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Master Thesis in Animal Science

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Investigation of Fermentation Kinetics and the Effect on Weight Gain

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Preface

This research project has the form of a master thesis and is written as the final part of the master program for Animal Science, University of Copenhagen. All laboratory work was conducted at or on the behalf of the Department of Veterinary and Animal Sciences, University of Copenhagen. This research project was carried out in the period from 30/09-2019 to 31/08-2020 and was funded by Kvægafgiftsfonden.

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Abstract

The growth of the world population is correlated with an increased demand for animal food products. In addition, an increased awareness for a more sustainable lifestyle is present, which is followed up by different environmental and sustainable goals set by governments and organizations worldwide. A measure to provide a more sustainable beef and milk production is to improve use of feeds in the cattle industry as an improved feed efficiency could result in a decrease in land use for feed production. Furthermore, an enhanced feed utilization could decrease the climate impact of the cattle industry.

The aim of this research project was to investigate if differences in fermentation kinetics of different feed components and rations are reflected in weight gain. Ten *in vitro* gas trials were preformed to examine 15 feed components and four rations. Fiber analyses were conducted on two feed components and four rations. In addition, two separate *in vivo* feeding trials were conducted.

The first feeding trial was performed at Assendrup Hovedgaard where two feed rations, with a feed component which had either a fast or slow *in vitro* fermentation rate in the early hours of fermentation (FAST; SLOW), were fed to 36 heifer calves of the breed Danish Red. The second feeding trial was performed at Grusgravgaard where two feed rations, with two feed components which had either fast or slow *in vitro* fermentation rates in the early hours of fermentation (FAST-FAST; SLOW-SLOW), were fed to 118 calves which were of mixed sex and of the breeds Danish Red, Holstein, and mixed breed.

The results from the *in vitro* gas trials show that the difference seen in fermentation kinetics was reflected in the weight gains from the *in vivo* feeding trials. No significant results were observed for the grain consumption by the animals along with the calculated gain-per-grain ratio. The experimental units fed the FAST ration gained significantly more than those fed the SLOW ration. A tendency was observed that the experimental units fed the FAST-FAST ration gained more than those fed the SLOW-SLOW ration. This indicates a decreased utilization of the slowly fermentable feed rations, although, the fiber analyses results show greater digestibility of the feed rations SLOW-SLOW. However, the standard fiber digestibility analyses used measure the digestibility after 48 h of fermentation while the greatest differences in the amount of produced gas were observed between 9 and 12 h of fermentation in the *in vitro* gas trials. This could indicate a faster passage rate than 48 h, which is currently used in feed evaluation systems.

Furthermore, an age depended difference, of the donor animals, in the early hours of fermentation was observed. These differences are presumed to be due to an age dependent variation in the rumen microbiome of the donor animals.

The hypothesis presented in this research project "*The weight results from the* in vivo *feeding trials will reflect the differences in the* in vitro *gas trials*" is accepted based on the results. However, further investigation is required.

Table of content

Abbreviations

Abbreviations	Meaning
ADF	Acid detergent fiber
ADG	Average daily gain
ADL	Acid detergent lignin
APO	Alfalfa pellets
BW	Body weight
CHO	Carbohydrate
CS1	Calf starter 1
CS2	Calf starter 2
dDM	Digested dry matter
DM	Dry matter
DMI	Dry matter intake
FAST	Feed ration with one fast fermentable feed component
FAST-FAST	Feed ration with two fast fermentable feed components
GC	Grain consumption
G/g	Gain-per-grain
GSP	Grass pellets
IVGPT	In vitro gas production technique
MS	Maize silage
NDF	Neutral detergent fiber
PEXP	Palme kern expeller
RSM	Rapeseed meal
RSC	Rapeseed cake
RSMh	Heat-treated rapeseed meal
SBC	Soybean cake
SBM	Soybean meal
SBP	Sugar beet pulp
SBP (FAST)	Sugar beet pulp, steam dried
SBP (SLOW)	Sugar beet pulp, drum dried
SEM	Standard error of the mean
SLOW	Feed ration with one slow fermentable feed component
SLOW-SLOW	Feed ration with two slow fermentable feed components
T50	Time point of which 50 % of total gas produced
TFB	Toasted fababeans
TW	Total weight
UFB	Untoasted fababeans
VFA	Volatile fatty acids
WWG	Weight gain between each week in the feeding trials

1 Introduction

In 2050, the world population will increase to almost 10 billion people, and the production and demand for animal- and plant-based food will therefore also increase. The increase in production will have a negative impact on the environment. The need for new and innovative solutions to create an agricultural sector, which can feed the population of the world and simultaneously care for the climate challenges of the world, have never been more urgent (Landbrug & Fødevarer, n.d.b).

In the recent years, the focus on the environment and sustainability has grown, and as a result, an increased consumer demand for a sustainable food production and products have occurred. Global organizations, governments and trade organizations have implemented strategies and goals to create a sustainable food production and minimize the impact on the climate in the future.

In 2015, The United Nations (UN) announced The Sustainable Development Goals, which set 17 goals that strive to minimize poverty, protect the planet, and ensure the people of the world a more prosperous future by 2030. Goal 2, Zero Hunger, and goal 12, Responsible Consumption and Production, are the two goals which directly impact the agricultural sector (United Nations Development Programme, 2020). The Food and Agriculture Organization of the United Nations (FAO) has created the 5 principles of Sustainable Development Goals, which aims to ensure sustainability across agriculture, forestry, and fisheries. The first principle states "*Improving efficiency in the use of resources is crucial to sustainable agriculture*" (FAO, 2020), which aligns with The Sustainable Development Goals set by the UN.

The European Union (EU) member countries have a joint agriculture policy to ensure a high food quality with an environmental and sustainable production (Miljø- og Fødevareministeriet, 2019a). In addition, the Danish government has set a goal for Denmark to minimize its greenhouse gas emission by 70 % in 2030, including a binding reduction goal for the Danish agricultural sector (Miljø- og Fødevareministeriet, 2019b). In alignment with the policy set by the EU, Landbrug & Fødevarer has created a strategy for the cattle sector. The goals set with the strategy are to create an increased focus on a more sustainable agriculture to obtain a more global competitive production and profitable investment, obtain a more environmental and climate conscious production, and implement a better recycling of nutrients. The overall goal for the Danish milk and beef production is to become the most sustainable production in the world by 2025 (Landbrug & Fødevarer, n.d.a) and to become climate neutral by 2050 (Landbrug & Fødevarer, n.d.b).

An improvement in the efficiency of resource consumption will according to the UN and FAO result in a more responsible agricultural production. This is consistent with Landbrug & Fødevarer, which aims to improve the feed efficiency and reduce feed loss to achieve a more sustainable production (Landbrug & Fødevarer SEGES, 2019). Furthermore, if the feed efficiency was improved, the amount of feed and land used for feed production would be reduced (LandbrugsInfo, 2019). The type of feed used in the production is also of great importance regarding the climate impact. A review showed that nine feed rations which consisted of more than 50 % concentrate had a 30 % lower Global Warming Potential compared to roughage feed rations. The lower Global Warming Potential was due to a lower methane excretion, but also due to the lower amount of land used to produce the concentrate rations compared to the roughage rations (Vries *et al*., 2015).

The purpose for prosperous beef calf production is to utilize the full growth potential of the calves to obtain a rapid production. Rapid weight gain and increased growth could result in an improved quality of the animals at slaughter along with an improved meat quality, both which have an impact on the settlement price (Hansen, 2002). One of the major expenses in modern cattle production is feed. The overall cost is affected by feed prices and the possible income for the produced amount of animal products. In addition, the utilization efficiency of the feed by the animal has an impact. An evaluation of feed quality is therefore important to insure profitability (Volden & Gustafsson, 2011). The evaluation of feeds could also give insight into which feeds should be used to achieve an increase in feed efficiency.

1.1 Background

Different degradation curves from *in vitro* gas trials were investigated by Sembach *et al*. (2018a) to evaluate the assumption that all concentrate feedstuff would follow similar degradation curves. This assumption was based on the Nordic feed evaluation system and evaluated by examining degradation patterns of 12 different concentrates at 9 and 48 h of fermentation. The study concluded that degradation curves for the concentrates differed, which could implicate the nutritional values.

A study conducted by Nielsen *et al.* (2017) investigated if the drying method applied, steam or drum drying, for sugar beet pulp (SBP) would influence its fermentability. This was examined by conducting two *in vitro* gas trials which used both fresh and dried SBP from two different factories, harvested on four different days (26.10.16, 08.11.16, 07.12.16, and 30.12.16) along with rumen fluid from two Jersey heifers. The study concluded that there were

no significant differences between fresh and dried SBP, nor did the results from the four harvest dates show any significant difference. However, results showed a 60 % increase in fermentability in steam dried SBP (SBP (FAST)) compared to drum dried SBP (SBP (SLOW)).

The difference in fermentation kinetics based on age of the animals was investigated by Hansen *et al.* (2019). *In vitro* gas trials were conducted using 10 different concentrates, and rumen fluid was obtained from either 2-year-old heifers and 5-7-week-old or 14-16-week-old calves. It was concluded that the age of the donor animal did have an impact on the early fermentation kinetics.

These studies have been considered prior to this research project and have been a source of inspiration for the present subject. Furthermore, some of the results and raw data from the studies will be referred to and used in this research project.

1.2 Aim of study

The aim of this research project is to investigate if differences in fermentation kinetics of different feed components and rations are reflected in weight gain. This was performed by *in vitro* gas production techniques (IVGPT), *in vitro* fiber analyses, and *in vivo* feeding trials. The results from this study could be applied to improve the evaluation of the nutritional values of feeds for calves.

1.3 Hypothesis and research questions

Based on results from previous *in vitro* gas trials which examined the fermentation kinetics for different feed components, four different feed rations were produced and consisted of one or two chosen feed components. Each component has either a fast or slow early fermentation pattern, but with similar endpoints for total gas produced after 48 h of fermentation. The four feed rations were used in two *in vivo* feeding trials to examine if a correlation between the *in vitro* gas trials and *in vivo* feeding trials are present. The following hypothesis will be investigated along with the research questions listed below.

"*The weight results from the* in vivo *feeding trials will reflect the differences in the* in vitro *gas trials*"

- Will the difference in fermentation kinetics result in different weight gain?
- Will the difference in fermentation kinetics affect the grain consumption?
- Will the difference in fiber content affect the weight of the calves?

1.4 Limitations

In both *in vivo* feeding trials, all the calves did have free access to a straw source. Due to uncertainties in the amount of straw intake, this factor was not included in this research project. Therefore, the factor feed conversion ratio was not considered, as information about total feed intake was unavailable. Instead, the gain-per-grain (G/g) ratio was calculated as it only includes the ration intake in relation to total weight (TW).

The feeding methods used for the calves prior to trial start were not considered in this research project due to unavailable information. However, it is plausible that this could influence the results.

2 Literature review

2.1 Estimation of degradation by *in vitro* techniques

A simple *in vitro* method for determination of digestibility of different feeds is developed by Tilley and Terry (1963). The endpoint method consists of two separate stages: (1) An incubation of 0.5 g feed in rumen fluid medium in a glass rod, where anaerobic conditions were crucial, (2) Followed by a pepsin solution added to the residue from the first stage. The dry weight of the residue from the two stages is used to calculate the digestibility of the given feed component. However, the calculated *in vitro* digestibility can only be considered as estimates as the *in vitro* conditions do not directly correspond to what occurs *in vivo*.

The Hohenheim Gas Test developed by Menke *et al*. (1979) uses piston-syringes in a motorized rotor placed into a heated oven which simulates the conditions of *in vivo* fermentation. Each syringe has a capacity of 100 mL and contains 0.2 g dry matter (DM) along with rumen fluid. The amount of gas produced is read manually on the syringes. The position of the pistons is read at the beginning of the trial at 6 to 8 h of fermentation and for the last time at 24 h at the end of the experiment. The gas production from the *in vitro* gas trials corresponds with the findings from *in vivo* digestibility trials conducted with sheep.

A computerized system developed, which records gas pressure, continuously examines the fermentation kinetics of forage digestion by measuring the gas production by the headspace pressure in the sample bottles. The headspace pressure is recorded by individual sensors and automatically transmitted to a computer. This *in vitro* gas method focuses on the production of the fermentation products, CO_2 and CH_4, and uses these as an expression for digestibility (Pell & Schofield, 1993).

A wireless automated system which measure fermentation gas production by a pressure senor with a three-way lock which are secured to the sample bottle cap was developed. The sensors record an hourly measurement for 96 h. Furthermore, the wireless system uses radio frequency signals to send information to a server which records the measurements. The method is a useful tool for feed characterization, due to correlation between gas production and degradability (Adesogan *et al*., 2005).

2.2 Rumen physiology

In the anaerobic environment of the rumen, degradation of organic matter occurs due to the symbiotic relationship between the animal and the microbes. These anaerobes consist of different microbes such as bacteria, protozoa, fungi, bacteriophages, and methanogenic archaea, which all have co-evolved with their host for millions of years. The role of microbiomes in utilization of feeds are essential for the health, development, and nutrition for the animal (Huhtanen *et al*., 2006).

At birth, the gastrointestinal tract of ruminants is sterile, however, a rapid colonization of microbiomes occurs within the first 24 h after birth. Rumen development is an ongoing physiological process in calves as the rumen at birth only represents 29 % of the total stomach compartments whereas it is 55 % in the adult animal (Li *et al*., 2012).

Suárez *et al*. (2006b) investigated the hypothesis that it would have beneficial properties later in life for veal calves to have an early stimulation of the rumen development. A complete randomized block design experiment with 160 bull calves of mixed breed (Holstein Friesian × Dutch Friesen) was undertaken in The Netherlands. The rumen development was evaluated by mucosal thickness and mucosa to serosa length. The calves were assigned one of three different pelleted concentrate diets: pectin-based, neutral detergent fiber-based, starch-based, mixed concentrate, and a milk replacer diet as a control group. After 8 or 12 weeks, the calves were culled, and the rumen was examined. The study concluded that all concentrate diets resulted in a more rapid development of the rumen compared to the control group. This was based on that all calves fed the concentrate diets did not differ significantly from each other in relation to ratio of mucosa length to serosa length in the dorsal part of the rumen. However, they had an increase when compared to the calves fed milk replacer ($P < 0.01$). In addition, an increase of the ratio of mucosa length to serosa length was present in the ventral part of the rumen within the group of calves fed mixed and pectin diets compared to the control group ($P < 0.05$). The mucosa thickness in the rumen did not differ between the concentrate diets for the dorsal part, but the calves fed the mixed and starch-based diets showed an increase in mucosa thickness in the ventral part ($P < 0.05$). The muscle thickness of the rumen showed an increase in the dorsal part compared to the ventral. A concentrate which differed in carbohydrates (CHO) composition promoted the rumen development compared to milk replacer. In addition, calves which were fed a concentrate had a heavier rumen and a greater level of rumen papillae with embedded hair.

A study applied metagenomic tools to examine the rumen microbiome in pre-weaned ruminants, and three 14-day-old calves were compared to three 42-day-old calves. The study was undertaken in Beltsville USA, all the calves were bulls, of the breed Holstein, and they were fed ad libitum milk replacer. Furthermore, samples from four 12-month-old calves fed a hay-based diet were also included. Seven different genera were found in the rumen fluid. A significant difference was found when the 14-day-old calves were compared with the 42-day-old calves (*Bacteroides* P = 0.012; *Fusobacterium* P = 0.013; *Ruminococcus* P = 0.015; *Turicibacter* P = 0.034; *Tessaracoccus* P=0.000; *Luteimonas* P = 0.020; *Mogibacterium* P = 0.025). Fifteen different phyla were identified, Bacteroidetes, Firmicutes, and Proteobacterium were found to be the most prominent. The microbial composition of the rumen fluid was significantly changed ($P < 0.1$) along with the development of the rumen as the level of e.g. Bacteroidetes increased from 47.7 % in the 14-day-old calves to 74.8 % in the 42-day-old calves. Both age groups of milk replacer fed calves differed from 12-month-old calves as their level of Bacteroidetes was 52.0 %. It was concluded that the composition of microbial diversity in the rumen was likely to be significantly dependent on the physiological changes and development in the animals (Li *et al*., 2012).

A study performed in Israel by Jami *et al*. (2013) investigated the composition of microbiome present in the rumen in different life stages of bovines. Samples collected from 21 animals, which were distributed among five age groups, all of the breed Israeli Holstein. The animals were fed with one of three different diets: three 1-day-old calves (colostrum), three 3-day-old calves (colostrum), five 2-month-old calves (milk and starter feed), five 6-month-old heifers (70 % concentrate, 30 % roughage), and five 2-year-old lactating cows (70 % concentrate, 30 % roughage). Of the 15 identified phyla Bacteroidetes, Firmicutes, and Proteobacteria were dominantly present despite age. However, the amount and genera differed between age groups, as the main genus in the 1-3-day-old calves were *Bacteroides*, whereas *Prevotella* was the most prominent in the older age groups. Furthermore, the diversity index along with operational taxonomic unit showed an increase when the calves grew older. Despite consuming the same diet, the microbiome of the 6-month-old calves differed significantly from the 2-year-old. These results indicate that the composition of microbiome is not solely dependent on diet but also on age.

