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Effects of wine grape by-products on ruminal fermentation and nutrient degradation in vitro

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Table of contents

1 Introduction

By-products are secondary products obtained by processing primary products or during the harvest of food crops (Grasser et al., 1995). In general, by-products do not have much direct value as a food source for humans but still have nutritional value as feed for animals.

Using by-products as animal feed has several ecologic and economic advantages, one being that by-products are mostly of relatively low cost compared to traditional commonly used feed sources. Livestock feeding costs are one of the most important variable costs when it comes to livestock farming. Thus, the possibility to turn by-products into cost effective feed sources, which do not negatively affect livestock productivity, holds a great value for successful production (Grasser et al., 1995). Another benefit is that in recovering such products as a feed source for livestock, the agro-industry is able to lower waste-management costs, as well as waste discharges. It also has a positive influence on the environment as it helps to lessen pollution. This aspect is of ever-growing importance, as the human population increases, and with it, the quantity of food-byproducts and food wastes. With a higher human population, it is important to lower the dependency of animals on food sources, like cereal grains, that could also be used by humans (Bampidis and Robinson, 2006; Grasser et al., 1995); mainly because feed production accounts for most of the water consumption in livestock farming (Mekonnen and Hoekstra, 2012; Pimentel et al., 2004). Using food wastes and by-products more efficiently would lower the consumption of valuable resources such as water, land, or fertilizer and reduce waste as well as negative effects on the environment, while at the same time providing feed for farm animals (Ajila, 2012; Dou et al., 2018; Foley et al., 2011; Grasser et al., 1995).

Almost any by-product can be used as animal feed, as long as it holds enough nutritional value (Grasser et al., 1995). In fact, a great variety of by-products and food wastes are already successfully used in livestock feeds. However, a lot of by-products are fiber-rich and therefore have more value as feed for ruminants than for monogastric animals due to their ability to process feeds with a high fiber content. Still, when selecting by-products, several factors should be considered, such as quantity, their nutritional value, palatability, shelf life, and possible toxicity or contamination. Notably, by-products can also vary in nutrient content due to different processing or variation of original materials, thus a batch-to-batch divergence must be considered.

Many fruits and vegetable by-products also contain functional compounds that may have health-promoting effects on livestock. In this context, grapes stand out in terms of availability and functional properties. In the process of winemaking, a considerable amount of solid waste namely grape pomace is generated. Around 0.3 tons of grape pomace are produced by processing one ton of grapes (Oliveira and Duarte, 2016). This waste material is either discarded, which is linked to high disposal costs, or used for grappa production, which leads to more solid waste after production. Also, grape seeds can be separated and used for grape seed oil extraction, leaving grape seed meal as the final by-products. Both grape pomace and grape seed meal hold potential as ruminant feeds. In fact, wine by-products are generated worldwide, and some feed suppliers already sell grape pomace commercially. Grape seeds are rich in biologically active compounds such as polyphenolic compounds, which are associated with antioxidant power, as discussed by Yilmaz and Toledo (2004). Research has shown some positive influences of grape pomace or grape seed meal on animal productivity (Ragni et al., 2014; Moate et al., 2014; Mokni et al., 2017), as well as on products from those animals by enriching health-beneficial fatty acids in tissue and milk (Ragni et al., 2014; Correddu, 2015; Correddu et al., 2016). Effects on ruminal fermentation have also been studied (Spanghero et al., 2009; Moate et al., 2014, Khiaosa-ard et al., 2015). It must be noted that it is more sustainable and cost-effective to promote the use of local by-products. In Austria, growing grape for wine production is an important agro-industrial factor. According to STATISTIK AUSTRIA (2021b), 2.40 million hectoliters wine were produced in Austria in 2020. Thereby, the land use accounted for a planted vineyard area of 46,165 hectare in the year of 2020 (STATISTIK AUSTRIA 2021a). According to FAOSTAT (2021), a total of 309,920 tons of grapes were produced in Austria in 2019. Properties of the by-products depend on the grape varieties and the way they are processed. It is likely that the same by-products could differ considerably from country to country or even region to region and thus local knowledge has to be acquired.

2 Research Questions, Hypothesis, and Aim

This thesis will focus on the common grape by-products: grape pomace and grape seed meal, locally produced in the Lower Austria region of Austria, and their effects on rumen fermentation and nutrient degradation. For this thesis, an *in vitro* experiment based on the Rumen Simulation Technique (Rusitec) was conducted to answer the following questions: What effects do these two different wine grape by-products have on ruminal fermentation and nutrient degradation parameters and are there any negative effects? It is hypothesized that wine grape by-products will not express negative effects on ruminal fermentation and nutrient degradation. However, they may possess different functional effects due to their differences in nutrient composition as well as their content of functional compounds.

3 Literature Review

3.1 Ruminal fermentation and its influence on health and productivity

3.1.1 The rumen: anatomy and function

The rumen serves ruminants as a part of the forestomach by degrading the plant-based diet, which could otherwise not be utilized, with the help of microorganisms (König et al., 2015; Salomon, 2015). Another important function in which the rumen takes part is the process of rumination, which is important for microbial fermentation because it gives the microorganisms a larger surface on the feed particles by finely masticating the feed as well as maintaining the physiological pH conditions via salivary buffering (Reece, 1997).

The rumen is divided into smaller sections on the inside by numerous pillars, the so called *pilae ruminis,* which move the contents within the rumen through contractions (Salomon, 2015; Reece, 1997). The ruminal mucosa consists of *papillae*, which expand the effective size of the rumen's inner surface by seven times to maximize the absorption of vitamins, water, short-chain fatty acids and non-protein nitrogen compounds (König et al, 2015). Their quantity and size are subjected to the type of feed (Salomon, 2015). Within the rumen, its contents are divided into three layers. A gas dome fills the most dorsal part. The layer underneath the gas consists of long fiber particles which float as a mat on top of a liquid layer. The mat is formed by forage that was taken in recently. The most ventral layer is comprised of liquid, smaller particles, and heavier particles, which sink to the bottom (Ishler et al., 1996; Krehbiel, 2014).

Ruminants cannot extract energy from plant cells themselves because their own enzymes are not able to break down the cell wall components (Reece, 1997). In order to properly ferment plant-based feeds inside the rumen, ruminants form a symbiosis with microorganisms like bacteria, protozoa and fungi. The host ensures a consistent feed intake, consistent body temperature and sufficient saliva secretion for a consistent flow. This environment could not be provided by the microbiota themselves (Breves, 2015). In turn, the microorganisms help the host by degrading feed components such as cellulose with their microbial enzymes and synthesizing energy and high-quality nutrients such as microbial protein or certain vitamins (Breves, 2015).

3.1.2 Rumen microorganisms

The rumen microbiota are responsible for many different tasks in the rumen; the most important ones being fermentation of carbohydrates, hydrolysis of proteins, hydrolysis of triglycerides and the production of B-vitamins (Reece, 1997). The microorganisms bypass the rumen and subsequently are digested in the small intestine and abomasum, therewith providing high quality protein for the host (Breves et al., 2015; Moran, 2005). The rumen microbiome consists of three main microorganisms which interact with each other to form functional communities. Bacteria and protozoa each account for about 10 % of the rumen volume. Anaerobic fungi are present as well, but to a lesser extent. Inside the rumen subsist different habitats for the microbiota. Some are attached to fibers, some are associated with rumen liquid, while others are affiliated with the rumen epithelium (Breves et al., 2015). Mizrahi and Jami (2018) discussed several studies which focus on the comparison between the different habitats. They summarized that the living spaces differ in microbiota composition, with the fiber adherent and the fluid-affiliated groups being rather similar, and the epithelium-associated group being almost entirely different from the other two.

The rumen itself is inactive in newborn calves and does not significantly contribute to digestion yet, as the milk physiologically evades the rumen by a reflex in the esophagus (Van Soest, 1994). Aerobic, facultative anaerobic and anaerobic microbiota colonize the rumen right after birth. The aerobes and facultative anaerobes continuously decrease during the first weeks of the young ruminant's life, while the anaerobes increase. Within the first week, cellulolytic bacteria and methanogenic bacteria can be found. Fungi are present from around the second and third week of life. Anaerobic community thrives when solid food is offered (Fonty et al., 1987). According to Jami et al. (2013), bacterial communities are especially affected by age, apart from diet, which they showed in groups from one day to two year old calves and cows.

The productivity and growth of ruminal microorganisms is heavily influenced by the environment of the rumen itself. For fermentation and thereby the production of symbiotic end products, the microbes need favorable conditions. Fermentation parameters such as pH-value, redox potential and osmolality, which will all be detailed later on, are considered important physiochemical factors and are required to be within a certain range for the microbes to thrive (Castillo-González et al., 2014; Herdt and Sayegh, 2012).

3.1.2.1 Bacteria

The post-natal bacterial colonization of the rumen takes place through the environment, the presented feed and through contact to other animals. The density of rumen bacteria is around 10^9 to 10^{11} per ml in adult ruminants. While aerobic and facultative aerobic bacteria are present, they only account for a small content of the total bacterial microbiome. The main content consists of anaerobe bacteria (Breves et al., 2015).

The larger part of bacteria, around 70 %, are attached to surfaces, mostly feed particles but also the rumen epithelium, while the other 30 % are linked to the rumen liquid (Zhou et al., 2020). Those bacteria form small colonies which can consist of different kinds of bacteria. Rumen epithelium-associated bacteria are especially connected with keeping the anaerobic environment inside the rumen by eliminating the oxygen which is brought to the rumen with digesta (Breves et al., 2015). They also recycle the rumen epithelium by assimilating disconnected cells and digesting them (Zhou et al., 2020). An important characteristic of rumen bacteria is their ability to synthesize vitamin K and B-vitamins which are important for the host and other ruminal microbes (Dehority, 2003). Diet has a large impact on the composition of the bacterial microbiome. Cellulolytic bacteria are more abundant when diets contain large amounts of fiber, whereas a large amount of starch in the ration is associated with a thrive in amylolytic bacteria (Breves et al., 2015). Dehority (2003) pointed out that bacteria also provide nutrients on which fungi and protozoa depend.

3.1.2.2 Archaea

Archaea are obligate anaerobes which, like the bacteria, colonize the rumen within the first days after birth. Their density is about 10^8 to 10^9 per ml (Breves et al., 2015). They mainly function as methanogens, producing methane by the reduction of carbon dioxide (Attwood et al., 2020). This process also limits the production of ethanol and lactate by keeping the partial pressure of hydrogen low. Thereby, they prevent the redox potential from becoming too negative (Breves et al., 2015). Carbon-rich and anaerobe surroundings with a low abundance of sulfate, nitrogen and oxidized iron are optimal for these archaea. Because of their methane production, they have been targeted by research for a solution to reduce greenhouse gas emissions from animal farms and to lower this negative effect of ruminants on the environment (Attwood et al., 2020).

3.1.2.3 Protozoa

Protozoa are divided into two groups, the ciliates and the flagellates. Like bacteria, they are mostly anaerobe, and their colonization of the rumen starts post-natal through contact to other animals. Ciliates have a density of 10^5 to 10^8 cells per ml. Flagellates account for a smaller part of the protozoa concentration with a density of 103 to 104 cells per ml (Breves et al., 2015). Rumen bacteria are higher in number than the protozoa, but protozoa are larger in size. This makes them take up to 50 % of the total microbial biomass within the rumen (Huws et al., 2020). Due to various parameters which have an impact on their concentration, their content in the rumen is more variable than the content of bacteria. Thereby, dietary concentrate content, frequency of feeding times and various physiochemical parameters play a part in protozoal abundances. Functionally, protozoa take part in the digestion of carbohydrates, fat and protein with their enzymes. They are also able to store easily fermentable carbohydrates, keeping them away from amylolytic bacteria, resulting in a reduced risk for rumen acidosis (Breves et al., 2015). Protozoa also provide fermentation products such as acetate, butyrate and lactate but not propionate. They provide carbon dioxide and hydrogen, which in turn can be used by some methanogenic archaea to produce methane (Huws et al., 2020). Their enzymes make the protozoa quite tolerant against antibiotics and pesticides and enable them to neutralize toxic substrates in the rumen. Furthermore, they are able to produce heavy-metal compounds that are difficult to absorb, which helps to prevent intoxications (Breves et al., 2015).

They feed on plant materials rich in protein and lipids, therefore retaining the plants' chloroplasts (Huws et al., 2020). However, they also prey upon other ruminal microorganisms like bacteria (Dehority, 2003; Huws et al., 2020). Thereby, they help to manage the population of those microbes and further their growth (Breves et al., 2015).

3.1.2.4 Fungi

Fungi in the rumen are strictly anaerobe and colonize the rumen within the first days of life. The density in adult ruminants is between 10^4 to 10^5 zoospores per ml. There is a positive correlation between the concentration of rumen fungi and the content of crude fiber in the diet. Their tolerance to temperature is quite limited at 33–41 °C (Breves et al., 2015). Fungi help other microbes in the colonization of plant-based feed particles by primary colonization and by

loosening up the fibrous materials through fibrolytic enzymes (Akin and Borneman, 1990; Hess et al., 2020). Some of them even have proteolytic properties (Breves et al., 2015).

3.1.3 Nutrient degradation and fermentation products in the rumen

The degradation of carbohydrates and proteins takes place in the rumen, which is particularly important for the function of the ruminal microbiota, as well as for the host itself, and they are closely linked together. For microbial protein synthesis, both the amount of degradable carbohydrates and protein are essential. Carbohydrates are needed as energy sources for microbes to transform nitrogen from ammonia and amino acids into microbial protein. Thus, when carbohydrates are deficient, ammonia is collected in the rumen and later excreted with urine. This results in an inefficient use of nitrogen. Protein can also be used as an energy source but the process is more energy-expensive than carbohydrates (Nocek and Russell, 1988; Seo et al., 2013).

Carbohydrates can be further divided into fibrous and non-fibrous carbohydrates. The least degradable contents of a ruminant diet are usually insoluble parts from forage plants. They mainly consist of cell wall components from higher plants (Breves et al., 2015). Those cell walls consist of polysaccharides, lignin, lipids, glycoproteins, and minerals (Naas and Pope, 2020). The most important polysaccharides of these plant cell walls are cellulose, hemicellulose, and pectin (Breves et al., 2015). The cell wall itself consists of three different layers: the primary cell wall, the secondary cell wall, and the middle lamella. Scheller and Ulvskov (2010) discussed several different cell wall models, but the primary cell wall's main components are cellulose fibers, which are in connection with pectin and hemicellulose. The secondary cell wall mainly consists of cellulose, hemicellulose and lignin (Zhong et al., 2019). The digestive value of forage plants derives from their degradability. These cell wall components are not digestible by the hosts' inherent enzymes and thus the hosts need microbial action. The rumen microbiota have generated certain tactics on how to make use of their carbohydrate-active enzymes to free the cellulose (Naas and Pope, 2020). Lignin is the least degradable component of all and its content increases within the cell wall with age (Breves et al., 2015), and so ruminal degradation of plant materials decreases with increased maturity. Due to its position within the cell wall, surrounded by hemicellulose and lignin, cellulose is quite resistant to degradation.

For the microbial degradation of fibrous (structural) carbohydrates, the feed particles must first be colonized by microorganisms. Fungi function as the pioneers and assist other microbes like bacteria in the process. However, most of the hydrolyzing enzymes come from bacteria or protozoa (Breves et al., 2015). Dehority (2003) compared several important cellulolytic bacterial species such as *Ruminococcus flavefaciens, Ruminococcus albus* and *Fibrobacter succinogenes*. According to Dehority (2003), the most important hemicellulose digesting bacteria are: *Butyrivibrio fibrisolvens, Prevotella ruminicola, Ruminococcus flavefaciens* and *Ruminococcus albus*. The most important pectinolytic bacteria are: *Butyrivibrio fibrisolvens, Prevotella ruminicola, Lachnospira multiparus* and *Peptostreptococcus*. It is worth mentioning that one ruminal bacterium carries out multiple functions. For instance, some fibrolytic bacteria are also amylolytic, such as: *Butyrivibrio fibrisolvens, Prevotella ruminicola, Fibrobacter succinogenes* and some *Clostridia* species. After the cell walls are hydrolyzed, the products are taken in by bacteria and are transformed to pyruvate via anaerobe glycolysis or the pentose phosphate pathway. Pyruvate is then further processed to fermentation gas and short-chain fatty acids. This happens so swiftly that pyruvate is almost undetectable in the rumen fluid (Breves et al., 2015). The degradation of carbohydrates is determined by the amount of feed intake, the passage rate, and the type of carbohydrate. Consequently, the breakdown of cell wall components decreases with higher feed intake or passage rate (Breves et al., 2015).

Non-fibrous carbohydrates like starch or sugars are also referred to as non-structural carbohydrates and are present in relatively high quantities within most concentrates. They are linked to increased ruminal fermentation rates, higher feed intake and a faster turnover of feed (Martin et al., 2010). Studies have shown that elevating the concentrate content in diets increases feed intake and promotes total tract digestibility and nitrogen-use-efficiency (Bayat et al., 2017; Vanhatalo and Halmemies-Beauchet-Filleau, 2020). Rations high in concentrate and low in forage also reduce methane emissions due to a higher ratio of propionate in the short-chain fatty acids and a lower pH in the rumen (Bayat et al., 2017; Martin et al., 2010), which affects microbiota composition and fiber degradation in the rumen. A study proposed that lowering the retention time of the feed within the rumen by feeding concentrate-rich diets could also play a part in reduced methane production, since concentrates are degraded quickly and methanogens need a longer time to grow sufficiently (Hales et al., 2020). However, a high content of easily fermentable carbohydrates leads to acidification of the rumen due to a fast rise

of high concentrations of short-chain fatty acids which in turn leads to a drop in pH (Breves et al., 2015). A low level of pH then restrains microbial cellulolysis and eventually harms the fiber degrading microflora. This leads to an inhibition of fiber degradation and to a decreased intake of dry matter. However, some amylolytic bacteria are still able to ferment under a lower pH down to 5.0 (Mould and Orskov, 1983). Important amylolytic bacteria in the rumen are: *Streptococcus bovis, Ruminobacter amylophilus, Succinimonas amylolytica* and *Selenomonas ruminantium* (Dehority, 2003).

In general, it is important to provide ruminal microbes with enough energy through carbohydrates for microbial growth. Otherwise, the degradation of carbohydrates might be too little to support their activity, resulting in confined microbial growth. By this, it also effects the quantity of microbial protein synthesis and the amount of needed rumen degradable protein (Mohamed and Chaudhry, 2008).

