, however no consistent link was observed between diet and antimicrobial resistance patterns in EC. In summary, the use of WB as cattle feed supplements did not alter the loads of EC by diet as well as the numbers of samples containing antimicrobial resistant organisms, or the diversity of fecal samples.

4.   PROJECT SUMMARY

4.1   Overview

This thesis has described the impacts of a wine by-product (WB)-supplemented cattle feed on feed intake, meat quality, temperament, as well as the changes to EC loads, associated antimicrobial resistance profiles and the bacterial diversity within cattle feces. Cattle fed WB feeds required less feeds to yield carcass weights similar to cattle fed the control ration. Further, feeding WB-supplemented feeds was not found to change the tenderness, chemistry or colours of meats, with the exception of ground steak meat that was found to be darker in colour in cattle fed WB finishing rations. As such, it is hypothesized that chemical compounds not evaluated in feeds, such as polyphenols or condensed tannins, may have caused the observed changes to feed intake in cattle.

Another aspect of this research was to evaluate the impacts that WB-supplemented feeds had on cattle behaviour. At the commencement of this project the marketers of the "wine-finished beef" product made un-validated claims suggesting that feeding WB to cattle altered their behaviour (Findlay 2010). To evaluate these claims, the flight speed of cattle was determined over the feeding period to objectively measure animal temperament. From this research it was determined that cattle became habituated to handling over time, which is in support of other published results (Petherick et al. 2009; Schwartzkopf-Genswein et al. 2012). However, WB-supplemented feeds were not found to alter the flight speeds of cattle.

The potential for feeding cattle WB-supplemented feeds to reduce the *Escherichia coli* (EC) loads in feces was evaluated also evaluated. When comparing the impacts of the WB-supplemented feeds on pre-harvest EC loads, fecal EC were found to increase over the feeding period in WB fed animals over time, when compared to the control. However, no changes between diets were observed when comparing the number of samples containing EC resistant to ampicillin or tetracycline antibiotics.

74

To further evaluate changes to antibiotic resistance in feces the minimum inhibitory concentration (MIC) of EC was monitored. The results of this study found that the resistance of EC towards antibiotics decreased with time on feed, which is in support of other published research (Alexander et al. 2008). The isolation method of EC was found to alter the MIC of EC over the experiment as changes between diets were not consistent throughout isolation methods. Of note, many pens were found to contain EC able to withstand ampicillin and tetracycline antibiotics at concentrations over 100 μg mL^{-1} indicating a great level of resistance in fecal EC even among cattle not fed antibiotics. As such, this stresses the need for further research to identify novel production methods that can reduce the antimicrobial resistance in fecal pathogens pre-harvest.

Recent advancements in sequencing technology have revealed that the diversity of fecal bacterial is directly related to animal health, as such it was important to understand if WB can alter the diversity of fecal bacterial (Mao et al. 2012). To accomplish this, repetitive extragenic palindromic-PCR (Rep-PCR) and pyrosequencing were used to evaluate changes to the diversity of EC and the diversity of global fecal bacterial communities respectively. The diversity of EC at harvest, was not found to be impacted by diet or isolation method, through multi-dimensional scaling comparisons. However, the communities of fecal bacteria were observed to change over time, but not between diets. Specifically, the diversity and species richness of samples increased with time on feed, however diversity did not increase after the d64 sampling event. These changes were supported in the principal component analysis of this data, which found the components at d1 to be different from the other sampling events with no changes observed between diets. When comparing the populations of bacteria, the numbers *Ruminococcus* spp., the dominant bacterial Genus, were found to increase over time, however, not between diets. No changes were observed within the dominant bacterial Phyla over time, indicating that feeding cattle WB-supplemented rations caused minimal changes to the bacterial diversity of their feces.

4.2   Concluding remarks

In conclusion, feeding cattle winery by-product (WB)-supplemented feeds appears to be a safe way to produce a novel niche beef product "wine-finished beef". This product offers economic incentives to producers in the form of feed reductions, additionally, the ground steak meat of WB finished animals was found to be darker in colour than animals fed the control, with no other changes observed to the tenderness, chemistry or colour of meats. WB-fed animals were found to have elevated EC loads compared to control animals, however no changes were observed to the antimicrobial resistant populations and their virulence between diets. Global fecal bacterial diversity changed over time, with no changes observed between diets. In conclusion, supplementing cattle rations with WB was not found to alter the behaviour of the cattle as measured by flight speed or antimicrobial resistance in EC while reducing the feed required to meet slaughter weights when compared to animals fed the control ration. The results of this study indicate that the practice of feeding WB supplemented rations to beef cattle to produce "wine-finished beef" appears to have altered the internal meat colour of the final beef product, without negatively impacting either food safety or animal behavior as determined by the measures utilized in this research project.