2.3 Carbohydrates impact on digestion factors

The intake of feed is constrained by available space in the digestive tract. The availability can be affected by factors such as the rate of degradation and retention time as feed needs to be digested or pass through the rumen cavity to make space for new fed (Krizsan *et al*., 2010). For instance, the digestion of insoluble cell wall CHOs is highly dependent on the passage rate as the degradability of CHOs is slow in the rumen (Huhtanen *et al*., 2006). To prevent ruminal acidosis and to reach an efficient conversion of energy into end products, an optimal level of starch and digestible fibers are desired (Poorkasegaran & Yansari 2014).

The rumen is the main cavity where degradation of CHO occurs, however, most CHOs are digestible in both the small intestine and hind gut, although only a limited amount off undigested CHOs will reach those parts of the gastrointestinal tract. Therefore, the rumen is also the main cavity for degradation of fiber, though some degradation might occur in the hind gut (Huhtanen *et al*., 2006). The degradation of fiber both soluble and insoluble is dependent on and limited by microbial fermentation.

2.3.1 Impact of concentrate feeds on digestion factors

An experiment was conducted in Iran on nine mid-lactation Holstein dairy cows, which were fed three different diets, to investigate how different sources of CHOs (barley, corn, and beet pulp) effect intake, chewing, digestibility, and performance. The trial period was 23 days, the first 14 days were an adaption period, and samples were collected the last seven days. The difference between the diets was the level of barley: (1) 34.19 %, (2) 18.87 %, and (3) 18.86 %. The barley in diet 1 was partly replaced with corn in diet 2, and with corn and beet pulp in diet 3. The chemical and physical characteristics for the three diets were similar. Between diet 1 and 3, the dry matter intake (DMI) ($P = 0.004$), neutral detergent fiber (NDF) intake ($P = 0.001$), and starch intake ($P = 0.002$) were significantly different. However, between diet 1 and diet 2 no significant difference for the three factors was observed. When the barley was replaced with beet pulp, the DMI increased due to the variation in non-fiber carbohydrates together with the NDF intake. The starch ($P = 0.002$), sugars ($P = 0.001$), and neutral detergent soluble carbohydrates ($P = 0.001$) differed among the different diets. Diets 1 and 2 had a greater starch intake compared to diet 3. Furthermore, diet 1 had the lowest sugar and neutral detergent soluble carbohydrates intake compared to the two other diets. The greatest DM ($P = 0.004$), NDF ($P = 1.028$), energy efficiency ($P = 0.024$), and significantly lower non-fiber carbohydrates digestibility ($P = 0.032$) were observed in diet 3. The total volatile fatty acids (VFA)

concentration was significantly different ($P < 0.001$) from diet 1 and diet 2, however, not from diet 3. The concentration of acetate, propionate, and the ration between the two fatty acids were significantly different ($P < 0.001$) from all three diets. However, the butyrate concentration was not significantly different ($P = 0.075$) between the three diets. Ruminal passage rate was significantly different ($P = 0.012$), together with mean retention time ($P = 0.002$), and total mean retention time ($P = 0.003$). The eating time for diet 1 was significantly different ($P = 0.045$) from diet 2, however, diet 3 did not differ from the two other diets. Rumination was significantly different ($P < 0.001$) for all three diets. Total chewing activity were significantly different ($P < 0.001$) between diet 1 and 2 and between diet 2 and 3, but not between diet 1 and 3. The results indicate that a change in dietary level of non-fiber carbohydrates, starch, and neutral detergent soluble carbohydrates due to the three differences CHOs source barley, corn, and beet pulp, which have a different level of degradability, may change intake, chewing activity, retention time, and passage rate (Poorkasegaran & Yansari 2014).

Four different diets were tested on 64 Holstein calves in an experiment carried out at National Institute of Animal Science in South Korea. The four diets were pelleted and contained either barley, corn, oat, or wheat. The diets were produced to contain an equal amount of starch from either barley, corn, oat, or wheat. The feed intake, body weight (BW), and skeletal growth of the calves were measured until 84-day-old. The results showed a greater hay consumption ($P < 0.05$), average daily solid DMI, daily intake of crude protein and starch, NDF consumption, BW, BW gain, and total DMI in the calves fed a corn-based diet followed by those fed on a wheat based diet and then on oat and barley diets (Khan *et al*., 2007).

In the same experiment, the effects of corn, wheat, oat, and barley on ruminal parameters, rumen development, and nutrient digestibility were investigated. Significant differences ($P < 0.05$) were found in the concentration of total VFA between calves fed barley and corn, barley and wheat, oat and corn, and lastly between the calves fed oat and wheat when the calves were 50- and 70-day-old. The ruminal concentration of acetate, propionate, and butyrate differed among diets, but generally a greater concentration of total VFA was found in calves fed corn and wheat diets compared to the animals fed the two other diets. The various starch sources in the different diets leads to variations in the VFA profile of the rumen. These different profiles could have a variable effect on the development of the rumen, as calves with a greater concentration of VFAs also had a more developed rumen. It has further been shown that calves fed on a corn- and wheat-based diet have a greater papillae growth and increased forestomach weight compared to calves fed a ration on barley and oat (Khan *et al*., 2008).

Suárez *et al*. (2006a) examined the effects of different CHO sources by feeding different diets which were either pectin-based, NDF-based, starch-based, or a mixed concentrate on growth performance and rumen fermentation characteristics. The experiment was carried out using the same method as described in Suárez *et al*. (2006b) which is previously described. The experiment showed that both daily DMI and average daily gain (ADG) were affected by diet ($P < 0.001$). The calves fed the starch diet had the lowest DMI, where the NDF diet had the greatest ($P < 0.05$) DMI compared to the pectin diet throughout the trial. The greatest ADG was observed in the calves fed the mixed diet together with the calves fed the NDF diet, compared to those fed pectin and starch diet ($P < 0.05$). The total VFA concentration was also affected by diet ($P < 0.001$). When compared to the other concentrate treatments, the lowest total VFA concentration was found in the calves fed a starch diet, where the greatest was observed in the calves fed the NDF diet ($P < 0.05$). The greatest acetate concentration was found in the NDF fed calves, where the lowest was found in the starch fed calves ($P < 0.05$). Furthermore, the starch fed calves showed the greatest propionate concentration, however, without any significant differences compared to the other concentrate treatments. The calves fed mixed, pectin, and starch diets had a significantly greater butyrate concentration compared to the calves fed the NDF diet. These results show that the CHO source in the concentrate feed influence the intake, growth, and parameters of rumen fermentation (Suárez *et al*., 2006a).

2.3.2 Impact of forage supplements on digestion factors

A Spanish study was carried out to determine the improvement of performance of calves related to the supplementation of hay to increase the total NDF content in the diet. A total of 63 Holstein male calves were randomly distributed to four different diets: low-NDF starter (18 %) with or without a hay supplement and high-NDF starter (27 %) with or without a hay supplement. Lower VFA concentration was observed in the calves supplemented with hay compared to calves which received no supplement. Furthermore, the NDF content of the pellets did not affect the rumen pH level. However, an increase in the VFA concentration along with an altered rumen fermentation profile were observed in calves fed pellets with a greater NDF content. It was further observed that the calves fed a hay supplement had an increased weight gain and feed efficiency compared to the calves fed no hay. The study concluded that a hay supplement, right after weaning, could improve the performance due to an improved starter intake. This improved starter intake could further lead to an increased ruminal pH, and ADG without the gain-to-feed ratio is affected compared to calves fed no hay supplement (Terré *et al*., 2013).

A feeding trial was undertaken at the University of Hohenheim, Germany, to investigate which impact the fiber content in hay and highly degradable concentrate have on rumen fermentation, digesta particle size, faecal particle size, chewing activity, passage rate, and apparent digestibility. Four rumen cannulated Holstein cows in late lactation were restrictively fed four different diets varying in hay quality and concentrate level. The four diets consisted of a concentrate feed with either 20 % or 50 % of ration DM, together with either a low-fiber hay (47% NDF in DM) or a high-fiber hay (62 % NDF in DM). Results showed that retention time was greatest for the low-fiber hay diet with 50 % concentrate. Furthermore, the amount of concentrate feed in the high-fiber diets showed no significance in relation to retention time. Diets with different fiber level influenced the composition of particles size both in rumen and faeces more than the level of concentrate in the diets, the same is apparent for rumen fermentation. An increase in rumination time along with rumination periods and chewing activity were found when high-fiber diets were compared to low-fiber diets ($P \leq 0.05$). The level of concentrate did not account for a significant difference ($P \leq 0.05$). The conclusion was that a concentrate supplement did not account for the properties of a high-fiber hay in low-fiber diets. However, feeding a high-fiber diet is an effective alternative if the concentrate contend is limited. Concentrate supplements to a hay diet could result in an increased utilization of the fiber fraction as the amount of fermentable organic matter will be increased, and more energy and nitrogen will be available to the rumen microbiota (Tafaj *et al*., 2005).

A study was carried out in Canada by Montoro *et al*. (2013) to determine the effect of two different physical forms of hay on performance, apparent digestibility and feeding behavior of young calves. A total of 20 Holstein bull calves were included in the study. The calves were randomly assigned one of two different diets: (1) A 90 % crumb concentrated starter feed supplemented with 10 % coarse grass hay (3 to 4 cm), or (2) A 90 % crumb concentrated starter feed supplemented with 10 % ground grass hay (2 mm). A gain-to-feed ratio was found to be 0.68 for diet 1 and 0.63 for diet 2, as a result, the calves gained more weight if fed a supplement of hay with a larger particle size. This aligns with the findings for ADG as it was 0.94 kg/day for diet 1 and 0.89 kg/day for diet 2. In addition, diet 2 had a greater DMI intake compared to diet 1. However, no significant difference was found for either ADG or DMI between the two diets.

Coverdale *et al.* (2004) investigated at Iowa State University, USA, the effect which different diets and hay sources could have on intake, growth, and feed efficiency in calves. The study consisted of two separate feeding trials. In the first feeding trial, four different diets were randomly distributed between 60 Holstein bull calves. The diets were either (1) A finely ground

starter, (2) A coarse ground starter, (3) A coarse ground starter supplemented with 7.5 % grass hay, or (4) A coarse ground starter supplemented with 15 % grass hay. In the second feeding trial, the same four diets were randomly distributed among 56 calves of the breeds Holstein, Jersey, Ayrshire, and Brown swiss. For trial 1, no significant differences were found in relation to the gain-to-feed ratio. In trial 2, no significant differences were found for BW, ADG, and gain-to-feed ratio for the whole duration of the trial. However, in trial 1, significant differences ($P = 0.01$) were found for ADG between diet 1 and 2, and a tendency was shown for BW and ADG when diet 1 was compared to diet 3 and 4 for the whole duration of the trial. The study concluded that both the amount of supplement hay and the type of concentrate diet could influence intake and growth of calves.

In a study conducted in Ithaca, New York, USA, 32 female and 32 male Holstein calves were used to examine the impact of withholding roughage on the growth and development of neonatal calves. The calves were randomly assigned one of four different diets: a low or high-fiber pelleted starter feed or a low or high-fiber mash feed. All feeds were produced to have a similar physical form and nutritional value which would cover the nutritional requirements of the calves. However, the fiber fractions were greater in the pelleted feeds. The study showed no implications in raising calves until 8 weeks of age without any forage supplements, as no negative influence on physiological development and growth was found. Furthermore, an increase in feed intake was found when the calves were fed mash instead of a pelleted fed. This was due to the lower particle size in the mash feed (Porter *et al*., 2007).

3 Method and Materials

3.1 Literature search

The scientific literature applied in this research project was obtained by systematic literature search in the following scientific databases: PubMed, AGRIS, AGRICOLA, and CAB Abstracts. The primary search words applied were chewing activity, *in vitro* gas production, neutral detergent fiber, passage rate, retention time, rumen development, rumen digestion, rumen fermentation, rumen microbiota, and ruminant.

The included literature has primarily origin from 2000 to 2020, with only a few exceptions. Furthermore, only literature in English and Danish were included.

3.2 *In vitro* trials

In this research project, two *in vitro* methods were carried out to examine the fermentation kinetics and fiber content of different feed components and rations.

3.2.1 *In vitro* gas production technique

The IVGPT trials were conducted as described in the protocol by Hansen (2019a). Furthermore, all substrates, including a micro- and macro mineral solution, a buffer solution, a redox indicator along with a reduction agent were prepared according to Menke & Steingass (1988). The ratio of the mixture was 2:1 of the combined substrates and rumen fluid. As seen in Table 3.1, the rumen fluid used in the buffer solution was collected from either rumen cannulated heifers of the breed Danish Jersey, rumen cannulated lactating cows of the breed Danish Red, or slaughter calves of either 5-7-week or 14-16-week of age. The incubation time for the trials ranged between 20-48 h.

Table 3.1. *The distribution of replicates and information regarding the* in vitro *gas trials. Wheat (WHT), alfalfa pellets (APO), grass pellets (GSP), calf starter 1 (CS1), calf starter 2 (CS2), maize silage (MS), palm kernel expeller (PEXP), rapeseed meal (RSM), rapeseed cake (RSC), heat-treated rapeseed meal (RSMh), soybean meal (SBM), soybean cake (SBC), soybean hulls (SOH), untoasted fababeans (UFB), toasted fababeans (TFB), FAST, SLOW, FAST-FAST, and SLOW-SLOW.*

Trial no.	**F103**	**F104**	**F105**	**F106**	**F108**	**F109**	**F116**	**F117**	**F128**	**F129**	
Donor	**Heifers**	**Heifers**	**Calves 5-7-week**	**Calves 5-7-week**	**Calves 14-16-week**	**Calves 14-16-week**	**Heifers** / **Calves 14-16-week**	**Heifers**	**Lactating cows**	**Lactating cows**	
Feed	**24 h**	**24 h**	**24 h**	**24 h**	**24 h**	**20 h/48 h**	**48 h**	**48 h**	**48 h**	**48 h**	**Total**
WHT	-	-	-	-	-	-	-	-	4	3	7
APO	4	4	3	3	4	4	-	-	-	-	22
GSP	4	4	3	4	4	4	-	-	-	-	23
CS1	4	4	3	4	4	4	-	-	-	-	23
CS2	4	4	2	4	4	4	-	-	-	-	22
MS	-	-	-	-	2	-	2[1] | 2[2]	2	4	4	16
PEXP	3	-	-	3	4	6	-	-	-	-	16
RSM	4	4	-	4	4	6	-	-	-	-	22
RSC	4	3	3	4	4	4	-	-	-	-	22
RSMh	4	4	2	4	4	4	-	-	-	-	22
SBC	4	4	3	4	4	3	-	-	-	-	22
SBM	4	4	3	4	4	4	-	-	-	-	23
SOH	-	-	-	-	4	6	-	-	4	4	18
TFB	4	4	3	4	4	4	-	-	4	3	30
UFB	4	4	3	4	4	4	-	-	4	3	30
FAST	-	-	-	-	-	-	3[1]	4	-	-	7
SLOW	-	-	-	-	-	-	3[1]	4	-	-	7
FAST-FAST	-	-	-	-	-	-	-	-	4	4	8
SLOW-SLOW	-	-	-	-	-	-	-	-	4	4	8
Total	47	43	28	46	54	57	10	10	28	25	348

[1] *Donors: Calves 14-16-week-old*
[2] *Donors: Heifers*

3.2.1.1 Materials and Equipment

The *in vitro* gas trials were conducted using the wireless gas production measurement system ANKOM RF (ANKOM Technology, 2020a). Each fermentation module consisted of a 100 mL glass BlueCap bottle along with an ANKOM RF1 head. Buffered rumen fluid (90 mL) was added to a bottle which contained a ground feed sample (~0.5 g) as shown in Figure 3.1. During the fermentation, all modules were placed in a thermoshaker (C. Gerhardt GMBH & Co. KG, 2015). F57 bags were used to filter the samples after end fermentation.

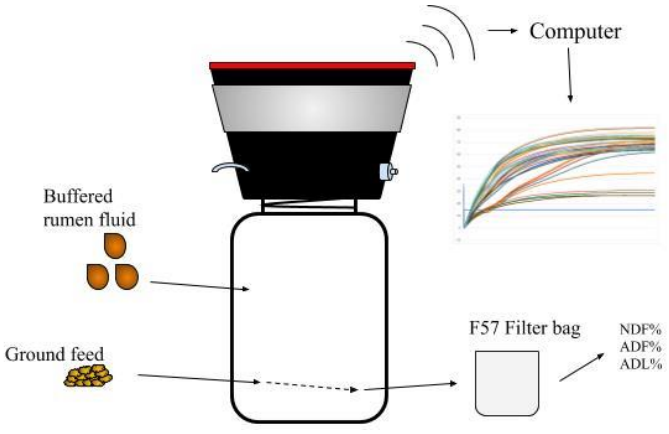


Figure 3.1. *Schematic overview which shows the ANKOM RF fermentation process. Made with inspiration from Hansen et al. (2013).*

3.2.2 *In vitro* fiber analyses

Fiber analyses were carried out to determine the cell wall content in both unfermented (raw) and fermented samples of feed components and rations. The NDF analysis determines the insoluble fiber fractions: hemicellulose, cellulose, and lignin. Whereas the acid detergent fiber (ADF) analysis determines the cellulose and lignin fractions. Lastly, the acid detergent lignin (ADL) analysis determines the lignin fraction. Insoluble ash is also present in the samples. However, it was chosen not to determine this fraction in this research project.

