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Effects of inclusion levels of dried grape pomace in high-quality hay based diets on rumen fermentation characteristics studied using RUSITEC

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Content

1 Introduction and literature review

The wine industry is an important part of Austrian agriculture. However, like other producers in the agro-industrial sector, the wine production produces a considerable amount of waste products in the form of grape pomace. There are several ways of recycling these solid wastes as they have negative effects on the environment when simply disposed. Due to the chemical composition of grape pomace, which is rich in fiber as well as phenolic compounds, its exploitation as ruminant feed is of good interest, thereby harnessing not only the energy from the fiber and other nutrients but also taking advantages of its natural bioactive compounds to improve rumen health. This strategy would contribute to the global initiative of reducing food waste and, at the same time, decrease the burden of cattle farming on the environment especially concerning methane (CH_4) and nitrogen emission. This chapter provides information regarding basic knowledge on ruminal fermentation and research data regarding forage feeding, the properties of grape pomace as feed and functional compounds sources, and how inclusion of grape pomace could beneficially modulate ruminal fermentation in cattle.

1.1 A brief overview of rumen fermentation

Mammals are incapable of digesting fibrous carbohydrates like cellulose and hemicellulose. Therefore, herbivores live in symbiosis with microorganisms in their digestive tract that are able to degrade these complex carbohydrates, resulting in compounds that can be absorbed and used by the host animal as the main source of energy and nutrients. In cattle, the major event of this process takes place inside the rumen.

Carbohydrates and proteins are the major nutrients needed by ruminal microorganisms (Hoover and Stokes 1991). Ruminal protein degradation is necessary for microbial metabolic activity inside the rumen (Bach et al. 2005), but excessive protein degradation could be undesirable. Dietary protein and non-protein nitrogen compounds are utilized by ruminal microbes by extracellular microbial proteases yielding peptides, amino acids and ammonia (NH_3). These N products are taken up by microbes for microbial protein synthesis or are absorbed in the rumen and transported to the liver via the portal vein. In the liver, the potentially toxic end product NH_3 is converted to urea or glutamine (Parker et al. 1995). Microbial degradation of branch-chain amino acids like leucine, valine, isoleucine and proline results in

iso butyrate, iso valerate and valerate which are considered as part of short chain fatty acids (SCFA) whose majority is produced by carbohydrate fermentation (Andries et al. 1987).

NH_3 can then either be used for microbial protein synthesis or recycled back to the liver. Microbial protein synthesis is the majority (over 50%) of proteins supplied for the small intestine and therefore for the host ruminant (Seo et al. 2013). Microbial protein synthesis is an energy and therefore carbohydrate dependent reaction; when insufficient carbohydrates are provided in the diet, NH_3 can accumulate in the rumen (Reynolds and Kristensen 2008). When NH_3 is transported back to the liver it can be converted to urea (Bach et al. 2005). Urea is then either excreted via urine or recycled to the rumen via salvia or blood (Alemneh 2019). This nitrogen cycle enables ruminants to maintain their nitrogen household in times of an undersupply of dietary proteins and is seen as an evolutionary advantage over other mammals (Reynolds and Kristensen 2008). However, when the amount of NH_3 produced outweighs the NH_3 utilized, large amounts of NH_3 have to be excreted as manure. As NH_3 uptake and therefore NH_3 emission is positively correlated with nitrogen intake in the diet, reducing excessive crude protein in the feed is desirable (Reynolds and Kristensen 2008). Furthermore, milk urea increases as well with a higher amount of crude protein in the diet (Frank and Swensson 2002). Another mentionable point is that protein degradability depends on the solubility, time spent inside the rumen and susceptibility to proteases of microorganisms (Parker et al. 1995).

Carbohydrates are polymers of sugar or sugar derivatives. In the diet, they mainly consist of structural (hemicellulose, cellulose, lignin) and non-structural (sugars, starch, pectin) carbohydrates. Structural carbohydrates are insoluble in neutral detergents and need to be degraded by microbial enzymes whereas non-structural carbohydrates such as starch and sugars are often soluble (Nocek and Russell 1988). Carbohydrates are the main source of energy for ruminal microbes and determine the energy content of the diet. The fermentation of plant carbohydrates takes place mainly in the rumen, where they are enzymatically hydrolyzed to oligosaccharides by the microbes (Hoover and Stokes 1991). The end products of carbohydrate fermentation are SCFA, mostly acetate, propionate and butyrate (Nozière et al. 2010). The composition of SCFA depends on the type of carbohydrate as well in which fibrous carbohydrates promote acetate production while starch promotes propionate production (Bauman et al. 1971, Mertens 1997).

Besides soluble products, ruminal fermentation also results in formation of gases. Because of the contribution of ruminants to the greenhouse gas their harm to the environment is widely discussed. They emit CH_4, carbon dioxide and nitrous oxide, though CH_4 emission is the

greenhouse gas attributed mainly to cattle farming (Beauchemin 2009). CH_4 is a by-product generated by methanogens during the anaerobic process of fermenting carbohydrates especially fibrous carbohydrates in the rumen (Nocek and Russell 1988). It must be noted that methanogenesis is important for the survival of ruminal microbes. They reduce carbon dioxide with hydrogen to CH_4 so that hydrogen cannot accumulate inside the rumen (Beauchemin 2009). Whilst natural CH_4 sources have remained stable, anthropogenic CH_4 emissions have increased in the past decades (Lassey 2008). The main anthropogenic emissions arise almost equally from using fossil fuels and agricultural waste. Enteric fermentation of livestock alone constitutes about 23% of the global CH_4 source (Jackson et al. 2020, Lassey 2008). Although methanogenesis is needed to protect ruminal microbiota, it is possible to reduce it. Therefore, dietary options to reduce CH_4 emission from cattle are required. Appealing ways for farmers are increasing the amount of grains, using lipids in the diet, or the supplementation with ionophores (e.g. monensin) because these ways also enhance production efficiency (Beauchemin et al. 2008). However, there are limitations too because these methods could lead to some disadvantages. For example, too much grain can result in acidosis and lipids can suppress fiber degradation. The EU has also banned the use of antibiotics (including monensin sodium) as feed additives to promote growth. More recent approaches are the inclusion of bioactive plant compounds like phenolic compounds and essential oils, as well as other rumen fermentation modifiers (Beauchemin 2009).

1.2 High-quality hay for cattle

Due to the bulkiness limiting dry matter intake and the relatively low energy contents, fibrous feeds often cannot keep up with high demands for energy and nutrients in high-producing animals such as cows in early lactation. Instead, high amounts of starch-rich concentrates are frequently fed to dairy cows to overcome their state of negative energy balance. This strategy has been severely questioned in the past. For once, concentrate has become more expensive over the years (Klevenhusen et al. 2017). Also, these easily digestible carbohydrates are quickly fermented to SCFA which can accumulate in the rumen and decrease ruminal pH, possibly leading to metabolic acidosis (Zebeli and Metzler-Zebeli 2012). Sufficient salivation is essential for ruminal health because its bicarbonate and phosphate buffers neutralize the acidity in the rumen induced by ruminal fermentation (Allen 1997). Feeding high amounts of grain reduces the structural carbohydrate intake and thus decreases sufficient chewing and

rumination that promotes salivation to maintain stable rumen conditions especially a stable pH. Therefore, alternative feeding strategies are requested.

One possibility is feeding high-quality forage-based diets which can satisfy the need for structural carbohydrates and at the same time energy and nutrients, and thus would be healthier for cows (Klevenhusen et al. 2017). In this context, high-quality hay is an important player. It is characterized by its richness in crude protein and water-soluble carbohydrates, mainly oligosaccharides (around 20% of dry matter (Kleefisch et al. 2017)) and therefore energy. Its fiber content is lower than low and medium-quality hay but is still greater than all cereal grains (Kleefisch et al. 2018). The high water-soluble carbohydrate content of high-quality hay supplies enough energy and at the same time the sufficient fiber content to ensure proper rumen function (Tafaj et al. 2005). High-quality hay is grown and dried in Austria and therefore available to Austrian farmers to use as feed for their cattle. There is a need to recognize its positive and negative aspects. It has been shown that using high-quality hay, which shifts the kind of carbohydrates to more water-soluble carbohydrates and increases the protein supply for microbes, modulates ruminal microbial community.