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APPENDIX A

6.   **SUPPLEMENTARY INFORMATION**

Table A.1. Fecal *E. coli* loads and proportions (%) of fecal samples containing *E. coli* capable of growth on membrane fecal coliform agar containing with the indicator BCIG with no antibiotics (mFc), 4 μg mL^{-1} ampicillin (mFc^A) or 4 μg mL^{-1} tetracycline (mFc^T) from cattle fed diets supplemented with 6-7 % of either winery by-products (WB) or water as a control (C). Letters represent statistically similar numbers (95 %), not compared between isolation methods, and numbers in brackets are sample size (n).

Diet	Media	Units	Time on feed (d)					P (repeated measures MANOVA)		
			1	36	64	98	143	Diet	Time	Diet x time
WB	mFc	Log CFU	7.62^{adeh} (12)	6.69^{bcfh} (12)	6.17^{bcfh} (12)	7.65^{acdeh} (12)	7.65^{adeh} (11)	0.0774	<0.0001	0.0498
C			7.57^{adeh} (12)	5.88^{bcfg} (12)	5.42^{cfg} (7)	7.68^{deh} (12)	7.06^{abcdeh} (10)			
WB	mFc^A	%	97.14^a (35)	97.14^a (35)	94.29^a (35)	97.14^a (35)	100^a (33)	0.0874	0.0647	0.4247
C			96.97^a (33)	84.85^a (33)	81.82^a (33)	93.55^a (31)	96.77^a (31)			
WB	mFc^T	%	100.00^a (35)	90.91^a (35)	96.97^a (35)	96.77^a (35)	100.00^a (33)	0.1008	0.0062	0.0806
C			93.94^a (33)	84.85^a (33)	72.73^b (33)	93.55^a (31)	96.77^a (31)			

Table A.2. Sequence numbers between samples from cattle fed winery by-product (WB) and water (C) supplemented feeds over time.