The method was performed as described in the protocol by Hansen (2019b) which is based on the technique described by ANKOM (ANKOM Technology, 2020b; ANKOM Technology, 2020c). The equipment used for this method included the ANKOM Fiber Analyzer A200 (ANKOM Technology, 2020d) for the NDF and ADF analyses, along with a Daisy Incubator (ANKOM Technology, 2020e) for ADL analyses. Fiber analyses for SBP (FAST) and SBP (SLOW) were desired, but as fermented samples were unobtainable, these were not included. A list of the analyzed samples can be seen in Table 3.2.

Table 3.2. *The distribution of samples analyzed in the fiber analyses.*

Sample type	No. of raw samples	No. of fermented samples	Trial no. of fermented samples	No. of replicates
Untoasted fababeans	3	4	F128, F129	7
Toasted fababeans	3	4	F128, F129	7
FAST	3	4	F116, F117	7
SLOW	3	4	F116, F117	7
FAST-FAST	3	4	F128, F129	7
SLOW-SLOW	3	4	F128, F129	7
Total	16	24	-	42

3.3 *In vivo* feeding trials

The *in vivo* feeding trials were conducted at the dairy farm Assendrup Hovedgaard, Assendrupvej 10, 4690 Haslev, from April to September 2019, and at the beef calf farm Grusgravgaard, Grusgravvej 3, Tommerup, 4660 Store Heddinge, from November 2019 to January 2020.

3.3.1 Trial designs and equipment

The *in vivo* feeding trial at Assendrup Hovedgaard, where two feed rations with a feed component with either a fast or slow fermentation rate in the early hours of fermentation (FAST; SLOW) were examined. The trial was a completely randomized block design conducted with six experimental units which consisted of 36 animals in total (Table 3.3). The *in vivo* feeding trial at Grusgravgaard, where two feed rations with two feed components with either fast or

slow fermentation rate in the early hours of fermentation (FAST-FAST; SLOW-SLOW) were examined. The trial was a randomized block design conducted with four experimental units which consisted of 118 animals in total as seen in Table 3.3. All calculations for the *in vivo* feeding trials were performed on pen level as each pen represented an experimental unit. All calves were weighed once a week for the duration of the trial. In the trial design for the *in vivo* feeding trial at Assendrup Hovedgaard, a 5±2 days adaption period was included. As the calves on Grusgravgaard originate from 14 different farms, the calves had previously experienced a sudden change of feed. Therefore, it was chosen to start weighing the calves from the first week of the trial period.

Table 3.3. *The distribution of experimental units and insertion information.*

Ration type	Experi-mental unit	Date of insertion	Mean weight at insertion (kg)	Age at insertion (days)	No. of animals	Distribution of sex	Distribution of breed
Assendrup Hovedgaard							
FAST	**1**	30.03.2019	97	67-81	6	6 heifers	6 Danish Red
	2	15.06.2019	110	62-97	6	6 heifers	6 Danish Red
	3	01.07.2019	94	57-88	6	6 heifers	6 Danish Red
SLOW	**1**	14.04.2019	84	67-77	6	6 heifers	6 Danish Red
	2	03.05.2019	93	62-81	6	6 heifers	6 Danish Red
	3	08.07.2019	90	54-59	6	6 heifers	6 Danish Red
Grusgravgaard							
FAST-FAST	**1**	11.11.2019	105	71-119	31	10 heifers, 21 bulls	8 Holstein, 11 Danish Red, 12 Mixed breeds
	2	18.11.2019	103	67-109	28	5 heifers, 23 bulls	10 Holstein, 6 Danish Red, 12 Mixed breeds
SLOW - SLOW	**1**	11.11.2019	105	71-115	30	9 heifers, 21 bulls	11 Holstein, 6 Danish Red, 14 Mixed breeds
	2	18.11.2019	101	61-108	29	5 heifers, 24 bulls	13 Holstein, 1 Danish Red, 15 Mixed breeds

3.3.2 Composition of the four feed rations

The feed plans which were applied in both of the *in vivo* feeding trials were created in DLBR by Mogens Vestergaard, senior researcher at the Department of Animal Science, Animal nutrition and physiology, Aarhus University, and Per Spelth, special consultant in cattle and beef production and SEGES in agreement with Associate Professor Hanne Helene Hansen, Department of Veterinary and Animal Sciences, University of Copenhagen.

Table 3.4 *The composition of the four feed rations (% of pelleted feed).*

Main feed compounds	**FAST**	**SLOW**	**Difference**
Wheat	22.00	22.00	0.00
Sugar beet pulp (SLOW)	-	46.00	46.00
Sugar beet pulp (FAST)	46.00	-	46.00
Soybean cake	25.11	25.11	0.00
Main feed compounds	**FAST-FAST**	**SLOW-SLOW**	**Difference**
Untoasted fababeans	40.01	-	40.01
Toasted fababeans	-	40.00	40.00
Wheat	30.76	36.92	6.16
Sugar beet pulp (FAST)	18.00	-	18.00
Soybean hulls	-	15.00	15.00
Rapeseed cake	6.84	3.43	3.41

The daily weight gain per animal was estimated to 800 g for the four different feed plans. FAST and SLOW rations were produced by Brødr. Ewers A/S, whereas FAST-FAST and SLOW-SLOW rations were produced by Vestjyllands Andel A.m.b.a.

DM, crude protein, crude fat, and crude ash were determined by Eurofins, whereas energy and NDF were obtained from the feed plans. The composition of the feed rations is shown in Table 3.4 and the feed analyses in Table 3.5.

Table 3.5. *Analyses of the four different feed rations. DM, crude protein, crude fat, and crude ash were determined by Eurofins. Neutral detergent fiber (NDF), starch, and energy were obtained from the feed plans (% of pelleted feed).*

Analytical compounds	**FAST**	**SLOW**	**Difference**
DM	89.10	88.40	0.70
Crude protein	18.20	18.71	0.51
Crude fat	3.70	3.70	0.00
Crude ash	7.70	7.20	0.50
NDF	18.62	19.36	0.74
Starch	14.61	14.06	0.55
Energy	0.68	0.67	0.01
Analytical compounds	**FAST-FAST**	**SLOW-SLOW**	**Difference**
DM	87.60	88.86	1.26
Crude protein	16.60	16.37	0.20
Crude fat	3.10	4.25	1.10
Crude ash	5.70	5.59	0.10
NDF	16.73	19.56	2.83
Starch	34.16	39.38	5.22
Energy	0.64	0.61	0.03

3.4 Data analyses

Two programs were used in the data analyses: Microsoft Excel for descriptive statistics and R for theoretical statistics. Statistical significance was declared at the p-value < 0.05, and a tendency when the p-value ≥ 0.05 ≤ 0.1.

3.4.1 Data processing for *in vitro* gas trials

The ideal gas law was used to convert the accumulated gas production recorded as psi into mL of gas at standard conditions for temperature and pressure. After preliminary model evaluation using the Box Cox Transformation, a linear mixed model was chosen. All interactions are tested and discarded when not found significant.

The final model presented below is used to test differences in fermentation kinetics depending on different donors for the feed components maize silage (MS), palm kernel expeller (PEXP), soybean meal (SBM), soybean cake (SBC), soybean hulls (SOH), untoasted fababeans (UFB), and toasted fababeans (TFB):

$$Y_i = \alpha_{donor(i)} + \gamma_{trial(i)} + e_i$$

Where Y_i is the gas production at i hour after trial start, α is the i type of donor as a main effect, and γ is the i trial number as a random effect. The variable e_i is the error term which is assumed to be independent and normally distributed, $N(0,\sigma^2)$.

Due to significant interactions between the factors feed and trial, regarding SBM, SBC, and SOH together with UFB, and TFB, it was not possible to test these feed components in relation to differences in fermentation kinetics between feed components with similar endpoints and different donors which was desired, therefore these feed components are tested individually.

The final model presented below is used to test differences in fermentation kinetics between feed components with similar endpoints and different donors for the feed components alfalfa pellets (APO) against grass pellets (GSP), rapeseed meal (RSM) against rapeseed cake (RSC), and heat-treated rapeseed meal (RSMh), calf starter 1 (CS1) against calf starter 2 (CS2), and FAST against SLOW:

$$Y_i = \alpha_{feed(i)} + \beta_{donor(i)} + \gamma_{trial(i)} + e_i$$

Where Y_i is the gas production at i hour after trial start, α is the i type of feed, β is the i type of donor, both as main effects. γ is the i trial number as a random effect. The variable e_i is the error term which is assumed to be independent and normally distributed, $N(0,\sigma^2)$.

The final model presented below is used to test differences in fermentation kinetics within donors for the four feed rations FAST against SLOW and FAST-FAST against SLOW-SLOW:

$$Y_i = \alpha_{feed(i)} + \gamma_{trial(i)} + e_i$$

Where Y_i is the gas production at i hour after trial start, α is the i type of feed as a main effect, and γ is the i trial number as a random effect. The variable e_i is the error term which is assumed to be independent and normally distributed, $N(0,\sigma^2)$.

The time points, which the models were calculated for, were selected from the fermentation patterns of the feed components and rations cumulative gas curves. Furthermore, the gas curves endpoints were obtained from the data, and the time points of which 50 % of total gas produced (T50) were subsequently calculated from the endpoints.

Bottles which were registered with a negative gas production throughout the IVGPT trials were not included in the statistical processing in R. No further selection was done on the grounds of standard error of the mean (SEM). Trial F117 and F129 have a duration at 47 h of fermentation. However, due to the assumption that the fermentation is stable between 47 and 48 h, these two trials are considered as trials with a duration at 48 h of fermentation.

Data for digested DM (dDM) at 24 h of fermentation are obtained from the *in vitro* gas trials F103, F105 and F108 and are included for the feed components PEXP, SBM, SBC, UFB, and TFB. No data are included from other components as the data was not available.

The final model presented below is used to test differences in the amount of dDM depending on different donors:

$$Y_{24} = \alpha_{donor(i)} + e_i$$

Where Y_{24} is the amount of dDM 24 h after trial start, α is the i type of donor as a main effect. The variable e_i is the error term which is assumed to be independent and normally distributed, $N(0,\sigma^2)$.

3.4.2 Data processing for *in vitro* fiber analyses

Eight out of 24 (4 TFB, 3 UFB, and 1 SLOW-SLOW) results for the calculation of percentages for ADL ranged between -0.0092 to -0.0002 %. It was chosen to set these values to zero due to the assumption that the ADL level in these two feed components and one feed ration are close to non-existent.

A Student's t-test was preformed to examine the data for any significant difference between the digested NDF, digested ADF, and dDM results. A two-way homoscedastic t-test

was chosen after a Breusch-Pagan Test showed no significance for heteroscedasticity. Due to the assumption that ADL is indigestible, no further statistical calculations have been made.

3.4.3 Data processing for *in vivo* feeding trials

The factors TW, weekly weight gain (WWG), grain consumption (GC), and gain-per-grain (G/g) ratio were calculated for each experimental unit. Subsequently, the average and SEM were calculated for each feed ration presented in each *in vivo* feeding trial. TW represents the total weight at a given date for an experimental unit. This factor is used to calculate WWG for a specific week, e.i. the factor WWG represent the weight gain in relation to the previous week. A week is defined by the number of days between two weighings. A week at Assendrup Hovedgaard was 6 to 9 days, whereas for Grusgravgaard the week length was 7 days. The ratio G/g defines the relationship between GC and TW. It is important to emphasize that G/g ratio is always in relation to trial start (week 0). Where WWG shows the weight gain in relation to the previous week and does not take the GC into consideration.

It was examined if the different feed rations had led to any difference in weight. Model validation was used to find the most suitable model for the data. Based on a Normal Q-Q plot, Residuals versus Fitted values plot, Scale-Location plot, and Residuals versus Leverage plot, a linear model was chosen. The four assumptions for a linear regression model: linearity, homoscedasticity, independence, and normality were met. Therefore, the linear model was found as the most suitable fit, although the data did not fit the linear model to the full. It could be assumed that the missing fit for the linear model is due to the fact that the animals in the end of the trial are past the linear part of the growth curve. All interactions are tested and discarded when not found significant.

The final model used to examine the data from the *in vivo* feeding trial at Assendrup Hovedgaard is presented below. The model is used to the test data from the entire trial period of eight weeks:

$$Y_i = \alpha_{feed(i)} + \beta_{group(i)} + \gamma_{day(i)} + e_i$$

Where Y_i is the TW at a i time, α is the i type of feed as a main effect, β is the i group, and γ is the i day. Both β and γ are included as random effects in the model. The variable e_i is the error term which is assumed to be independent and normally distributed, $N(0,\sigma^2)$.

The final model used to examine the data from the *in vivo* feeding trial at Grusgravgaard is presented below. It was chosen to exclude the first two weeks of the trial period from the statistical analysis due to the observed low WWG in week 2. The model is used to test data from week 2 to week 8 of the trial period:

$$Y_i = \alpha_{feed(i)} + \beta_{group(i)} + \gamma_{farm(i)} + e_i$$

Where Y_i is the TW at a i time, α is the i type of feed as a main effect, β is the i group, and γ is the i farm. Both β and γ are included as random effects in the model. The variable e_i is the error term which is assumed to be independent and normally distributed, $N(0,\sigma^2)$.

To examine the G/g ratio and the GC individually for any significant difference, a Student's t-test was undertaken. As a Breusch-Pagan Test showed no significance for heteroscedasticity, a two-way homoscedastic t-test was chosen. It is important to emphasize that the G/g ratio is calculated for the entire trial period for both Assendrup Hovedgaard and Grusgravgaard, as it was not possible to calculate a separate GC from week 2 to week 8 but only a total GC for the entire trial period for Grusgravgaard. However, a sensitivity analysis was carried out to investigate if GC the first two weeks of the trial period, at Grusgravgaard, had any significant importance. A two-way homoscedastic Student's t-test was chosen to examine the sensitivity analysis for any significant difference.

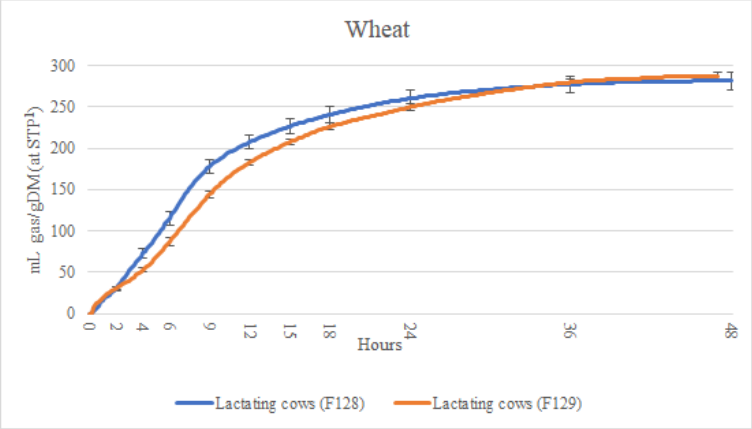
Furthermore, a two-way homoscedastic Student's t-test was also chosen to examine the WWG data, both from Assendrup Hovedgaard and Grusgravgaard, for each week between each experimental unit. However, no significant differences or tendencies were found, and therefore these results are not presented in this research project.

4 Results

In this results section, three main sections with subsections will be presented. The first main section contains results from the *in vitro* gas trials, and is has four subsections which present the following results: (1) Fermentation kinetics for wheat, (2) Differences in fermentation kinetics depending on different donors, (3) Differences in fermentation kinetics between feed components with similar endpoints and different donors, and (4) Differences in fermentation kinetics within donors for the four feed rations. To describe the fermentation kinetics from the in *vitro gas* trials the shape of the cumulative gas curves, the total amount of produced gas along with T50, are used as descriptive parameters. The second main section presents the results from the fiber analyses, and the third main section shows the results from the two *in vivo* feeding trials.

4.1 Fermentation kinetics for wheat

For the feed component wheat (WHT), only a single type of donor is used in the *in vitro* gas trials, and therefore no standard for comparison depending on different donor is possible. Furthermore, no other feed component is found suitable based on composition and applied donor to compare with WHT. Therefore, WHT is presented alone without any statistical calculations.



[1] *STP: Standard conditions for temperature and pressure.*
Figure 4.1. *Cumulative gas curves for wheat. Bars indicate standard error of the mean.*

The cumulative gas curves in both trials for WHT have an exponential shape (Figure 4.1). T50 for trial F128 is reached 2 h 10 min earlier than T50 is reached for trial F129. However, after both 24 and 48 h of fermentation, the donors in both trials have produced a similar amount of gas (Table 4.1).