One concern regarding feeding high-quality hay is whether the low fiber content offers enough structural effectiveness to induce sufficient chewing and consequently salivation to maintain stable rumen conditions especially a stable pH (Tafaj et al. 2005). This point was validated by a recent study in dry cows (Kleefisch et al. 2017). They showed that in comparison to control (normal hay plus 60% concentrate), though the time spent eating was reduced when high-quality hay as the only component of the diet was used. The high-quality hay feeding still ensured a sufficiently long time spent ruminating and chewing and therefore led to a stable pH in the rumen of dry cows possibly due to enough salivation as buffer. This effect on chewing and pH was not seen when the high-quality hay was combined with energy concentrate (25 or 40% of the diet DM) as the combination led to a less structural effectiveness and lowered the pH. Nevertheless, despite some differences on chewing activity, the changes on the ruminal pH were still within a physiological range, i.e. the pH was above 6.0 (Kleefisch et al. 2017). Besides, cows in lactation appear to utilize high-quality hay more efficiently than dry cows. According to a more recent study by Klevenhusen et al. (2018) performed in cows in early lactation, using 100% high-quality hay or in combination with concentrate up to 40% does not impair chewing activity and productivity of the animals. Another study showed that when small amounts of concentrate (under 20%) are combined with high-quality hay, it leads to better rumen conditions for fiber digestion than when combined with high-fiber hay (Tafaj et al. 2005).

All in all, using high-quality hay is not detrimental to sufficient chewing and rumination in dairy cows.

Because of its high protein content (up to 20%) (Kleefisch et al. 2017) which is highly digestible (Kleefisch et al. 2018), one major drawback of feeding high-quality hay to ruminants is excessive ruminal degradation of the protein (Klevenhusen et al. 2017). The researchers reported a substantial increase of NH_3 concentration with an increasing level of high-quality hay in the diet of dry cows; the concentration was up to three times higher than when using normal-quality hay plus concentrate in the ruminal liquid fraction and up to five times in the solid associated fraction. The rise of NH_3 in the rumen could be seen as early as two hours after feeding. The same team studied cows in lactation and reported that while there was no difference between body conditioning score and body fat thickness in comparison to the control group (high-fiber hay and concentrate), the oversupply of dietary nitrogen due to the high crude protein led to an increased milk urea when 100% high quality-hay was fed without the concentrate (Kleefisch et al. 2018). Milk urea reflects a protein and energy balance in the diet and therefore its availability for ruminal microbial protein synthesis. In the 100% high quality-hay group, the energy concentration was below the recommended concentration which led to an overall energy deficit for these cows. The overload of NH_3 due to the increased crude protein intake worsened the negative energy state and due to higher amounts of non-esterified fatty acids (NEFA) the risk of ketosis increased. They, however, did not investigate ruminal NH_3 concentration (Kleefisch et al. 2018).

In line with the effect on N-compounds in the rumen and in milk, including more high-quality hay shifted the population of the epimural bacteria from *Firmicutes* to *Proteobacteria* (Petri et al. 2018) but this change was not seen in the liquid and solid-associated population in the rumen (Klevenhusen et al. 2017). Furthermore, Kleefisch et al. (2018) underlined some effects on the metabolic status of the early lactation cows fed high-quality hay only. They concluded that the hay can decrease the dependency on concentrates but cannot completely replace it in feeding of early lactation cows. Solely feeding high-quality hay is, however, not a problem in dry cows that have a lower requirement for energy and nutrients (Kleefisch et al. 2017).

Hard evidence for an effect of high-quality hay in producing CH_4 is generally lacking. However, its ability to promote digestibility of fiber (neutral detergent fiber, acid detergent fiber as well as crude fiber) compared to the fibers in hay with lower quality and concentrates (Kleefisch et al. 2018) suggests its potency to promote CH_4 formation in the rumen considering fibrous highly digestible feeds lead to a higher availability of nutrients for microbes including microbes

involved in methanogenesis (Hindrichsen et al. 2004) and could therefore lead to a higher CH_4 emission (Olivares-Palma et al. 2013).

It can be seen, that feeding high-quality hay to cattle is a promising approach, yet some negative effects must be taken into consideration. Especially the increasing amount of NH_3 is an undesirable effect. Additional feeding strategies might be the solution. For example, natural functional compounds like the polyphenols, which are able to bind protein in the rumen, could consequently balance the nitrogen-pool (Tayengwa and Mapiye 2018).

1.3 Exploitation of winery by-products as functional feed for cattle

The agricultural sector of wine production is of great importance in Austria. Being the second most popular alcoholic beverage after beer, 2.4 million hectoliters of grapevine (*Vitis vinifera*) were harvested only in 2020, mostly in Lower Austria, Styria and Burgenland but also in Vienna (Statistik Austria 2021). During the process of wine production around 25% of the grapes are classified as winery waste products. These are the solid wastes that remain after the pressing of the wine before (white wine) or after (red wine) the fermentation, called grape pomace. It consist mostly of seeds, skins and stems and is rich in fiber. When simply disposed they pose a serious threat for the environment as they pollute the soil and groundwater and attract vectors that could spread diseases (Dwyer et al. 2014). Exploitation of these winery by-products is of general interest, aiming to reduce global food waste.

Grape pomace is often recycled as compost, having a sufficient carbon to nitrogen ratio that is demanded for composting substrates. But due to high tannin contents that could potentially have a negative impact on the soil and a lack of nutrients needed for the compost process this solution is not optimal. Other approaches are turning solid wastes into spirits (Grappa) or protein powder. Grape seeds can be separated from pomace and processed to grape seed oil which can further be exploited in the food, pharmaceutical or cosmetic industry. However, the production of mentioned products still results in other by-products and the entire grape pomace is not completely utilized (Dwyer et al. 2014, Soceanu et al. 2021).

Grape pomace is not only rich in fiber but also in natural functional compounds, the phenolic compounds which are categorized as secondary metabolites that are produced during the plant's intermediary metabolism (Jayanegara et al. 2012). They are an inhomogeneous group of molecules or polymers that are characterized by an aromatic ring with one or more hydroxyl

groups that are directly attached to the aromatic ring and can be roughly divided into flavonoids and non-flavonoids (Barcia et al. 2015). The main polyphenols are phenolic acids, proanthocyanidins or condensed tannins, flavonols, anthocyanins and flavan-3-ols (Waghorn and McNabb 2003). Approximately 70% of the total phenolic content remains in the grape pomace after wine production (Dwyer et al. 2014).

Polyphenols can be found not only in wine but in many other forage plants, legumes, cereals and seeds such as citrus, green tea, cranberry or acacia (Sinz et al. 2019, Tayengwa and Mapiye 2018). Driven by the global aim of using natural substances and reducing food waste, there are many studies on their beneficial impacts and how phenolic compounds can be utilized. Polyphenols have antioxidant, anti-helmintic and antimicrobial properties (Chedea et al. 2017, Peixoto et al. 2018) and prevent bloats (Patra A. and Saxena J. 2011, Waghorn and McNabb 2003).

Several studies have investigated the effect of grape pomace and polyphenols on rumen fermentation and animal performance. Polyphenols have the ability to form bonds with proteins by phenolic hydroxyl groups (Frutos et al. 2004) -which also occurs under the ruminal condition (Tayengwa and Mapiye 2018). Thus polyphenols decrease ruminal protein degradation resulting in a higher protein flow to the small intestine which can then be utilized by the host and in consequence decreased urine NH_3 excretion (Tayengwa and Mapiye 2018).

Polyphenols, especially tannins, are able to mitigate CH_4 emission by suppressing the activity of methanogens (Beauchemin 2009, Moate et al. 2014, Sinz et al. 2019). However, high dosages seem to be required since a different effect was found when low doses of polyphenols were included in the diet (Jayanegara et al. 2012).