| Sample ID	Diet	Pen	Date	Sequences
A1	WB	A	1	5233
B1	WB	B	1	4091
X1	C	X	1	4477
Y1	C	Y	1	2777
A3	WB	A	63	4539
B3	WB	B	63	3722
X3	C	X	63	3745
Y3	C	Y	63	4550
A6	WB	A	143	3864
B6	WB	B	143	3618
X6	C	X	143	4660
Y6	C	Y	143	4302
			Total	49578
			Average	4131.5
			Stdev	637.5
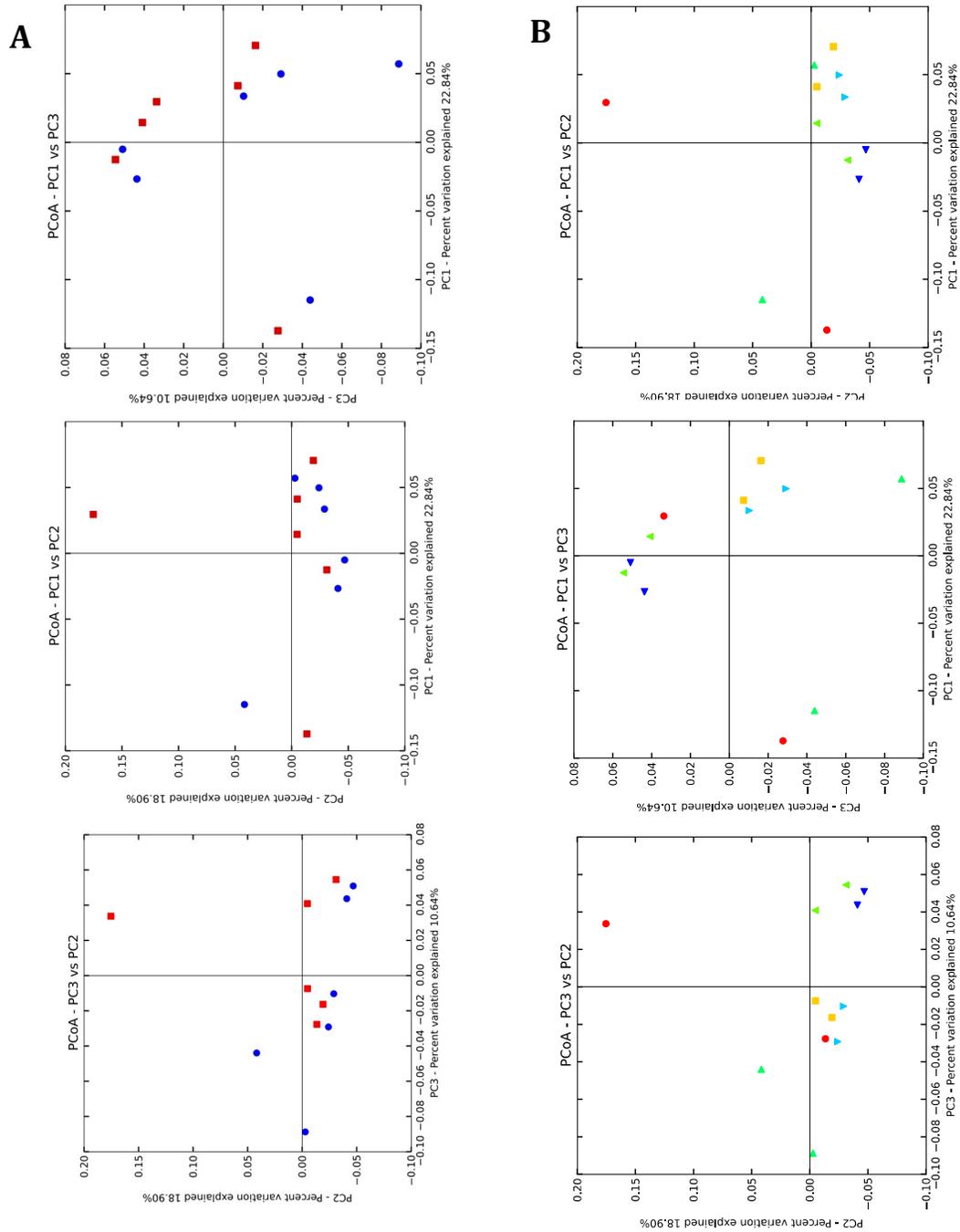
Figure A.1 Principal component analysis of bacterial diversity between fecal samples from cattle fed experimental diets using discrete un-weighted UNIFRAC parameters. A. Diversity between cattle fed a diet supplemented with 6-7 % winery by-products (●) and cattle fed a diet supplemented with 6-7 % water (■) at d1, 64 and 143 on feed. B. Sample diversity between cattle fed 6-7 % winery by-products (d1►, d64▼ and ◄ d143) and cattle fed 6-7 % water as the control (d1●, d64■ and d143▲)
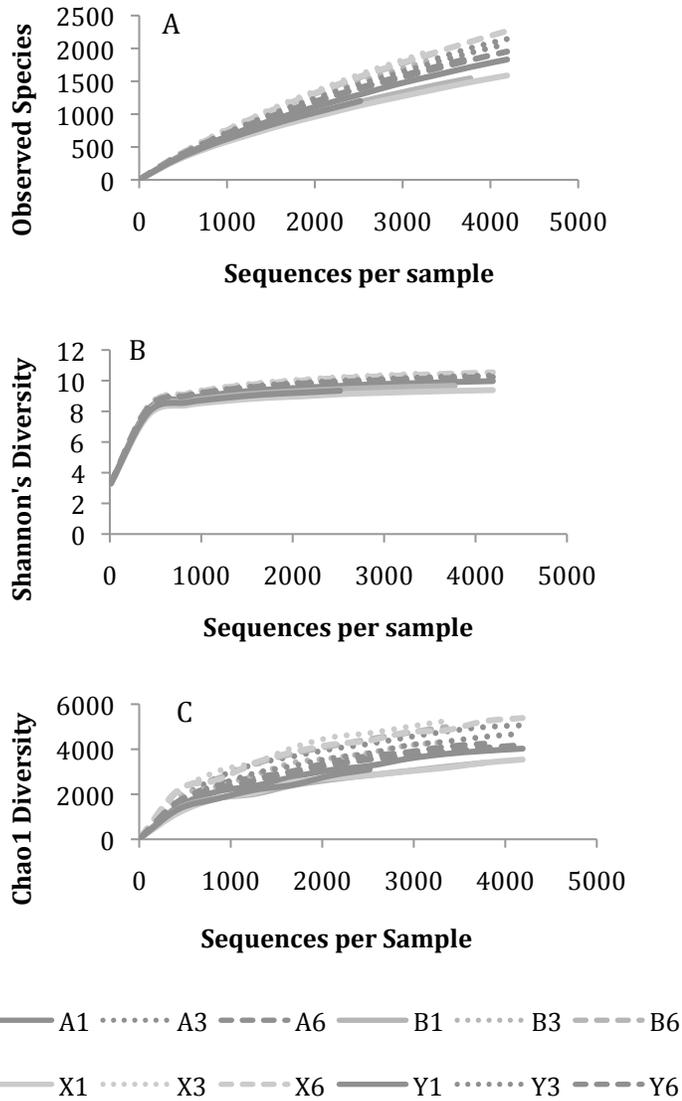
Figure A.2. Bacterial diversity and species richness between samples from cattle fed experimental diets. A) Changes between observed species; B) changes to Shannon's diversity; and C) Chao1 richness index.