Proteolytic microbes are able to produce proteases which degrade protein to oligopeptides, dipeptides and amino acids. While protozoa and fungi can produce such enzymes, the majority of these proteases in the rumen come from bacteria (Breves et al., 2015). Dehority (2003) discussed various studies which identified the most important proteolytic bacteria: *Ruminobacter amylophilus, Prevotella ruminicola, Selenomonas ruminantium, Butyrivibrio fibrisolvens* and *Streptococcus bovis*. However, it is debated whether plant derived proteases also take a part in the ruminal protein break down, as protein from fresh grass shows a ruminal degradability of almost 100 %, whereas other sources of protein show a ruminal degradability between 30–70 %. The structure of the disulfide bonds as well as the solubility have a large impact on dietary protein degradation (Breves et al., 2015). Rumen degradable protein is hydrolyzed by the microorganisms and gets broken down into peptides and amino acids. These amino acids and small peptides get taken in by microbes and are transformed to ammonia, carbon dioxide and carboxylic acids. In the rumen fluid, ammonia is the main nitrogen consisting soluble component. A great number of ruminal bacteria are able to use ammonia as their main nitrogen source for building their cellular components. Ruminal microbes can also utilize non-protein nitrogen sources like urea that is quickly hydrolyzed to ammonia and carbon dioxide as well through urease (Reece, 1997, Mohamed and Chaudhry, 2008). Because of that, ruminal microbes can turn low-quality protein as well as non-protein nitrogen sources into microbial protein, which is also a major source of protein supply for the ruminant host.

Thus, adequate amounts of rumen degradable protein are important to keep up microbial protein synthesis and microbial growth for a sufficient level of fiber degradation (Mohamed and Chaudhry, 2008). Lee et al. (2012) observed a decrease of total-tract fiber digestibility when a deficit of microbial protein occurred. Undegradable dietary protein in the rumen is also important because high producing ruminants cannot sufficiently cover their protein demand with only the protein from the microbial protein synthesis (Schwab et al., 1992). However, excessive protein that is digested but is not utilized by the host is excreted in urine. Therefore, feeding more ruminally undegradable dietary protein than needed has no benefits, except for extra energy supply.

Fats can be partially degraded by rumen microorganisms, or they can pass the rumen without digestion. In the rumen, triglycerides are hydrolyzed by lipase and phospholipase to free fatty acids and glycerol. These fatty acids can be assimilated by the microbes and used as structural components. Unsaturated fatty acids are transformed by bacteria capable of biohydrogenation. The glycerol can be fermented to short-chain fatty acids by the rumen microbes. The rest of the dietary fat and the fatty acids is transported to the small intestines for further digestion and absorption by the host (Breves et al., 2015; Moran, 2005; Reece, 1997).

3.1.4 Ruminal fermentation parameters

3.1.4.1 Ruminal pH

Ruminal pH is physiologically slightly acidic to neutral, in the range from 5.5 to 6.8 (Herdt and Sayegh, 2012). After a meal, there is a physiological decrease in pH as a result of increased short-chain fatty acid concentration (Dijkstra et al., 2012). The pH decrease is more severe with rations high in easily fermentable carbohydrates due to a faster rise of short-chain fatty acid concentration (Breves et al., 2015).

Ruminal pH is influenced by many different factors. The type of feed itself, as already mentioned, plays a major role along with the clearance rate of short-chain fatty acids from the rumen and the different systems which lead to a secretion of buffer into the rumen. The microbial fermentation within the rumen leads to the production and sometimes accumulation of short-chain fatty acids and lactic acids, which causes a decrease in ruminal pH (Dijkstra et al., 2012). These acids can be removed from the rumen either through the rumen wall via

absorption or through passage via the liquid phase (Aschenbach et al., 2009). Both are influenced by differences in feed composition and feed intake (Dijkstra et al., 2012).
Bicarbonate is another important factor for the regulation of ruminal pH. It is mainly administered to the rumen through saliva and neutralizes the acids within. The saliva flow is stimulated by chewing and rumination. Thus, a certain amount of effective fiber is crucial to feed rations because it ensures a sufficient secretion of buffer. Rations short in fiber lead to reduced chewing activity and thereby to a lower ruminal pH as a consequence of decreased saliva secretion (Mertens, 1997).

3.1.4.2 Redox Potential

The redox potential is an electron transfer potential (Breves et al., 2015). In the rumen, redox potential is important to indicate whether oxidation or anaerobic fermentation takes place, which has an influence on metabolism and digestion of feed (Huang et al., 2018). A positive potential indicates a strong oxidative force, while a negative potential indicates a strong reductive force (Breves et al., 2015).
Typical ruminal redox potential is between -250 mV and -450 mV (Herdt and Sayegh, 2012), which indicates that oxidation-reduction reactions take place with a high reducing power (Marden et al., 2005). Anaerobe bacteria are adapted to a potential between under -250 mV and up to +100 mV (Ray, 2004). This is due to the properties of their enzymes which enable them to ferment under these conditions (Baldwin and Emery, 1960). Because of the negative potential of short-chain fatty acids in the rumen, they are not completely fermented in the rumen and therefore are available to ruminants to use them as an energy source instead (Breves et al., 2015). According to Marden et al. (2005), as well as Baldwin and Emery (1960), facultative organisms in the rumen are responsible for the redox stability by using the greatly oxidative compounds which make their way into the rumen through feeding or blood and serve as hydrogen acceptors, therewith keeping the potential at a low enough level for the anaerobe bacteria. Technical issues have been discussed to be a source for errors. Marden et al. (2005) showed in their study that it needs to be strictly measured under anaerobic conditions; otherwise, the present oxygen could distort the measurements.

3.1.4.3 Osmolality

Rumen osmolality is physiologically at around 280 mOsm/kg and is generated by osmotically active solutes. These are composed of electrolytes from the diet or the saliva, and organic acids. During fermentation, the osmolality increases due to an elevation of short-chain fatty acid concentration. Because the osmolality of extra cellular fluids and blood is slightly higher than the osmolality of the rumen, the osmotic water flow is directed outwards (Herdt and Sayegh, 2012). This normally leads to continuous water resorption throughout the day. When the rumen osmolality is elevated above 340 mOsm/kg, which can happen in high yielding dairy cows, the water flow changes its direction from the blood into the rumen (Breves et al., 2015).

3.1.4.4 Ammonia

There are two main sources of nitrogen which ruminants take in through their diet: proteins and non-protein nitrogen. Apart from the feed, there are several other sources which supply the ruminal microbes with nitrogen. This mainly takes place via secretion. Saliva for example is a source for mucoprotein and urea. There is also urea coming from the rumen wall along with exfoliations of epithelial cells (Breves et al., 2015). Microbes break down these nitrogen compounds to ammonia for the subsequent use for their protein synthesis (Reece, 1997). The ammonia is released into the rumen fluid and the concentration is physiologically at a range of 3–15 mmol/l. Ammonia can be eliminated from the rumen by absorption through the rumen wall, by passage through the digestive tract or by the already mentioned assimilation through the rumen microbes (Breves et al., 2015).

After the ammonia is absorbed through the rumen wall, it is recycled to urea by the liver. The urea then is returned to the alimentary tract through saliva or the rumen wall (Breves et al., 2015). According to Lapierre and Lobley (2001), up to 40 % of the digested nitrogen is returned to the gut as urea. With around 50 % of the urea getting transformed to mainly amino acids, this gives the ruminant an excellent way of preserving nitrogen. Huntington and Archibeque (2000) discussed that recycling urea enables ruminants to deal with a diet low in protein, while at the same time, they can manage high amounts of ammonia.

3.1.4.5 Fermentation gas

Fermentation gas is produced by microbes during their metabolic processes in the rumen. The main content is carbon dioxide (40–70 %), followed by methane (20–40 %). Oxygen, hydrogen, and nitrogen can also be present, but only in very little amounts. These three gases are not considered fermentation gases per se. Some parts of them are rather considered to be part of the atmospheric air (Breves et al., 2015). Carbon dioxide comes from several different sources. It is formed through the deamination of amino acids, as well as through carbohydrate fermentation. The neutralization of acids by bicarbonate from saliva is also a source to produce carbon dioxide. Methane is generated by bacteria which reduce carbon dioxide. Up to one liter of gas is produced in the ruminoreticulum per minute by a dairy cow (Reece, 1997). Methane production is considered to be a loss of feed energy. Furthermore, it is also relevant for the environment. It is estimated that 30 % of the global annual methane emissions originate from microbial fermentation inside the digestive tract (Breves et al., 2015).

Gas can exit the rumen in two different ways. Approximately once per minute, gas is transported through the esophagus and pharynx by eructation. Some of this gas flows into the lungs during inspiration where it is used as a carbon source (Reece, 1997). The second way is by passing through the rumen wall. While it is impossible for the ruminant to use methane as an energy source, the carbon dioxide can partly be utilized to keep a steady bicarbonate concentration in the saliva (Moran, 2005). Furthermore, carbon dioxide is used by some bacteria for both the generation of short-chain fatty acids and the assimilation into bacterial cells (Huhtanen et al., 1954).

3.1.4.6 Short-chain fatty acids

The three most abundant short-chain fatty acids in the rumen are acetate, followed by propionate and butyrate. However, to a lower degree, also valerate, isovalerate and isobutyrate are present (Breves et al., 2015). The ratio and concentration of these volatile fatty acids can be influenced by diet (Breves et al., 2015; Reece, 1997). Furthermore, the acid concentration is also increased shortly after feeding but it decreases again under physiological conditions after one to three hours. The short-chain fatty acids need to leave the rumen again to prevent long periods of low pH. Some acids pass the rumen into the abomasum, while the majority of

produced short-chain fatty acids (50–85 %) are absorbed through the rumen wall (Breves et al., 2015). The undissociated forms are able to pass the luminal membrane by diffusion due to their lipophilic properties (Dijkstra, 1994). However, the dissociated forms can only be absorbed via active transport by a short-chain fatty acid/bicarbonate-antiporter. Once the acids are inside the rumen epithelium, they are metabolized to different extents (Breves et al., 2015).

Baldwin and Barry (1996) discussed the metabolism of the different acids and their interactions. They found in their study that the metabolism of butyrate is influenced by the abundance of other volatile fatty acids. More than 90 % of butyrate are transformed to ketone bodies such as beta-hydroxybutyrate and acetoacetate (Weigand et al., 1975). Propionate is metabolized in a lesser extent to lactate (Weeks, 1974, as cited in Baldwin and Barry, 1996), while acetate is only transformed to ketone bodies to a low degree (Stevens, 1969, as cited in Baldwin and Barry, 1996). Shen et al. (2019) discovered in their study that a moderate increase of short-chain fatty acids has a positive effect on the epimural microbiota and their gene pool by increasing their variety. Furthermore, the opportunistic pathogens were shifted to live as commensals, while at the same time, the misplaced immune reaction of the host got restrained. Other positive effects were an increase of cell refreshing and tight junctions. A further enhancement of the acids and thereby acidic conditions in the rumen had the opposite effect. Epimural microbiota gene pool and diversity were reduced, opportunistic pathogens were shifted to a pathogenic life, host immune response got induced and tight junctions were reduced along with epithelial cell overgrowth.

3.1.5 Influence of diet on rumen health and overall cow productivity

There are various different dietary factors influencing rumen health as well as cow health and productivity. However, the most prominent one is the balance between structural and non-structural carbohydrates in rations for cattle. While ruminants are physiologically adapted to fibrous feeds, high concentrate diets are used to increase the animal's productivity. Such a high-nutrition diet also has negative outcomes on the animal's wellbeing. Therefore, this part of the thesis will describe the advantages and disadvantages of such a feeding strategy and its alternative options.

Especially in early lactation, the energy that cows need for milk production often exceeds the energy that the cows can take in through their conventional forage-rich diets. This can lead to

health issues and a loss of productivity (Block et al., 2001; Humer et al., 2016; Reist et al., 2002). To meet the increased energy requirements, starch rich concentrates are commonly fed in high amounts due to their ability to give large amounts of energy and a lower rumen fill effect resulting in more dry matter intake. Starch-rich feeds are also rapidly digestible in the rumen. The quick fermentation in the rumen produces high amounts of short-chain fatty acids and helps with speedy weight gain and high milk yields (Zebeli and Metzler-Zebeli, 2012; Klevenhusen et al., 2017). Furthermore, while rations which are high in structural carbohydrates are considered to produce higher amounts of enteric methane, an inclusion of nonstructural carbohydrates such as starch rich feeds can lower methane production (Chagunda et al., 2010; Johnson and Johnson, 1995). This is the result of an increased content of propionate which competes with methanogens for the utilization of hydrogen (Hales et al., 2020; Terry et al., 2020), along with inhibited methanogen growth. Thereby, a higher content of propionate also leads to an increased amount of energy accessible to the ruminant (Hales et al., 2020). However, high contents of concentrate often lead to a swift aggregation of those short-chain fatty acids and subsequently can lead to acidotic conditions inside the rumen, thereby increasing the risk of health issues for the cows (Breves et al., 2015; Zebeli et al., 2012; Kleefisch et al., 2018). It has already been shown in several studies that high amounts of concentrate can influence the rumen microbiome population in a way which can be the cause of several rumen health issues as well (Zebeli and Metzler-Zebeli, 2012; McCann et al., 2016). Huang et al. (2018) found that the content of concentrate within rations showed positive correlations with redox potential, thereby decreasing reducing conditions.

In order to maintain sufficient dietary structural properties, while supplying elevated energy and nutrients, feeding high-quality hay as an alternative to starch rich concentrates may pose as a valuable option. High-quality hay has a high amount of crude protein as well as a high content of water-soluble carbohydrates (up to 20 % of dry matter), which act as an energy source to support high milk yields while at the same time saving gut health (Kleefisch et al., 2017). According to a study from Kleefisch et al. (2017), dry matter intake as well as dry matter digestibility can be increased with an elevating content of high-quality hay in the ration, as compared to a diet with typical fibrous forage. Interestingly, the content of structure in the high-quality hay was enough to stimulate chewing and ruminating for a sufficient flow of buffering saliva despite the lower fiber content. In another study in early lactating cows, Kleefisch et al.

(2018) showed that feeding a diet consisting of high-quality hay with an inclusion of 25 % concentrate was able to enhance energy balance and had a similar milk yield compared to a diet consisting of 60 % typical hay and 40 % concentrate. This shows that the inclusion of high-quality hay could help to decrease the amount of concentrate in ruminant feed, thereby having a positive influence on rumen health. A study from Klevenhusen et al. (2017) discovered increased overall in situ nutrient degradation and a quicker increase of short-chain fatty acid concentration with rations including high-quality hay compared to rations with fiber-rich, low-sugar hay. However, they also showed that feeding high-quality hay leads to high ruminal ammonia concentrations caused by the high protein content in the feed along with the increased nutrient degradability, which is the major drawback of this feeding strategy. High-quality hay also had a significant effect on the rumen microbiota (Klevenhusen et al., 2017). For instance, relative *Ruminobacter* abundance decreased in the rumen fluid with rising content of high-quality hay. Most abundant in the solid rumen fraction were *Fibrobacter* and *Prevotella*. A study from Petri et al. (2018) showed how the population of rumen epimural bacteria also reacts to a change of carbohydrate source from concentrate to high-quality hay and an elevation of protein in the diet. Accordingly, the inclusion of high-quality hay modified the epimural microbial population from the most abundant being *Firmicutes* to *Proteobacteria*. In total, the diversity was reduced when high-quality hay was part of the ration compared to the control diet with typical hay.

3.2 Wine by-products

Grapevine (*Vitis vinifera*) is an important crop plant that is used worldwide and to a large extent for winemaking. Of around 60 vine species, *Vitis vinifera* is the one species that most of the worldwide wine production derives from (Letaief, 2016). The process of winemaking leaves several by-products such as stems, leaves, skins, pulp, and seeds. However, the physical composition of these by-products is largely influenced by the type of wine production system used (red wine or white wine, degree of maturation, equipment, etc.), which influences the nutritional and chemical composition (Bekhit et al., 2016). In general, while most by-products are often not used any further and considered as waste, some have the potential to be used as livestock feeds, which has several advantages. Since humans are not able to use most by-

products as a direct food source, there is no competition for human digestible foods as there is with cereal grains (Grasser et al., 1995). In animal production, most of the water is used for the growing of feed plants such as grains (Mekonnen and Hoekstra, 2012; Pimentel et al., 2004). Using by-products and food wastes could therefore lower the use of all valuable resources linked to feed plant production such as water, land, and fertilizer. This would also help the environment by lessening negative impacts and reducing wastes. Furthermore, it would also be an economic way to reduce waste-management costs for the agro-industry and lower feed costs, as by-products are mostly inexpensive (Ajila et al., 2012; Dou et al., 2018; Foley et al., 2011; Grasser et al., 1995). Wine by-products, for example, need very little processing to be used as animal feed (Bekhit et al., 2016), which makes them cheaper in comparison to conventional feed sources. These by-products make up around 30 % of the total grape, of which grape pomace and stems have the largest share (Makris et al., 2007). Since wine production also uses up a lot of valuable resources as mentioned above (Bordiga, 2016a), especially with such a large quantity of wastes, those resources should not be lost in vain. Importantly, since discarded wine by-products can be a source of environmental pollution. Moldes et al. (2008) found that wine production wastes like grape marc and lees might have phytotoxic effects when administered to crop fields. Stems, seeds, and grape skins on the other hand are not harmful for the environment themselves. However, because of their seasonal production and because they consist to a large degree of organic matter with a high demand of oxygen, pollution could still be an issue (Letaief, 2016; Spigno et al., 2008). Thus, it is extremely important to find a solution for both an environmentally friendly and economically effective way to make further use of wine wastes (Bekhit et al., 2016). Since by-products often are rich in fiber, when intended to be used as animal feed, they are appropriate for ruminants due to their capability of digesting feeds with a high fiber content (Grasser et al., 1995). Thus, this chapter will focus on wine by-products that are already used or could have the potential to be used as ruminant feeds with a focus on grape pomace and grape seed meal.

3.2.1 Types of by-products used in ruminant feeding

In the production chain of winemaking, vine prunings are the first by-product to occur. As discussed in Ye et al. (2016), leaves, shoots and canes are the largest part of the wastes generated by pruning. The canes and spurs are pruned in winter, while the leaves and shoots

are pruned in summer. In a study by Reynolds et al. (1995), cane pruning weight was between 0.56 and 2.04 kg per vine. Most of the pruning waste is either mulched into the vineyard or burned on-site (Letaief, 2016), which has a negative impact on the environment (Estrellan and Iino, 2010). Especially the leaves and shoots have a potential as ruminant feed (Kamalak, 2005; Magnier, 1991).

Grape stems nowadays are separated before crushing to lower the absorption of phenolic contents into the must (Jackson, 2014). However, for the production of certain premium red wines or white wines, pressing and crushing takes place with the stems still attached (Letaief, 2016). After destemming, the stems are disposed of via landfills, distilleries or in rural areas. There are several different alternatives to make further use of this by-product such as heavy metal removal from aqueous solutions, or composting (Letaief, 2016, Martínez et al., 2006; Bertran et al., 2004). Due to the high fiber content (Llobera and Cañellas, 2007), when used as animal feed (Anastasiadi et al., 2012), the stems are mostly suited for ruminants.