It is known that the content and composition of the functional compounds of grape wine (*Vitis* spp.) depends on breed, agro-technical factors, soil and climate zone as well as how grape pomace is obtained. For example, the skin of red wine is richer in total phenolics and fiber than white wine, which on the other hand contains higher total sugar. Anthocyanins are only found in red wine (Dwyer et al. 2014, Hüthmayr 2012) which also contains more grape seeds than white wine (Böchzelt et al. 2003). Grape seeds generally have higher concentrations of polyphenols than other parts of the grapevine except for anthocyanins which are mainly found in the skins of red wine (Peixoto et al. 2018). Due to their diversity, the impact of polyphenols depends on the type, molecular size and concentration (Frutos et al. 2004). While especially hydrolysable tannins could potentially have harmful effects on the host's health, other

polyphenols like condensed tannins or anthocyanins could be beneficial when fed in the right dosage. The intake of high amounts of grape pomace (>150 g/kg DM) has been shown to reduce dry matter intake (Frutos et al. 2004, Tayengwa and Mapiye 2018), probably due to their low palatability and taste and might be related to decreased digestibility. For instance, according to a meta-analysis, ruminal digestibility of organic matter is reduced by increasing dietary condensed tannin concentration (Jayanegara and Palupi 2010). The reason for this could be related to their tendency to bind natural polymers such as proteins and carbohydrates. The pH value remained stable after grape pomace intake (Tayengwa and Mapiye 2018).

Aforementioned factors suggest variations in the properties of grape pomace as cattle feed and functional compound sources. To the author's best knowledge, no study has evaluated the potential of using grape pomace from Austrian viticulture as functional feed for cattle.

It is clear that grape pomace could only be partially used in the diet of cattle because high amounts could affect the intake as well as ruminal fermentation as explained before. The effective dosages, however, would depend on the basal diet, which may, in turn, interfere with the dominant effect of grape pomace. Due to the property of grape polyphenols, it is convincing that grape byproducts should be supplemented in feed sources that are highly digestible rich in protein such as high-quality hay to reduce excessive ruminal protein degradation, which is undesirable due to losses of good quality protein of the diet and the body's energy cost to handle excessive NH_3 as well as nitrogen emission. Furthermore, an additional desired property related to their inhibiting effect on methanogenesis (Beauchemin 2009, Moate et al. 2014) could be anticipated when combining highly digestible hay with grape pomace.

Considered these facts, combining high-quality hay with high-polyphenolic plants could be a strategy to keep the benefits of feeding high-quality hay whilst overcoming its negative aspects. However, optimal inclusion levels of grape pomace in such diets have yet to be evaluated.

2 Hypothesis and aim of this thesis

This thesis explored the favorable effects of using different inclusion levels of wine byproduct grape pomace in high-quality hay-based diets on ruminal fermentation in vitro. Both high-quality hay and grape pomace were Austrian-produced. The grape pomace came locally from a producer in the Lower Austria region. Using an in vitro method to simulate the rumen fermentation called RUSITEC, the effect of two different dosages of grape pomace in combination with high-quality hay were investigated.

This thesis tested the hypothesis that, due to the properties of phenolic compounds to bind macromolecules especially protein in the rumen, inclusion of grape pomace in a dairy cow diet can help reducing an excessive ruminal protein degradation and mitigate ruminal CH_4 formation. As such, grape pomace represents a ruminant's feed source with added functional effects. Altogether, the present study evaluated the effective dosage of grape pomace that overcomes the downsides of feeding high-quality hay whilst maintaining sufficient ruminal fermentation.

3 Materials and Methods

3.1 Experimental design and dietary treatments

Two RUSITEC (rumen simulation technique) systems owned by the University of Veterinary Medicine Vienna were used to conduct this experiment. RUSITEC is a well-established in vitro method that simulates fermentation of the rumen including a continuous flow of salivary buffer and outflow of fermentation liquid (Wetzels et al. 2018). Each RUSITEC system consists of six fermenters, therefore a total of twelve fermenters were available for each experimental run but only eight fermenters were included in the present work as the remaining four fermenters were used for a different experiment including grape seed meal.

Four different types of diets were tested: the negative control (CON), the positive control (EXT), as well as low (GP low) and high (GP high) doses of grape pomace as described below.

- Negative control: 70% high-quality hay + 30% concentrate mix (dry matter (DM) basis)
- Positive control: 70% high-quality hay + 30% concentrate mix + commercial grape extract as top-dressing at 3.7% of the diet (DM basis)
- Low level grape pomace: 65% high-quality hay + 25% concentrate mix + 10% grape pomace (DM basis)
- High level grape pomace: 56% high-quality hay + 24% concentrate mix + 20% grape pomace (DM basis)

The concentrate mix used in all dietary treatments contained 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). The commercial extract was an OPC product (Nature Love® OPC Grape Seed Extract, Tauron Ventures GmbH, Düsseldorf, Germany).

Each diet was used in two fermenters in each run, therefore a total of eight fermenters were needed. For each diet, feed ingredients were weighed and mixed before putting into nylon bags prior to use.

Table 1: Chemical composition of the dietary treatments

	CON / EXT	GP low	GP high
Dry matter (%)	89.21	89.54	90.81
Organic matter (OM, % of DM)	92.29	92.57	92.54
Ash (% of DM)	7.71	7.43	7.46
Crude protein (CP, % of DM)	18.7	19.13	18.3
Ether extract (EE, %)	2.58	3.66	3.39
Neutral detergent fiber (NDF, % of DM)	49.26	48.31	52.56
Acid detergent fiber (ADF, % of DM)	20.88	24.68	28.46
Hemicellulose (% of DM)	28.38	23.63	28.64
Non-fiber carbohydrates (NFC, % of DM)	21.75	21.47	18.29
Total phenol contents (% of DM)	2.9/6.4	4.0	5.0
Thereof EXT or GP (% of DM)	0/3.4	1.3	2.7

OM = DM – Ash
Hemicellulose = NDF – ADF
NFC = OM – CP – EE – NDF
Total phenols were expressed as catechin equivalents

The experiment took place from June 2nd to August 14th 2020 and was divided into four independent runs each consisting of ten days (Table 2). Each run was divided into an adaptation period (first five days) and a sampling period (last five days) (Figure 1).

Table 2: Timetable of the experiment

Run 1	2.06-12.06
Run 2	23.06-3.07
Run 3	14.07-24.07
Run 4	4.08-14.08

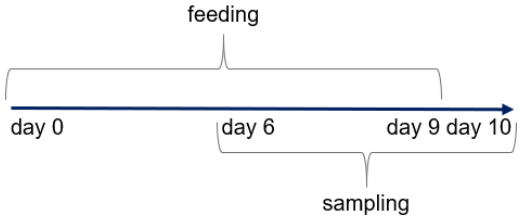

Figure 1: Schedule of each run

3.2 RUSITEC procedure

The setup of RUSITEC including a description can be seen in Figure 2. On day 0 of each experimental run, fluid and solid rumen digesta from two non-lactating donor cows owned by the University of Veterinary Medicine Vienna were collected. The cows' diet consisted mainly of hay and they were kept at the University Clinic for Ruminants (University of Veterinary Medicine Vienna) according to the Austrian guidelines for animal welfare. The rumen fluid was obtained by a rumen fistula, strained through medical gauze with 1 mm pore size and pooled. Each of the 12 fermenters was filled with 600 mL of the pooled ruminal fluid as well as 100 mL of the McDougall's buffer (Table 4). Additionally, two nylon bags (140 mm x 70 mm, 150 µm pore size) were put into each fermenter inside the rumen fluid: one containing pooled solid rumen digesta and one with the respective diet. On day 1, the bag with solid rumen digesta was replaced with a bag containing the diet and on each following day the oldest bag would be removed and replaced with a new bag containing the diet, thus every feed bag remained inside the fermenter for 48 hours (3.2.1 Feed bag exchange). The RUSITEC procedure was performed according to a standard procedure (Humer et al. 2018).

Fermenters were submerged inside a water bath at a controlled temperature of 39.5 °C. The content of fermenters was constantly stirred vertically, simulating the movement of the fermentation fluid during the ruminal cycle. To simulate salivation and regulate the pH, McDougall's buffer (Table 3*)* was constantly flowing into each fermenter with a rate of approximately 375 mL per day. A 12-channel peristaltic pump (Model ISM932, ISMATEC, IDEX Health & Science GmbH, Wertheim, Germany) ensured an even infusion. Each fermenter was connected to bottles to collect outflow liquid as well as aluminium bags (TECOBAG 8 L, Tesseraux Spezialverpackungen, Bürstadt, Germany) in order to collect fermentation gas. The effluent bottles were cooled in an refrigerator at 1 °C to prevent the outflow from further fermentation.