Table A.3 Diversity within bacterial Phyla and Genera populations between cattle fed experimental diets. Numbers in sample ID indicate sampling session: 1 at d1, 3 at d63 and 6 at d143. Letters indicate statistically similar numbers between each Phyla or Genera (P < 0.05).

Phylum Genus	WB1	C1	WB3	C3	WB6	C6	ANOVA
Firmicutes	67.41	65.07	70.03	68.42	70.99	69.60	0.7787
Ruminococcus	22.87^{ab}	29.78^a	28.11^a	19.74^b	25.40^{ab}	29.88^a	0.0974
o_Clostridiales; Other	3.01^c	4.34^a	4.29^{ab}	3.35^{bc}	4.69^a	3.78^{abc}	0.0309
c_Clostridia; Other	5.14^a	3.55^{ab}	3.25^{ab}	4.22^{ab}	3.36^{ab}	2.81^b	0.2894
g_Oscillospira	2.42^a	1.31^b	2.45^a	2.25^{ab}	1.25^b	2.25^{ab}	0.0872
c_Clostridia;g_ unnamed	0.68^a	0.42^{ab}	0.36^b	0.55^{ab}	0.55^{ab}	0.51^{ab}	0.2907
g__Dorea	0.44^{bc}	0.58^{abc}	0.64^{ab}	0.30^c	0.32^c	0.78^a	0.0413
Bacteroidetes	23.80	29.15	21.95	21.56	21.75	23.69	0.4946
Tenericutes	1.40	1.11	1.50	2.59	1.40	1.53	0.7307
g__Sutterella	0.04^b	0.09b	0.73a	0.15b	0.11b	0.60a	0.0011
Proteobacteria	1.27	1.09	0.31	0.63	1.34	1.28	0.6356
Spirochaetes	0.69	0.45	1.65	0.44	0.27	0.38	0.5866
Cyanobacteria	0.71^a	0.04^c	0.34^{abc}	0.62^{ab}	0.41^{abc}	0.25^{bc}	0.0749
o__YS2;g_other	0.71^a	0.33^{abc}	0.40^{abc}	0.04^c	0.59^{ab}	0.25^{bc}	0.0615
Fibrobacteres	0.84	0.41	0.00	0.31	0.00	0.19	0.6008
Verrucomicrobia	0.48	0.01	0.27	0.40	0.32	0.11	0.5861
Lentisphaerae	0.18a^b	0.02^c	0.04bc	0.23^a	0.08^{bc}	0.13^{bc}	0.0657
Elusimicrobia	0.01^b	0.00^b	0.04^{ab}	0.09^a	0.01^b	0.01^b	0.0843
Planctomycetes	0.09	0.00	0.00	0.05	0.00	0.00	0.3864
Fusobacteria	0.00	0.00	0.00	0.00	0.03	0.00	0.4894
TM7	0.00	0.00	0.01	0.00	0.00	0.01	0.5865
Chloroflexi	0.00	0.00	0.00	0.00	0.00	0.02	0.4894
Actinobacteria	0.00	0.00	0.00	0.00	0.01	0.00	0.4894
Acidobacteria	0.00	0.00	0.00	0.01	0.00	0.00	0.4894
Other	3.11	2.65	3.86	4.64	3.39	2.78	0.3368
Unclassified	0.01	0.00	0.00	0.00	0.00	0.00	0.4894