Grape pomace is the solid part that is left after pressing and consists of fruit pulp, fruit skin, and sometimes also seeds and stems. Pomace accounts for up to 20 % of the total grape weight (Letaief, 2016). The chemical and physical composition depends on several factors such as grape variety, grape maturity, the type of wine being produced, equipment and technique (Bekhit et al., 2016), along with whether stems and seeds are still present or already removed. Grape pomace is sometimes synonymously called grape marc or pulp, although on occasion those two terms are used for the product without seeds and stems. Grape pomace is often composted by the winery and thus utilized for the vineyard (Bordiga, 2016b). However, its high tannin content can affect the soil quality and ecosystem (Kraus et al., 2003). Another use for this by-product is the extraction of anthocyanins, which is an antioxidant (Letaief, 2016). Furthermore, grape pomace is used for grappa production in Italy, where ethanol is extracted, and exhausted pomace is left as a by-product (Ye et al., 2016; Bekhit et al., 2016). It also has the potential to be used as ruminant feed (Basalan et al., 2011; Molina-Alcaide et al., 2008). However, fresh grape pomace is perishable and needs to be either dried or ensiled to be stored over a longer period.

Fresh and wet grape pomace has a seed content of up to 30 % (Letaief, 2016). Two seeds are typically present in a grape, but this depends on the berry size (May, 2000). The seeds can be separated from the pomace and can be utilized on a large scale due to a huge range of

compounds (Bordiga, 2016c). Utilization of this by-product is within the health and cosmetic industry as grape seed extract (Bordiga, 2016b), which can be used to produce laccase, or for oil extraction via different methods (Bekhit et al., 2016). The oil can be further used to produce biodiesel, or as a high-quality dietary oil (Fernández et al., 2010; Letaief, 2016). The solid residue of oil extraction is grape seed meal, which has similar characteristics to grape seeds apart from the oil content. Both grape seeds and grape seed oil meal are being used as parts of ruminant feeds (Magnier, 1991; Nudda et al., 2015). It is important to look into further uses for these by-products and how to maximize their potential as ruminant feed because worldwide, over three million tons of seeds are discarded every year (Fernandes et al., 2013).

Lees (also called dregs) are the by-product that is generated during wine fermentation. They are the remainder which is left at the bottom of the container in which the wine was fermented, or the sediment, which is formed while storing, or after filtration and centrifugation (Letaief, 2016; Bordiga, 2016c). Dead yeasts and other microorganisms, along with plant cell scraps, tartaric acids, phenolic compounds, and inorganic matter are the main contents of lees. However, the composition may differ considerably and is subjected to many factors regarding wine production (Letaief, 2016; Ye et al., 2016). They can be used as parts of ruminant feed, as nutritional media for *Lactobacillus* species or for the distillation of ethanol. The tartaric acid can be used in the cosmetic, the pharmaceutical, the construction, and the food industry (Moote et al., 2014; Letaief, 2016; Bekhit et al., 2016; Cardona and Calix, 2016).

3.2.2 Nutritional aspects

As already mentioned before, the nutrient composition of wine by-products varies depending on the winemaking process and the type of by-product. Thus, their nutritional value should be determined on a case-to-case basis. According to Molina-Alcaide et al. (2008), a combination of different wine by-products could provide sufficient protein and energy for ruminants. However, this chapter will focus primarily on grape pomace and grape seed meal, as they were the two by-products used in this study. Naziri et al. (2014) discussed that grape seeds are mainly composed of non-digestible carbohydrates, oil, protein and phenolic compounds. Their carbohydrate content is up to 70 %, the protein content is around 11 % and phenolic content is up to 8 %, which makes it the highest phenolic content within the grape by-products. The oil content makes up between 8 and 20 % according to Rombaut et al. (2015). This is not only due

to different genotypes, but the oil content also varies as the grapes mature along with a change in fatty acid composition (Rubio et al., 2009; Sabir et al. 2012). Grape seed meal has a similar nutrient composition as whole grape seeds except for the oil content, which is much lower since it is the by-product of grapeseed oil production. The high fiber and phenol contents along with the low protein content make grape seeds a poorly digestible feed source. However, according to Magnier (1991), both grape seeds (up to 20 %) and grape seed meal (up to 10 %) can be included in ruminant rations. Grape pomace can be composed of grape seeds, pulp, skins, and stems (Letaief, 2016). Depending on the different components, the chemical composition may vary accordingly. The main components of grape pomace are carbohydrates, protein, fat, and phenolic compounds. The neutral detergent fiber content is up to 60 %, the protein content is up to 15 %, the sugar content is up to 31 %, the fat content is up to 7 %, and the phenol content is up to 6 % (Baumgärtel et al., 2007; Molina-Alcaide et al., 2008; Spanghero et al., 2009). Due to the high lignin content, the digestibility of grape pomace is rather low (Hanušovský et al., 2020). However, Guerra-Rivas et al (2017) found in their study on grape pomace of red wine that grape pulp (without seeds) has better values for digestibility compared to the seed fraction. The pulp fraction also showed lower contents of structural carbohydrates (neutral detergent fiber, acid detergent fiber, acid detergent lignin) which is believed to be the cause for the enhanced digestibility values compared to the seed fraction. Based on their results, they suggested that separating the seeds from the pulp would increase the value of grape pomace as a nutrient source for ruminants. Winkler et al. (2015) found in their study that ensiled grape pomace has higher digestibility of organic matter, crude fiber, neutral detergent fiber, acid detergent fiber and crude protein than dried grape pomace. Furthermore, they showed that the phenol content of grape pomace was lowered when ensiled, which could explain its positive effect on ruminant productivity. Also, grape color had an effect on digestibility. Baumgärtel et al. (2007) found in their study that red grape pomace had a higher content of fiber and therefore lower organic matter digestibility than white grape pomace (32 % and 56 %). Crude protein (8 % and 30 %) and crude fat (70 % and 81 %) digestibility on the other hand was higher in red grape pomace. They concluded that grape pomace could be considered as a nutritional source for ruminants, particularly in phases when there is no need for high performance.

3.2.3 Functional compounds

Phenolics are described as substances which have one or more hydroxyl groups attached to an aromatic ring. They have numerous different functions within plants, including natural antibiotic and pesticidal properties, coloration, protection against UV-light, insulation against gas or water, signaling functions for pollinators or symbiosis with rhizobia, as well as plant stabilization (Shahidi and Naczk, 2004). Different plants contain a great diversity of phenolic compounds which can be divided into groups according to their chemical structure. As mentioned in Bekhit et al. (2016), flavonoids and non-flavonoids are the two most important groups of phenolics present in grapes and their by-products. Each of them can be further divided into three main subgroups. Non-flavonoids consist of hydroxycinnamic acids, hydroxybenzoic acids and stilbenes. Hydroxycinnamic acids are present and esterified with coutaric acids, caftaric acids and tartaric acids. The main hydroxybenzoic acid in grape by-products is gallic acid. Piceid and resveratrol are the main stilbenes.

The group of flavonoids consist of flavan-3-ols, anthocyanins, and flavonols. Flavan-3-ols present in grape by-products are catechins, epicatechins, epigallocatechins, procyanidins and tannins. The anthocyanins consist of delphinidins, cyanidins, petunidins, peonidins and malvidins. Quercetins, myricetins and kaempferols belong to the group of flavonols. The phenolic content of grape products differs depending on various factors. Ye et al. (2016) mentioned that the extraction time during the winemaking process influences the content of phenols in grape pomace. Less extraction time therefore leads to higher phenol contents within the by-products. According to Bekhit et al. (2016), phenol content declines after de-oiling of grape seeds because some of the phenols are extracted along with the oil. This leads to the conclusion that the phenol content in grape seed meal should be lower than in whole grape seeds or grape pomace. Furthermore, the process of winemaking, the type of pretreatment, grape variety, and the type of product itself all have an influence on phenol concentration. The highest contents are thereby found in the skin (285–550 mg/kg) and in the seeds (5000–8000 mg/kg) (Pinelo et al., 2005; Bekhit et al., 2016). In general, red grapes tend to have higher concentrations of phenolics than white grapes. They also differ in the main functional compounds. Shahidi and Naczk (2004) discussed that while the most abundant phenolics in white table grapes are flavan-3-ols, the more dominant phenolics in red table grapes are anthocyanins. Peixoto et al. (2018) showed in their study that the composition of phenolics

differs within the different parts of the grape. Anthocyanins, for instance, are mainly present in the skin, while flavan-3-ols are present in the seeds and in grape pomace mix. Ianni and Martino (2020) give an account of several studies on phenolic compounds as well as their *in vitro* and *in vivo* effects in their review. Such as the antioxidant, anti-carcinogenic and anti-inflammatory effects of procyanidins, which are most abundant in the seeds. Resveratrol, which is mainly present in pomace, has positive effects on inflammations and infections, the cardiovascular system, neoplastic conditions, as well as plasma cholesterol, as shown in the review. They also discussed catechins to function as antioxidants and reactive oxygen scavengers among other effects. Shahidi and Naczk (2004) also discussed that whilst some phenolics have shown beneficial effects on animal health in several studies, others, such as tannins, have shown some antinutritive effects. There are two types of tannins, hydrolysable and condensed tannins. Ianni and Martino (2020) discussed that the type of tannin and the amount of intake have an effect on the digestibility and feed intake in ruminants. Furthermore, Tayengwa and Mapiye (2018) discussed that especially tannins are able to form bonds with carbohydrates and proteins, thus resulting in decreased digestibility of those dietary compounds.

3.2.4 Effects on performance and productivity of ruminants

Several studies have already shown inconclusive effects of grape by-products on animal performance and productivity. Nielsen and Hansen (2004) demonstrated in their study that dairy cows supplemented with 4.5 g grape pomace per cow and per day showed a tendency towards an increase in milk yield of 0.4 kg per cow/day in contrast to cows fed with the control diet. It should be noted that the dosage of grape pomace supplementation, as reported by the author, is a very small amount compared to most other studies, but surprisingly still seemed to show an effect. Similar to these findings are the results of a study by Nistor et al. (2014), in which dairy cows were fed grape pomace in addition to their rations and also showed a tendency towards an increased milk yield but no significant effects. However, Gessner et al. (2015) reported that Holstein cows supplemented with 1 % of grape seed and grape marc meal extract (in regard to dry matter content) had a significantly increased milk yield. These findings are supported by Mokni et al. (2017), who supplemented dairy ewes with 20 % grape seed and skin supplement. Moate et al. (2013) who supplemented dried grape marc to dairy cow rations showed that dried grape marc showed a small increase in milk yield compared to the control diet, whereas ensiled

grape marc supplementation showed the opposite effect. Contrary to these studies, Scuderi et al. (2019) did not observe an effect of grape marc (1.5 kg dry matter/cow/day) in Holstein cows. This is supported by the findings of Nudda et al. (2015) who also reported no effects on milk yield after the supplementation of grape seed meal to the diets of dairy ewes.

Apart from daily milk yield, many studies have been conducted on the influence of grape by-products on milk composition, also showing different results. Most studies show no effect of feeding wine by-products (grape seed and skin supplement, grape pomace, or grape seed meal) on milk protein, milk fat, or lactose of dairy cows and ewes (Nudda et al., 2015; Mokni et al., 2017; Ianni et al., 2019; Nielsen and Hansen, 2004). Rolinec et al. (2021) reported that an addition of 116 g of grape pomace per cow per day to the ration of dry dairy cows had no effect on fatty acid or nutrient composition of the colostrum. Mokni et al. (2017) described an increase of urea, calcium, and free iron concentration in the milk of ewes when supplementing the diet with grape seed and skin supplement. Scuderi et al. (2019) reported that while protein yield did not change from the control diet, grape marc supplementation affected the milk proteome. In contrast to the results of the studies mentioned above, Gessner et al. (2015) showed an increase of daily protein yield in transition cows supplemented with 1 % of grape seed and grape pomace extract over 12 weeks. Furthermore, Moate et al. (2013) reported a reduction in milk fat when supplemented with dried grape marc, while the supplementation of ensiled grape marc had no effect on milk nutrient composition. Collectively, these data have indicated that milk protein seems more responsive to wine by-product feeding compared to lactose and fat. Furthermore, high dosage and long duration of supplementation may play a role.

While milk fat content seems unresponsive to wine by-product feeding, milk fatty acid composition can be affected. Moate et al. (2013) showed an effect of both grape marc varieties on milk concentrations of monounsaturated fatty acids, polyunsaturated fatty acids and cis-9,trans-11 linoleic acid, which were all enhanced through supplementation. In contrast to these findings, Resconi et al. (2018) showed that supplementation with grape pomace and grape seeds decreased α-linolenic acid and monounsaturated fatty acid concentration compared to the control diet. Correddu et al. (2016) supplemented dairy ewes with grape seed (300 g/day/animal) and reported that the content of unsaturated fatty acids and polyunsaturated fatty acids within the milk increased, which was mainly caused by an increase of linoleic acid. However, the content of saturated fatty acids decreased in their study. According to Correddu

(2015), the supplementation of grape seeds can also help to reduce lipid oxidation in milk. These findings are supported by Ianni et al. (2019) who also showed an increase of linoleic acid, vaccenic acid and rumenic acid in milk and cheese, as well as lower oxidation products in cheese when grape pomace was included.

There are also some studies which looked at the fatty acid composition of meat from animals fed with grape by-products. The study of Resconi et al. (2018) showed no variations in meat fatty acid concentrations of suckling lambs when the mothers were supplemented with 10 % grape pomace or 5 % of grape seed in comparison to the control group. However, the meat of the grape by-product treatment group showed some divergence in metallic and spicy flavor for sensory evaluation. The findings of a study by Ragni et al. (2014), however, show contrasting results to that. They reported an increase of unsaturated fatty acids, while the content of saturated fatty acids in the meat was decreased when grape seed meal was added to fattening lambs' diets. Furthermore, the indices of thrombogenicity and atherogenicity suggested better results regarding consumer health when grape seed meal was included.

Amaral et al. (2019) reported that grape pomace did not lower growth or feed intake of lambs at an inclusion of 300 g/kg dry matter. These results are in agreement with Clifford (2015) who found no significant effects of grape marc on feed intake, bodyweight and average weight gain at an addition of up to 30 %. Contrary to these studies are the findings of Nistor et al. (2014), who showed improved growth rates for lambs with a grape pomace supplementation of 100 g/day. However, a higher supplementation did not improve weight gain and final body weight any further, instead it eventually showed lower results compared to the control group. Ragni et al. (2014) reported similar findings in their study. Accordingly, grape seed meal addition also resulted in an increase of feed intake. The inclusion of up to 30 % of grape seed meal had a positive effect on live weight and average daily weight gain. These findings suggest that wine by-products can be incorporated in ruminants' diets to a certain inclusion level. Too high inclusion rates can adversely affect the intake and performance of the animal.

A study by Nudda et al. (2015) showed that grape seeds potentially could have immunomodulatory effects since their inclusion in the diet leads to a decrease of some immunological parameters. Furthermore, they reported that grape seeds had no effect on liver, and kidney parameters, as well as hematological parameters. Gessner et al. (2017) found a down-regulation of several genes, which are connected to endoplasmic reticulum stress and

consequently unfolded protein response and inflammatory processes, when feeding grape seed and grape marc meal extract.

3.2.5 Effects on rumen variables of ruminants

Several *in vitro* and *in vivo* studies have been conducted on ruminal fermentation and nutrient digestibility following grape by-product inclusion, but their findings are often controversial. For example, some studies showed that ruminal pH was not affected by grape pomace or grape seed meal supplementation (Abarghuei et al., 2010; Khiaosa-ard et al., 2015; Correddu, 2015; Guerra-Rivas et al., 2017). However, Vinyard et al. (2021) reported that maximum pH increased with grape pomace content, while mean and minimum ruminal pH were not affected by diet. This is in contrast with the findings of Moate et al. (2014), who found a decrease in ruminal pH when dried grape marc was added in comparison to the control group. Khiaosa-ard et al. (2015) showed no further significant effect on redox potential and ruminal ammonia concentration *in vitro* when dried distillers grains plus solubles were fortified with grape seed meal compared to the effect of the dried distillery grains alone. Similar to these findings, Moate et al. (2014) also found no effect on ruminal ammonia when grape marc was included. However, Guerra-Rivas et al. (2017) and Abarghuei et al. (2010) found that grape pomace addition reduced ruminal ammonia concentration, while Correddu (2015) discovered an increase of ruminal ammonia concentration with an inclusion of grape seed to the diet. Similar to this, Vinyard et al. (2021) showed that ruminal ammonia concentration had a tendency to be higher with a higher amount of grape pomace in the ration but was not affected by diet over time.

Spanghero et al. (2009) showed the differences in *in vitro* ruminal gas production between pulp and seeds of different grape cultivars and the effect of ensiling. Overall, seeds showed lower gas production than pulp. Ensiled grape by-products decreased gas production for seeds at 24 and 48 hours and at 48 hours for pulp. Moate et al. (2014) discovered that treatment had no effect on ruminal headspace gas composition. However, the supplementation of grape marc led to a decrease in methane emissions and methane yield with even lower values for dried grape marc in comparison to ensiled grape marc. This result is similar to the *in vitro* study of Khiaosa-ard et al. (2015), where methane variables including the concentration in fermentation gas, absolute methane formation, and methane production per gram of degraded organic matter/neutral detergent fiber were lowered by grape seed meal inclusion.

While some studies showed no effect of grape by-products on ruminal total short-chain fatty acid concentration (Vinyard et al., 2021; Khiaosa-ard et al., 2015; Correddu, 2015; Moate et al., 2014), Guerra-Rivas et al. (2017) showed in their study that concentration of total short-chain fatty acids was reduced by grape pomace inclusion. Furthermore, they showed that contents of acetate, propionate, butyrate, isobutyrate, valerate, and isovalerate were all reduced as an effect of grape pomace inclusion compared to the control diet. Moate et al. (2014) reported that dried grape marc supplementation led to an increase of caproate content along with a decrease of acetate content. Vinyard et al. (2021) showed in their study that the contents of isovalerate, valerate and isobutyrate were not affected by diet. However, grape pomace did affect the contents of acetate and propionate, as well as acetate:propionate ratio, along with an increase of butyrate content. Contrary to these results, Correddu (2015) discovered that the contents of acetate and propionate, along with the acetate:propionate ratio were not influenced by diet, while butyrate content was lowered by an inclusion of grape seed mixed with linseed. Similar results were obtained by Khiaosa-ard et al. (2015), with a slight increase in acetate and a decrease in butyrate.