Table 4.1. *Fermentation parameters for wheat.*

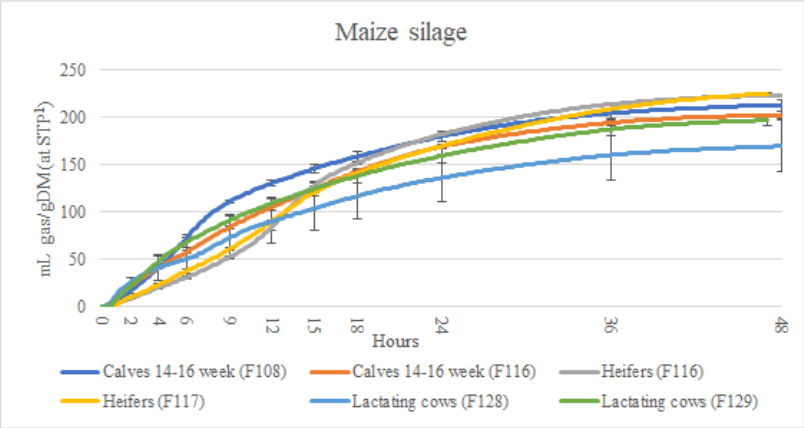
Trial no.	Donor	Gas produced at 24 h at STP[1] (mL gas/gDM)	Gas produced at 48 h at STP[1] (mL gas/gDM)	Time point for 50 % of total gas produced at STP[1]
F128	Lactating cows	260.23	281.65	6 h 50 min
F129	Lactating cows	249.92	287.18	9 h

[1] *STP: Standard conditions for temperature and pressure.*

4.2 Differences in fermentation kinetics depending on different donors

4.2.1 Fermentation kinetics for maize silage

The cumulative gas curves for MS with either 14-16-week-old calves or the lactating cows as donor have an exponential shape (Figure 4.2). Whereas the curves for MS with heifers as donors are sigmoidal in shape. Furthermore, the cumulative gas curves for the MS with heifers as donors show a slower early fermentation compared to the MS with either the 14-16-week old calves or the lactating cows as donors. However, the MS with heifers as donors has produced more gas at the end of fermentation compared to all other donors. A remarkable large variation for trial F128 is present (Figure 4.2).



[1] *STP: Standard conditions for temperature and pressure.*
Figure 4.2. *Cumulative gas curves for maize silage. Bars indicate standard error of the mean.*

In trial F108 and F116, the time point for T50 differs despite that 14-16-week-old calves are used as donors in both trials. The MS with heifers as donors reaches T50 last compared to MS with the other donors. Furthermore, the total amount of produced gas differs in trial F128 and F129, despite that lactating cows are used as donors in both trials (Table 4.2). However, MS in both trials reached T50 almost simultaneously.

Table 4.2. *Fermentation parameters for maize silage.*

Trial no.	Donor	Gas produced at 48 h at STP[1] (mL gas/gDM)	Time point for 50 % of total gas produced at STP[1]
F108	Calves 14-16-week	212.81	8 h 30 min
F116	Calves 14-16-week	202.30	11 h 20 min
F116	Heifers	223.76	13 h 40 min
F117	Heifers	225.34	14 h 10 min
F128	Lactating cows	169.66	10 h 50 min
F129	Lactating cows	197.35	10 h10 min

[1] *STP: Standard conditions for temperature and pressure.*

As seen in Table 4.3, a significant difference in the amount of produced gas for MS is found after 4 h of fermentation between MS with heifers and lactating cows as donors. However, no significant difference is found between donors for the remaining chosen time points.

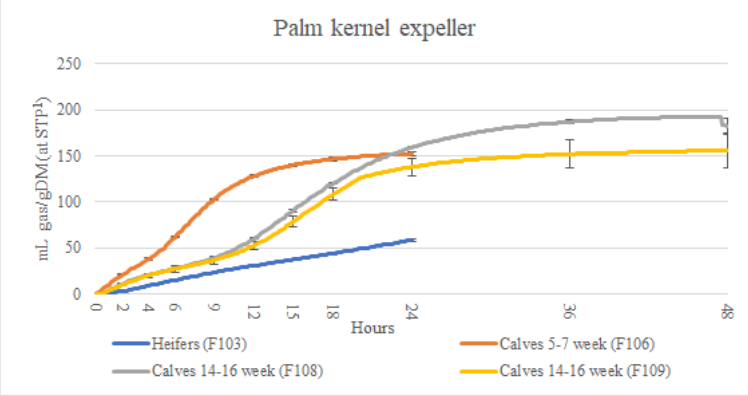
Table 4.3. *The effect of different donors for maize silage (MS) fermentation. Values in a column with different superscripts are significantly different (P < 0.05)[1] (standard error of the mean).*

	Donor	Hours of fermentation			
		4 h	**6 h**	**9 h**	**12 h**
		Total gas production at STP[2] (mL gas/gDM)			
MS	**Calves 14-16-week**	41.26 [ab] (0.29)	65.63 [a] (2.29)	97.54 [a] (3.96)	118.06 [a] (3.67)
	Heifers	21.60 [a] (0.53)	34.43 [a] (1.04)	56.50 [a] (1.21)	87.19 [a] (1.03)
	Lactating cows	44.56 [b] (2.38)	59.57 [a] (3.86)	81.91 [a] (4.09)	99.79 [a] (4.21)

[1] *A linear mixed model with donor as a main effect and trial as a random effect.*
[2] *STP: Standard conditions for temperature and pressure.*

4.2.2 Fermentation kinetics for palm kernel expeller

The cumulative gas curves for PEXP in trials with either 5-7-week-old calves (F106) or 14-16-week-old calves (F108; F109) as donors are sigmoidal in shape. Whereas the curve for the trial with heifers (F103) as donors is linear in shape as it might not have reached its point of inflection before the end of fermentation (Figure 4.3). The early fermentation rate for trial F106, where 5-7-week-old calves are used as donors, is greater compared to all other donors.



[1] *STP: Standard conditions for temperature and pressure.*
Figure 4.3. *Cumulative gas curves for palm kernel expeller. Bars indicate standard error of the mean.*

A remarkable difference is seen in amount of produced gas at 24 h of fermentation between PEXP with heifers as donor, compared to all other donors (Table 4.4).

Table 4.4. *Fermentation parameters for palm kernel expeller.*

Trial no.	Donor	Gas produced at 24 h at STP[1] (mL gas/gDM)	Gas produced at 48 h at STP[1] (mL gas/gDM)	Time point for 50 % of total gas produced at STP[1]
F103	Heifers	58.50		11 h 10 min
F106	Calves 5-7-week	152.43		7 h
F108	Calves 14-16-week	159.08	182.28	15 h
F109	Calves 14-16-week	137.98	155.66	14 h 50 min

[1] *STP: Standard conditions for temperature and pressure.*

As seen in Table 4.5, significant differences for PEXP between all three types of donors were found at 4 to 18 h of fermentation. At 24 h, a significant difference is found between PEXP with heifers as donors and PEXP with either 5-7-week-old calves or 14-16-week-old calves as donors. However, no significant difference for PEXP is found between the donors 5-7-week-old calves and 14-16-week-old calves at 24 h of fermentation.

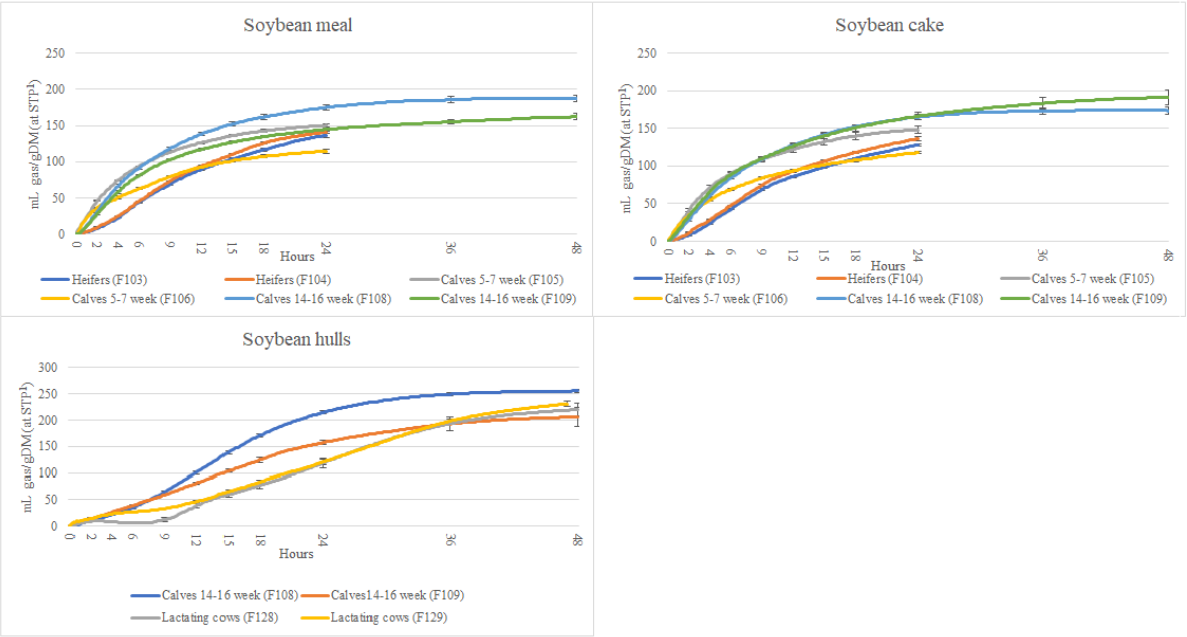
Table 4.5. *The effect of different donors for palm kernel expeller (PEXP) fermentation. Values in a column with different superscripts are significantly different (P < 0.05)[1] (standard error of the mean).*

	Donor	Hours of fermentation						
		4 h	6 h	9 h	12 h	15 h	18 h	24 h
		Total gas production at STP[2] (mL gas/gDM)						
PEXP	**Calves 5-7-week**	38.39 [c] (0.64)	61.96 [c] (0.33)	103.37 [c] (0.48)	128.17 [c] (0.73)	140.11 [c] (0.89)	146.71 [c] (1.17)	152.43 [b] (1.32)
	Calves 14-16-week	20.50 [b] (0.47)	27.49 [b] (0.58)	37.83 [b] (0.76)	55.86 [b] (1.00)	83.54 [b] (1.25)	112.86 [b] (1.37)	155.64 [b] (2.20)
	Heifers	9.30 [a] (0.10)	15.04 [a] (0.08)	23.61 [a] (0.11)	31.07 [a] (0.23)	37.66 [a] (0.41)	44.13 [a] (0.62)	58.50 [a] (1.03)

[1] *A linear mixed model with donor as a main effect and trial as a random effect.*
[2] *STP: Standard conditions for temperature and pressure.*

4.2.3 Fermentation kinetics for soybean meal, cake, and hulls

The feed components appear to have a similar fermentation kinetics, except for SOH, as the cumulative gas curves for SBM and SBC are remarkably similar in shape. The cumulative gas curves for SBM and SBC in trial F103 and F104, which both have heifers as donor, have a sigmoidal shaped. Whereas the cumulative gas curves for the SBM and SBC in trial F105 and F106 with 5-7-week-old calves as donors, and trial F108 and F109 with 14-16-week-old calves as donors, have exponential shaped gas curves. The cumulative gas curves for SOH in trial F128 and F129 with lactating cows as donor both have a sigmoidal shaped curve. SOH in trial F108 with 14-16-week-old calves as donors also has a sigmoidal shaped cumulative gas curve. Whereas the SOH in trial F109 which also have 14-16-week-old calves as donors has an exponential shaped cumulative gas curve (Figure 4.4).



[1] *STP: Standard conditions for temperature and pressure.*
Figure 4.4. *Cumulative gas curves for soybean meal, soybean cake, and soybean hulls. Bars indicate standard error of the mean.*

The cumulative gas curves indicate a slower early fermentation for both SBM and SBC in trial F103 and F104 where heifers are used as donors compared to the other trials. Furthermore, the cumulative gas curves for SOH in trial F128 and F129 where lactating cows are used as donors also show a slower early fermentation compared to SOH in trial F108 and F109 where 14-16-week-old calves are used as donors (Figure 4.4). These differences are also seen in Table 4.6, where the SBM and SBC in trial F103 and F104 with heifers as donors reached the T50 last compared to the other trials. The SOH with lactating cows as donors in trial F128 and F129 also reached the T50 at the latest time point compared to the other trials where SOH is tested. Furthermore, the SOH with lactating cows as donors in trial F128 and F129 reached the T50 at the latest time point compared to all the other trials where soybean products have been tested.

Table 4.6. *Fermentation parameters for soybean meal, soybean cake, and soybean hulls.*

Trial no.	Donor	Gas produced at 24 h at STP[1] (mL gas/gDM)	Gas produced at 48 h at STP[1] (mL gas/gDM)	Time point for 50 % of total gas produced at STP[1]
	Soybean meal			
F103	Heifers	136.24	-	8 h 50 min
F104	Heifers	141.00	-	8 h 40 min
F105	Calves 5-7-week	150.35	-	4 h
F106	Calves 5-7-week	114.78	-	5 h
F108	Calves 14-16-week	175.09	187.64	6 h 10 min
F109	Calves 14-16-week	144.77	162.35	6 h
	Soybean cake			
F103	Heifers	127.94	-	8 h 20 min
F104	Heifers	136.20	-	8 h 20 min
F105	Calves 5-7-week	147.98	-	4 h 10 min
F106	Calves 5-7-week	117.98	-	4 h 30 min
F108	Calves 14-16-week	165.16	173.44	6 h 10 min
F109	Calves 14-16-week	166.23	191.29	6 h 50 min
	Soybean hulls			
F108	Calves 14-16-week	214.64	255.49	14 h 10 min
F109	Calves 14-16-week	157.16	206.09	15 h
F128	Lactating cows	118.37	219.93	22 h 50 min
F129	Lactating cows	120.78	230.65	23 h 10 min

[1] *STP: Standard conditions for temperature and pressure.*

Significant difference is found for SBM after 4 and 6 h of fermentation between heifers and all other donors (Table 4.7). Furthermore, a significant difference is found after 9 h of fermentation for SBM between the donors, heifers and 14-16-week-old calves. However, SBM does not differ significantly in the amount of produced gas after 9 h of fermentation between 5-7-week-old calves and the other donors. No significant differences are found for the donors in the remaining chosen time points for SBM.

Table 4.7. *The effect of different donors for soybean meal (SBM) fermentation. Values in a column with different superscripts are significantly different (P < 0.05)[1] (standard error of the mean).*

	Donor	Hours of fermentation						
		4 h	6 h	9 h	12 h	15 h	18 h	24 h
		Total gas production at STP[2] (mL gas/gDM)						
SBM	**Calves 5-7-week**	61.11 [b] (1.85)	76.22 [b] (2.35)	94.32 [ab] (2.58)	107.32 [a] (2.58)	116.36 [a] (2.67)	122.71 [a] (2.72)	130.02 [a] (2.81)
	Calves 14-16-week	62.68 [b] (0.71)	86.53 [b] (0.77)	110.87 [b] (1.11)	127.79 [a] (1.51)	139.77 [a] (1.77)	148.33 [a] (1.91)	159.93 [a] (2.14)
	Heifers	23.93 [a] (0.19)	44.80 [a] (0.24)	71.26 [a] (0.38)	91.79 [a] (0.43)	107.19 [a] (0.53)	121.36 [a] (0.74)	138.62 [a] (0.60)

[1] *A linear mixed model with donor as a main effect and trial as a random effect.*
[2] *STP: Standard conditions for temperature and pressure.*

In Table 4.8, it is apparent for SBC that heifers as donors differ significantly from both age groups of calves at 4, 6, and 9 h of fermentation. However, no significant differences are found for SBC between 5-7-week-old and 14-16-week-old calves as donors at 4, 6, and 9 h of fermentation. A significant difference is found for SBC between 14-16-week-old calves and

heifers as donors at 12, 15, and 18 h of fermentation. Whereas at 24 h of fermentation for SBC, 14-16-week-old calves differ significantly from all other donors.

Table 4.8. *The effect of different donors for soybean cake (SBC) fermentation. Values in a column with different superscripts are significantly different (P < 0.05)*[1] *(Standard error of the mean).*

	Donor	Hours of fermentation						
		4 h	6 h	9 h	12 h	15 h	18 h	24 h
		Total gas production at STP[2] (mL gas/gDM)						
SBC	Calves 5-7-week	62.14 [b] (1.33)	78.08 [b] (1.67)	94.65 [b] (1.99)	106.14 [ab] (2.20)	114.72 [ab] (2.43)	121.68 [ab] (2.53)	130.84 [a] (2.43)
	Calves 14-16-week	62.80 [b] (0.70)	86.00 [b] (0.61)	109.48 [b] (0.53)	127.13 [b] (0.64)	141.00 [b] (0.68)	151.80 [b] (0.75)	163.82 [b] (0.82)
	Heifers	26.46 [a] (0.40)	45.12 [a] (0.38)	71.69 [a] (0.51)	89.52 [a] (0.60)	102.69 [a] (0.65)	114.11 [a] (0.67)	132.07 [a] (0.73)

[1] *A linear mixed model with donor as a main effect and trial as a random effect.*
[2] *STP: Standard conditions for temperature and pressure.*

For all the chosen time points in Table 4.9, a significant difference for SOH between the 14-16-week-old calves and lactating cows as donors is found. SOH with 14-16-week-old calves as donors has produced the greatest amount of gas for all the chosen time points compared to SOH with lactating cows as donors.