Table 3: Components of the McDougall's buffer

Component	mmol/L
$NaHCO_3$	116.5
Na_2HPO_4 x 2 H_2O	26.3
NaCl	8.04
KCl	7.64
$CaCl_2$ x 2 H_2O	0.37
$MgCl_2$ x 6 H_2O	0.63

3.2.1 Feed bag exchange

Each day of the run except for day 10 when only the samples were taken, the feed bag exchange took place at around 10 a.m. Prior to the feeding, the feed bags and buffer had to be prepared. The following steps were conducted with each fermenter one after the other. Gloves were changed between the fermenters to avoid cross microbial contamination.

First the motor for stirring fermentation fluid was switched off. Then nitrogen gas flushed the system for 30 s to push all fermentation gas remaining in the overhead of the fermenter and in the effluent bottle into the gas bag. After the nitrogen gas flushing the bag was removed and exchanged. The effluent bottle was emptied into a measuring cylinder so the amount of outflow was measured to check the buffer-inflow volume. The emptied bottle was then reattached. Subsequently, the fermenter was opened and the feed bags replaced, simulating the daily feed intake. There were always two feed bags in one fermenter. The older feed bag was exchanged with a new one so that again two feed bags would remain in the system. Therefore each bag was incubated for 48 hours. Before removal, the older feed bag was rinsed with a syringe with 40 mL of the prewarmed buffer and the excess liquid was squeezed back into the fermenter. Also, the new and the old feed bag were pressed together to transfer feed-associated microbes into the new bag. When the lid was closed again, the system was flushed 3 min with nitrogen gas to re-establish anaerobic conditions. Immediately after flushing, the new empty gas bag was attached to the system.

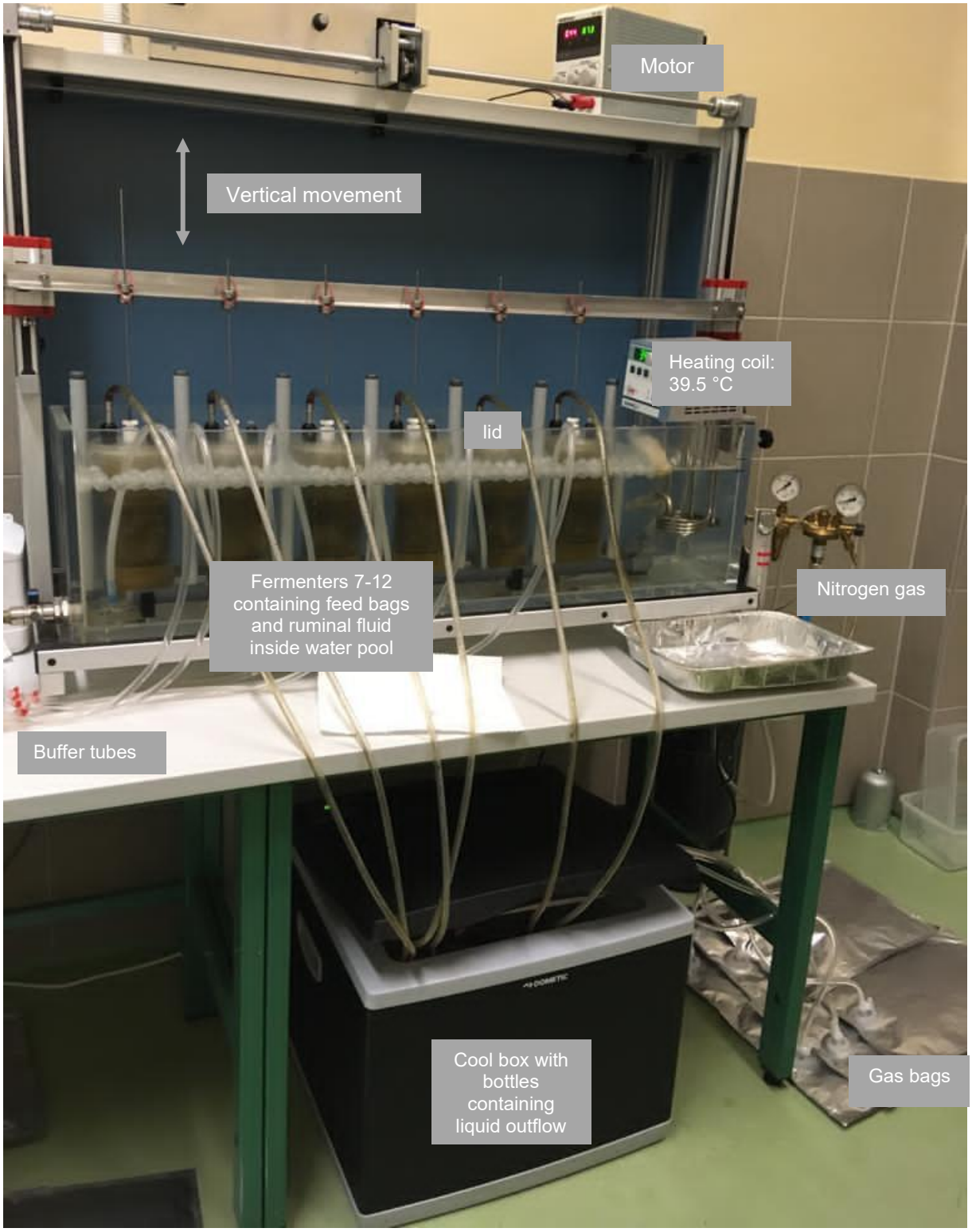

Figure 2: RUSITEC (Rumen simulation technique) system with 6 fermenters

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3.3 Sampling

On day 0, redox potential and pH of the rumen fluid obtained from the donor cows were measured individually as well as of the pooled rumen liquid sample representing the inoculum. On average across all experimental runs, the pH and redox potential of the inoculum were 6.69 ± 0.40 and -304 ± 33 mV, respectively (mean ± SD).

During the sampling period, fermentation fluid, fermentation gas and incubated feed residues were taken. Fermentation fluid was extracted by aspirating it directly out of the fermenter with a 20 mL syringe prior to the feeding process. Every day of each run, pH and redox potential of the rumen fluid were determined using a pH meter (Seven Multi TM, Mettler-Toledo GmbH, Schwerzenbach, Switzerland) consisting of separate electrodes (InLab Expert Pro-ISM for pH and Pt4805-DPA-SC-S8/120 for redox; Mettler-Toledo GmbH, Schwerzenbach, Switzerland). Before use, the electrode was calibrated every day with standard solutions with a pH of 4 and 7. In between the fluids of different fermenters, the electrode was rinsed thoroughly with aqua bidest and padded dried with kitchen roll. The rest of the rumen fluid was preserved at - 20 °C for the analysis of NH_3 (indophenol reaction method) and SCFA (gas chromatography method).

The composition of fermentation gas was measured using a portable biogas analyzer (ATEX Biogas monitor Check BM 2000, Ansyco, Karlsruhe, Germany). It was calibrated in fresh air every day before and after measuring the gas and measured the percentage content of CO_2, O_2 and CH_4. For determining gas outflow volume, a water replacement method was used. The incubated feed bags were machine-washed (cold wash, gentle cycle, and no spin) and the excessive water was squeezed out before stored at -20 °C until later analysis of chemical composition to determine DM, OM, CP, EE and NDF using the protocols of (VDLUFA 2012). The chemical composition of original diets was also determined and the nutrient degradation was calculated from the difference in the supply and remaining amount after 48-h fermentation.

3.4 Statistical analysis

Daily data (fermentation liquid and gas) were averaged per fermenter per run and then subjected to statistical analysis. The data were analyzed using the MIXED procedure of SAS (version 9.4, SAS Institute Inc., Cary, NC, USA). The mixed model tested the fixed factor “dietary treatment” with consideration of random factors namely experimental run and

fermentation vessel. The results presented in the thesis are as least squares means and standard error of the mean (SEM). The comparisons among least squares means were done according to the Tukey's method (Engineering Statistics Handbook 2018). Two types of P values are reported and described in the thesis: one is related to the overall effect of treatment and the other one is related to pairwise comparisons of the least squares means (always indicated with $P < 0.05$). The significant effect and pairwise differences were defined when $P \leq 0.05$ and tendency for difference when $0.05 < P < 0.10$.