Only a handful of studies investigated an effect of wine by-products on ruminal microorganisms. Moate et al. (2014), using sequencing technique, described that ruminal bacterial community was affected by both ensiled and dried grape marc supplementation, while only dried grape marc affected archaeal community, and a small effect on protozoa community was found with ensiled grape marc addition. The changes in bacterial and archaeal communities were connected to a decrease in enteric methane yield in this study. Abarghuei et al. (2010) found that grape pomace decreased the abundance of protozoa as well as proteolytic and cellulolytic bacteria in their study. Khiaosa-ard et al. (2015) quantified total abundances of rumen protozoa, archaea and bacteria as well as shifts in the relative abundances of target bacterial and archaea species using qPCR. They reported an effect of grape seed meal mainly on total fungi and protozoa abundances. Due to the small body of research but high variation in the nature of experiment as well as the analytical perspective, it is not possible to conclude from the existing data how the rumen microbial community reacts to wine by-products or the bioactive compounds.

More is known about the ruminal degradability of wine by-products. Winkler et al. (2015) showed the difference in ruminal digestibility of dried and ensiled grape pomace. Specifically,

organic matter, crude protein, crude fiber, neutral detergent fiber, and acid detergent fiber digestibility were lower for dried grape pomace. Ether extract, however, showed no difference between the grape pomace variants. The researchers also reported higher organic matter, crude protein, ether extract and crude fiber digestibility of dried red grape pomace than dried white grape pomace. In accordance with these results, Basalan et al. (2011) found that *in vitro* disappearance of dry matter and neutral detergent fiber after four hours was higher for red grape pomace compared to white grape pomace. However, color related differences declined at later times. Contrary to those two studies, Baumgärtel et al. (2007) found that, with the exception of crude protein, all parameters were lower for red grape pomace compared to the white kind. Nevertheless, they showed that digestibility in general, with the exception of crude fat, was lower when grape pomace was added to the ration. Guerra-Rivas et al. (2017) showed ruminal degradation of different fractions of grape pomace in their study. According to their results, dry matter degradability was higher in pulp fraction than in the seed fraction, while crude protein degradability was lower in pulp and higher in seeds. But when comparing grape pomace to control diet, the effects on dry matter, organic matter, and crude protein degradation were only minor. Vinyard et al. (2021) showed a decrease in apparent total tract digestibility of dry matter, organic matter, neutral detergent fiber, nitrogen, and acid detergent fiber when grape pomace was included. Furthermore, Abarghuei et al. (2010) discovered in their study that grape pomace supplementation decreased total tract digestibility of organic matter, neutral detergent fiber, and crude protein, as well as microbial protein production and retained nitrogen. Inclusion of grape seed meal also lowered ruminal degradation of dry matter and organic matter *in vitro* but no effect was observed for neutral detergent fiber (Khiaosa-ard et al., 2015).

Altogether, it can be seen that studies have revealed both positive and negative effects of wine by-products and data are often inconclusive. The data on microbiota are still limited. The discrepancies observed could be related to various factors. Differences in basal diet, inclusion of different kinds of grape by-products, agricultural differences in wine production, different inclusion levels, even different locations for growing grape vines can all have an impact on the nutritional and functional properties of the by-products and thus the results. Therefore, it is important for this thesis to focus on locally produced grape pomace and grape seed meal from the region of Lower Austria. Furthermore, high-quality hay, also produced in Austria, was part

of the basal diet. The approach was to investigate whether the wine by-products would be able to mitigate the excessive ruminal ammonia build up which is usually induced by high-quality hay diets (Klevenhusen et al., 2017).

4 Material and methods

4.1 Rusitec

4.1.1 Overview

Rumen simulation technique (RUSITEC) is a widely accepted *in vitro* model which simulates the environment of the rumen to study fermentation processes under a standardized and controlled environment. As shown in Figure 1, the system used in this thesis includes gas-tight reaction vessels, which contain a solid phase of feed as well as a liquid phase of rumen inoculum-buffer mix. Both phases need to be stirred constantly by a motor. The fermentation units are connected to bags for the collection of fermentation gas as well as to flasks for the outflow collection and permit a continuous flow of artificial saliva. The system is held at a temperature similar to that of the rumen at between 39–39.5 °C through a water bath, surrounding the fermenters. On the first day, the system is inoculated with rumen fluid and solid rumen content. A bag containing a test diet is then placed alongside the inoculum. On the second day, the solid rumen content gets replaced by a second bag of the respective diet. Feed bag exchange takes place every day. With this system, feed bags are incubated for 48 hours.

Figure 1: One of the RUSITEC systems used in this experiment (photo by the author 2020).

The RUSITEC method is used regularly to evaluate differences between treatments of varying composition (Czerkawski and Breckenridge, 1977; Martínez et al., 2010). Test conditions as well as the apparatus differ among studies. Details of RUSITEC setup and conditions specific to the work of this thesis are given below.

4.1.2 Experimental design, dietary treatment, and RUSITEC condition

This specific experiment was conducted over the course of three months and consisted of four independent runs, each run lasting for ten days. The first five days were used to adapt and monitor the system to reach its equilibrium before sampling, taken during the last five days of each run.

Four diets were tested in every run consisting of high-quality hay and typical energy concentrate without (CON, serves as negative control) or with grape pomace (GP) or grape seed meal (GS) at the same inclusion level of 10 % of diet dry matter (Figure 2). The last diet was the same as the CON diet, additionally top-dressed with 3.7 % of a commercial grape seed extract (Nature Love® OPC Grape Seed Extract, Tauron Ventures GmbH, Düsseldorf, Germany), which served as the positive control (EXT).

Figure 2: from left to right: Grape seed meal, whole grape pomace and ground grape pomace used in this experiment. (pictures by Dr. Khiaosa-ard, 2020)

The exact diet composition is shown in Table 1. There was a rotation among the treatments arranged to the fermenters to ensure no bias on the treatment's effect that might have been related to the performance and location of fermenters.

Prior to fermentation, feed bags were prepared according to the diet composition, aimed at supplying 12 g feed dry matter per bag. To do so, single components were first weighed, then mixed together and filled into previously sewed nylon bags (140 × 70 mm, 150 µm pore size, Fa. Linker Industrie-Technik GmbH, Kassel, Germany), which were closed by differently colored cable ties to enable a differentiation between the days of the experiment.

Table 1: Diet ingredients, chemical composition and content of total phenols (% of diet dry matter, otherwise stated)

Ingredients	**CON**	**EXT**	**GP**	**GS**
Hay	70	70	65	65
Energy concentrate mix[1]	30	30	25	25
Grape pomace	0	0	10	0
Grape seed meal	0	0	0	10
Grape seed extract	0	3.7	0	0
Chemical composition	**CON**	**EXT**	**GP**	**GS**
Dry matter (DM, %)	89.21	89.21	89.54	89.78
Organic matter (OM)	92.29	92.29	92.57	92.92
Ash	7.71	7.71	7.43	7.08
Crude protein (CP)	18.70	18.70	19.13	18.84
Ether extract (EE)	2.58	2.58	3.66	2.15
Neutral detergent fiber (NDF)	49.26	49.26	48.31	51.67
Acid detergent fiber (ADF)	20.88	20.88	24.68	24.16
Non-fiber carbohydrates (NFC)	21.75	21.75	21.47	20.26
Total phenol content [2]	2.9	6.4	4.0	4.1
Phenol content from grape product	0	3.4	1.3	1.4

[1]Energy Concentrate Mix1: contains on dry matter basis (g/kg): 216 barley; 216 wheat; 517 maize; and 52 vitamin and mineral supplement (Rindavit TMR 11 ASS-CO + ATG; H. Wilhelm Schaumann GmbH & Co KG, Brunn/Gebirge, Austria)
[2]expressed as catechin equivalents

On the first day (day 0) of each experimental run, rumen fluid and solid rumen content were collected through rumen fistulas from the same two donor cows kept at the Ruminant Clinic of the University of Veterinary Medicine Vienna. The cows were fed mainly hay ad libitum with a daily allowance of 0.5 kg of a commercial concentrate. Ruminal fluid and solid were used as the inoculum for the RUSITEC system.

For inoculation of vessels, the collected rumen fluid was filtered through four layers of medical gauze and the equal portions of the strained ruminal fluid from the donor cows were then pooled together. The solid rumen contents of all donor cows were pooled as well and about 40 g were filled into each nylon bag. The pH and redox potential of the inoculum were 6.69 ± 0.40 and -304 ± 33 mV, respectively (mean ± SD).

The same two RUSITEC systems, each equipped with six fermenters, were used for all four runs of this experiment. Each of the twelve fermenters was filled with 600 mL of the liquid inoculum, along with 100 mL of artificial saliva (McDougal's buffer, Table 2).

Table 2: McDougal's buffer composition

Components	**mmol/L**
$NaHCO_3$	116.5
$Na_2HPO_4 \cdot 2\ H_2O$	26.3
NaCl	8.04
KCl	7.64
$CaCl_2 \cdot 2\ H_2O$	0.37
$MgCl_2 \cdot 6\ H_2O$	0.63

After the liquid contents were set up, the nylon bags filled with the pooled solid rumen content were added, one bag in each fermenter. At the same time, the prepared feed bags for day 0 were put inside the assigned fermenters, thus there were always two nylon bags per fermenter. After the fermenters were closed, the system was flushed with a stream of nitrogen gas to ensure the necessary anaerobic environment for the fermentation to take place. The fermenters were kept at a temperature of 39.5 °C through a water bath. An electric motor which was attached to metal bars on top of the fermenters (Figure 1) was operated to keep the fermentation fluid and feed bags inside the fermenters in motion. A continuous inflow of the buffer (Table 2) was

administered by a peristatic pump (model ISM932, Ismatec, Idex Health & Science GmbH, Wertheim, Germany) at a rate of 375 ml/d throughout the experiment. The outflow was continuously collected in flasks, which were kept in a refrigerator (1 °C) to prevent ongoing fermentation of the outflow. The fermentation gas was collected in gas-tight aluminum bags (TECOBAG 8 L, Tesseraux Spezialverpackungen, Bürstadt, Germany).
From the second day (day 1) on, feedbag exchange was performed daily. Accordingly, the rumen solid bag was exchanged with a respective diet. On the third day, the spent feedbag inserted in day 0 was replaced and so on. In this way, all feedbags were incubated for 48 h. Before feedbag exchange, the fermenter was flushed with a stream of nitrogen gas for 30 s to collect the fermentation gas. The outflow bottles were emptied, and the volume was measured. After the exchange of the feed bags and closing of the vessels, each fermenter was flushed 3 min with nitrogen gas to reestablish the anaerobic milieu.

On the first five days of each run, only the pH and the redox potential of the fermentation fluid and the volume of outflow were checked daily to ensure the stability of the system and sufficient buffer flow. Data collected on the sampling days were used for this thesis. On these adaptation days, approximately 5 ml of fermentation fluid was taken out from each of the twelve fermenters. For the pH measurement, a pH electrode (InLab Expert Pro-ISM, Mettler-Toledo GmbH, Schwerzenbach, Switzerland) attached to a meter (Seven Multi TM, Mettler-Toledo GmbH, Schwerzenbach, Switzerland) was used. Before the measurement of the samples, the electrode was calibrated with two standard pH solutions, the first of a lower pH of 4.0 and the second of a higher pH of 7.0. For the redox potential, another electrode (Pt4805-DPA-SC-S8/120, Mettler-Toledo GmbH, Schwerzenbach, Switzerland) attached to the same meter mentioned above was used. The performance of redox electrode was checked using a standard solution before measuring the samples.
The last five days of each individual run served as the sampling and measurement period. During these days, about 20 ml of fermentation fluid was taken from each of the fermenters. Part of the sample was used for the pH and the redox potential, those parameters were recorded daily using the same electrodes as was described for the adaptation period. The remaining portion of the fluid samples were taken to measure other fermentation parameters including the ammonia concentration using indophenol reaction method, the concentration and composition

of short-chain fatty acids using gas chromatography, and the sequencing of the microbiota. Those samples were put into a freezer at -20 °C immediately after being portioned into tubes. The tubes for the microbiota analysis first needed to be shock-frozen with liquid nitrogen before being put into the freezer with the rest of the fluid samples. Apart from the fluid samples, also the fermentation gas and the volume of the outflow were measured daily for each fermenter. For the gas measurement, the composition (methane, carbon dioxide, and oxygen) was measured using a portable infrared detector (ATEX Biogas monitor Check BM 2000, Ansyco, Karlsruhe, Germany). The gas volume was measured using a water replacement method.

The 48-h bags were collected and machine-washed before storage at -20 °C until the analysis of the chemical composition. Original diets were also collected for the same chemical composition analysis. The contents of dry matter, ash, crude protein, neutral detergent fiber and ether extract of diets and incubated diets were determined following the procedures of VDLUFA (2012). On the last day, the 24-h feedbags were taken from the fermenter, immediately snap-frozen with liquid nitrogen and stored at -20 °C for later use for the sequencing of the solid-associated microbiota.

4.2 Statistical Analysis

Averaged daily data (fermentation liquid and gas per fermenter per run) were used for statistical analysis. The data were analyzed using the MIXED procedure of SAS (version 9.4, SAS Institute Inc., Cary, NC, USA). The mixed model included the fixed factor (dietary treatment) and random factors (experimental run and fermenters). Values presented in the thesis are least squares means and standard error of the mean (SEM). The Tukey's method was used to compare the least squares means among dietary treatments. There were two types of P values described in the thesis: one is for the overall effect of treatment and the other one related to pairwise comparisons of the least squares means following the Tukey's method. The latter is identified by superscripts designated to mean values. The significant treatment effect and pairwise differences were considered when $P \leq 0.05$ and a tendency for an effect or differences when $0.05 < P < 0.10$.

5 Results

5.1 Fermentation parameters

The addition of both grape by-products had a significant effect on ruminal pH (P = 0.004). As shown in Figure 3, the pH was lowest in CON, which was lower than that of GS and GP (P < 0.05), while EXT showed an intermediate value.

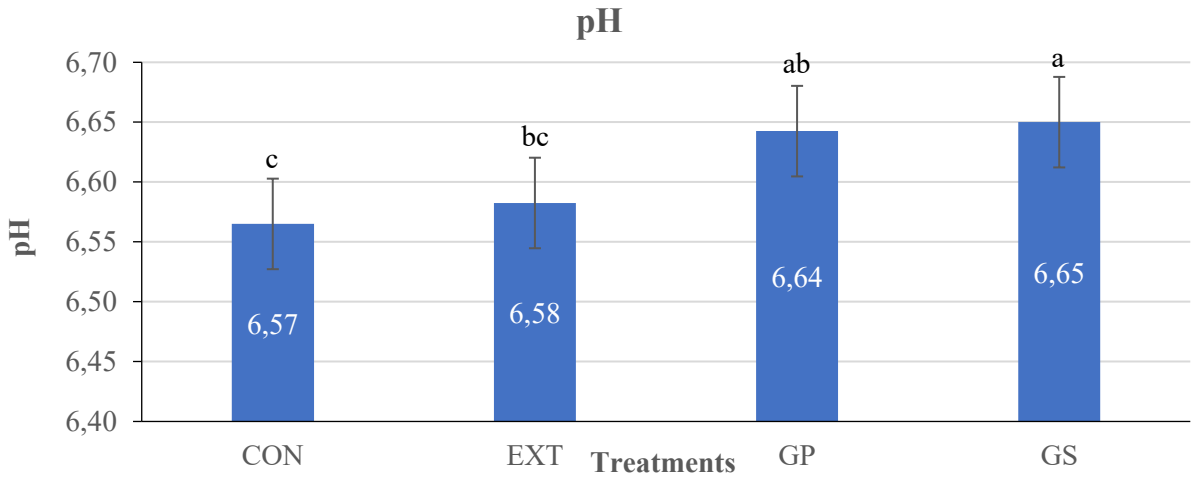

Figure 3: pH in relation to the different treatments. Error bars indicate standard error of the mean (±0.04). [abc]Treatments sharing no common superscripts differ significantly (P ≤ 0.05) according to the Tukey's test.

No significant difference was found for the redox potential between CON, EXT, GP or GS (P = 0.568), as shown in Figure 4. The redox potential ranged from -244 to -232 mV.

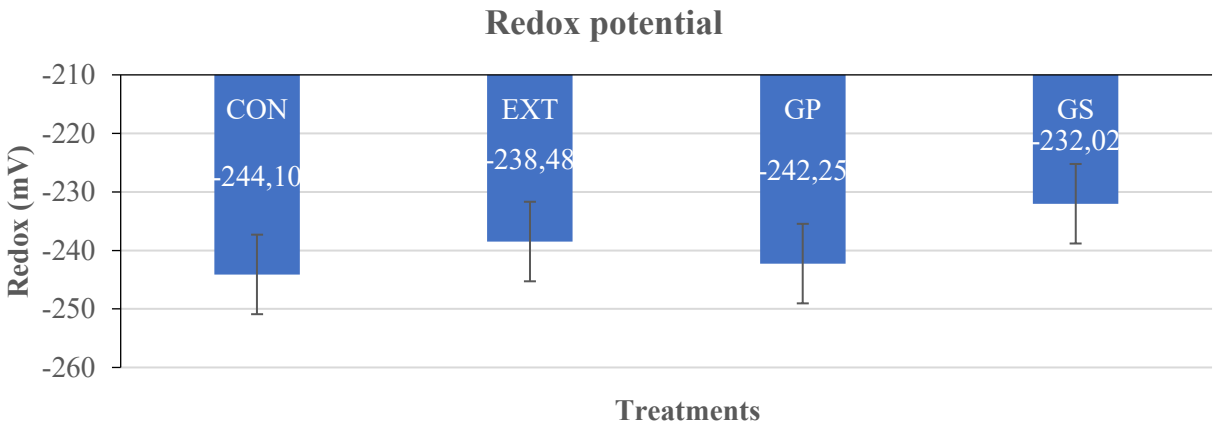

Figure 4: Redox potential in relation to the treatments. Error bars indicate standard error of the mean (± 6.8). [abc]Treatments sharing no common superscripts differ significantly (P ≤ 0.05) according to the Tukey's test.

The addition of both grape by-products and EXT had a significant effect on ruminal ammonia concentration ($P < 0.001$). CON resulted in an average of 14.45 mmol ammonia/L. As shown in Figure 5, EXT showed the lowest concentration (12.00 mmol/L), being significantly lower than CON and GP according to Tukey's method ($P < 0.05$) but not different when compared to GS. Overall, treatment decreased ammonia concentration between 10 – 17 % from that of CON.