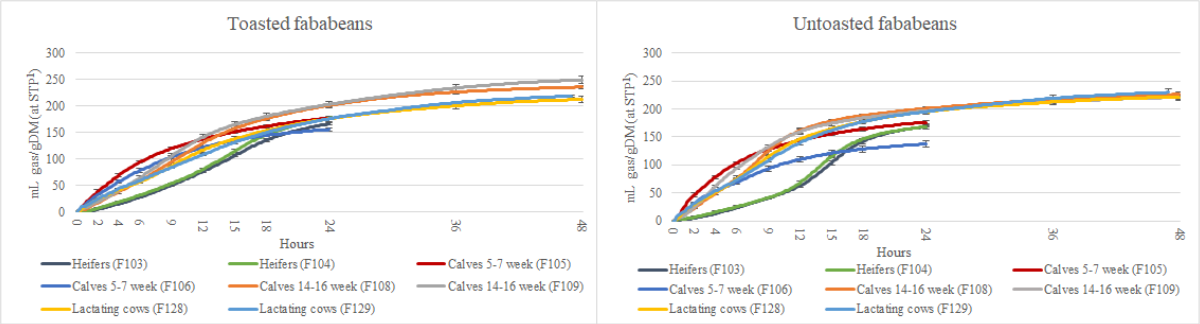
Table 4.9. *The effect of different donors for soybean hulls (SOH) fermentation. Values in a column with different superscripts are significantly different (P < 0.05)*[1] *(standard error of the mean).*

	Donor	Hours of fermentation						
		6 h	9 h	12 h	15 h	18 h	24 h	36 h
		Total gas production at STP[2] (mL gas/gDM)						
SOH	Calves 14-16-week	37.54 [b] (0.36)	61.18 [b] (0.53)	88.91 [b] (1.15)	117.53 [b] (1.95)	143.03 [b] (2.57)	187.53 [b] (3.78)	231.60 [b] (2.53)
	Lactating cows	16.38 [a] (1.43)	22.89 [a] (1.58)	42.19 [a] (0.93)	62.50 [a] (0.94)	80.38 [a] (1.23)	119.57 [a] (1.72)	195.66 [a] (2.12)

[1] *A linear mixed model with donor as a main effect and trial as a random effect.*
[2] *STP: Standard conditions for temperature and pressure.*

4.2.4 Fermentation kinetics for fababeans

The cumulative gas curves show a similarity in fermentation kinetics for both UFB and TFB despite the different treatments of the feed components (Figure 4.5). The cumulative gas curves for both UFB and TFB in trial F103 and F104 where heifers are used as donors show a sigmoidal shaped gas curve. The rest of the cumulative gas curves for both UFB and TFB are exponential shaped instead (Figure 4.5).



[1] *STP: Standard conditions for temperature and pressure.*

Figure 4.5. *Cumulative gas curves for untoasted fababeans and toasted fababeans. Bars indicate standard error of the mean.*

The endpoint at 24 h for both UFB and TFB also shows a similarity in fermentation kinetics. For both the UFB and TFB where 14-16-week-old calves are used as donors, the greatest amount of gas is produced. The least amount of gas is produced when the 5-7-week-old calves (F106) are used as donors for UFB and when the heifers are used as donors (F103) for TFB (Table 4.10).

Table 4.10. *Fermentation parameters for untoasted fababeans and toasted fababeans.*

Trial no.	Donor	Gas produced at 24 h at STP[1] (mL gas/gDM)	Gas produced at 48 h at STP[1] (mL gas/gDM)	Time point for 50 % of total gas produced at STP[1]
	Untoasted fababeans			
F103	Heifers	168.42	-	13 h 40 min
F104	Heifers	168.50	-	13 h 10 min
F105	Calves 5-7-week	176.31	-	4 h 40 min
F106	Calves 5-7-week	137.70	-	6 h
F108	Calves 14-16-week	201.04	225.95	8 h 20 min
F109	Calves 14-16-week	196.44	220.29	7 h 10 min
F128	Lactating cows	194.76	221.48	8 h 40 min
F129	Lactating cows	195.23	229.60	9 h 40 min
	Toasted fababeans			
F103	Heifers	166.64	-	12 h 40 min
F104	Heifers	178.05	-	12 h 40 min
F105	Calves 5-7-week	177.31	-	5 h 30 min
F106	Calves 5-7-week	155.65	-	6 h
F108	Calves 14-16-week	201.47	236.18	10 h 40 min
F109	Calves 14-16-week	203.54	249.58	10 h 30 min
F128	Lactating cows	176.13	212.67	10 h 30 min
F129	Lactating cows	176.35	218.97	12 h 10 min

[1] *STP: Standard conditions for temperature and pressure.*

The similar fermentation kinetics between UFB and TFB is shown in Table 4.11 and Table 4.12. The amount of produced gas for UFB and TFB differs at the different hours of fermentation between the different donors. However, significant differences in the amount of produced gas for UFB and TFB are found at the same hours between the same donors.

Table 4.11. *The effect of different donors for untoasted fababeans (UFB) fermentation. Values in a column with different superscripts are significantly different (P < 0.05)[1] (standard error of the mean).*

	Donor	Hours of fermentation						
		4 h	6 h	9 h	12 h	15 h	18 h	24 h
		Total gas production at STP[2] (mL gas/gDM)						
UFB	**Calves 5-7-week**	63.92 ^{c} (1.96)	83.60 ^{c} (2.74)	107.81 ^{c} (2.86)	124.39 ^{bc} (2.84)	135.63 ^{bc} (2.94)	143.88 ^{a} (3.02)	154.25 ^{a} (3.20)
	Calves 14-16-week	55.65 ^{b} (1.01)	84.56 ^{b} (1.46)	128.32 ^{bc} (1.26)	160.17 ^{c} (1.03)	176.51 ^{c} (0.95)	186.24 ^{b} (0.91)	198.74 ^{b} (0.89)
	Heifers	14.40 ^{a} (0.41)	24.24 ^{a} (0.43)	40.77 ^{a} (0.44)	65.55 ^{a} (0.64)	109.44 ^{a} (1.08)	144.21 ^{a} (0.82)	168.46 ^{a} (0.71)
	Lactating cows	52.31 ^{b} (0.39)	74.19 ^{b} (0.39)	111.92 ^{b} (0.84)	143.21 ^{b} (0.89)	163.21 ^{b} (0.77)	177.37 ^{a} (0.72)	194.96 ^{a} (0.82)

[1] *A linear mixed model with donor as a main effect and trial as a random effect.*
[2] *STP: Standard conditions for temperature and pressure.*

At 4 and 6 h of fermentation for UFB and TFB, a significance difference between the donors 5-7-week-old calves, 14-16-week-old calves, heifers, and lactating cows is found. However, no significance difference for UFB and TFB is found at 4 and 6 h of fermentation where 14-16-week-old calves and the lactating cows were used as donors. At 9 h, significant differences are found for UFB and TFB where the 5-7-week-old calves, heifers, and lactating cows are used as donors. However, no further significant differences are found between UFB and TFB where 5-7-week-old calves, 14-16-week-old calves, and the lactating cows are used as donors. Furthermore, at 12 and 15 h of fermentation, no significant difference is found between UFB and TFB where 5-7-week-old calves, 14-16-week-old calves, and the lactating cows are used as donors. However, a significant difference is found between UFB and TFB with heifers and the rest of the donors after 12 and 15 h of fermentation. At 18 and 24 h of fermentation, the UFB and TFB with 14-16-week-old calves as donors differ significantly from all the other donors.

Table 4.12. *The effect of different donors for toasted fababeans (TFB) fermentation. Values in a column with different superscripts are significantly different (P < 0.05)[1] (standard error of the mean).*

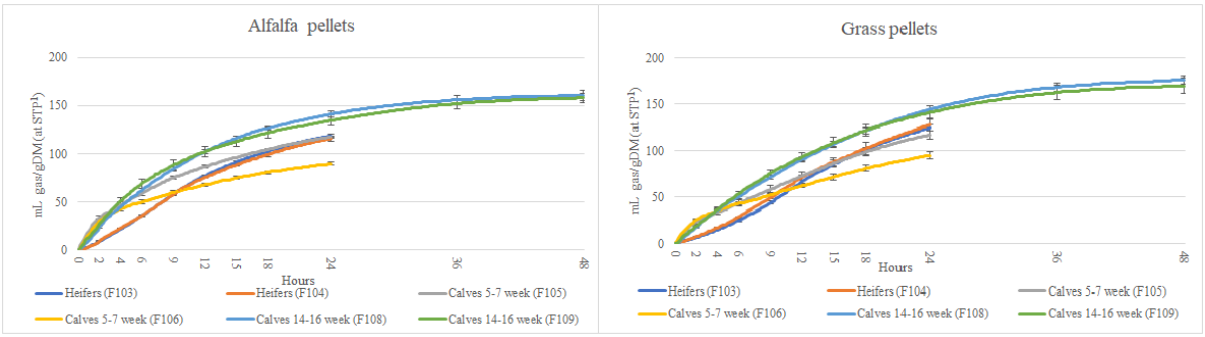
	Donor	Hours of fermentation						
		4 h	6 h	9 h	12 h	15 h	18 h	24 h
		Total gas production at STP[2] (mL gas/gDM)						
TFB	**Calves 5-7-week**	62.79 ^{c} (1.14)	85.32 ^{c} (1.41)	110.51 ^{c} (1.44)	128.93 ^{bc} (1.35)	142.12 ^{bc} (1.35)	152.10 ^{a} (1.44)	164.93 ^{a} (1.77)
	Calves 14-16-week	39.55 ^{b} (0.71)	61.91 ^{b} (0.99)	99.88 ^{bc} (1.38)	135.97 ^{c} (1.47)	161.20 ^{c} (1.29)	178.52 ^{b} (1.19)	202.50 ^{b} (1.14)
	Heifers	17.62 ^{a} (0.38)	30.41 ^{a} (0.41)	52.50 ^{a} (0.45)	79.54 ^{a} (0.57)	111.75 ^{a} (0.92)	141.27 ^{a} (0.92)	172.35 ^{a} (0.88)
	Lactating cows	42.88 ^{b} (0.61)	58.11 ^{b} (0.73)	85.63 ^{b} (1.05)	113.42 ^{b} (1.29)	134.91 ^{b} (1.16)	152.11 ^{a} (1.08)	176.22 ^{a} (1.05)

[1] *A linear mixed model with donor as a main effect and trial as a random effect.*
[2] *STP: Standard conditions for temperature and pressure.*

4.3 Differences in fermentation kinetics between feed components with similar endpoints and different donors

4.3.1 Fermentation kinetics for alfalfa and grass pellets

The cumulative gas curves for both feed components have an exponential shaped curve when 5-7-week-old and 14-16-week-old calves are used as donors. However, when heifers are used as donor, the cumulative gas curves for both feed components have a sigmoidal shape (Figure 4.6). After 24 h of fermentation, both feed components have produced the least amount of gas with 5-7-week-old calves as donor in trial F106. Furthermore, both feed components have produced the greatest amount of gas with 14-16-week-old calves as donors in trial F108 and F109.



[1] *STP: Standard conditions for temperature and pressure.*

Figure 4.6. *Cumulative gas curves for alfalfa pellets and grass pellets. Bars indicate standard error of the mean.*

APO appears to have a greater early fermentation compared to GSP in all applied donors as seen in Figure 4.5. This is supported by the T50, where APO regardless of donor reaches this point earlier than GSP except in trial F105 (Table 4.13).

Table 4.13. *Fermentation parameters for alfalfa pellets and grass pellets.*

Trial no.	Donor	Gas produced at 24 h at STP[1] (mL gas/gDM)	Gas produced at 48 h at STP[1] (mL gas/gDM)	Time point for 50 % of total gas produced at STP[1]
	Alfalfa pellets			
F103	Heifers	118.30	-	9 h
F104	Heifers	115.50	-	9 h
F105	Calves 5-7-week	11721	-	9 h
F106	Calves 5-7-week	89.69	-	2 h 20 min
F108	Calves 14-16-week	141.46	160.60	8 h 30 min
F109	Calves 14-16-week	134.85	157.88	7 h 30 min
	Grass pellets			
F103	Heifers	124.80	-	11 h 20 min
F104	Heifers	127.92	-	11 h
F105	Calves 5-7-week	117.41	-	9 h
F106	Calves 5-7-week	95.37	-	7 h 40 min
F108	Calves 14-16-week	144.59	175.92	11 H 30 min
F109	Calves 14-16-week	141.26	169.61	10 h 30 min

[1] *STP: Standard conditions for temperature and pressure.*

After 6 h of fermentation, the amount of produced gas for both feed components is significantly different for all donors. From 9 to 18 h of fermentation, a significant difference in the amount of produced gas for both feed components is found between 5-7-week-old calves and 14-16-week-old calves, and between 14-16-week-old calves and heifers. However, no significant difference is found between 5-7-week-old calves and heifers in the amount of produced gas for both feed components after 9 to 18 h of fermentation. Lastly, after 24 h of fermentation, a significant difference is found in the amount of produced gas for both feed components between the 5-7-week-old calves and 14-16-week-old calves. However, no significant difference in the amount of produced gas for both feed components is found between the donors 5-7-week-old calves, 14-16-week-old calves, and heifers at 24 h (Table 4.14).

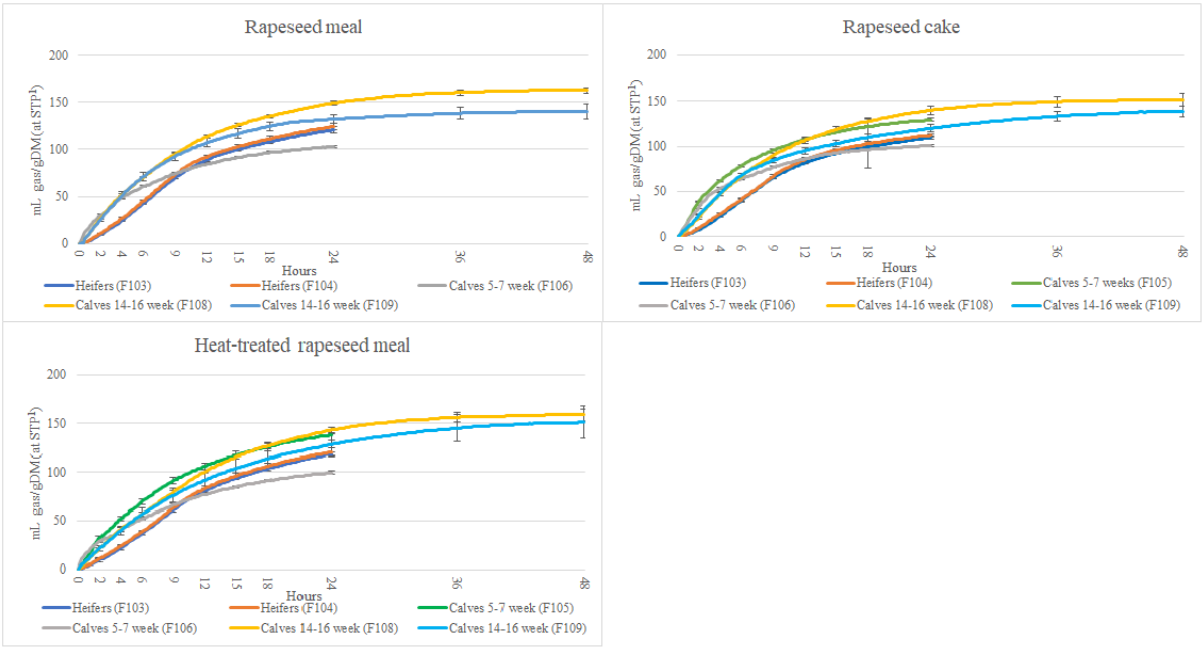
Table 4.14. *The effect of different donors for alfalfa pellets (APO) and grass pellets (GSP) fermentation. Values in a column with different superscripts are significantly different (P < 0.05)[1] (standard error of the mean).*

	Donor	Hours of fermentation					
		6 h	9 h	12 h	15 h	18 h	24 h
		Total gas production at STP[2] (mL gas/gDM)					
APO GSP	**Calves 5-7-week**	48.75 [b] (0.57)	60.70 [a] (0.73)	71.69 [a] (0.79)	81.52 [a] (0.86)	90.58 [a] (0.91)	104.18 [a] (1.05)
	Calves 14-16-week	58.79 [c] (0.58)	79.84 [b] (0.59)	97.30 [b] (0.55)	111.38 [b] (0.53)	122.94 [b] (0.54)	140.54 [b] (0.61)
	Heifers	31.42 [a] (0.30)	52.47 [a] (0.41)	72.53 [a] (0.33)	88.40 [a] (0.34)	101.49 [a] (0.42)	121.63 [ab] (0.53)

[1] *A linear mixed model with donor and feed as main effects and trial as a random effect.*
[2] *STP: Standard conditions for temperature and pressure.*

4.3.2 Fermentation kinetics for rapeseed meal, and cake

The feed components RSM, RSC, and RSMh appear to have a similar fermentation kinetics as all the cumulated gas curves have a similar shape. The cumulated gas curves for all three feed components where 5-7-week-old and 14-16-week-old calves are used as donors have an exponential shape. Whereas the other gas curves for all three feed components with heifers as donors have a sigmoidal shape. Furthermore, all three feed components produce less gas in the early hours of fermentation with heifers as donors compared to 5-7-week-old and 14-16-week-old calves. RSC and RSMh appear to have similar fermentation patterns in 5-7-week-old and 14-16-week-old calves. However, RSC and RSMh fermentation patterns in trial F105 and trial F106 differ from each other despite that 5-7-week-old calves are used as donors in both trials. A remarkable variation is observed after 18 h of fermentation for RSC in trial F104 (Figure 4.7).