4 Results

4.1 Rumen physiochemical factors

The pH and redox potential of fermentation liquid were affected by dietary treatment (P = 0.026 and 0.003, respectively, Table 4). Compared to the pH of CON, only the GP low treatment showed a statistically significant difference to CON but only with a difference of + 0.07 units. The redox potential of GP high was significantly increased in comparison to CON (+13.69 mV) whereas that of EXT slightly increased but not statistically relevant.

Table 4: Effect of dietary treatments on pH and redox

	CON	EXT	GP low	GP high	SEM	P value
pH	6.57^{b}	6.58^{ab}	6.64^{a}	6.62^{ab}	0.04	0.026
Redox potential (mV)	-244.62^{b}	-238.97^{ab}	-242.20^{b}	-230.93^{a}	6.17	0.003

^{ab} values sharing no common superscripts are significantly different (p value < 0.05)

4.2 NH_3

Concentrations of NH_3 were highly affected by dietary treatments (P <.0001; SEM = 0.24). As shown in Figure 3, EXT as well as inclusion of grape pomace led to lower NH_3 concentrations than CON (P < 0.05). Interestingly, there were also differences among EXT and GP treatments. GP high resulted in the lowest NH_3 concentration (11.25 mmol/L; -3.20 mmol/L compared to CON), followed by EXT (12.01 mmol/L, -2.44 mmol/L compared to CON), while GP low had the smallest decrease of NH_3 (12.92 mmol/L, -1.53 mmol/L compared to CON).

Compared to CON, all dietary treatments led to decreased levels NH_3 per degraded crude protein (P <.0001; SEM = 0.30). However, here EXT and both GP treatments showed similar results with approximately 20% reduction from that found in CON (Figure 4).

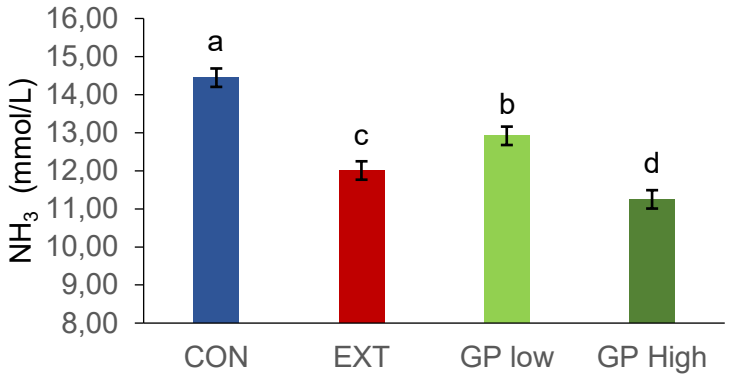

Figure 3: Effect of dietary treatments on NH_3

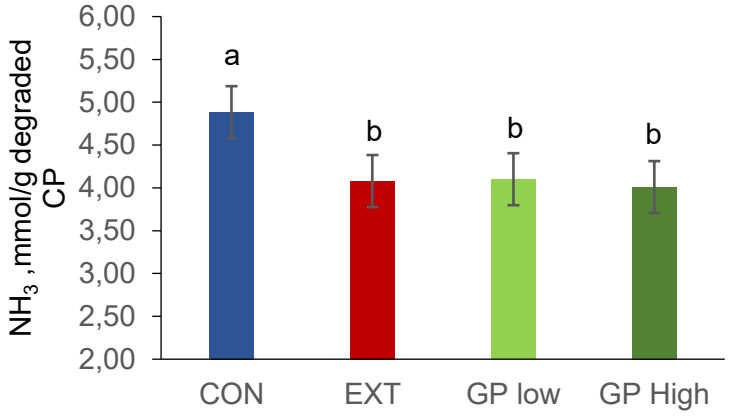

Figure 4: Effect of dietary treatments on NH_3 per degraded crude protein

4.3 Gas production

Overall, dietary treatment affected gas production (P = 0.023). CON resulted in 870.63 mL of fermentation gas (CO_2 + CH_4 + O_2) per day, and only the GP high diet resulting in the lowest average amount of fermentation gas (777.16 mL/d) differed from CON (-93.47 mL/d, P < 0.05). The other two treatments EXT and GP low showed intermediate values (815 and 810 mL/d, respectively) being insignificant from CON or GP high. The SEM was 54 mL/d.

There was a tendency of dietary effect on CH_4 concentration (P = 0.056). But this was related to the difference between GP low and EXT, that the value of GP low was significantly higher than EXT whose value was the lowest among all treatments (P < 0.05, Figure 5). None of the

GP treatments and EXT differed significantly from CON. The amount of emitted CH_4 per degraded organic matter (P = 0.043, Figure 6) complied with the values measured for CH_4 in % of total gas production. The effect of the diets on CO_2 was negligible (P = 0.162, SEM 0.49), with all values falling within 83 - 85% of total gas production.

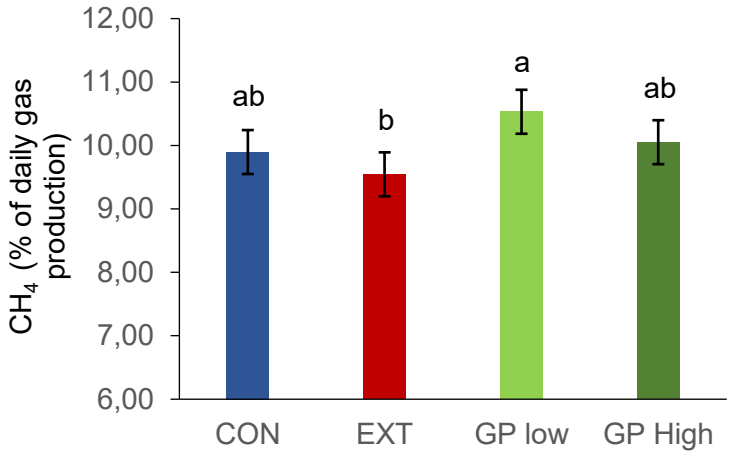

Figure 5: Effect of dietary treatments on CH_4 percentage

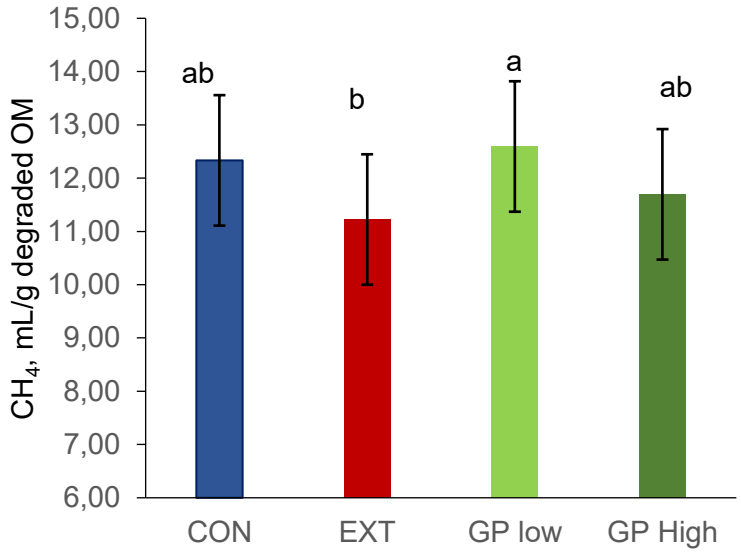

Figure 6: Effect of dietary treatments on CH_4 produced relative to the amount of degraded OM

4.4 SCFA

All test diets showed a reducing effect on total SCFA concentration ($P = 0.003$, Figure 7) but the effect was stronger with GP diets. Compared to CON (128.7 mmol/L). EXT only lowered the amount of SCFA marginally (-4.19 mmol/L), both treatments with grape pomace showed a significant reduction (GP low: -8.98 mmol/L; GP high: -8.55 mmol/L) of total SCFA content ($P < 0.05$).