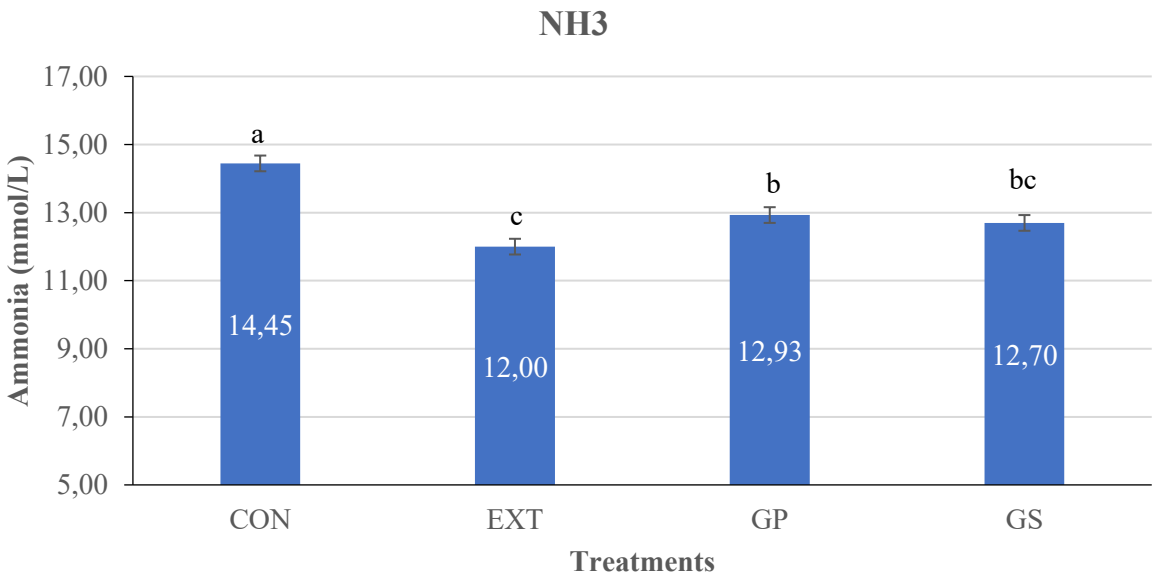

Figure 5: Ammonia concentration in relation to the different treatments. Error bars indicate standard error of the mean (± 0,23). [abc]Treatments sharing no common superscripts differ significantly ($P \leq 0.05$) according to the Tukey's test.

For the overall volume of fermentation gas, no significant difference ($P = 0,128$) was found between the by-product treatments GP (809.75 mL/d ± 59.16) and GS (775.15 mL/d ± 59.16), EXT (814.65 mL/d ± 59.16) and CON (870.53 mL/d ± 59.16).

This was also the case for the overall carbon dioxide gas content ($P = 0.079$), EXT (84.20 % ± 0.49), GP (83.54 % ± 0.49) and GS (83.65 % ± 0.49) showed no significant difference ($P > 0.05$) compared to CON (84.31 % ± 0.49). Treatments also showed no significant difference ($P = 0.106$) for the carbon dioxide gas volume compared to CON (733.21 mL/d ± 47.43) with EXT, GP and GS resulted in carbon dioxide volume of 685.54 mL/d ± 47.43, 676.02 mL/d ± 47.43 and 648.64 mL/d ± 47.43, respectively.

The addition of EXT (6.24 % ± 0.28), GP (5.93 % ± 0.28) and GS (5.95 % ± 0.28) did not influence ($P = 0.116$) the oxygen gas content, with CON showing an average of 5.80 % ± 0.28.

The grape by-products and EXT also showed no significant effect on the oxygen gas volume (P = 0.248). Compared to CON (50.31 mL/d ± 4.44), they all had P-values above 0.05. The oxygen volume of EXT, GP and GS resulted in 51.09 mL/d ± 4.44, 47.77 mL/d ± 4.44 and 46.35 mL/d ± 4.44.

The methane gas content (%) showed a significant difference among the treatments (P = 0.026), but only for the comparison between EXT and GP (P = 0.0306) (Figure 6). EXT, GP, and GS showed no significant difference (P > 0.05) compared to CON. Methane gas volume showed no significant difference (P = 0.217) between the treatments either, where the methane volume of CON, EXT, GP, and GS resulted in 86.68 mL/d ± 8.30, 78.15 mL/d ± 8.30, 85.57 mL/d ± 8.30 and 80.78 mL/d ± 8.30.

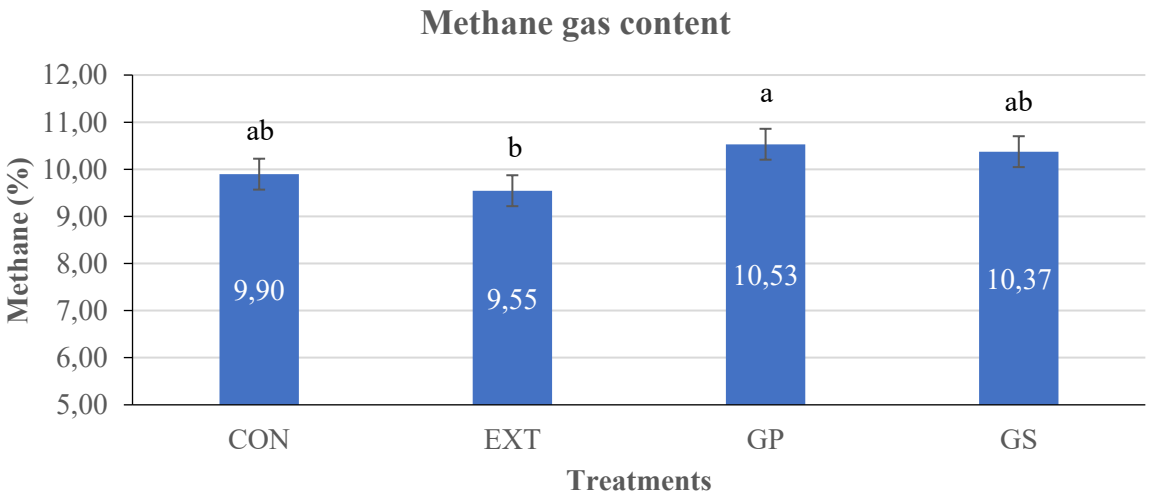

Figure 6: Methane gas content in relation to the treatments. Error bars indicate standard error of the mean (± 0.33). [abc]Treatments sharing no common superscripts differ significantly (P ≤ 0.05) according to the Tukey's test.

As shown in Table 3, a significant influence (P = 0.0008) on total short-chain fatty acid concentration from the addition of grape seed meal and grape pomace to the ration was detected. CON, with an average concentration of 128.68 mmol/L was 7–8 % higher than both GP, and GS, (P ≤ 0.05). No significant difference was found between CON and EXT, but EXT significantly lowered the short-chain fatty acid concentration as compared to GS (P ≤ 0.05).

Table 3: Short-chain fatty acid composition (%) in relation to the different treatments; [abc]Treatments sharing no common superscripts differ significantly (P ≤ 0.05) according to the Tukey's test.

Variable	CON	EXT	GP	GS	SEM	P Value
Short-chain fatty acids (mmol/L)	128.68[a]	124.49[ab]	119.70[bc]	117.76[c]	5.21	0.001
Acetate (%)	48.53[b]	50.46[a]	49.22[ab]	49.48[ab]	0.57	0.007
Propionate (%)	24.61	26.50	25.05	24.68	0.95	0.179
Butyrate (%)	10.84[a]	9.73[b]	10.76[a]	10.37[ab]	0.19	0.002
Isobutyrate (%)	0.96[a]	0.77[b]	0.92[a]	0.94[a]	0.04	<.0001
Valerate (%)	8.10[a]	6.79[b]	7.59[ab]	7.82[ab]	0.40	0.022
Isovalerate (%)	2.94	2.74	2.68	2.90	0.11	0.060
Caproate (%)	3.10[a]	2.35[b]	2.93[ab]	2.84[ab]	0.28	0.029
Heptanoate (%)	0.95[a]	0.69[b]	0.84[ab]	0.91[a]	0.11	0.008

The composition of individual short-chain fatty acids expressed (% of total short-chain fatty acids) was affected by treatment (Table 3). The treatments had a significant effect (P = 0.007) on the percentage of acetate. However, this was only because EXT had a significantly higher acetate (P ≤ 0.05) compared to CON with an increase of 4 %. The propionate percentages were similar among treatments (P = 0.179). The maximum change was detected with EXT showing an increase of 7 % compared to CON, but the difference was not significant (P > 0.05). Butyrate percentage differed significantly between the treatments (P = 0.002). CON and GP showed very similar values, which were about 10 % higher than that of EXT (P < 0.05), while GS was intermediate. The isobutyrate percentage was highly affected by treatment (P < 0.0001). EXT showed a significant decrease of isobutyrate percentage compared to CON and the by-product treatments (P ≤ 0.05). For valerate percentage, the treatments had a significant effect (P = 0.022), but this was only due to EXT, which had a significantly lower valerate content (P ≤ 0.05) compared to CON (decrease of 16 %). Both GP and GS had similar values. The treatments had no significant effect on isovalerate percentage (P = 0.06). CON and GS showed similar values, while EXT and GP reduced isovalerate content by 7–9 %, but not to a significant degree (P > 0.05). The treatments showed a significant effect (P = 0.029) on caproate percentage. CON was the highest, followed by GP and GS. However, only EXT showed a significant difference

($P \leq 0.05$) and decreased caproate content by 24 % compared to CON. The treatments also had a significant effect on heptanoate content ($P = 0.008$). But again, only EXT showed a significant difference ($P \leq 0.05$) compared to CON with a reduction of 27 %.

5.2 Nutrient degradation

Table 4: Nutrient degradation in relation to the different treatments; [abc]Treatments sharing no common superscripts differ significantly ($P \leq 0.05$) according to the Tukey's test.

Variable	CON	EXT	GP	GS	SEM	P value
Dry matter (%)	65.02[a]	62.50[b]	62.68[b]	62.74[b]	0.63	<0.001
Organic matter (%)	63.07[a]	60.34[b]	60.72[b]	60.86[b]	0.68	<0.001
Ash (%)	88.49[a]	88.30[a]	87.03[b]	87.29[b]	0.26	<0.001
Crude Protein (%)	69.49[a]	66.90[b]	69.44[a]	70.71[a]	1.13	<0.001
Fat (%)	45.57[b]	39.53[c]	52.98[a]	43.21[bc]	2.89	<0.001
Neutral detergent fiber (%)	47.22[a]	46.27[a]	39.73[b]	45.09[a]	2.43	<0.001
Acid detergent fiber (%)	36.68[a]	33.70[ab]	32.94[b]	34.79[ab]	1.07	0.024
Short-chain fatty acids (mmol/g degraded Organic matter)	9.60	9.39	8.87	9.05	0.25	0.066
CH4 (mL/g degraded Organic matter)	12.33	11.22	12.59	11.78	1.28	0.145
NH3 production efficiency (mmol NH3/g degraded Crude protein)	4.88[a]	4.08[b]	4.10[b]	4.15[b]	0.30	<0.001

The nutrient degradation for the different treatments can be seen in Table 4. All treatments had a significant effect on dry matter and organic matter degradation, with EXT, GP and GS being lower than CON (decrease of 3.5–3.9 % and 3.5–4.3 %). Only GP and GS also had a lowered ash degradation (1.4–1.7 %) compared to CON ($P < 0.05$). Crude protein showed a significant decrease with EXT (decrease of 3.7 %) only. EXT showed a significant decrease in fat degradation (decrease of 13 %), while GP showed a significant increase (increase of 16 %) in comparison to CON. The neutral detergent fiber fraction was affected by GP causing a decrease of 16 %, while EXT and GS showed no significant difference from CON. Furthermore, GP decreased the acid-detergent fiber fraction (decrease of 10 %) while EXT and GS had no significant effect. No significant effects were detected for short-chain fatty acid yield and methane yield per gram of degraded organic matter. However, ammonia production efficiency was affected by treatment ($P < 0.001$). All treatments and EXT decreased the value by about 15–16 % from that of CON ($P < 0.05$).

5.3 Microbiota

Table 5: Microbiota diversity in relation to the treatments; [abc]Treatments sharing no common superscripts differ significantly ($P \leq 0.05$) according to the Tukey's test. ASV = Amplicon sequence variants.

Diversity index	CON	EXT	GP	GS	SEM	P value
Liquid						
Observed ASVs	926	795	872	986	74	0.3264
Chao 1	932	800	880	994	75	0.3253
Shannon	4.79	4.69	4.67	4.79	0.15	0.8036
Simpson	0.971	0.965	0.963	0.965	0.006	0.7607
Solid						
Observed ASVs	627^{ab}	597.5^{b}	562.3^{b}	716^{a}	43	0.0739
Chao 1	631^{ab}	600^{b}	565^{b}	720^{a}	44	0.0739
Shannon	4.28^{ab}	4.06^{b}	4.42^{a}	4.31^{a}	0,19	0.0412
Simpson	0.960	0.938	0.969	0.956	0.014	0.0807

The alpha diversity indices are shown in Table 5. They were measured in the fermentation fluid and in the solid part taken from the feed bags. No significant difference in the alpha diversity of liquid microbiota was detected among the treatments. An effect of treatment on the diversity indices of solid-associated microbiota was apparent, showing a tendency ($P < 0.10$) for Observed ASVs, Chao 1 and Simpson, and a significance for Shannon ($P = 0.041$). According to Tukey's method, EXT decreased the Shannon index compared to GP and GS ($P < 0.05$).

Figure 7 presents the bacterial composition for both the liquid and the solid samples at the phylum level.

The figure shows that, although *Firmicutes* is the most abundant followed by *Bacteroidetes* in both liquid and solid associated bacteria, *Actinobacteria, Proteobacteria, Euryarchaeota*, and *Patescibacteria* were more abundant in the fluid microbiota, while *Bacteroidetes, Spirochaetes* and *Tenericutes* were more prevalent in the solid sample.

There are also noticeable shifts in bacterial composition caused by the treatments. In the fluid sample, all treatments showed a decrease in *Actinobacteria* abundance. Furthermore, EXT and GS both showed a decrease in *Bacteroidetes*. EXT also showed an increase of *Spirochaetes*, while GS and GP both seemed to increase *Proteobacteria*. In the solid sample, mainly GP and

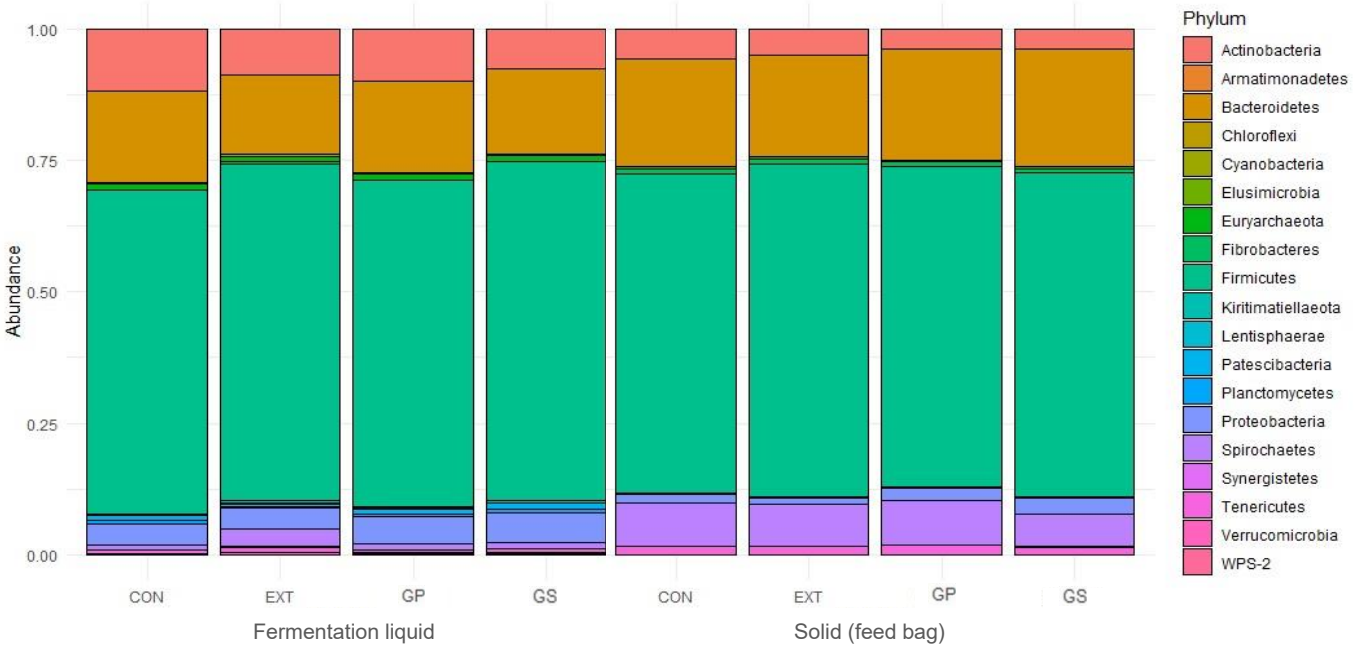

Figure 7: The bacterial composition at the phylum level of fermentation liquid and solid (feed bag).

GS seemed to influence bacterial composition, as both increased *Proteobacteria*, while GS also decreased *Spirochaetes*.

Figure 8 shows a heatmap of the most abundant bacterial families in both the solid and the liquid samples. *Lactobacillaceae* and *Lachnospiraceae* were the most abundant families in both liquid and solid-associated microbiota, together occupying over 30 % of the read abundance in liquid-associated microbiota and up to 40 % of the solid-associated microbiota. Other families with high abundances in the liquid microbiota were *Ruminococcaceae* and *Prevotellaceae*, and in the solid microbiota, *Prevotellaceae*, *Veillonellaceae* and *Spirochaetaceae*.

The shift in bacterial composition was mainly affected by EXT. Especially, EXT induced an increase in *Lactobacillaceae, Prevotellaceae, Spirochaetaceae* and *Streptococcaceae*, while *Ruminococcaceae, Christensenellacaea, Bacteroidales* and *Methanobacteriaceae* were reduced by EXT compared to CON.