[1] *STP: Standard conditions for temperature and pressure.*
Figure 4.7. *Cumulative gas curves for rapeseed meal, rapeseed cake, and heat-treated rapeseed meal. Bars indicate standard error of the mean.*

After 24 h of fermentation, similar fermentation kinetics are present. All three feed components have produced more gas in trial F108 which has 14-16-week-old calves as donors. Furthermore, all three feed components have produced less gas in trial F106 which has 5-7-week-old calves as donors (Table 4.15).

Table 4.15. *Fermentation parameters for rapeseed meal, rapeseed cake, and heat-treated rapeseed meal.*

Trial no.	Donor	Gas produced at 24 h at STP[1] (mL gas/gDM)	Gas produced at 48 h at STP[1] (mL gas/gDM)	Time point for 50 % of total gas produced at STP[1]
	Rapeseed meal			
F103	Heifers	121.11		8 h
F104	Heifers	124.61		7 h 40 min
F106	Calves 5-7-week	103.14		4 h 20 min
F108	Calves 14-16-week	149.23	162.63	7 h 10 min
F109	Calves 14-16-week	132.18	140.62	6 h
	Rapeseed cake			
F103	Heifers	109.49		7 h 40 min
F104	Heifers	111.89		7 h 40 min
F105	Calves 5-7-week	129.17		4 h 20 min
F106	Calves 5-7-week	100.71		3 h 20 min
F108	Calves 14-16-week	139.80	151.70	7 h 40 min
F109	Calves 14-16-week	119.92	138.59	6 h 10 min
	Heat-treated rapeseed meal			
F103	Heifers	118.21		8 h 50 min
F104	Heifers	120.69		8 h 40 min
F105	Calves 5-7-week	138.19		6 h
F106	Calves 5-7-week	99.26		5 h 30 min
F108	Calves 14-16-week	143.35	158.88	9 h
F109	Calves 14-16-week	128.10	151.20	9 h

[1] *STP: Standard conditions for temperature and pressure.*

At 4 and 6 h of fermentation, the use of heifers as donors differ significantly from the donors 5-7-week-old and 14-16-week-old calves. However, after 9 and 12 h of fermentation, no significant difference is found in the amount of produced gas for the three feed components for all three types of donors (Table 4.16).

Table 4.16. *The effect of different donors for rapeseed meal (RSM), rapeseed cake (RSC), and heat-treated rapeseed meal (RSMh) fermentation. Values in a column with different superscripts are significantly different (P < 0.05)[1] (standard error of the mean).*

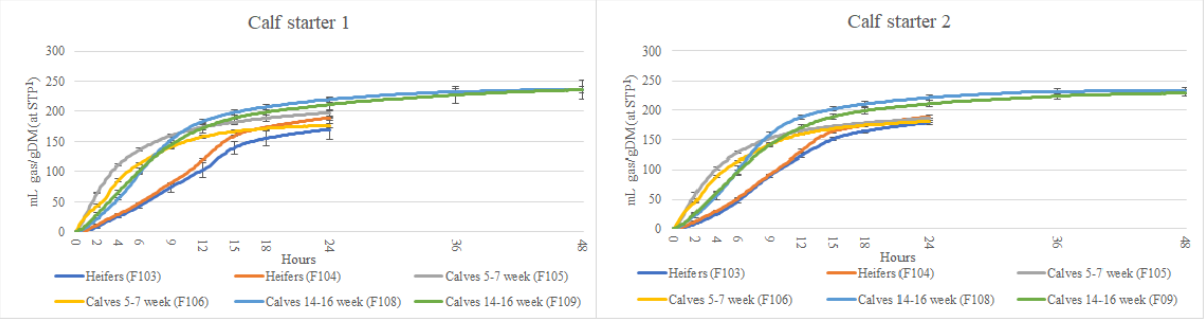
	Donor	Hours of fermentation			
		4 h	6 h	9 h	12 h
		Total gas production at STP[2] (mL gas/gDM)			
RSM	**Calves 5-7-week**	50.67 [b] (0.45)	63.59 [b] (0.54)	78.61 [a] (0.65)	89.64 [a] (0.71)
RSC	**Calves 14-16-week**	46.80 [b] (0.28)	65.35 [b] (0.36)	86.59 [a] (0.40)	102.66 [a] (0.44)
RSMh	**Heifers**	24.08 [a] (0.11)	40.55 [a] (0.15)	66.40 [a] (0.22)	85.28 [a] (0.23)

[1] *A linear mixed model with donor and feed as main effects and trial as a random effect.*
[2] *STP: Standard conditions for temperature and pressure.*

4.3.3 Fermentation kinetics for calf starter

The cumulative gas curves for both CS1 and CS2 show that both feed components have a similar fermentation pattern with the used donors. The cumulative gas curves for both feed components when 5-7-week-old and 14-16-week-old calves are used as donors have an exponential shape, where the cumulative gas curves when heifers are used as donor have a sigmoidal shape. Both feed components produce less gas in the early hours of fermentation

when heifers are used as donor compared to when 5-7-week-old and 14-16-week-old calves are used (Figure 4.8).



[1] *STP: Standard conditions for temperature and pressure.*
Figure 4.8. *Cumulative gas curves for calf starter 1 and calf starter 2. Bars indicate standard error of the mean.*

After 24 h of fermentation, similar fermentation patterns for the donors are shown. Both feed components have produced less gas in trial F103 where heifers are used as donors. Furthermore, both feed components have produced the greatest amount of gas in trial F108 where 14-16-week-old calves are used as donors. The T50 is also reached similar for both feed components as the time point for trial F105, F106, F108, and F109 only differs with ±10 min for CS1 and CS2. However, between the reached T50 for both feed components when heifers are used as donors, a difference of ±3 h is observed, where CS1 is the fastest fermentable feed component compared to CS2 (Table 4.17).

Table 4.17. *Fermentation parameters for calf starter 1 and calf starter 2.*

Trial no.	Donor	Gas produced at 24 h at STP[1] (mL gas/gDM)	Gas produced at 48 h at STP[1] (mL gas/gDM)	Time point for 50 % of total gas produced at STP[1]
	Calf starter 1			
F103	Heifers	169.91	-	6 h 10 min
F104	Heifers	189.46	-	6 h 10 min
F105	Calves 5-7-week	197.90	-	3 h 20 min
F106	Calves 5-7-week	176.66	-	4 h 10 min
F108	Calves 14-16-week	219.63	232.66	7 h
F109	Calves 14-16-week	211.38	227.20	7 h
	Calf starter 2			
F103	Heifers	178.86	-	9 h
F104	Heifers	188.91	-	9h 10 min
F105	Calves 5-7-week	185.99	-	3 h 30 min
F106	Calves 5-7-week	180.86	-	4 h 10 min
F108	Calves 14-16-week	221.61	231.81	6 h 50 min
F109	Calves 14-16-week	211.21	224.03	7h 10 min

[1] *STP: Standard conditions for temperature and pressure.*

For the two feed components, a significant difference between in the amount of produced gas for all donors after 4 and 6 h of fermentation is found. After 15 h of fermentation, CS1 and CS2 show a significant difference in the amount of produced gas between 14-16-week-

old calves and heifers. However, at this time point, no significant difference is found between 5-7-week-old calves compared to 14-week-old calves and heifers. After 18 and 24 h, a significant difference is found in the amount of produced gas for CS1 and CS2 between 5-7-week-old calves compared to 14-16-week-old calves, and 5-7-week-old calves compared to heifers as donors. However, no significant difference is found for the feed components between 5-7-week-old calves and heifers as donors after 18 and 24 h of fermentation (Table 4.18).

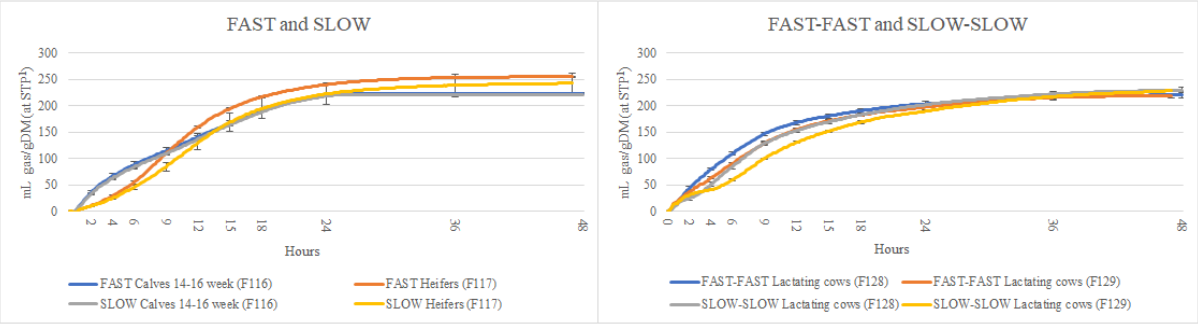
Table 4.18. *The effect of different donors for calf starter 1 (CS1) and calf starter 2 (CS2) fermentation. Values in a column with different superscripts are significantly different (P < 0.05)*[1] *(standard error of the mean).*

	Donor	Hours of fermentation				
		4 h	6 h	15 h	18 h	24 h
		Total gas production at STP[2] (mL gas/gDM)				
CS1 CS2	Calves 5-7-week	94.96 [c] (0.85)	121.13 [c] (0.81)	173.47 [ab] (0.53)	179.12 [a] (0.59)	184.29 [a] (0.77)
	Calves 14-16-week	59.50 [b] (0.55)	98.59 [b] (0.56)	194.53 [b] (0.77)	203.92 [b] (0.78)	215.96 [b] (0.82)
	Heifers	27.50 [a] (0.21)	47.39 [a] (0.33)	153.58 [a] (0.85)	167.00 [a] (0.91)	181.79 [a] (1.06)

[1] *A linear mixed model with donor and feed as main effects and trial as a random effect.*
[2] *STP: Standard conditions for temperature and pressure.*

4.4 Differences in fermentation kinetics within donors for the four feed rations

Different donors are used in the *in vitro* gas trials for the feed rations FAST and SLOW. The differences in fermentation kinetics between different donors are examined for FAST and SLOW and are included in this section. However, it is not possible to examine differences in fermentation kinetics between different donors for FAST-FAST and SLOW-SLOW as only one type of donor is used in the *in vitro* gas trials.



[1] *STP: Standard conditions for temperature and pressure.*
Figure 4.9. *Cumulative gas curves for FAST, SLOW, FAST-FAST, and SLOW-SLOW. Bars indicate standard error of the mean.*

The feed rations FAST, SLOW, FAST-FAST, and SLOW-SLOW have similar fermentation kinetics. All cumulative gas curves have an exponential shape, with exception of

the two gas curves for trial F117 for FAST and SLOW which have heifers as donor. These cumulative gas curves have a more sigmoidal shape (Figure 4.9).

Table 4.19. *Fermentation parameters for FAST, SLOW, FAST-FAST, and SLOW-SLOW.*

Trial no.	Donor	Gas produced at 24 h at STP[1] (mL gas/gDM)	Gas produced at 48 h at STP[1] (mL gas/gDM)	Time point for 50 % of total gas produced at STP[1]
	FAST			
F116	Calves 14-16-week	220.66	223.48	8 h 40 min
F117	Heifers	240.28	255.34	10 h
	SLOW			
F116	Calves 14-16-week	218.51	221.22	9 h
F117	Heifers	222.70	242.82	11 h 30 min
	FAST-FAST			
F128	Lactating cows	203.48	220.76	6 h
F129	Lactating cows	197.43	219.09	7 h 30 min
	SLOW-SLOW			
F128	Lactating cows	201.84	229.56	7 h 50 min
F129	Lactating cows	189.64	228.34	10 h 10 min

[1] *STP: Standard conditions for temperature and pressure.*

The endpoints for FAST and SLOW show a greater amount of gas produced when heifers are used as donors compared to the 14-16-week-old calves in trial F116 and F117. The T50 is reached similar for both FAST and SLOW also in regard to donor. However, for FAST-FAST and SLOW-SLOW, a greater variation for the reached T50 is seen, especially in trial F129, despite that both trials have used lactating cows as donor (Table 4.19).

Table 4.20. *The effect of different donors for the rations FAST and SLOW fermentation. Values in a column with different superscripts are significantly different (P < 0.05)[1] (standard error of the mean).*

	Donor	Hours of fermentation			
		4 h	6 h	9 h	12 h
		Total gas production at STP[2] (mL gas/gDM)			
FAST	**Calves 14-16-week**	64.79 [b] (1.19)	86.08 [b] (1.31)	112.11 [a] (1.48)	139.09 [a] (1.26)
SLOW	**Heifers**	27.01 [a] (0.46)	49.92 [a] (0.94)	97.40 [a] (2.15)	144.69 [a] (2.91)

[1] *A linear mixed model with donor and feed as main effects and trial as a random effect.*
[2] *STP: Standard conditions for temperature and pressure.*

In the fermentation kinetics different between donors, a significant difference in the amount of produced gas after 4 and 6 h of fermentation for FAST and SLOW is found when 14-16-week-old calves and heifers are used as donors. No further significant differences in the amount of produced gas for FAST and SLOW are found (Table 4.20).

The fermentation kinetics within donors for FAST and SLOW did not show any significant differences after 4 and 6 h of fermentation. The only significant differences are found at 9 and 12 h after fermentation for FAST and SLOW (Table 4.21).

Table 4.21. *The effect within donors for the rations FAST and SLOW fermentation. Values in a column with different superscripts are significantly different (P < 0.05)[1] (standard error of the mean).*

	Hours of fermentation			
	4 h	**6 h**	**9 h**	**12 h**
	Total gas production at STP[2] (mL gas/gDM)			
FAST	45.29 [a] (3.00)	69.09 [a] (2.73)	111.92 [b] (1.14)	151.62 [b] (1.72)
SLOW	41.11 [a] (2.94)	61.75 [a] (3.08)	95.49 [a] (2.53)	132.96 [a] (2.68)

[1] *A linear mixed model with feed as a main effect and trial as a random effect.*
[2] *STP: Standard conditions for temperature and pressure.*

The fermentation kinetics within donors for FAST-FAST and SLOW-SLOW showed a significant difference in the amount of produced gas at all the chosen time points (Table 4.22).

Table 4.22. *The effect within donors for the rations FAST-FAST and SLOW-SLOW fermentation. Values in a column with different superscripts are significantly different (P < 0.05)[1] (standard error of the mean).*

	Hours of fermentation					
	4 h	**6 h**	**9 h**	**12 h**	**15 h**	**18 h**
	Total gas production at STP[2] (mL gas/gDM)					
FAST -FAST	71.47 [b] (1.22)	99.78 [b] (1.36)	137.82 [b] (1.34)	161.50 [b] (1.07)	176.00 [b] (0.95)	186.34 [b] (0.90)
SLOW-SLOW	46.06 [a] (1.08)	71.82 [a] (1.85)	114.16 [a] (2.02)	141.81 [a] (1.68)	160.94 [a] (1.38)	175.84 [a] (1.18)

[1] *A linear mixed model with feed as a main effect and trial as a random effect.*
[2] *STP: Standard conditions for temperature and pressure.*

4.5 Digestible dry matter depending on different donors

For PEXP, a significant difference for dDM is found between the donors 14-16-week-old calves and heifers. No significant differences for dDM between donors are found for SBC and UFB. For SBC, a significant difference for dDM is found between 14-16-week-old calves and the other donors, however, no significant difference is found between 5-7-week-old calves and heifers. For TFB, a significant difference is found between the donors 5-7-week-old-calves and 14-16-week-old calves, where no significant differences are found between the donors for UFB (Table 4.23)

Table 4.23. *Amount of digested dry matter (DM) for the different feed components palm kernel expeller (PEXP), soybean cake (SBC), soybean meal (SBM), toasted fababeans (TFB), and untoasted fababeans (UFB). Values in a column with different superscripts are significantly different (P < 0.05)[1].*

		Digested DM (%)				
Trial no.	**Donor**	**PEXP**	**SBC**	**SBM**	**TFB**	**UFB**
F105	**Calves 5-7-week**	-	76.01 [a]	88.10 [b]	82.33 [b]	80.30 [a]
F108	**Calves 14-16-week**	38.00 [b]	68.57 [a]	66.51 [a]	70.25 [a]	71.86 [a]
F103	**Heifers**	27.04 [a]	76.92 [a]	83.83 [b]	78.50 [ab]	79.83 [a]

[1] *A linear model with feed as a main effect.*

4.6 Fiber analyses

No significant differences are found in the fiber analyses between UFB and TFB in any aspect, the same applies for FAST and SLOW (Table 4.24).