Besides total SCFA concentration, the composition of SCFA also differed among treatments, whereby all SCFA except propionate was affected by dietary treatment (Table 5). Both EXT and GP high led to an increased acetate percentage compared to CON ($P < 0.05$). The percentages of butyrate as well as iso-butyrate, valerate, caproate and heptanoate decreased with EXT and GP high diets compared to CON ($P < 0.05$). The percentage of propionate showed a similar pattern as for acetate but with a higher variation and therefore it did not reach significance ($P = 0.242$). The ratios of acetate to propionate were similar among all treatments ($P = 0.878$). The percentage of iso-valerate was reduced with all test diets compared to CON, however, only GP reached a statistical difference ($P < 0.05$).

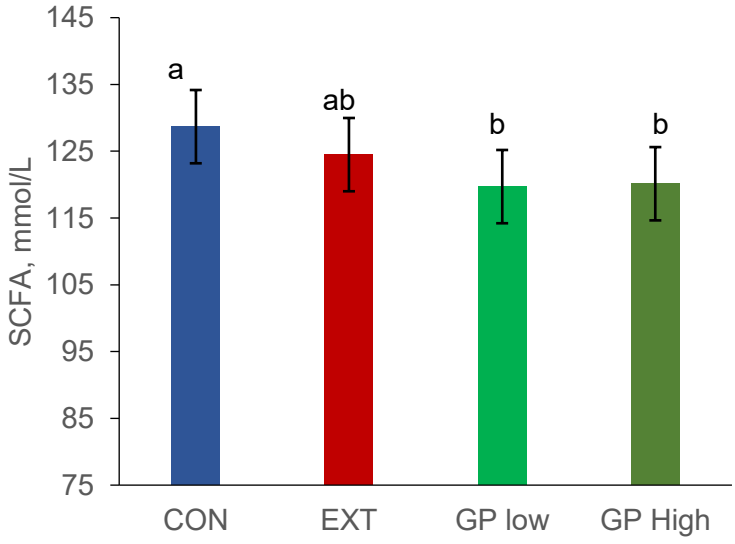

Figure 7: Total SCFA concentration

Table 5: Effect of dietary treatments on the distribution of SCFA

	CON	EXT	GP low	GP high	SEM	p value
SCFA (in mmol/L)	128.68^{a}	124.49^{ab}	119.70^{b}	120.13^{b}	5.49	0.003
Acetate (in %)	48.55^{c}	50.47^{ab}	49.20^{bc}	50.65^{a}	0.66	0.002
Propionate (in %)	24.50	26.41	25.11	26.27	1.01	0.242
Butyrate (in %)	10.83^{a}	9.74^{b}	10.78^{a}	9.64^{b}	0.18	<.0001
Isobutyrate (in %)	0.96^{a}	0.77^{c}	0.92^{ab}	0.87^{b}	0.04	<.0001
Valerate (in %)	8.14^{a}	6.80^{b}	7.57^{ab}	6.91^{b}	0.47	0.017
Isovalerate (in %)	2.95^{a}	2.75^{ab}	2.67^{ab}	2.57^{b}	0.11	0.029
Caproate (in %)	3.12^{a}	2.37^{b}	2.92^{ab}	2.35^{b}	0.28	0.016
Heptanoate (in %)	0.98^{a}	0.68^{b}	0.85^{ab}	0.76^{ab}	0.11	0.032
Acetate to propionate ratio	2.00	1.93	1.98	1.95	0.09	0.878

^{ab} values sharing no common superscripts are significantly different ($P < 0.05$)

4.5 Nutrient degradation

Nutrient degradation was affected by diet, although for crude protein it was only a tendency (P = 0.055, Table 6). Specifically, dry matter degradation was significantly reduced with all treatments compared to CON (P < 0.05). The influence of EXT and GP low were nearly the same (EXT: 3.9%; GP low: 3.8% relative reduction from that of CON), whereas GP high led to an even lower amount of dry matter degradation (5.7% reduction compared to CON) that was also significantly lower from those of EXT and GP low ($P < 0.05$). This effect could also be seen with organic matter degradation (EXT: 4.4%; GP low: 4.0%; GP high: 6.1% relative reduction from that of CON). Ash degradation was reduced with both grape pomace treatments (GP low: 1.5%; GP high: 1.7% reduction compared to CON), while the of EXT percentage remained nearly the same as in CON.

Although the degradation of crude protein stayed at a lower end with EXT and GP treatments, the changes were not as severe as the other nutrients and did not reach significance. GP low showed almost identical value of crude protein degradation as CON. Both GP diets led to

significantly higher ether extract degradation than EXT, however EXT as well as the GP diets did not differ from CON (treatment effect, P = 0.001). Especially GP low but also GP high significantly reduced neutral detergent fiber (NDF) degradation (GP low: 15.9%; GP high: 10.5% reduction from that of CON), while EXT did not have any impact (2.1% reduction from that of CON). The same trend could be seen in acid detergent fiber (ADF) degradation, however, it was not as clear as in neutral detergent fiber degradation. Though EXT as well as the GP treatments showed some reduction in ADF, only GP low significantly differed from CON (P < 0.05; 10.2% reduction from that of CON).

Table 6: Nutrient degradation

Degradation in %	**CON**	**EXT**	**GP low**	**GP high**	**SEM**	**p value**
Dry matter degradation	65.05^{a}	62.51^{b}	62.56^{b}	61.33^{c}	0.67	<0.0001
Organic matter degradation	63.09^{a}	60.35^{bc}	60.59^{b}	59.27^{c}	0.73	<0.0001
Ash degradation	88.49^{a}	88.30^{a}	87.03^{b}	86.79^{b}	0.22	<0.0001
Crude protein degradation	69.49	66.90	69.44	67.14	1.03	0.055
Ether extract degradation	45.58^{ab}	39.55^{b}	52.55^{a}	51.27^{a}	3.38	0.001
NDF degradation	47.24^{a}	46.24^{a}	39.74^{c}	42.29^{b}	2.48	<0.0001
ADF degradation	36.68^{a}	33.70^{ab}	32.94^{b}	34.48^{ab}	0.99	0.026

NDF = neutral detergent fiber
ADF = acid detergent fiber
^{ab} values sharing no common superscripts are significantly different (P < 0.05)

5 Discussion

5.1 Grape pomace as feed for cattle

One major question arising from including grape pomace in the diet of cattle was whether by combining it with high-quality hay, ruminal health would be compromised and if so at what degree. Tafaj et al. (2005) and Kleefisch et al. (2017) have validated that the decreased fiber of high-quality hay offered enough structural effectiveness to induce sufficient salivation hence maintaining a stable pH in the rumen. Furthermore, high-quality hay meets the cattle's requirements for energy and nutrients, albeit small substitutions of concentrates are still needed in early lactation to avoid intensive body mobilization (Kleefisch et al. 2018). Following these previous studies, we studied a diet containing 70% high-quality hay. With the in vitro set-up used in the current experiment, we did not see any disadvantages of the high-quality hay (70% in diet DM) as well as the inclusion of grape pomace up to 20% of diet DM on fermentation pH and redox potential.

Ruminal pH varies depending on chewing activity, meals and time since the last feed intake (Allen 1997), however a range between 6.2 and 7 is considered physiologically (Department of Agriculture and Fisheries 2013). Comparably, in the present experiment, the pH values of all treatments remained within the physiological range with the highest mean value being 6.64 with the GP low diet (Table 4). Oxidation-reduction reactions are highly crucial for the survival of microorganisms (Huang et al. 2018). The anaerobic microbes inside the rumen are adapted to a redox potential of -115 to -300 mV (Huang et al. 2018). The redox potential of the grape pomace treatments was increased, however this effect was only of statistical significance not of physiological significance. With – 230.93 mV being the lowest value, all diets resulted in a redox potential that remained within the norm for ruminal health. Nevertheless, one should keep in mind that the roles of chewing, stimulated salivation as well as ruminal absorption on regulation of physiochemical condition of the rumen cannot be accounted for in vitro.