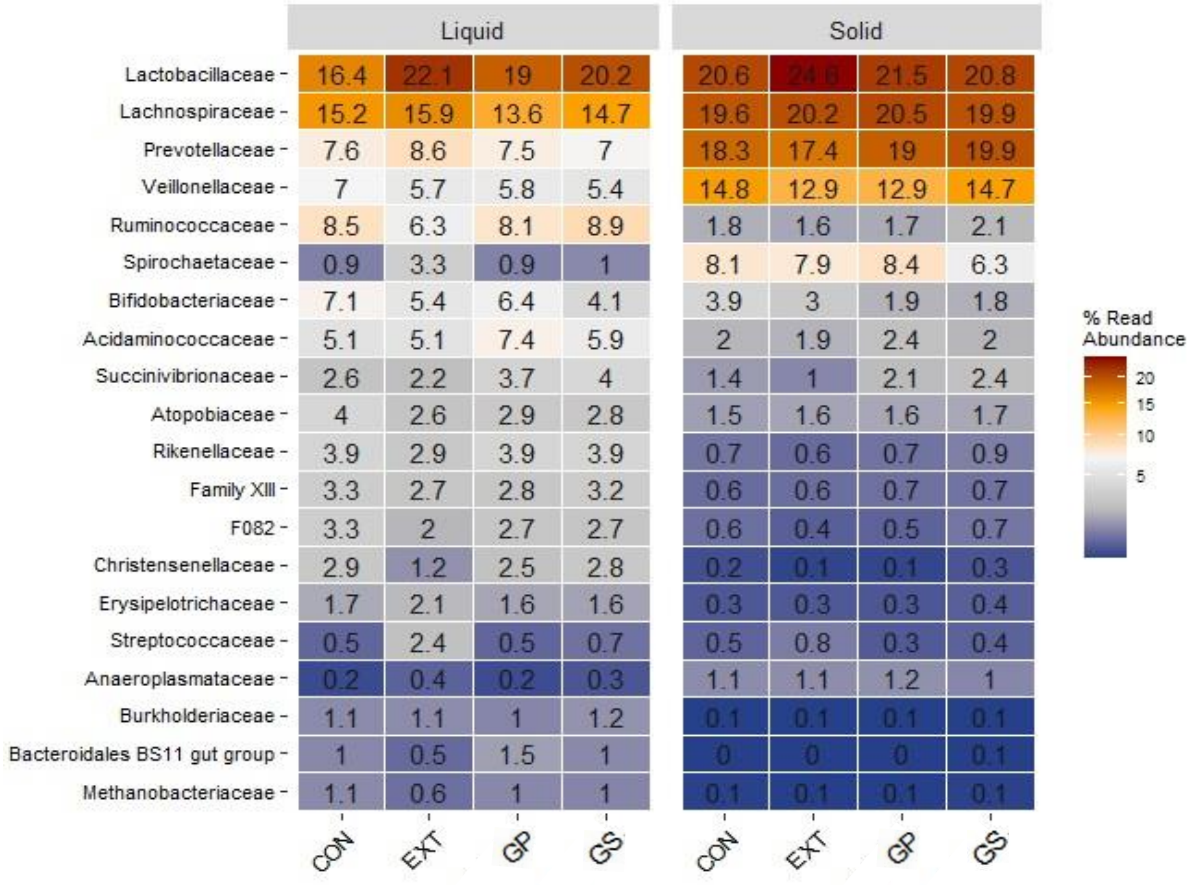

Figure 8: The abundances of the top 20 families in the liquid and solid.

In general, the bacteria of the solid samples were more resistant to all treatments than the bacteria in the fluid samples. The noticeable changes were *Lactobacillaceae*, being increased by EXT, and *Spirochaetaceae*, being decreased by GS.

6 Discussion

Based on the previous chapter, this discussion will compare the results of this study with the found literature.

6.1 Effects of grape pomace and grape seed meal inclusion

This part is dedicated to answer the main thesis questions: what effects do grape seed meal and grape pomace have on ruminal fermentation and nutrient degradation parameters, and are there any negative effects associated with these winery by-products?

Rumen microbes fulfill several important tasks within the rumen. They play a large role, especially in the fermentation of carbohydrates, because they are able to break down structural carbohydrates with their enzymes, which could otherwise not be digested by the host itself. In turn, they require certain physiochemical parameters in a specific range to survive and for proper activity. The stability of those parameters, the most important ones being pH and redox potential, is provided by the host ruminant (Castillo-González et al., 2014; Herdt and Sayegh, 2012). The physiological range for ruminal pH varies between 5.5 to 6.8 (Herdt and Sayegh, 2012) and the type of feed is critical for ruminal pH (Breves et al., 2015; Dijkstra et al., 2012). From the literature research, most studies displayed no effect of grape by-products on ruminal pH (Abarghuei et al., 2010; Khiaosa-ard et al., 2015; Correddu, 2015; Guerra-Rivas et al., 2017). The effect detected by some other studies was considered not detrimental since the values were all never outside the optimal range (Vinyard et al., 2021; Moate et al., 2014). The study by Moate et al. (2014) showed a decrease in ruminal pH to a value of 6.54 when 5 kg of alfalfa hay was replaced by 5 kg of dried grape marc. The same replacement of hay with ensiled grape marc showed a tendency to decrease ruminal pH at 6.6. They discussed that low ruminal pH can potentially impede methanogenesis. Contrastingly, the ruminal pH in the study by Vinyard et al. (2021) showed no effect on mean or minimal pH, only maximum pH was elevated with grape pomace inclusion at an inclusion rate of both 15 % and 30 %. Both values stayed within physiologic range, although the effect seemed stronger for the lower inclusion rate. Similarly, all pH values of the present study remained in physiological range above 6.5, while both treatments with grape by-product inclusion showed an increase in ruminal pH compared to the control diet. In animals, besides the type of feed, different feed intake (Breves et al., 2015) as well as chewing activity, which is in correlation to saliva secretion, affect ruminal pH

(Mertens, 1997). In the present *in vitro* experiment, both factors were kept constant in all fermenters. The increased pH by grape by-product treatments was clearly associated with a lower short-chain fatty acid concentration in both grape seed meal and grape pomace, as well as a slight decrease of nutrient degradation. As stated by Naziri et al. (2014), grape seeds, which are also present in grape pomace, have a high content of non-digestible carbohydrates. Part of both the hay and the concentrate were substituted for grape by-products within the rations, thus replacing rapidly fermented carbohydrates with more structural carbohydrates. While the ratio of hay to concentrate used in this study had enough structure to promote a physiological ruminal pH, the substitution with structural carbohydrates could still have been a factor to elevate ruminal pH in comparison to both control diets, especially since total short-chain fatty acid concentration was also decreased with both by-product treatments. The redox potential within the rumen indicates whether anaerobic fermentation can take place and thus has an influence on the digestion of feed (Huang et al., 2018). Typical values for ruminal redox potential are between -250 mV and -450 mV (Herdt and Sayegh, 2012). While the values of this study are slightly higher with a range from -232 mV to -244 mV, these results should still be within a range which does not affect rumen health in a negative way, as anaerobe bacteria require a redox potential between +100 mV and under -250 mV (Ray, 2004). No significant difference on redox potential was found with the inclusion of grape pomace or grape seed meal to the ration, which is in agreement with the findings of Khiaosa-ard et al. (2015).

The physiological concentration of ammonia in the rumen is in a range of 3–15 mmol/l (Breves et al., 2015). It either leaves the rumen through its wall or the digestive tract or is used as a valuable compound for microbial protein synthesis (Breves et al., 2015; Reece, 1997). Because the *in vitro* system does not account for the absorption, the ammonia concentration recovered is mainly from microbial activity in the system. In the present study, rumen ammonia concentration was in the normal range for all treatments with the highest value being 14.45 mmol/l for the control diet. The addition of grape seed meal and grape pomace led to a significantly lower ruminal ammonia concentration. Overall, ammonia concentration was decreased between 10 – 13 % compared to the control diet. Previous studies have reported different results of feeding grape by-products *in vivo* as well as *in vitro*. These findings are supported by the *in vivo* studies of Guerra-Rivas et al. (2017), who supplemented sheep with an inclusion level of 7.5 % of grape pomace, and Abarghuei et al. (2010), in which sheep were

also supplemented with grape pomace but at a much higher inclusion level of up to around 76 %. Those studies are in contrast with the *in vivo* study of Correddu (2015), in which sheep were supplemented with grape seed (1 g/d per sheep of total phenolics). Other result of an *in vivo* study by Vinyard et al. (2021) also showed elevated values when grape pomace was included into the rations of beef heifers (15–30 % inclusion level). However, Khiaosa-ard et al. (2015) detected no effects on ammonia concentration when grape seed meal fortified with dried distillers grains plus solubles was added to the diets in an *in vitro* study. The highest fortification level (20 % in dried distillers grains plus solubles) resulted in about 5 % grape seed meal in the total diet dry matter. Similarly, Moate et al. (2014) showed in an *in vivo* study that inclusion of around 27 % of grape marc to dairy cow diets showed no effect on ruminal ammonia concentration. The discrepancies could be related to several factors such as ruminant species, different inclusion levels of by-product, different locations from which the by-products were obtained, different nutrient composition, *in vitro* vs. *in vivo*, etc. In the present work, the by-products were incorporated into high-protein diets from the use of high-quality hay, which is often associated with a higher rumen ammonia build up (Klevenhusen et al., 2017). The results clearly demonstrated that, at the same inclusion rate of 10 % of diet dry matter, grape seed meal and grape pomace reduced the ammonia production. Thus, it can be assumed that grape pomace and grape seed meal could be a viable option to mitigate dietary protein loss to ammonia. Interestingly, the ammonia reduction was not accompanied by decreased crude protein degradation and branched chain short-chain fatty acids, which are products of microbial metabolism of amino acids (Andries et al., 1987). Decreased ammonia concentration and a decrease in those iso-acids are often linked to a reduction of protozoa but can also be linked to inhibition of microbes and their deaminase activity (Bodas et al., 2012). However, our results show exclusively ammonia reduction along with a reduction in ammonia production efficiency and no major shifts in microbiota which would otherwise explain the lowered ammonia concentration. A dilution effect of drinking water or ammonia flow to the lower digestive tract can also affect the ruminal ammonia concentrations. However, this factor can be ruled out in this study as well. This leads to the conclusion that the reduced ammonia concentration is most likely the result of increased microbial utilization instead.

The main negative effect of feeding grape seed meal and pomace can be linked to decreased degradation of dry matter and organic matter, which were mainly attributed to the ash and fiber

fractions of the diet. The reduction in ruminal degradation was in line with the decrease in fermentation acid short-chain fatty acids concentration. These results are in agreement with several other studies. For instance, Winkler et al. (2015) found a reduction of organic matter degradation upon grape pomace addition and Khiaosa-ard et al. (2015) found lowered *in vitro* ruminal degradation of dry matter and organic matter with grape seed meal inclusion. Baumgärtel et al. (2007) showed that digestibility in general was lowered when grape pomace was added to the ration. Guerra-Rivas et al. (2017) discovered that dry matter degradation was higher in the pulp fraction of their grape pomace than in the seed fraction. Based on their findings, a lower dry matter degradation of grape seed meal compared to grape pomace could be anticipated but was not supported by our results. Moreover, the decrease in degradation of neutral detergent fiber and acid detergent fiber degradation was apparent with the grape pomace diet. The grape seed meal diet had a minor effect, but not enough to be significant. These results may be explained by the presence of stems within the grape pomace which are harder to digest due to their carbohydrate structure. Additionally, a different composition of phenolic compounds could also explain why the fiber of grape pomace was less digestible than the fiber fraction of grape seed meal. These results are in accordance with Winkler et al. (2015) and Vinyard et al. (2021), who discovered a decrease in neutral and acid detergent fiber *in vivo*. Furthermore, Abarghuei et al. (2010) reported a decrease in total tract digestibility of organic matter, neutral detergent fiber, and crude protein after supplementation with grape pomace at an inclusion level of around 76 %, which, except for the crude protein digestibility, can be aligned with our results. It should be noted that stronger effects are expected with higher inclusion rates.

In the present work, the degradation of fat was higher in the diet with grape pomace, which may be related to the higher oil content since grape seed meal is the product which is left after the de-oiling process. The different effect of grape pomace on ruminal degradation compared to grape seed meal might be related to slightly different nutritional properties and potentially a difference in phenol composition. In addition, processing of grape by-products can also influence its degradability in the rumen. For instance, Winkler et al. (2015) showed in their study that there are differences in nutrient digestibility between dried and ensiled grape pomace. According to their results, ensiled grape pomace has higher digestibility of organic matter, crude fiber, neutral and acid detergent fiber and crude protein, along with a lower phenol

content when compared to dried grape pomace. This marks a potential for future research, as different processing of the feed seems to lead to varying results regarding both phenol content of the product and ruminal digestion.

Taken all together, the results of the present study indicate that both grape pomace and grape seed meal at an inclusion level of 10 % can be safely incorporated into cattle diets without any severe detrimental effects to ruminal nutrient degradation or fermentation parameters.

6.2 Are the observed effects contributed to the functional compounds of the grape by-products?

Besides the nutritive values for ruminants, grape by-products also contain flavonoids (Peixoto et al., 2018). Thus, another important question to answer is whether the observed effects of the by-products can be attributed to their functional compounds. With that in mind, discussion in relation to the positive control (EXT vs. CON) to justify the effect of the grape phenols is necessary. Furthermore, because the supplementation of grape seed extract provided a substantially higher phenol content (3.4 %) compared to GS or GP (1.3–1.4 %), comparisons between EXT and the grape by-product treatments may offer some perspectives of the dosage effect. Bekhit et al. (2016) mentioned that the phenol content of grape seeds declines after de-oiling since part of the phenols are extracted along with the oil. Therefore, depending on the de-oiling process, grape seeds are expected to have much higher values of phenolic compounds as well as an elevated oil content, at least when compared to other parts of the grape. Both GS and GP supplied similar contents of total phenols. Thus, the de-oiling process for the test grape seed meal must have been quite strong, as both parameters were not elevated in the present study. Secondly, different profiles of the phenolic compounds cannot be ruled out since different parts of the grape itself contain different phenolic compounds. Peixoto et al. (2018) discussed in their study that grape seeds in general showed the highest content of phenols. However, anthocyanins are mainly present in the grape skin. Flavanol derivates were the most abundant phenolic compounds detected in skins but were also present in seeds and the grape pomace. The skin samples showed no flavan-3-ol derivates, those were the main compounds found in the seeds and in grape pomace mixture, to name just a few examples. Correlating with these results are the findings of Shahidi and Naczk (2004), who discussed that white and red

table grapes show different compositions of phenolic compounds. Thereby, flavan-3-ols are more prevalent in white table grapes, while anthocyanins are more abundant in red table grapes.

One of the main properties described for phenolic compounds like tannins is the ability to bind macronutrients like carbohydrates and proteins (Tayengwa and Mapiye, 2018), thereby decreasing the microbial access and fermentation of these nutrients. The extract-including diet clearly decreased crude protein degradation, as well as branched-chain short-chain fatty acid percentages, and it showed the strongest reduction in ammonia concentration. This indicates that grape phenols can modulate the ruminal protein metabolism by binding dietary protein, resulting in an increased passage of protein from the rumen into the further digestive tract (Tayengwa and Mapiye, 2018). This effect could pose as a strategy to target protein loss in the rumen, especially in combination with feedstuffs rich in ruminally degradable proteins such as the hay used in the present work. The extract diet showed the lowest values of ammonia concentration while the grape by-products showed a similar direction but ultimately a weaker effect. However, when the values are adjusted per unit of degraded crude protein, both by-products and the extract diet performed at a similar level. The fact that GS and GP only decreased ammonia production shows that while the phenols still showed an effect at 10% inclusion level, the dosage might be too low to show a decrease in crude protein degradation as well. Thus, it is more likely that the observed effect can be associated with the functional compounds in the by-products and the strength of the effect is dose dependent.

The data in this work suggests that the form (i.e., as an extract or in the plant matrix) and dosage of grape phenols determines the effect on ruminal microbiota. While the grape seed meal and the grape pomace treatments showed some effects on the ruminal microbiota, their effects were rather small compared to those of the grape seed extract diet. Most notable are the changes in bacterial family abundance of *Spirochaetaceae*, which were increased by almost three times compared to the other treatments and the control diet, as well as *Ruminococcaceae*, which were significantly decreased by the extract diet, compared to the other diets within the liquid samples. *Lactobacillaceae* were increased by all treatments in both the liquid and the solid samples, which indicates their tolerance towards different forms and dosages of phenolic compounds. Altogether, those results suggest that the solid-associated bacterial families seem to be more resistant to the phenolic compounds than the fluid-associated bacteria. Due to the high water

solubility of the grape seed extract, its phenolic compounds may have been in contact more with liquid microbiota as compared to the solid microbiota. Nevertheless, while the extract-including diet was able to alter the ruminal microbiota more than the other two diets with grape byproducts, their microbial functions were still relatively similar.

Enteric methane is not only considered to be a loss of feed energy, but it also has a big impact on the environment, as approximately 30 % of the annual global methane emissions originate from microbial fermentation in the digestive tract (Breves et al., 2015). The methane mitigating effect of grape by-products is shown in several other studies. Moate et al. (2014) showed that grape pomace (at an inclusion level of around 27 % in diet dry matter) decreased both methane yield and methane emissions, which were connected to changes in the bacterial and archaeal communities within the rumen, in dairy cows. Similarly, Khiaosa-ard et al. (2015) reported that methane formation decreased in Rusitec with increasing inclusion levels of grape seed meal in the diet. The dosages they used were much lower (maximum 5 % grape seed meal in the diet dry matter) compared to that of Moate et al. (2014). The present study, however, showed no decrease in ruminal methane production with the addition of grape by-products or grape seed extract to the ration. However, these results might not be related to dosage effects alone as a high forage diet was used in this study, whereas previous works mostly used a moderate to high content of forage as part of their diets. The abundance of archaea in the liquid samples was strongly decreased by the extract-including diet, while the grape by-products showed no different effect from the control diet. Furthermore, the archaea abundance in the solid samples was the same for all diets. This suggests that not only dosage but also other factors such as phenol form or sample type (liquid vs. solid) determine an effect of grape's phenols on methanogenesis.

The results of this thesis suggest that an inclusion level of grape by-products grape pomace and grape seed meal of 10 % in the ration could be used without an adverse effect on rumen microbiota and their activity in the rumen. However, decreased nutrient degradation was evident, but not too dramatic. Literature data indicate that higher inclusion rates would likely show stronger effects on nutrient degradation. Thus, there will be a limit for the inclusion of grape by-products, as a severe decrease of nutrient degradation would not be beneficial. Additionally, the results suggested that at a 10% inclusion rate, the by-products were able to alleviate any excessive ruminal ammonia build up, likely attributed to the effect of the phenols.

This indicates that they are a viable addition to rations with feed ingredients containing high contents of ruminally degradable protein like high-quality hay. Using grape by-products and grape seed extract at the test dosages in high forage diets were not sufficient to elicit a methane-mitigating effect. Notably, since this study was an *in vitro* experiment, several host factors such as ruminal absorption, the recycling of urea and the selection of feed by the animal were not taken into account, and thus, *in vivo* experiments will be required to confirm the potency of grape by-products as an alternative functional feed for cattle.

7 Summary

Winery by-products have no nutritional value as a food source for humans but still have value as a feed source for animals. Especially ruminants are able to make use of the high content of fiber due to their ruminal microbes. Moreover, grape by-products are rich in phenolic compounds, which have a potential to modulate ruminant health and productivity. Using those by-products as animal feed poses several economic and ecologic advantages.

The present study investigated the potential use of two grape by-products, grape pomace and grape seed meal, as an alternative feed source for cattle. Their effects on rumen microbiota, fermentation variables and nutrient degradation were studied via an *in vitro* experiment based on the Rumen Simulation Technique (RUSITEC). Grape seed extract was used as a positive control, which was added to a diet consisting of 70 % high quality hay and 30 % energy concentrate (on a dry matter basis). Test diets consisted of 65 % high quality hay, 25 % energy concentrate and 10% of grape pomace or grape seed meal (dry matter basis). Both by-products were locally produced in the region of Lower Austria. The phenol content from the by-products ranged from 1.3–1.4 %. The phenol content of the grape seed extract was 3.4 %. It was hypothesized that including grape by-products would not express any negative effects on ruminal microbial and fermentation parameters. However, they may possess different functional effects due to their differences in nutrient composition as well as the content of functional compounds.