Table 4.24. *Fiber composition for two feed components and four feed rations. (standard error of the mean).*

	Mean dDM[1] (%)	**Mean NDF[2] (%/DM in raw sample)**	**Mean dNDF[3] (%/gNDF in raw sample)**	**Mean dADF[4] (%/gADF in raw sample)**	**Mean ADL[5] (%/DM)**
Untoasted fababeans	93.5 (0.01)	27.8 (0.01)	37.5 (0.00)	29.4 (0.01)	0 (0)
Toasted fababeans	90.5 (0.02)	20.8 (0.02)	35.9 (0.01)	29.6 (0.02)	0 (0)
FAST	83.5 (0.04)	27.2 (0.01)	32.7 (0.04)	38.5 (0.02)	2.9 (0.02)
SLOW	81.7 (0.08)	28.0 (0.02)	31.9 (0.07)	37.4 (0.04)	2.3 (0.01)
FAST-FAST	88.6 (0.02)	20.8 (0.00)	35.0 (0.01)	36.9 (0.00)	0.4 (0.00)
SLOW-SLOW	91.2 (0.01)	26.6 (0.02)	38.3 (0.01)	39.9 (0.01)	0.3 (0.00)

[1] *dDM: Digested dry matter*
[2] *NDF: Neutral detergent fiber*
[3] *dNDF: Digested neutral detergent fiber*
[4] *dADF: Digested acid detergent fiber*
[5] *ADL: Acid detergent lignin*

For FAST-FAST and SLOW-SLOW, no significant difference is found for dDM (Table 4.25), even though SLOW-SLOW has a marginally greater digestibility than FAST-FAST. Furthermore, SLOW-SLOW has a greater NDF percentage in the raw feed sample than FAST-FAST (Table 4.24). However, a significant difference is found between SLOW-SLOW and FAST-FAST for digested NDF (P = 0.02) and digested ADF (P = 0.01) (Table 4.25).

Table 4.25. *P-values for the results of the fiber analysis between two feed components and four feed rations.*

	P-value[1]		
	dDM[2]	**dNDF[3]**	**dADF[4]**
Untoasted fababeans/Toasted fababeans	0.23	0.14	0.91
FAST/SLOW	0.85	0.93	0.82
FAST-FAST/SLOW-SLOW	0.31	0.02	0.01

[1] *A two- way homoscedastic Student's t-test*
[2] *dDM*: *Digested dry matter*
[3] *dNDF: Digested neutral detergent fiber*
[4] *dADF*: *Digested acid detergent fiber*

4.7 Feeding trials

4.7.1 Feeding trial with the feed rations FAST and SLOW

Throughout the trial period of 8 weeks, both the animals fed SLOW and FAST have a TW gain of 411 kg. However, there is a greater variation for TW for the animals fed FAST compared to the animals fed SLOW. The animals fed FAST weighed 68 kg more than the animals fed SLOW at the beginning of the trial (Figure 4.10). A significant difference (P = 0.01) was found in TW at trial start (week 0) between the experimental units fed FAST and the experimental units fed SLOW.

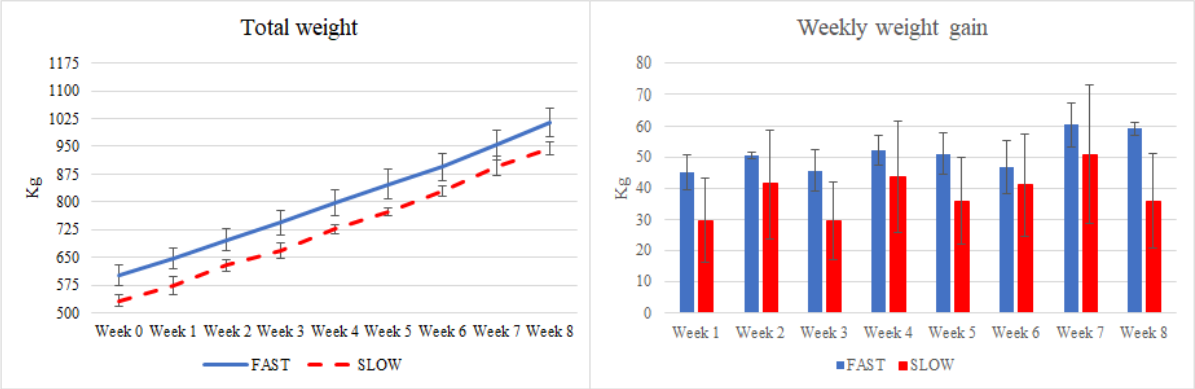


Figure 4.10. *Data from Assendrup Hovedgaard. Total weight (mean) and weekly weigh gain (mean) between each week, for the calves fed the feed rations FAST and SLOW. Bars indicate standard error of the mean.*

Every week throughout the trial period, the animals fed FAST had a greater WWG compared to the animals fed SLOW (Figure 4.10; Table 4.26). Furthermore, the variation for WWG is greater every week for the animals fed SLOW compared to the animals fed FAST, which is the opposite compared to the variation for TW (Figure 4.10).

Table 4.26. *Data from Assendrup Hovedgaard. Total weight (TW) and weekly weight gain (WWG) for the six experimental units fed the feed rations FAST and SLOW (standard error of the mean).*

Calculations (kg)		Experimental unit	Week								
			0	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
FAST	**TW**	**1**	584	619	669	711	755	793	856	903	965
		2	658	706	755	813	866	926	967	1033	1089
		3	566	619	671	708	768	823	859	928	988
	WWG	**1**	-	34	50	42	44	38	64	46	62
		2	-	48	49	58	52	61	41	67	55
		3	-	53	53	36	60	55	36	69	61
	TW (mean)	**1, 2, 3**	603 (28)	648 (29)	698 (28)	744 (35)	796 (35)	847 (40)	894 (36)	955 (40)	1014 (38)
	WWG (mean)	**1, 2, 3**	-	45 (6)	51 (1)	46 (7)	52 (5)	51 (5)	47 (9)	61 (7)	59 (2)
SLOW	**TW**	**1**	504	527	600	629	703	754	809	853	912
		2	560	610	655	694	741	787	852	940	973
		3	538	584	631	682	735	782	828	899	951
	WWG	**1**	-	22	74	28	74	51	54	45	59
		2	-	50	45	38	47	46	65	88	32
		3	-	46	46	51	54	47	46	71	52
	TW (mean)	**1, 2, 3**	534 (16)	574 (25)	629 (16)	668 (20)	726 (12)	775 (10)	830 (13)	897 (25)	945 (18)
	WWG (mean)	**1, 2, 3**	-	30 (14)	41 (18)	29 (13)	44 (18)	36 (14)	41 (16)	51 (22)	36 (15)

4.7.2 Feeding trial with the feed rations FAST-FAST and SLOW-SLOW

Throughout the trial period of 8 weeks, the animals fed FAST-FAST have a TW gain of 1889 kg compared the animals fed SLOW-SLOW which have a TW gain of 1840 kg (Table 4.27). Furthermore, the animals fed FAST-FAST have a greater variation for TW compared to the animals fed SLOW-SLOW throughout the trial period. At the beginning of the trial, the animals fed FAST-FAST weighed 31 kg more than the animals fed SLOW-SLOW. However, no significant difference (P = 0.63) was found in TW at trial start (week 0) between the experimental units fed FAST-FAST and the experimental units fed SLOW-SLOW. Lastly, in week 2, a lower WWG is observed, for the animals fed both feed rations compared to the WWG observed in week 1. However, the lowest WWG is observed for the animals fed FAST-FAST compared to the animals fed SLOW-SLOW (Figure 4.11; Table 4.27).

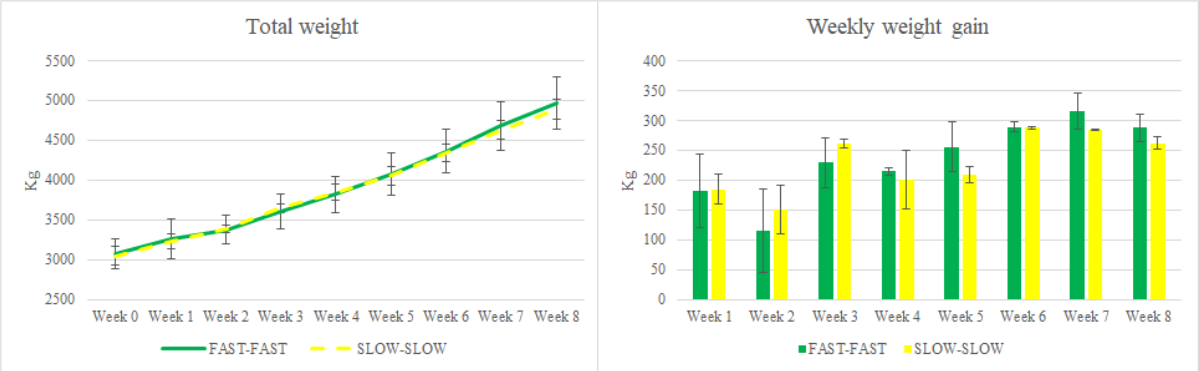


Figure 4.11. *Data from Grusgravgaard. Total weight (mean) and weekly weigh gain (mean) between each week, for the calves fed the feed rations FAST-FAST and SLOW-SLOW. Bars indicate standard error of the mean.*

The greatest WWG is observed for the animals fed SLOW-SLOW in week 1 to 3. The rest of the trial period, week 4 to 8, the greatest WWG is observed for the animals fed FAST-FAST. For the entire trial period, the greatest variation for WWG is observed for the animals fed FAST-FAST compared to the animals fed SLOW-SLOW (Figure 4.11; Table 4.27).

Table 4.27. *Data from Grusgravgaard. Total weight (TW) and weekly weight gain (WWG) for the four experimental units fed the feed rations FAST-FAST and SLOW-SLOW (standard error of the mean).*

Calculations (kg)		**Experimental unit**	**Week**								
			0	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
FAST - FAST	**TW**	**1**	3264	3507	3552	3822	4043	4341	4638	4983	5293
		2	2892	3012	3197	3385	3594	3808	4089	4375	4640
	WWG	**1**	-	243	45	270	221	298	297	345	310
		2	-	120	185	188	209	214	281	286	265
	TW (mean)	**1, 2**	3078 (186)	3250 (248)	3375 (178)	3604 (219)	3819 (225)	4075 (267)	4364 (275)	4679 (304)	4967 (327)
	WWG (mean)	**1, 2**	-	182 (62)	115 (70)	229 (41)	215 (6)	256 (42)	289 (8)	316 (30)	288 (23)
SLOW - SLOW	**TW**	**1**	3163	3322	3431	3700	3949	4172	4458	4742	5014
		2	2932	3143	3335	3589	3740	3936	4225	4510	4762
	WWG	**1**	-	159	109	269	249	223	286	284	272
		2	-	211	192	254	151	196	289	285	252
	TW (mean)	**1, 2**	3048 (116)	3233 (90)	3383 (48)	3645 (56)	3845 (105)	4054 (118)	4342 (17)	4626 (116)	4888 (126)
	WWG (mean)	**1, 2**	-	185 (26)	151 (42)	262 (8)	200 (49)	210 (14)	288 (2)	285 (1)	262 (10)

4.7.3 Total weight and gain-per-grain for the four feed rations

In relation to the TW for the animals, a significant difference ($P = 3.24e^{-0.5}$) is found between the animals fed FAST and the animals fed SLOW for the entire trial period. Furthermore, a tendency ($P = 0.06$) for TW is found between the animals fed FAST-FAST and the animals fed SLOW-SLOW, for the last six weeks of the trial period (Table 4.28).

Table 4.28. *P-values for total weight between the animals fed FAST and SLOW, along with FAST-FAST and SLOW-SLOW.*

	P-value
FAST/SLOW	$3.24e^{-0.5}$ [1]
FAST-FAST/SLOW-SLOW	0.06 [2]

[1] *A linear mixed model with feed as a main effect and group and day as random effects.*
[2] *A linear mixed model with feed as a main effect and group and farm as random effects.*

All four types of feed rations show a similar G/g ratio. SLOW has on average a greater G/g ratio compared to FAST. Similarly, SLOW-SLOW has on average a greater G/g ratio compared to FAST-FAST. Therefore, no significant difference is found between the G/g ratio for the animals fed FAST, SLOW, FAST-FAST, and SLOW-SLOW (Table 4.29).

When the GC was examined individually for all eight weeks, no significant difference was found neither between FAST and SLOW (P = 0.63) or between FAST-FAST and SLOW-SLOW (P = 0.75). Furthermore, a sensitivity analysis was conducted to investigate if a significant difference would occur if the animals fed FAST-FAST and SLOW-SLOW had consumed either 10.0 %, 12.5 %, 15.0 %, 18.5 %, 21.0 %, 23.5 %, or 26.0 % of the total GC in the first two weeks of the trial period. The sensitivity analysis showed no significant difference (P = 0.75) for the total GC between the experimental units fed FAST-FAST and SLOW-SLOW.

Table 4.29. *Gain-per-grain (G/g) ratio calculated from the total grain consumption (GC) and total weight (TW) gain from all eight weeks. (standard error of the mean).*

Experimental unit		**GC (kg)**	**GC p-value[1]**	**TW gain (kg)**	**G/g ratio**	**G/g ratio p-value[1]**	**Mean G/g**
FAST	**1**	1205	0.63	381	0.32	0.41	0.32 (0.00)
	2	1302		430	0.33		
	3	1290		423	0.33		
	Total	3797		1234	-		
SLOW	**1**	1214		408	0.34		0.33 (0.00)
	2	1262		413	0.33		
	3	1267		413	0.33		
	Total	3743		1234	-		
FAST-FAST	**1**	7165	0.77	2029	0.28	0.74	0.28 (0.00)
	2	6107		1748	0.29		
	Total	13272		3777	-		
SLOW-SLOW	**1**	7157		1851	0.26		0.30 (0.04)
	2	5344		1830	0.34		
	Total	12501		3681	-		

[1] *A two- way homoscedastic Student's t-test.*

5 Discussion

5.1 *In vitro* techniques for estimation of degradation

In ruminant nutrition, *in vitro* gas methods are applied to estimate degradation of feed components and rations. The main advantages are that the methods are inexpensive, less time consuming, and makes it possible to maintain more constant experimental conditions compared to *in vivo* trials (Makkar, 2004; Bueno *et al*., 2005).

The *in vitro* method developed by Tilley & Terry (1963) is an endpoint method as it provides an estimate for degradation based on the residues of the samples. The fermentation of a feed incubated with buffered rumen fluid will result in the production of microbial growth, H_2, CO_2, and CH_4 along with acetate, propionate, and butyrate (Makkar, 2004). The IVGPT applied in this research project records gas pressure to estimate the fermentability of potential degradable substrates. As the recordings occur automatically according to specified settings, this method could have a greater accuracy compared to methods which are manually recorded, such as The Hohenheim Gas Test where syringes are used to measure mL gas produced. Furthermore, it is important that the manual recordings are obtained rapidly to avoid a sudden drop in temperature in the incubator. In this research project, the applied IVGPT makes it possible to avoid opening the incubator during the fermentation and thereby maintain a constant temperature. In addition, The Hohenheim Gas Test has the disadvantage that it is limited by the amount of gas which can be obtained in the syringes which, in turn, depends on the amount of sample and the size of the syringes (Menke *et al*., 1979). The IVGPT applied in this research project does not have a restriction for the possible amount of gas to be measured as it releases the gas at a chosen and fixed pressure. No release of pressure can result in a negative effect on the microbial fermentation as an increased pressure can change the solubility of gases in the medium (Getachew *et al*., 1998). The IVGPT used in this research project will not have a negative feedback which could occur in the method developed by Pell & Schofield (1993).

The system presented by Adesogan *et al*. (2005) applies radio frequency signals to send the recorded information to a server which makes it possible to obtain the data at any location which has access to the internet. Furthermore, this also makes it theoretically possible to perform the incubation without supervision except in the beginning and the end of fermentation. The IVGPT applied in this research project sends absolute gas pressure at chosen intervals to a computer located in the laboratory. It is also possible to perform the IVGPT without constant supervision, only checks of batteries are needed during the fermentation.

In this research project, rumen fluid was collected from either rumen cannulated Jersey heifers, rumen cannulated Danish Red cows in lactation, or from culled calves (Table 3.3). Bueno *et al*. (2005) reports that the quality of the rumen fluid inoculum is dependent on the preparation method. Furthermore, its microbial population composition can differ between donor animals and within the liquid and solid phases of the inoculum. In the IVGPT method applied in this research project the variations are minimized as the rumen fluid used in the *in vitro* gas trials where obtained from the same heifers and lactating cows. In addition, the rumen fluid samples were collected around the same time point for each trial. For the trials with calves as donors, the animals originated from the same farm, were culled at the same slaughterhouse, and were declared free from infectious diseases. Furthermore, all rumen fluid was handled and prepared according to the same guidelines and protocol, which will minimize variation. Rymer *et al*. (2005) report that different housing and diets, under the same management, could have an impact on the individual difference in the rumen liquid for the donors. Furthermore, a difference in the rumen microbiome between two similar animals in age and physiology are reported by Li *et al*. (2012) and Jami *et al*. (2013). This could explain the differences observed on the results between the 5-7-week-old donors (e.g. Table 4.13; Table 4.15).