The overall influence of grape pomace on rumen health parameters has been outlined in previous works (Jayanegara and Palupi 2010, Kleefisch et al. 2017, Tafaj et al. 2005) and can be confirmed in this thesis. Jayanegara and Palupi (2010) stated that with rising levels of condensed tannins in the diet, overall digestibility was compromised due to the polyphenols' tendency to bind polymers like proteins and carbohydrates. As expected, in this experiment dry matter degradation decreased with EXT, GP low and GP high diets (Table 6). However, the diets containing grape pomace led to an even more reduced organic matter degradation

than EXT, which was more related to the reduction found with fiber fractions (NDF and ADF). The decreased degradation was in line with the reduced SCFA concentration in GP diets. Hence, not only the polyphenols have an impact on degradability but also the fibrous carbohydrates of grape pomace itself that have lower degradability than those of high-quality hay and concentrate constituting the diets. Comparably, a decreased rumen degradation of DM, OM, CP and NDF with levels of grape pomace more than 150 g/kg DM has been mentioned before (Tayengwa et al. 2020, Tayengwa and Mapiye 2018). Compared to these studies, GP low contained a lower inclusion rate (100 g/kg DM) and the reduced ruminal degradation of DM, OM and NDF was already evident. Nevertheless, grape pomace in the present study replaced feed components having better quality (high-quality hay and cereal grains) whose nutrients are highly degradable. However, the decrease in degradation was within acceptable levels. Therefore grape pomace appears to be suitable as a partial replacement of typical feed components for dairy cattle.

Apart from the question whether grape pomace is an adequate feed for cattle, the practicability of inclusion levels of grape pomace needs to be evaluated as well. Considering that grape pomace is generally considered a waste product that can cause environmental pollution and economic loss (Chowdhary et al. 2021), exploiting this byproduct as a low cost and available functional feed is undoubtedly a practical approach (Chedea et al. 2017), saving local wine producers the struggle from managing the disposal of the wastes. Due to its high amount of dietary fiber and concomitant reduction of nutrient degradation, it should be combined with the high-quality forages to maintain sufficient ruminal nutrient degradation. This strategy was also recommended when including tannin-rich feed sources (Jayanegara et al. 2012). The present study indicates that it is feasible to include grape pomace up to 20% in high-quality forage based diets. However, challenges like palatability, economical drying and storage methods for the optimal polyphenol retention need to be faced and further researched (Tseng and Zhao 2012).

5.2 Functional effects of grape pomace

5.2.1 Protein metabolism

Sparing dietary protein from microbial fermentation in the rumen is one of the main effects of polyphenols discussed by previous research (Frutos et al. 2004, Tayengwa and Mapiye 2018). NH_3 emission as well as milk urea arise with a higher nitrogen intake (Reynolds and Kristensen

2008). Therefore feeding cattle with high-quality hay with a large amount of highly digestible crude protein as feed for cattle has a major drawback due to extensive ruminal degradation of protein (Kleefisch et al. 2017, Kleefisch et al. 2018, Klevenhusen et al. 2017). For this thesis, to accurately address an in vitro effect of treatments, it must be taken into account that RUSITEC only involves microbial metabolism of protein and N compounds. Therefore the NH_3 concentration that was measured in this thesis represents a balance between NH_3 production from breaking down dietary protein and microbial protein minus the NH_3 utilization by the microbes. Under this condition, it was hypothesized that by including grape pomace into the diet, its polyphenols would decrease ruminal protein degradation, hence reduce ruminal NH_3 concentration resulting from the oversupply of dietary nitrogen in the high-quality hay based diets.

The hypothesis was confirmed by the current data. All tested diets resulted in NH_3 concentrations significantly lower than CON (Figure 3). GP high led to the lowest NH_3 concentration (11.25 mmol/L), followed by EXT (12.01 mmol/L) and GP low (12.92 mmol/L). With respect to the dosage of total phenols, the GP high contained 5.0% of DM, whereas GP low contained 4.0% and EXT 6.4% of DM, therefore the treatments with the higher phenol concentration had the strongest effect on NH_3 reduction. The diet formulation kept the crude protein contents similar among diets, especially the EXT diet that was identical to CON. Thus, the NH_3-reducing effect was not confounded by the level of dietary supply of crude protein.

Regarding crude protein degradation, EXT as well as GP high showed a slight decrease in degradability, but less dramatic compared to their effect on the NH_3 concentration, while the crude protein degradation of GP low was almost the same as in CON. When expressed in relation to CP degraded, all GP and EXT diets performed similarly showing about 20% reduction of NH_3 production. Therefore the drastic decrease of NH_3 with all treatments cannot completely be explained by reduced ruminal protein degradation due to the polyphenols. Interestingly, the EXT and GP high treatments also resulted in decreased proportions of the branched-chain fatty acids valerate, isobutyrate and isovalerate (Table 5). These are the products of branch-chain amino acid degradation synthesized by rumen microbes through deaminase (Andries et al. 1987). The decrease of these fatty acids is associated not only with a reduction in degradation of dietary proteins but also with an inhibition of microbes (Bodas et al. 2012). Thus, the discrepancy between the significant NH_3 reduction and the less obvious decrease in crude protein degradation might be explained by the inhibiting effect of polyphenols on microbes and their deaminase activity. As NH_3 could be poisoning to animals

when present in the circulatory system (Antonelli et al. 2006, Randall and Tsui 2002) and when excreted, urine NH_3 of ruminants is a significant environmental pollutant (Wang et al. 2017), the current in vitro data suggests an advantage of incorporating grape pomace in cattle's diets when using high degradable protein feed sources such as high-quality hay.

Using RUSITEC, Khiaosa-Ard et al. (2015) tested different fortification levels (0, 1, 5, 10, 20% of DM) of grape seed meal (GSM) in dried distillers grains plus solubles (DDGS), which is a byproduct of ethanol production that is rich in protein and low in fiber. Neither of the diets had an effect on NH_3 concentration, perhaps because the dosages were too low. The 20% GSM in DDGS was equal to 5% of GSM in total diet DM and total dietary phenol content was 2.78% (expressed as gallic acid equivalents). In the present study, the GP low treatment (10% in diet DM) already supplied a higher content total phenols at about 4.0% of diet DM (expressed as catechin equivalents), which was enough to express an effect in this study. The effect was stronger in the GP high treatment with 20% (therefore double the phenol dosage) and EXT treatment that supplied the highest phenol content. Comparing the previous Khiaosa-Ard et al. (2015) and current data, it may suggest that the functional effect of grape pomace on reducing NH_3 production in the rumen requires a minimum dosage of 10% grape pomace in the diet. Without confounding effects from host (ruminal absorption and urea recycling), the current in vitro data proved that functional compounds of grapes have a direct effect on microbial metabolism of protein and amino acids. However, transferring of the in vitro outcome to in vivo should be done with extra caution due to the contribution of host factors. For instance, Jayanegara and Palupi (2010) showed that the decrease of ruminal NH_3 with rising levels of dietary condensed tannins led to an exponential decrease of ruminal NH_3 in vitro, whereas in vivo studies showed a linear decrease. Thus, an effective dose could differ between in vivo and in vitro.

5.2.2 CH_4 emission

Several studies have reported the suppressing influence of polyphenols from various botanical species on methanogen activity and CH_4 formation in the rumen (Beauchemin 2009, Moate et al. 2014, Sinz et al. 2019). Therefore, it was expected that CH_4 emission would be reduced with the presence of vine grapes' phenols.

Contrary to our hypothesis, neither GP treatments nor EXT significantly reduced CH_4 formation, either as CH_4 emission in percent or CH_4 in mL per gram degraded organic matter

(Figure 5 and Figure 6). Increasing GP in the diet increased dietary fiber contents and thus may confound the CH_4 results, because forages rich in structural carbohydrates lead to a higher CH_4 production (Mirzaei-Aghsaghali and Maheri-Sis 2001, Sauvant and Giger-Reverdin 2020). However, when comparing EXT to CON, both diets had identical chemical compositions, still EXT did not express the effect either. Hence, the natural polyphenols of vine grapes did not show the CH_4-mitigating effect we had expected.

The absent effect of grape phenols might also be related to dosage. A meta-analysis investigated the influence of dietary tannins from various botanical origins on CH_4 emission in ruminants using both in vitro and in vivo data (Jayanegara et al. 2012). The authors concluded that though increasing levels of tannins in the diet generally lead to a reduced CH_4 emission, this effect was more inconsistently at low dietary tannin concentration. Specifically, they found that dietary concentrations of tannins < 20 g/kg DM led to bigger variability in vivo. On the other hand, they stated that very high amounts of dietary tannins (> 200 g/kg DM) neither showed a proportional reduction of CH_4 due to the drawbacks in limiting digestibility. In the present study, EXT and GP high contained a total phenol content of 64 and 50 g/kg DM, respectively (expressed as catechin equivalents). Although not analyzed, the tannins levels must be lower because tannins are part of total phenols. Thus, while these dosages were enough to abate NH_3 production, this was not sufficient for the anti-methanogenic effect.