All ruminal fermentation parameters stayed within physiological range. The main benefit of feeding grape by-products was lowering ruminal ammonia concentration, in total concentration as well as per unit of protein degraded. None of the treatments affected the methane production. The extract addition altered rumen microbiota composition more than other treatments despite similar effects on most of ruminal fermentation variables compared to grape seed and grape pomace diets. A negative effect on decreasing ruminal degradation of dry matter and organic matter was evident, especially for grape pomace from decreased fiber degradation.

The results of this thesis suggest that an inclusion of grape pomace and grape seed meal at 10 % to the ration would have no negative effects on ruminal fermentation parameters. Both grape by-products can express a significant effect on lowering ammonia production likely due to their functional compounds. This suggests that they could be a viable addition to rations with feed ingredients like high-quality hay, which are rich in ruminally degradable protein. However, as

this study was conducted *in vitro*, further *in vivo* experiments will be required to assess the value of grape seed meal and grape pomace as a functional feed substitution to cattle rations.

8 Zusammenfassung

Nebenprodukte aus der Weinproduktion haben keinen Nährwert als Nahrungsquelle für Menschen, sind aber dennoch eine potenziell wertvolle Futterquelle für Tiere. Besonders Wiederkäuer können diese Nebenprodukte trotz des hohen Fasergehaltes, aufgrund ihrer symbiotischen Beziehung mit den Mikroorganismen des Pansens, nutzen. Darüber hinaus sind Nebenprodukte von Weintrauben reich an phenolischen Verbindungen, die das Potenzial haben, die Gesundheit und Produktivität von Wiederkäuern zu beeinflussen. Dabei bietet die Verwendung dieser Nebenprodukte als Tierfutter mehrere ökonomische und ökologische Vorteile.

Die vorliegende Studie untersuchte die potenzielle Verwendung von zwei bestimmten Nebenprodukten der Weinproduktion, Weintrester und Traubenkernmehl, als alternative Futterquelle für Rinder. Ihre Auswirkungen auf die Pansenflora, verschiedene Fermentationsparameter und den Nährstoffabbau im Pansen wurden in einem *in vitro* Experiment basierend auf der Pansensimulationstechnik (RUSITEC) untersucht. Als Positivkontrolle diente Traubenkernextrakt, welches dem Basisfutter, bestehend aus 70 % hochwertigem Heu und 30 % Energiekonzentrat (auf Basis der Trockenmasse), zugesetzt wurde. Den Nebenprodukt-Rationen wurden dem Basisfutter, bestehend aus 65 % hochwertigem Heu und 25 % Energiekonzentrat, Weintrester bzw. Traubenkernmehl im Ausmaß von jeweils 10 % (bezogen auf die Trockenmasse) zugesetzt.

Dabei lag der Phenolgehalt der beiden Nebenprodukte zwischen 1,3 und 1,4 %. Der Phenolgehalt des Traubenkernextrakts lag bei 3,4 %. Es wurde die Hypothese aufgestellt, dass die Zugabe von Weintrester bzw. Traubenkernmehl keine negativen Auswirkungen auf die Pansenflora und die Fermentation haben würde. Die Nebenprodukte könnten jedoch aufgrund ihrer unterschiedlichen Nährstoffzusammensetzung und ihrer unterschiedlichen funktionellen Verbindungen verschiedene Auswirkungen hervorrufen.

Alle Fermentationsparameter blieben im physiologischen Bereich. Der Hauptvorteil der Verfütterung der Nebenprodukte der Weinproduktion war die Senkung der Ammoniakkonzentration im Pansen, sowohl der Gesamtkonzentration als auch pro Einheit an abgebautem Protein. Keine der Rationen zeigte eine Wirkung auf die Methanproduktion. Die Zugabe des Traubenkernextraktes hatte einen stärkeren Einfluss auf die Pansenflora als die anderen Rationen, obwohl die meisten Fermentationsparameter der Positivkontrolle ähnlich zu

denen der Nebenprodukt-Rationen war. Es konnte ein negativer Effekt auf den Nährstoffabbau von Trockenmasse und organischer Substanz im Pansen festgestellt werden, vor allem durch die Weintrester-Ration, welche einen deutlichen Rückgang des Faserabbaus zeigte.

Die Ergebnisse dieser Diplomarbeit legen nahe, dass eine Zugabe von Weintrester und Traubenkernmehl von 10 % zur Ration keine negativen Auswirkungen auf die Fermentationsparameter im Pansen hätte. Die Nebenprodukte zeigten eine signifikante Senkung der Ammoniakproduktion, wahrscheinlich aufgrund ihrer phenolischen Substanzen. Diese Ergebnisse deuten darauf hin, dass sie eine interessante Ergänzung zu Futterinhaltsstoffen wie hochwertigem Heu, welche reich an im Pansen abbaubarem Eiweiß sind, sein könnten.

Da es sich bei dieser Studie jedoch um eine *in vitro* Studie handelt, werden weitere *in vivo* Experimente benötigt, um den geeigneten Einsatz und den Wert von Traubenkernmehl und Weintrestern als funktionelles Futtermittel für Wiederkäuer weiter zu untersuchen.

9 References

Abarghuei MJ, Rouzbehan Y, Alipour D. 2010. The influence of the grape pomace on the ruminal parameters of sheep. Livestock Science, 132(1):73–79.

Ajila CM, Brar SK, Verma M, Tyagi RD, Godbout S, Valéro JR. 2012. Bio-processing of agro-byproducts to animal feed. Critical Reviews in Biotechnology, 32 (4): 382–400.

Akin DE, Borneman WS. 1990. Role of Rumen Fungi in Fiber Degradation. Journal of Dairy Science, 73(10):3023–3032.

Amaral FP, Grazziotin RCB, Machado MC, Hasse L, Frata M, Blanco CS, Gonçalves FM, Ribeiro-Filho HMN, Bermudes RF, Del Pino FAB, Corrêa MN, Brauner CC. 2019. Limits of grape byproduct inclusion in diets for lambs: Zinc supplementation to prevent copper poisoning. Research in Veterinary Science, 124:334-337. doi: 10.1016/j.rvsc.2019.04.005.

Anastasiadi M, Pratsinis H, Kletsas D, Skaltsounis AL, Haroutounian SA. 2012. Grape stem extracts: Polyphenolic content and assessment of their in vitro antioxidant properties. LWT - Food Science and Technology, 48(2):316–322.

Andries JI, Buysse FX, De Brabander DL, Cottyn BG. 1987. Isoacids in ruminant nutrition: Their role in ruminal and intermediary metabolism and possible influences on performances - A review. Anim Feed Sci Technol 18:169-180.

Aschenbach JR, Bilk S, Tadesse G, Stumpff F, Gäbel G. 2009. Bicarbonate-dependent and bicarbonate-independent mechanisms contribute to nondiffusive uptake of acetate in the ruminal epithelium of sheep. American Journal of Physiology Gastrointestinal and Liver Physiology, 296(5):G1098–107.

Attwood GT, Leahy SC, Kelly WJ. 2020. The Rumen Archaea. In: McSweeney CS, Mackie RI, ed. Improving rumen function. Cambridge: Burleigh Dodds Science Publishing Limited, 133–189.

Baldwin RL, Emery RS. 1960. The Oxidation-Reduction Potential of Rumen Contents. Journal of Dairy Science, 43(4): 506–511.

Baldwin RL, Barry J. 1996. Propionate Modulation of Ruminal Ketogenesis. Journal of Animal Science, 74(7):1694–700.

Bampidis VA, Robinson PH. 2006. Citrus by-products as ruminant feeds: A review. Animal Feed Science and Technology, 128 (3–4): 175–217.

Basalan M, Gungor T, Owens FN, Yalcinkaya I. 2011. Nutrient content and in vitro digestibility of Turkish grape pomaces. Animal Feed Science and Technology, 169(3–4):194–198. doi:10.1016/j.anifeedsci.2011.07.005.

Baumgärtel T, Kluth H, Epperlein K, Rodehutscord M. 2007. A note on digestibility and energy value for sheep of different grape pomace. Small Ruminant Research, 67(2):302–306.

Bayat A, Ventto L, Kairenius P, Stefanski T, Leskinen H, Tapio I, Negussie E, Vilkki J, Shingfield KJ. 2017. Dietary forage to concentrate ratio and sunflower oil supplement alter rumen fermentation, ruminal methane emissions, and nutrient utilization in lactating cows. Translational Animal Science, 1(3):277–286.

Bekhit AEA, Cheng VJ, Harrison R, Ye Z, Bekhit AA, NG TB, Kong L. 2016. Technological aspects of by-product utilization. In: Bordiga M, ed. Valorization of Wine Making By-Products. Boca Raton, Florida: CRC Press Taylor & Francis Group, 118–198.

Bertran E, Sort X, Soliva M, Trillas I. 2004. Composting winery waste: sludges and grape stalks. Bioresource Technology, 95(2):203–208.

Block SS, Butler WR, Ehrhardt RA, Bell AW, Van Amburgh ME, Boisclair YR. 2001. Decreased concentration of plasma leptin in periparturient dairy cows is caused by negative energy balance. Journal of Endocrinology, 171(2):339–348.

Bodas R, Prieto N, García-González R, Andrés S, Giráldez FJ, López S. 2012. Manipulation of rumen fermentation and methane production with plant secondary metabolites. Animal Feed Science and Technology, 176(1–4):78–93.

Bordiga M. 2016a. Overview. In: Bordiga M, ed. Valorization of Wine Making By-Products. Boca Raton, Florida: CRC Press Taylor & Francis Group, 1–25.

Bordiga M. 2016b. Regulatory and Legislative Issues. In: Bordiga M, ed. Valorization of Wine Making By-Products. Boca Raton, Florida: CRC Press Taylor & Francis Group, 199–225.

Bordiga M. 2016c. Future Perspectives. In: Bordiga M, ed. Valorization of Wine Making By-Products. Boca Raton, Florida: CRC Press Taylor & Francis Group, 293–344.

Breves G, Leonhard-Marek S, Martens H. 2015.Vormägen. In: von Engelhardt W, Breves G, Diener M, Gäbel G, ed. Physiologie der Haustiere. 5., vollständig überarbeitete Auflage. Stuttgart: Enke Verlag, 387–404.

Cammack KM, Austin KJ, Lamberson WR, Conant GC, Cunningham HC. 2018. RUMINNAT

NUTRITION SYMPOSIUM: Tiny but mighty: the role of the rumen microbes in livestock production. Journal of Animal Science, 96(2):752—770.

Cardona JA, Calix TF. 2016. Marketing Potential. In: Bordiga M, ed. Valorization of Wine Making By-Products. Boca Raton, Florida: CRC Press Taylor & Francis Group, 271–291.

Castillo-González AR, Burrola-Barraza ME, Domínguez-Viveros J, Chávez-Martínez A. 2014. Rumen microorganisms and fermentation. Archivos de Medicina Veterinaria, 46:349–361.

Chagunda MGG, Flockhart JF, Roberts DJ. 2010. The effect of forage quality on predicted enteric methane production from dairy cows. International Journal of Agricultural Sustainability, 8(4):250–256.

Clifford HA. 2015. Ensiled grape marc and the impact on ruminal function and digestion in sheep [Thesis]. Adelaide: The University of Adelaide, Faculty of Sciences, School of Animal and Veterinary Sciences, Roseworthy Campus.

Correddu F. 2015. Utilization of grape seeds in ruminant nutrition: effects of this by-product on health conditions, milk production and quality, and ruminal metabolism in Sarda dairy sheep [Dissertation]. Sassari: Università degli Studi di Sassari.

Correddu F, Gaspa G, Pulina G, Nudda A. 2016. Grape seed and linseed, alone and in combination, enhance unsaturated fatty acids in the milk of Sarda dairy sheep. Journal of Dairy Science, 99: 1725–1735.

Czerkawski JW, Breckenridge G. 1977. Design and development of a long-term rumen simulation technique (Rusitec). British Journal of Nutrition, 38 (3): 371–384.

Dehority BA. 2003. Rumen Microbiology. Nottingham: Nottingham University Press.

Dijkstra J. 1994. Production and absorption of volatile fatty acids in the rumen. Livestock Production Science, 39:61–69.

Dijkstra J, Ellis JL, Kebreab E, Strathe AB, López S, France J, Bannink A. 2012. Ruminal pH regulation and nutritional consequences of low pH. Animal Feed Science and Technology, 172(1–2):22–33.

Dou Z, Totha JD, Westendorf ML. 2018. Food waste for livestock feeding: Feasibility, safety, and sustainability implications. Global Food Security 17: 154–161.

Ertl P, Zebeli Q, Zollitsch W, Knaus W. 2016. Feeding of wheat bran and sugar beet pulp as sole supplements in high forage diets emphasises the potential of dairy cattle for human food

supply. Journal of Dairy Science, 99(2):1228–1236.

Estrellan CR, Iino F. 2010. Toxic emissions from open burning. Chemosphere, 80(3):193–207.

FAOSTAT. 2021. http://www.fao.org/faostat/en/#data/QC (Zugriff: 29.12.2022).

Fernandes L, Casal S, Cruz R, Pereira JA, Ramalhosa E. 2013. Seed oils of ten traditional Portuguese grape varieties with interesting chemical and antioxidant properties. Food Research International, 50(1):161–166.

Fernández CM, Ramos MJ, Pérez A, Rodríguez JF. 2010. Production of biodiesel from winery waste: Extraction, refining and transesterification of grape seed oil. Bioresource Technology, 101(18):7019–7024.

Foley JA, Ramankutty N, Brauman KA, Cassidy AS, Gerber JS, Johnston M, Mueller ND, O'Connell C, Ray DK, West PC, Balzer C, Bennett EM, Carpenter SR, Hill J, Monfreda C, Polasky S, Rockström J, Sheehan J, Siebert S, Tilman D, Zaks DPM. 2011. Solutions for a cultivated planet. Nature, 478: 337–342.

Fonty G, Gouet P, Jouany JP, Senaud J. 1987. Establishment of the Microflora and Anaerobic Fungi in the Rumen of Lambs. Microbiology,133(7):1835–1843.

Gessner DK, Koch C, Romberg FJ, Winkler A, Dusel G, Herzog E, Most E, Eder K. 2015. The effect of grape seed and grape marc meal extract on milk performance and the expression of genes of endoplasmic reticulum stress and inflammation in the liver of dairy cows in early lactation. Journal of Dairy Science, 98(12):8856¬¬–68. doi: 10.3168/jds.2015-9478.

Gessner DK, Winkler A, Koch C, Dusel G, Liebisch G, Ringseis R, Eder K. 2017. Analysis of hepatic transcript profile and plasma lipid profile in early lactating dairy cows fed grape seed and grape marc meal extract. BMC Genomics, 18(1):253. doi: 10.1186/s12864-017-3638-1.

Grasser LA., Fadel JG, Garnett I, DePeters EJ. 1995. Quantity and Economic Importance of Nine Selected By-Products Used in California Dairy Rations. Journal of Dairy Science, 78 (4): 962–97.

Guerra-Rivas C, Gallardo B, Mantecón AR, del Álamo-Sanza M, Manso T. 2017. Evaluation of grape pomace from red wine by-product as feed for sheep. Journal of the Science of Food and Agriculture, 97(6):1885–1893.

Hales K, Lourenco J, Seidel DS, Koyun OY, Davis D, Welch C, Wells JE, Callaway TR. 2020. The use of feedlot/cereal grains in improving feed efficiency and reducing by-products such as methane in ruminants. In: McSweeney CS, Mackie RI, ed. Improving rumen function. Cambridge: Burleigh Dodds Science Publishing Limited, 693–725.

Hanušovský O, Gálik B, Bíro D, Šimko M, Juráček M, Rolinec M, Zábranský L, Philipp C, Puntigam R, Slama JA, Gierus M. 2020. The nutritional potential of grape by-products from the area of Slovakia and Austria. Emirates Journal of Food and Agriculture, 32(1):1–10.

Herdt TH, Sayegh AL. 2012. Digestion: The Fermentative Processes. In: Klein BG, ed. CUNNINGHAM'S TEXTBOOK OF VETERINARY PHYSIOLOGY. FIFTH EDITION. St. Louis, Missouri: Elsevier Saunders, 320–341.

Hess M, Fliegerova K, Paul S, Puniya AK. 2020. The anaerobic rumen fungi. In: McSweeney CS, Mackie RI, ed. Improving rumen function. Cambridge: Burleigh Dodds Science Publishing Limited, 221–279.

Huang Y, Marden JP, Julien C, Bayourthe C. 2018. Redox potential: An intrinsic parameter of the rumen environment. Journal of Animal Physiology and Animal Nutrition, 102(2):393–402.

Huhtanen CN, Carleton FJ, Roberts HR. 1954. CARBON DIOXIDE UTILIZATION BY RUMEN MICROORGANISMS. Journal of Bacteriology, 68(6):749–751.

Humer E, Khol-Parisini A, Metzler-Zebeli BU, Gruber L, Zebeli Q. 2016. Alterations of the Lipid Metabolome in Dairy Cows Experiencing Excessive Lipolysis Early Postpartum. PLoS ONE, 11(7):e0158633. doi:10.1371/journal.pone.0158633.

Huntington GB, Archibeque SL. 2000. Practical aspects of urea and ammonia metabolism in ruminants. Journal of Animal Science, 77(suppl_E):1–11.

Huws SA, Williams CL, McEwan NR, Gordon R. 2020. Ruminal-ciliated protozoa. In: McSweeney CS, Mackie RI, ed. Improving rumen function. Cambridge: Burleigh Dodds Science Publishing Limited, 191–220.

Ianni A, Di Maio G, Pittia P, Grotta L, Perpetuini G, Tofalo R, Cichelli A, Martino G. 2019. Chemical-nutritional quality and oxidative stability of milk and dairy products obtained from Friesian cows fed with a dietary supplementation of dried grape pomace. J Sci Food Agric, 99(7):3635–3643. doi: 10.1002/jsfa.9584.

Ianni A, Martino G. 2020. Dietary Grape Pomace Supplementation in Dairy Cows: Effect on Nutritional Quality of Milk and Its Derived Dairy Products. Foods, 9(2): 168, doi:10.3390/foods9020168.

Ishler VA, Heinrichs AJ, Bánné Varga G. 1996. From feed to milk: understanding rumen function. University Park, Pa.: Pennsylvania State University.

Jackson RS. 2014. Fermentation. In: Jackson RS ed. Wine Science: Principles and Applications. Fourth Edition. London, Waltham, San Diego: Academic Press, 427–534.

Jami E, Israel A, Kotser A, Mizrahi I. 2013. Exploring the bovine rumen bacterial community from birth to adulthood. The ISME Journal, 7:1069–1079.