5.2 Variation in fermentation kinetics for different feed components

An age dependent difference was observed in the early fermentation patterns for different feed components. This occurred despite similar endpoints after 24 and 48 h of fermentation for all donors. Hansen *et al*. (2019) also report this age dependent difference in early fermentation kinetics by incubation of the same feed components, between 5-6-week-old, 10-12-week-old calves, and 1-1.5-year-old heifers as donors.

As the *in vitro* gas method used by Sembach *et al*. (2018b) and the method used in this research project are performed after the same protocol and with the same donor, it is possible to compare the cumulative gas curves for PEXP. Based on the shape of the cumulative gas curves, presented in this research project for PEXP, it is assumed that the point of inflection is not reached, as the fermentation was stopped at 24 h. This is supported by the cumulative gas curves for PEXP presented by Sembach *et al*. (2018b) as the point of inflection is reached after 24 h of fermentation.

After 24 h of fermentation, the amount of gas produced by PEXP was similar for all donors. However, in the early stages of fermentation significant differences were observed in

the amount of produced gas between the different donors (Table 4.5). A greater variation is observed in the first 24 h of fermentation between the cumulative gas curves for the feed components SBM and SBC (Figure 4.4), UFB and TFB (Table 4.5). The same variation is not observed in the amount of dDM between different donors (Table 4.23), as the dDM percentage only reflects the digestion after 24 h of fermentation whereas the cumulative gas curves show the fermentation kinetics throughout the 24 h. The variation in the *in vitro* gas trials for PEXP, SBM, SBC, UFB and TFB indicates a variation in the degradation of the same feed component by the different donors probably due to variations in the microbiota. This is supported as some significant differences were found in the amount of dDM between different donors (Table 4.23). This is further supported by Jami *et al*. (2013) who found a noticeable difference in the microbial composition between the age groups 1-3-day-, 2-month-, 6-month-, and 2-year-old. The rumen development had a significant impact on the microbial diversity of the rumen microbiome. This development is not solely influenced by the diet but also by the age of the animal. The phylum Bacteroidetes was found to be one of the most abundant in the rumen for all age groups. Furthermore, the amount of Bacteroidetes increases with an increase of age (Li *et al*., 2012; Jami *et al*., 2013). Within this phylum, the cellulolytic bacteria *Ruminococcu*s was present in the 3-day-old calves. In addition, the cellulolytic bacteria *Butyrivibrio* was also found in the 2-month-old calves. An increase in amount of both bacteria was found with an increase in age (Jami *et al*., 2013). An increase in fiber degradation with an increase in age of the calves could therefore be assumed. This is reflected in the total gas accumulation from the *in vitro* gas results. As the results generally show a greater amount of gas produced in the early hours of fermentation for the younger donors compared to the older donors, but with similar endpoints. The similar endpoints between different donors is also observed in the similar amounts of dDM (Table 4.23). However, as the percentage of dDM only show the amount digestion after 24 h of fermentation the variation in the early hours of fermentation is not reflected in these percentages. It is important to emphasize that a greater microbiome content does not result in a greater degradation. In addition, it is important to emphasize that the *in vitro* gas method does not account for the passage rate in the animal which conceivably depends on the physical size of the animals.

The NDF content of a feed component is shown to have an impact on the early fermentation patterns. The cumulative gas curves for the feed components PEXP (Figure 4.3) and SOH (Figure 4.4) are the only curves where a sigmodal shape is observed when calves are used a donor. All other curves where calves are used as donors followed a simple exponential curve. The slower early fermentation could be due to the greater content of NDF observed for

PEXP and SOH (63.0 - 64.0 %/DM) compared to UFB and TFB (20.8 - 27.8 %/DM), which both have less NDF content and greater early fermentation (Figure 4.5) when the same donors are used. This is supported by Sembach *et al*. (2018a) who reported a delayed gas production during degradation of PEXP, and SOH compared to other feed components when heifers were used as donors.

The feed ration SLOW-SLOW is the only one out of the four feed rations which contains SOH. However, the cumulative gas curves for SLOW-SLOW (Figure 4.9) do not show similar fermentation patterns with the slower early fermentation as the cumulative gas curves for SOH (Figure 4.4). It is assumed that it is due to the impact from the fermentation patterns of the other feed components which are included in the feed ration. SOH represents 15.00 % of the prepared pellets where the two primary feed components are TFB and WHT which represent 40.00 % and 36.92 % of the prepared pellets (Table 3.4). However, the altered fermentation patterns for SLOW-SLOW could also be due to donor differences as only lactating cows were used as donors in the *in vitro* gas trials. These results suggest that the age of the donor used in the *in vitro* gas trials is extremely important in determination of degradation.

The study by Jami *et al*. (2013) observed a rapid change in the bacterial communities between 1-day-old calves and 3-day-old calves. It was assumed that the compositional change was due to the change from aerobic to anaerobic environment in the rumen. A greater amount of unknown microbiome in younger calves compared to older calves is also reported (Li *et al*., 2012). A more diverse microbial composition was also shown in the rumen of 1-3-day-old calves compared to the older age groups, this could indicate a less diverse rumen microbiota in older animals compared to the rumen microbiota in younger animals (Jami *et al*., 2013). This is supported by the age dependent difference in fermentation found by Hansen *et al.* (2019). These age dependent differences are also shown in the results from the *in vitro* gas trials in this research project. For several of the feed components, when heifers and lactating cows were donors, a similar amount of produced gas is observed after 24 and 48 h of fermentation (e.g. Table 4.1; Table 4.2; Table 4.17). Based on these results it could be suggested that fermentations kinetics for different feeds might give more information about feed degradation than the *in vitro* gas endpoint values at 24 to 48 h.

5.3 Feeding trials with four feed rations

No significant difference was found for G/g ratio between the experimental units fed FAST and SLOW (P = 0.41), or between the experimental units fed FAST-FAST and SLOW-

SLOW (P = 0.74). This lack of difference could be due to variation between the experimental units fed all four feed rations (Table 3.3). However, significant differences in TW was observed for the calves fed FAST and SLOW ($P = 3.24e^{-0.5}$) along with a tendency for the calves fed FAST-FAST and SLOW-SLOW (P = 0.06). No significant differences were observed for GC between the animals fed FAST and SLOW (P = 0.63) or between FAST-FAST and SLOW-SLOW (P = 0.75). This indicates a decreased utilization by the animals fed the rations SLOW and SLOW-SLOW, compared to those fed the rations FAST and FAST-FAST. Properly, this is due to the slower fermentable feed components in the feed composition for the feed rations SLOW and SLOW-SLOW (Figure 4.9). These slow fermentable feed components and the decrease in utilization by the animals could be the reason for the lack of significant differences in the G/g ratios. This further supports the investigated hypothesis as the differences observed in the fermentation kinetics between the feed rations FAST and SLOW and between FAST-FAST and SLOW-SLOW are reflected in the weight results.

It is important to emphasize that the GC is measured for the entire trial period, and the results of TW between the experimental units fed FAST-FAST and SLOW-SLOW (P = 0.06) are calculated for week 2 to 8. To estimate a GC for the first two weeks of the trial period, an assumption was made that if the animals have had a linear GC for the entire trial period, the calves would have consumed a maximum of 25 % of the total GC, even though this is probably an overestimation of intake in the first two weeks. Therefore, a sensitivity analysis was conducted which showed no significant difference (P = 0.75) for the total GC between the experimental units fed FAST-FAST and SLOW-SLOW. The sensitivity test for GC shows that the adaption period has no influence on the calculations for G/g ratio.

In both *in vivo* feeding trials, presented in this research project, a supplement of low-quality straw was fed *ad libitum*. However, as no results for the straw consumption was obtained for the experimental units it is difficult to predict if this straw supplement had an impact on the TW for the experimental units and thereby influenced the G/g ratio. A published study emphasizes that a hay supplement increases the starter feed intake which results in a greater BW gain and feed efficiency compared to calves fed no hay (Coverdale *et al*., 2004). However, Terré *et al*. (2013) report no significant differences in weight gain between calves fed a hay supplement and those fed no hay supplement. As no clear indication can be found in the literature for the effect of a hay supplement, it is difficult to predict the effect of the hay supplement. Furthermore, Coverdale *et al*. (2004) report that the particle size of both pelleted feed and the hay supplement have an influence on the weight gain. This is supported by Porter *et al*. (2007), who observed less daily gain and less consumption for calves fed a starter feed

with large particle size compared to calves fed a starter feed with smaller particle size. Lastly, calves fed a chopped hay supplement tended to have a greater gain-to-feed ratio together with a greater DMI compared to calves fed a finely ground hay supplement. However, the particle size of the hay supplement is reported to have no influence on the BW gain (Montore *et al*., 2013). No particle size for the four pelleted feed rations was obtained, however, the straw supplement was observed to have a large particle size. As the literature is contradictive, the effect of straw and particle size are unknown. As all experimental units in both *in vivo* feeding trials had *ad libitum* access to a low-quality straw supplement with a large particle size the effect of these two factors are presumed to be equal for all experimental units.

An increase in fermentability of concentrate feed is reported to increase the passage rate along with a decreased retention time and fiber degradation (Tafaj *et al*., 2005). This suggests that calves fed FAST have an increased passage rate compared to the feed ration SLOW, as feed ration FAST contains SBP (FAST) which is more easily fermentable compared to SBP (SLOW) (Nielsen *et al*., 2017). This is supported by the cumulative gas curves presented in this research project (Figure 4.9). Despite no significant difference in GC ($P = 0.63$), between the experimental units fed FAST and SLOW, a significant difference was found in TW ($P = 3.24e^{-0.5}$). A faster fermentability for the fed rations FAST was observed in the *in vitro* gas trials as the T50 was reached earlier for FAST than for SLOW (Table 4.19). Along with the greatest difference in produced gas after 12 h of fermentation (Table 4.21). However, after 24 and 48 h of fermentation a minor variance on the amount of produced gas was observed (Table 4.19). Furthermore, no significant difference was found for the dDM ($P = 0.85$), nor were any significant differences found for digestible NDF ($P = 0.93$) or digestible ADF ($P = 0.82$) in the fiber analyses (Table 4.25). As no significant difference was found in the GC between the experimental units fed FAST and those fed SLOW, this suggest that the faster fermentability of the feed ration FAST not affects the GC. The results for FAST and SLOW further suggests a greater weight gain for animals fed a feed ration with a faster fermentable feed component compared to animals fed a feed rations with a slower fermentable feed component, which supports the hypothesis investigated in this research project.

The differences in content of NDF between the feed rations FAST-FAST (20.8 %/DM) and SLOW-SLOW (26.6 %/DM) indicate a slower passage rate for the fed ration SLOW-SLOW due to the greater level of NDF. This is supported by Poorkasegaran & Yansari (2014) who report a decrease in passage rate due to an increase in NDF content. Tafaj *et al*. (2005) report that a greater fermentability of concentrate feed increases the passage rate and decreases the retention time and fiber degradation. The feed ration FAST-FAST contains SBP (FAST)

(18 %/pellets), and the feed ration SLOW-SLOW does not contain any types of SBP. This would further increase the passage rate for the feed ration FAST-FAST as SBP (FAST) is fast fermentable (Nielsen *et al*., 2017), along with easily fermentable fibers in beet pulp (Poorkasegaran & Yansari 2014).

As seen in the results from the *in vivo* feeding trial, a tendency (P = 0.06) was present for TW between FAST-FAST and SLOW-SLOW. Both feed rations had a similar GC (P = 0.75). The lower weight gain for the experimental units fed SLOW-SLOW could be due to the greater NDF content in SLOW-SLOW (19.56 %/pellets) compared to FAST-FAST (16.73 %/pellets) as this would lower the digestibility of SLOW-SLOW. Furthermore, the *in vitro* gas trials show a faster fermentability for FAST-FAST compared to SLOW-SLOW as the T50 is reached earlier for FAST-FAST than for SLOW-SLOW (Table 4.19). The greatest differences in total gas production between donors for the rations FAST-FAST and SLOW-SLOW were observed after 9 h of fermentation (Table 4.22). However, a similar amount of gas was observed at both 24 and 48 h of fermentation (Table 4.19), and no significant difference in dDM for FAST-FAST and SLOW-SLOW (P = 0.31). As no significant difference was observed in the GC between the experimental units fed FAST-FAST and SLOW-SLOW this suggest that the faster fermentability for the feed ration FAST-FAST not affects the GC. As the results from the *in vitro* gas trials are reflected in the results from the *in vivo* feeding trial, the investigated hypothesis in the research project is supported.

The results from the fiber analyses show that SLOW-SLOW has a greater level of digested NDF (P = 0.02) and digested ADF (P = 0.01), compared to FAST-FAST, which indicate a greater digestibility of SLOW-SLOW. However, as the fiber analyses are based on a 48 h fermentation of the feed rations, this indicates that SLOW-SLOW needs a long fermentation period to reach this level of digestibility, which is also supported by the results from the *in vitro* gas trials (Figure 4.9). As the calves fed the feed ration SLOW-SLOW have a lower TW compared to the calves fed the feed ration FAST-FAST, it suggests that the calves' retention time is so low that the slow fermentable fed are passed through the rumen before fully degraded, as the greatest differences in the amount of gas produced is observed after 9 h of fermentation.

As the passage rate for the four feed rations was not examined in this research project, this factor is unknown. However, the results from the fiber analyses, the significant results from the *in vivo* feeding trials, and as the greatest differences in the amount of produced gas were observed after 9 and 12 h of fermentation in the *in vitro* gas trials, could indicate that calves have a faster passages rate than the 48 h currently used in the feed evaluation systems.

6 Conclusion

The results from the *in vitro* gas trials together, with the results from the *in vivo* feeding trials show that the difference seen in fermentation kinetics results in differences in weight gain patterns in heifer and slaughter calves.

No significant differences were observed for the grain consumption between the experimental units fed FAST and SLOW or between those fed FAST-FAST and SLOW-SLOW. This is further supported by a sensitivity analysis for the experimental units fed FAST-FAST and SLOW-SLOW. These results show that differences in fermentation kinetics do not affect the grain consumption.

The calculated gain-per-grain ratio shows no significant differences, neither between the experimental units fed FAST and SLOW nor between those fed FAST-FAST and SLOW-SLOW. This lack of significant differences for the gain-per-grain ratio and grain consumption along with the significant differences found between the total weight gains suggests that the animals fed the feed rations SLOW and SLOW-SLOW have a decreased utilization of the fed.

The results from the fiber analyses show a greater digestibility for the feed rations SLOW-SLOW compared the feed rations FAST-FAST. However, these results reflect the digestibility after 48 h of fermentation in contrast to results from the *in vitro* gas trials which show the fermentation kinetics throughout the hours for the entire fermentation. The *in vitro* gas results show the greatest difference in the amount of gas produced after 9 and 12 h of fermentation for all four feed rations. This suggest that the calves have a faster passage rate than the 48 h with a low retention time which could limit the full utilization potential of feeds. The cumulative gas curves for the feed rations FAST and FAST-FAST showed a greater amount of produced gas compared to the feed rations SLOW and SLOW-SLOW. Therefore, it is concluded that calves fed a fast fermentable feed rations will have a greater utilization of feed, which will be reflected in the greater total weight, compared to the calves fed the slower fermentable feed rations.

Finally, an age dependent differences in fermentation were observed between the donors used in the *in vitro* gas trials. These differences are presumed to be due to an age dependent variation in the rumen microbiome.

The investigated hypothesis "*The weight results from the* in vivo *feeding trials will reflect the differences in the* in vitro *gas trials*" is based on the results present in this research project accepted. However, is it important to emphasize that further examination of different feed components and rations with different fermentation kinetics in early fermentation but with

similar end points, is required to fully understand the importance and influence of this factor on weight gain in calves.

7 Future perspectives

As the *in vitro* gas trials do not account for passage rate, and as this factor are not investigated in this research project further investigation of this matter would be needed to better understand how the feed rations behaves in the gastrointestinal tract. A better understanding of passage rate for different feed components and rations could help to improve the feed composition to maximize the utilization potential of the different feed components and rations. This could decrease the amount of feed used in the production and the amount of land needed for feed production. A reduction of these factors would make the Danish agricultural sector a more sustainable production and help get closer to the goal of becoming climate neutral by 2025. Furthermore, a decreased amount of feed used in the production could further improve the profitability for the agricultural sector.

Volatile fatty acid analyses were not conducted in this research project. However, the literature research indicates that volatile fatty acid has an influence in relation to physiological development of the rumen and weight gain. In future studies the inclusion of volatile fatty acid profiles could be used as a tool for further examination of the age dependent early fermentation patterns. Furthermore, the volatile fatty acid profiles would help to understand the degradation of the feed components, based on the proportions of the different volatile fatty acids. In addition, an investigation of the rumen microbiome of the different donors, could also contribute to further understand the age dependent difference in the early fermentation of different feed components and rations.

The results in this research project suggests a need for change in the feed evaluation systems, as the results for the feed components after 48 h of fermentation does not seem to be representative for the fermentation which occurs within the animal, as the feed could have passed though the rumen before 48 h. Furthermore, the results show an age dependent difference in fermentation, which could indicate that feed evaluations for the feed components should be performed based on age and passage rate rather than 48 h. This could change the way calves should be fed in the future. However, further studies on this matter are required to support such a change in the feed evaluation systems.

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