Hindrichsen et al. (2004) tested eight diets and investigated among other things CH_4 emission using RUSITEC. They found that a decreased CH_4 release could be the result of a reduced nutrient availability for methanogenetic microbes. With that in mind, the effect dosages for mitigating CH_4 is difficult to determine. A balance between effective reduction of CH_4 emission without compromising ruminal balance is yet to be found (Beauchemin et al. 2008).

Interestingly, Khiaosa-Ard et al. (2015) reported a decrease in CH_4 formation in vitro with GSM fortified at 5, 10 and 20% in dried DDGS that actually supplied lower phenol contents than did the GP treatments in the present study. It is possible that the type and composition of phenolic compounds in the diet make a difference in this matter. As mentioned before, the content and composition of polyphenols of grape wine depends on breed, agro-technical factors, soil and climate zone. Furthermore different polyphenols have a different impact on methanogenesis than others and differ from grape pomace to grape seed meal. However, in this thesis the profile of phenolic compounds in total phenols was not characterized. It is not clear whether the fortification, thus a closer contact between fiber and functional compound, could aid the

CH_4 -abating effect. This question should be addressed because this would spare the need for high dosages that otherwise could be detrimental to nutrient degradation.

Previous research (Beauchemin et al. 2020, Holtshausen et al. 2009) has shown that diets effective in reducing CH_4 formation often shift the SCFA to more propionate at the expense of acetate. This is because propionate functions as an alternative sink for hydrogen inside the rumen. Starches in cereal grains lead to a higher propionate formation, where fibrous carbohydrates lead to more acetate formation that results in a release of metabolic hydrogen. Consequently, high-grain diets lead to lesser amounts of hydrogen available for methanogenesis, hence leading to a decreased CH_4 production and acetate-propionate ratio (Janssen 2010). In the present study the percentage of propionate as well as the acetate propionate ratio were similar among the treatments. In line with that CH_4 was unaffected by dietary treatment.

Furthermore, basal diets may contribute to the effect of functional compounds on CH_4 formation. For instance, Klevenhusen et al. (2012) indicates that synchronization between protein and carbohydrate availability may alter the effects of dietary supplementation of bioactive compounds. Diets could determine the microbial population and profile, some of which may or may not respond to the functional compounds or respond differently. Hindrichsen et al. (2004) state that a high sugar content in the diet leads to an increased methanogenesis when the pH in the rumen is high. As mentioned before, starchy concentrates promote propionate production that competes for hydrogen with methanogenesis, thereby leading to decreased CH_4 (Beauchemin et al. 2020). In the thesis, however, the diets were based on the same high-quality hay and concentrate mix and the forage to concentrate ratio did not differ substantially (24, 25 and 30% for GP high, GP low and CON/EXT, respectively) to drive the effect. Thus, the contribution of basal diets cannot be proven here and the microbiota was not studied. Data on the effect of high-quality based diets on methanogenesis is currently lacking. Still, high degradability of the nutrients in high-quality hay (Kleefisch et al. 2017, Klevenhusen et al. 2017) may have lessened the effect of functional compounds in grape pomace on CH_4 formation. The results did not confirm the hypothesis concerning the CH_4 mitigation, at least the fiber of grape pomace did not drive a CH_4 emission potency of the diet.

The results indicate that levels of GP up to 20% can be included in diets with high-quality forages without a severe reduction in nutrient degradation. With this approach, excessive ruminal NH_3 production can be decreased, however, research on the dietary treatments' influence on CH_4 formation should be a goal in the future.

6 Summary

Wine production results in a considerable amount of waste in the form of grape pomace, which is both rich in fiber and functional compounds, the polyphenols. Polyphenols are known for their ability to bind macromolecules especially protein in the rumen. In this thesis, different inclusion levels of grape pomace in high-quality hay based diets were tested on rumen fermentation in an in vitro experiment using RUSITEC. It was hypothesized that the downsides that come with feeding high-quality hay regarding their high amount of crude protein would be overcome by the properties of polyphenols in grape pomace. The expectation was that by combining these two components in the diet, ruminal health would not be compromised and ruminal NH_3 and CH_4 production would be reduced.

The experiment was divided into four independent runs each consisting of ten days (day 1-5: adaptation period; day 6-10: sampling period). Four different types of diets were compared: the negative control (CON: 70% hay, 30% concentrate), the positive control (EXT: 70% hay, 30% concentrate, grape pomace extract) as well as low (GP high: 65% hay, 25% concentrate, 10% grape pomace) and high (GP low: 56% hay, 24% concentrate, 20% grape pomace) doses of grape pomace.

NH_3 production decreased (up to -20%) with all tested diets, especially with rising levels of polyphenols in the EXT and GP high diets. However, GP and EXT treatments did not abate CH_4 formation. Redox and pH were changed minimally. The GP treatments decreased nutrient degradation especially related to the fiber fractions, (-15% with GP low; -10% with GP high) and decreased total SCFA concentration by GP was evident (-4% with GP high). The composition of SCFA was shifted to more acetate at the expense of butyrate by EXT and GP high.

Including levels of GP up to 20% in diets with high-quality forages seems to be a promising approach to reduce excessive ruminal NH_3 production without a strong reduction in nutrient degradation. However, its relation to CH_4 formation has to be further investigated.

7 Zusammenfassung

Bei der Weinproduktion fallen beträchtliche Mengen des Abfallstoffes Weintrester an. Dieser hat einen hohen Fasergehalt und ist reich an funktionellen Wirkstoffen, den Polyphenolen, welche für ihre Fähigkeit, Makromoleküle wie Proteine im Pansen zu binden, bekannt sind. In dieser Diplomarbeit wurden unterschiedliche Dosierungen an Weintrester in Qualitätsheu-basierten Rationen getestet und die Pansenfermentation mittels RUSITEC in vitro untersucht. Es wurde die Hypothese aufgestellt, dass die Nachteile, die die Fütterung mit hochqualitativem Heu hinsichtlich des hohen Rohproteingehaltes mit sich bringt, durch die Polyphenole im Weintrester kompensiert werden könnte. Es wurde erwartet, dass die Kombination beider Komponenten die Pansengesundheit nicht beeinträchtigt und die NH_3- und CH_4-Produktion im Pansen reduziert.

Das Experiment war in vier unabhängige jeweils zehn Tage dauernde Durchgänge aufgeteilt (Tag 1-5: Adaptionsphase; Tag 6-10: Probeentnahmezeitraum). Es wurden vier Rationen im RUSITEC getestet: die Negativkontrolle (CON: 70% Heu, 30% Konzentrat), die Positivkontrolle (EXT: 70% Heu, 30% Konzentrat, Weintresterextrakt), sowie Rationen mit niedriger (GP high: 65% Heu, 25% Konzentrat, 10% Weintrester) und hoher (GP low: 56% Heu, 24% Konzentrat, 20% Weintrester) Dosierung von Weintrester.

Die NH_3 Produktion verringerte sich (bis zu -20%) mit allen Rationen, insbesondere aber mit steigenden Mengen an Polyphenolen in den Rationen EXT und GP high. Jedoch konnten weder die Weintresterrationen, noch EXT die CH_4-Bildung vermindern. Redoxpotential und pH wurden nur minimal verändert. Die Weintresterrationen führten zu einer Abnahme des Nährstoffabbaus, insbesondere der Faserfraktion (-15% mit GP low; -10% mit GP high), sowie der Konzentration an Gesamt-SCFA (-4% mit GP high). Die Zusammensetzung der SCFA wurde durch EXT und GP high verändert, welche zu mehr Azetat und weniger Butyrat führten. Demnach scheint der Einsatz von bis zu 20% Weintrester in Rationen mit qualitativ hochwertigem Grundfutter ein vielversprechender Ansatz zu sein, um eine exzessive NH_3-Produktion im Pansen zu reduzieren, ohne dabei den Nährstoffabbau drastisch zu reduzieren. Die Zusammenhänge zur ruminalen CH_4-Produktion sollten weiter untersucht werden.

8 References

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9 Appendix: Tables, Charts and Figures