Johnson KA, Johnson DE. 1995. Methane emissions from Cattle. Journal of Animal Science, 73(8):2483–2492.

Kamalak A. 2005. Chemical composition and in vitro dry matter digestibility of leaves of Vitis vinifera. Livestock Research for Rural Development, 17(1):Art. #3. Retrieved October 21, 2021, from http://www.lrrd.org/lrrd17/1/kama17003.htm (21.10.2021).

Khiaosa-ard R, Metzler-Zebeli BU, Ahmed S, Muro-Reyes A, Deckardt K, Chizzola R, Böhm J, Zebeli Q. 2015. Fortification of dried distillers grains plus solubles with grape seed meal in the diet modulates methane mitigation and rumen microbiota in Rusitec. Journal of Dairy Science, 98(4):2611-2626.

Kleefisch MT, Zebeli Q, Humer E, Kröger I, Ertl P, Klevenhusen F. 2017. Effects of the replacement of concentrate and fibre-rich hay by high-quality hay on chewing, rumination and nutrient digestibility in non-lactating Holstein cows. Archives of Animal Nutrition, 71(1):21–36.

Kleefisch MT, Zebeli Q, Humer E, Gruber L, Klevenhusen F. 2018. Effects of feeding high-quality hay with graded amounts of concentrate on feed intake, performance and blood metabolites of cows in early lactation. Archives of Animal Nutrition, 72(4):290–307.

Klevenhusen F, Petri RM, Kleefisch MT, Khiaosa-ard R, Metzler-Zebeli BU, Zebeli Q. 2017. Changes in fibre-adherent and fluid-associated microbial communities and fermentation profiles in the rumen of cattle fed diets differing in hay quality and concentrate amount. FEMS Microbiology Ecology, 93(9):doi: 10.1093/femsec/fix100.

König HE, Sotonyi P, Ruberte J, Liebich HG. 2015. Verdauungsapparat, (Apparatus digestorius). In: König HE, Liebich HG, ed. Anatomie der Haussäugetiere, Lehrbuch und Farbatlas für Studium und Praxis. 6. überarbeitete und erweiterte Auflage. Stuttgart: Schattauer GmbH, 307–372.

Kraus TEC, Dahlgren RA and Zasoski RJ. 2003. Tannins in nutrient dynamics of forest ecosystems - a review. Plant Soil, 256:41–66.

Krehbiel CR. 2014. INVITED REVIEW: Applied nutrition of ruminants: Fermentation and digestive physiology. The Professional Animal Scientist, 30(2):129–139.

Lapierre H, Lobley GE. 2001. Nitrogen Recycling in the Ruminant: A Review. Journal of Dairy Science, 84(Supplement): E223-E236.

Lee C, Hristov AN, Cassidy TW, Heyler KS, Lapierre H, Varga GA, de Veth MJ, Patton RA, Parys C. 2012. Rumen-protected lysine, methionine, and histidine increase milk protein yield in dairy cows fed a metabolizable protein-deficient diet. Journal of Dairy Science, 95(10): 6042–6056.

Letaief H. 2016. Wine Making Process. In: Bordiga M, ed. Valorization of Wine Making By-Products. Boca Raton, Florida: CRC Press Taylor & Francis Group, 28–72.

Llobera A, Cañellas J. 2007. Dietary fibre content and antioxidant activity of Manto Negro red grape (Vitis vinifera): pomace and stem. Food Chemistry, 101(2):659–666.

Magnier L. 1991. Utilisation des sous-produits de la vigne dans l'alimentation animale. In: Tisserand JL, ed. Alibés X. (ed.). Fourrages et sous-produits méditerranéens. Zaragoza: CIHEAM, 89–99. (Options Méditerranéennes: Série A. Séminaires Méditerranéens; n. 16). Fourrages et Sous-Produits Méditerranéens, 5–6 Jul 1990, Montpellier (France). http://om.ciheam.org/om/pdf/a16/91605050.pdf

Makris DP, Boskou G, Andrikopoulos NK. 2007. Polyphenolic content and in vitro antioxidant characteristics of wine industry and other agri-food solid waste extracts. Journal of Food Composition and Analysis, 20(2):125–132.

Marden JP, Bayourthe C, Enjalbert F, Moncoulon R. 2005. A New Device for Measuring Kinetics of Ruminal pH and Redox Potential in Dairy Cattle. Journal of Dairy Science, 88(1): 277–281.

Martin C, Morgavi DP, Doreau M. 2010. Methane mitigation in ruminants: From microbe to the farm scale. animal, 4(3):351–65.

Martínez M, Miralles N, Hidalgo S, Fiol N, Villaescusa I, Poch J. 2006. Removal of lead(II) and cadmium(II) from aqueous solutions using grape stalk waste. Journal of Hazardous Materials, 133(1–3):203–211.

Martínez ME, Ranilla MJ, Tejido ML, Ramos S, Carro MD. 2010. Comparison of fermentation of diets of variable composition and microbial populations in the rumen of sheep and Rusitec fermenters. I. Digestibility, fermentation parameters, and microbial growth. Journal of Dairy Science, 93 (8): 3684–98.¬

May P. 2000. From bud to berry, with special reference to inflorescence and bunch morphology in Vitis vinifera L. Australian Journal of Grape and Wine Research, 6(2):82–98.

McCann JC, Luan S, Cardoso FC, Derakhshani H, Khafipour E, Loor JJ. 2016. Induction of Subacute Ruminal Acidosis Affects the Ruminal Microbiome and Epithelium. Frontiers in Microbiology, 7(169):Article 701.

Mekonnen MM, Hoekstra AY. 2012. A Global Assessment of the Water Footprint of Farm Animal Products. Ecosystems, 15: 401–415.

Mertens DR. 1997. Creating a System for Meeting the Fiber Requirements of Dairy Cows. Journal of Dairy Science, 80(7):1463–1481.

Mizrahi I, Jami E. 2018. Review: The compositional variation of the rumen microbiome and

its effect on host performance and methane emission. Animal, 12(s2):s220–s232.

Moate PJ, Williams SRO, Torok VA, Hannah MC, Ribaux BE, Tavendale MH, Eckard RJ, Jacobs JL, Auldist MJ, Wales WJ. 2014. Grape marc reduces methane emissions when fed to dairy cows. Journal of Dairy Science, 97(8):5073–5087.

Mohamed RAI, Chaudhry AS. 2008. Methods to study degradation of ruminant feeds. Nutrition Research Reviews, 21(1):68–81.

Mokni M, Amri M, Limam F, Aouani E. 2017. Effect of grape seed and skin supplement on milk yield and composition of dairy ewes. Tropical Animal Health and Production, 49:131–137.

Moldes AB, Vázquez M, Domínguez JM, Díaz-Fierros F, and Barral MT. 2008. Negative effect of discharging vinifcation lees on soils. Bioresource Technology, 99(13):5991–5996.

Molina-Alcaide E, Moumen A, Martín-García I. 2008. By-products from viticulture and the wine industry: Potential as sources of nutrients for ruminants. Journal of the Science of Food and Agriculture, 88(4):597–604.

Moote PE, Church JS, Schwartzkopf-Genswein KS, Van Hamme JD. 2014. Effect of Fermented Winery By-Product Supplemented Rations on the Temperament and Meat Quality

of Angus Hereford X Steers During Feeding in a British Columbia Feedlot. Journal of Food Research, 3(6):124–135.

Moran J. 2005. How the rumen works. In: Moran J, ed. Tropical Dairy Farming: Feeding Management for Small Holder Dairy Farmers in the Humid Tropics. Collingwood VIC 3066: Landlinks Press, 41–49.

Mould FL, Orskov ER. 1983. Manipulation of rumen fluid pH and its influence on cellulolysis in sacco, dry matter degradation and the rumen microflora of sheep offered either hay or concentrate. Animal Feed Science and Technology, 10(1):1–14.

Naas AE, Pope PB. 2020. Ruminal fibre digestion. In: McSweeney CS, Mackie RI, ed. Improving rumen function. Cambridge: Burleigh Dodds Science Publishing Limited, 349–381.

Naziri E, Nenadis N, Mantzouridou FT, Tsimidou MZ. 2014. Valorization of the major agrifood industrial by-products and waste from Central Macedonia (Greece) for the recovery of compounds for food applications. Food Research International, 65(C):350–358.

Nielsen B, Hansen H. 2004. Effect of grape pomace rich in flavonoids and antioxidants on production parameters in dairy production. Journal of Animal and Feed Sciences, 13(1):535–538.

Nistor E, Dobrei A, Dobrei A, Bampidis V, Ciolac V. 2014. Grape pomace in sheep and dairy cows feeding. Journal of Horticulture, Forestry and Biotechnology, 18(2):146–150.

Nocek JE, Russell JB. 1988. Protein and Energy as an Integrated System. Relationship of Ruminal Protein and Carbohydrate Availability to Microbial Synthesis and Milk Production. Journal of Dairy Science, 71(8): 2070–2107.

Nudda A, Correddu F, Marzano A, Battacone G, Nicolussi P, Bonelli P, Pulina G. 2015. Effects of diets containing grape seed, linseed, or both on milk production traits, liver and kidney activities, and immunity of lactating dairy ewes. Journal of Dairy Science, 98(2):1157–1166.

Oliveira M, Duarte E. 2016. Integrated approach to winery waste: waste generation and data consolidation. Frontiers of Environmental Science & Engineering, 10 (1): 168–176.

Peixoto CM, Dias MI, Alves MJ, Calhelha RC, Barros L, Pinho SP, Ferreira ICFR. 2018. Grape pomace as a source of phenolic compounds and diverse bioactive properties. Food Chemistry, 253:132-138.

Petri RM, Kleefisch MT, Metzler-Zebeli BU, Zebeli Q, Klevenhusen F. 2018. Changes in the Rumen Epithelial Microbiota of Cattle and Host Gene Expression in Response to Alterations in Dietary Carbohydrate Composition. Applied and Environmental Microbiology, 84(12):e00384–18.

Pimentel D, Berger B, Filiberto D, Newton M, Wolfe B, Karabinakis E, Clark S, Poon E, Abbett E, Nandagopal S. 2004. Water Resources: Agricultural and Environmental Issues. BioScience, 54 (10): 909–918.

Pinelo M, Rubilar M, Jerez M, Sineiro J, Nunez MJ. 2005. Effect of Solvent, Temperature, and Solvent-to-Solid Ratio on the Total Phenolic Content and Antiradical Activity of Extracts from Different Components of Grape Pomace. Journal of Agricultural and Food Chemistry, 53(6):2111-7.

Ragni M, Vicenti A, Melodia L, Marsico G. 2014. Use of Grape Seed Flour in Feed for Lambs and Effects on Performance and Meat Quality. APCBEE Procedia, 8:59–64.

Ray, B. 2004. Factors influencing microbial growth in food. Fundamental food microbiology (3rd ed., pp. 75–76). Boa Raton, FL, London, New York, NY, Washington, DC: CRC Press.

Reece WO. 1997. Physiology of Domestic Animals. Baltimore, Philadelphia, London, Paris, Bangkok, Buenos Aires, Hong Kong, Munich, Sydney, Tokyo, Wroclaw: Williams & Wilkins.

Reist M, Erdin D, von Euw D, Tschuemperlin K, Leuenberger H, Chilliard Y, Hammon HM, Morel C, Philipona C, Zbinden Y, Kuenzi N, Blum JW. 2002. Estimation of Energy Balance at the Individual and Herd Level Using Blood and Milk Traits in High-Yielding Dairy Cows. Journal of Dairy Science, 85(12):3314–3327.

Resconi VC, Pascual-Alonso M, Aguayo-Ulloa L, Miranda-de la Lama GC, Alierta S, Campo MM, Olleta JL, Villarroel M, María GA. 2018. Effect of Dietary Grape Pomace and Seed on Ewe Milk and Meat Quality of Their Suckling Lambs. Journal of Food Quality, 2018:1–8. DOI:10.1155/2018/2371754.

Reynolds AG, Wardle DA, Naylor AP. 1995. Impact of training system and vine spacing on vine performance and berry composition of Chancellor. American Journal of Enology & Viticulture, 46(1):88–97

Rolinec M, Bíro D, Šimko M, Juráček M, Hanušovský O, Schubertová Z, Chadimová L, Gálik B. 2021. Grape Pomace Ingestion by Dry Cows Does Not Affect the Colostrum Nutrient and Fatty Acid Composition. Animals, 11(6):1633. doi: 10.3390/ani1106163.

Rombaut N, Savoire R, Thomasset B, Castello J, Van Hecke E, Lanoisellé JL. (2015). Optimization of oil yield and oil total phenolic content during grape seed cold screw pressing. Industrial and Crops Production, 63:26–33.

Rubio M, Alvarez-Ortí M, Alvarruiz A, Fernández E, Pardo JE. (2009). Characterization of oil obtained from grape seeds collected during berry development. Journal of Agricultural and Food Chemistry, 57(7):2812–2815.

Sabir A, Unver A, Kara Z. 2012. The fatty acid and tocopherol constituents of the seed oil extracted from 21 grape varieties (Vitis spp.). Journal of the Science of Food and Agriculture, 92:1982–1987.

Salomon FV. 2015. Verdauungsapparat, Apparatus digestorius. In: Salomon FV, Geyer H, Gille U, ed. Anatomie für die Tiermedizin. 3., aktualisierte und erweiterte Auflage. Stuttgart: Enke Verlag, 248–340.

Scheller HV, Ulvskov P. 2010. Hemicelluloses. Annual Review of Plant Biology, 61(1):263–89.

Schwab CG, Bozak CK, Whitehouse NL, Mesbah MMA. 1992. Amino Acid Limitation and Flow to Duodenum at Four Stages of Lactation. 1. Sequence of Lysine and Methionine Limitation. Journal of Dairy Science, 75(12):3486–3502.

Scuderi RA, Ebenstein DB, Lam YW, Kraft J, Greenwood SL. 2019. Inclusion of grape marc in dairy cattle rations alters the bovine milk proteome. Journal of Dairy Research, 86(2):154–161. doi: 10.1017/S0022029919000372.

Seo JK, Kim MH, Yang JY, Kim HJ, Lee CH, Kim KH, Ha JK. 2013. Effects of Synchronicity of Carbohydrate and Protein Degradation on Rumen Fermentation Characteristics and Microbial Protein Synthesis. Asian-Australasian Journal of Animal Sciences, 26(3): 358–365.

Shahidi F, Naczk M. 2004 (edition 2006). Phenolics in Food and Nutraceuticals. Boca Raton, London, New York, Washington, D.C.: CRC Press LLC.

Shen H, Xu Z, Shen Z, Lu Z. 2019. The Regulation of Ruminal Short-Chain Fatty Acids on the Functions of Rumen Barriers. Frontiers in Physiology, 10:1305.

Spanghero M, Salem AZM, Robinson PH. 2009. Chemical composition, including secondary metabolites, and rumen fermentability of seeds and pulp of Californian (USA) and Italian grape pomaces. Animal Feed Science and Technology, 152(3–4): 243–255.

Spigno G, Pizzorno T, De Faveri DM. 2008. Cellulose and hemicelluloses recovery from grape stalks. Bioresource Technology, 99(10):4329–4337.

STATISTIK AUSTRIA. 2021a. Weinbau. https://www.statistik.at/statistiken/land-und-forstwirtschaft/pflanzenbau/wein/weinbau (Zugriff: 29.12.2022).

STATISTIK AUSTRIA. 2021b. Weinernte und Weinbestand, Kalenderjahr 2020. (Zugriff: 29.12.2022).

Tayengwa T, Mapiye C. 2018. Citrus and Winery Wastes: Promising Dietary supplements for Sustainable Ruminant Animal Nutrition, Health, Production, and Meat Quality. Sustainability, 10, 3718; doi:10.3390/su10103718.

Terry SA, Romero CM, Chaves AV, McAllister TA. 2020. Nutritional factors affecting greenhouse gas production from ruminants: implications for enteric and manure emissions. In: McSweeney CS, Mackie RI, ed. Improving rumen function. Cambridge: Burleigh Dodds Science Publishing Limited, 505–545.

Vanhatalo A, Halmemies-Beauchet-Filleau A. 2020. Optimising ruminal function: the role of silage and concentrate in dairy cow nutrition to improve feed efficiency and reduce methane and nitrogen emissions. In: McSweeney CS, Mackie RI, ed. Improving rumen function. Cambridge: Burleigh Dodds Science Publishing Limited, 651–691.

Van Soest PJ. 1994. Nutritional ecology of the ruminant. New York: Cornell University Press.

VDLUFA, Handbuch der Landwirtschaftlichen Versuchs-und Untersuchungsmethodik (VDLUFA-Methodenbuch), Bd. III Die Chemische Untersuchung von Futtermitteln. 8. Erg. 2012. VDLUFA-Verlag, Darmstadt, Germany.

Vinyard JR, Myers CA, Murdoch GK, Rezamand P, Chibisa GE. 2021. Optimum grape pomace proportion in feedlot cattle diets: ruminal fermentation, total tract nutrient digestibility, nitrogen utilization, and blood metabolites. Journal of Animal Science, 99(2):1–11. doi:10.1093/jas/skab044.

Weigand E, Young JW, McGilliard AD. 1975. Volatile Fatty Acid Metabolism by Rumen Mucosa from Cattle Fed Hay or Grain. Journal of Dairy Science, 58(9): 1294-1300.

Winkler A, Weber F, Ringseis R, Eder K, Dusel G. 2015. Determination of polyphenol and crude nutrient content and nutrient digestibility of dried and ensiled white and red grape pomace cultivars. Archives of Animal Nutrition, 69(3):1–14.

Ye Z, Harrison R, Cheng VJ, Bekhit AEA. 2016. Wine Making By-Products. In: Bordiga M, ed. Valorization of Wine Making By-Products. Boca Raton, Florida: CRC Press Taylor & Francis Group, 73–115.

Yilmaz Y, Toledo RT. 2004. Health aspects of functional grape seed constituents. Trends in Food Science & Technology, 15(9):422–433.

Zebeli Q, Aschenbach JR, Tafaj M, Boguhn J, Ametaj BN, Drochner W. 2012. Invited review: Role of physically effective fiber and estimation of dietary fiber adequacy in high-producing dairy cattle. Journal of Dairy Science, 95(3):1041–1056.

Zebeli Q, Metzler-Zebeli BU. 2012. Interplay between rumen digestive disorders and diet-induced inflammation in dairy cattle. Veterinary Science, 93(3):1099–1108.

Zhong R, Cui D, Ye ZH. 2019. Secondary cell wall biosynthesis. New Phytologist, 221: 1703–1723.

Zhou M, Liu J, Guan LL. 2020. The rumen wall microbiota community. In: McSweeney CS, Mackie RI, ed. Improving rumen function. Cambridge: Burleigh Dodds Science Publishing Limited, 321–345.

10 List of figures and tables