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Rumen Development in Veal (Preruminant) Calves

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ABSTRACT. Suárez, B.J. Rumen Development in Veal (Preruminant) Calves.
Traditionally, veal calves were fed until slaughter weight with milk replacer without the provision of concentrates. However, the absence of solid feed limited the physiological development of the forestomachs leading to abnormal oral behaviors. In 1997, a new EU legislation stipulated that a minimum amount of solid feed (fiber) has to be provided for the welfare of the calves; however, no specifications were made about the type and source of feed. Two in vivo experiments were performed to examine the effects of solids feeds supply (either as concentrate or concentrates plus roughage), differing in the carbohydrate source, on growth performance, rumen development and rumen fermentation characteristics of male veal calves. Additionally, the potential interaction effect of solid feed supply with milk replacer was also investigated. Finally, techniques for estimating fermentation characteristics of different substrates, to facilitate the choice of feed ingredient to be included in a complete ration for veal calves were developed and evaluated. The first experiment showed that feeding concentrates differing in carbohydrate composition to veal calves promoted rumen development compared with calves fed milk replacer only. Variation in the carbohydrate composition of the diets led to variation in total rumen VFA concentrations and its molar proportions; however the variation caused in rumen development, was small. A high incidence of coalescing rumen papillae with embedded hair and feed particles (plaque) was observed in calves fed concentrate. The second experiment showed that roughage and intake level affects rumen fermentation and rumen development of veal calves. Leakage of milk into the rumen (21 to 35 %) was observed in all dietary treatments. Roughage addition decreased the incidence of plaque formation and the incidence of calves with poorly developed rumen mucosa. However, morphometric parameters of the rumen wall were hardly influenced by the type and level of roughage. In both experiments the rumen fermentation of calves fed solids feeds (concentrate or a concentrate/roughage combination) was characterized by low pH (4.9 – 5.3), VFA concentrations between 100 and 150 mmol/L and high concentrations of reducing sugars (between 20 to 61 g/kg DM). Furthermore, also in both experiment, the activities of the ruminal polysaccharide degrading enzyme (PDE) reflected the adaptation of the microorganisms to the diets. From the in vitro technique (gas production technique) it was concluded that the kinetics and end products of fermentation of the selected feed ingredients varied with the diet fed to calves the inocula was obtained from. Presence of substrate and inocula interactions demonstrated the importance of using inocula from calves adapted to the substrate of interest.

Key Words: Veal Calf, Feed Composition, Fermentable Carbohydrates, Rumen Fermentation, Enzyme Activity, Gas Production Technique.
In Memory of My Grandmother “Josefa”
Dedicated to those whom I care for
Table of Contents

CHAPTER 1 ............................................................................................................................. 9
  General Introduction ........................................................................................................... 9

CHAPTER 2 ........................................................................................................................... 19
  Effects of Supplementing Concentrates Differing in Carbohydrate Composition to Veal Calf Diets: I. Animal Performance and Rumen Fermentation Characteristics ................................................................. 19

CHAPTER 3 ........................................................................................................................... 45
  Effects of Supplementing Concentrates Differing in Carbohydrate Composition to Veal Calf Diets: II. Rumen Development .................................................................................................................... 45

CHAPTER 4 ........................................................................................................................... 71
  Effect of Roughage Source and Roughage:Concentrate Ratio on Animal Performance and Rumen Development in Veal Calves ........................................................................................................... 71

CHAPTER 5 ......................................................................................................................... 101
  The Importance of Using Adapted Microflora as Inoculum for Testing Fermentation Characteristics of Carbohydrate Sources for Veal Calves, Using the In Vitro Cumulative Gas Production Technique 1 ................................. 101

CHAPTER 6 ......................................................................................................................... 127
  General Discussion ............................................................................................................ 127

SUMMARY ........................................................................................................................... 155

SAMENVATTING ............................................................................................................. 161

PUBLICATIONS AND ORAL PRESENTATIONS ......................................................... 168

ACKNOWLEDGMENTS .................................................................................................... 171

ABOUT THE AUTHOR ...................................................................................................... 172

TRAINING AND SUPERVISION PLAN WIAS ............................................................. 173
CHAPTER 1

General Introduction
**VEAL PRODUCTION**

Veal production has been practiced since ancient agriculture, when fattening a calf with the excess of milk obtained from the house cow was a common practice. However, industrial veal production as it is known today has its origins in Europe, as in the late 50s large surpluses of skim milk were imported inexpensively from the American market to The Netherlands. Dutch veal producers quickly found that feeding their veal calves a diet of skim milk, whey and vegetable fat improved the growth rates of the calves and the quality of the meat. As veal quality improved, the demand for veal meat increased and nowadays in the European Union (EU), veal calf production is an important part of the beef meat sector, closely related to the dairy systems and in some countries fully integrated with the milk replacer producers.

In the EU, only five countries are responsible of nearly 90 % of the total veal production, namely France ≈ 31%, Italy ≈ 18%, The Netherlands ≈ 27%, Germany ≈ 6 % and Belgium ≈ 6 %. Furthermore, especially in The Netherlands, veal production is economically important because over 90% of its production is exported. However, in the EU, public opinion has always been influencing the agricultural policies and with regard to veal production, in the last decade the increased public concern on animal welfare has resulted in stricter regulations on veal production (Directive 97/2/EC; Directive by the Council of Europe, 1997). In the past, the provision of solid feeds (either concentrate or roughage) to veal calves was deliberately avoided by believing that this practice would have a negative effect on milk intake, feed conversion and mainly meat color and quality. But as a result of the new EU policies, since 1997 feeding of solid feeds has become mandatory for the veal industry. The reason for the new EU legislation was the potential improvement in health and well being of the calves when fed diets stimulating rumen function (Directive 97/2/EC; Directive by the Council of Europe, 1997). But only little knowledge was available on the effects of low quantities of solid feeds on animal health, rumen development, animal performance and meat quality in veal calves. Consequently, Blokhuis et al. (2001) investigated the effects of supplementing milk replacer based diets with solid feed (different sources of chopped roughage) on animal performance, behavior and health of the gastro-intestinal tract in veal calves. Results showed that small quantities of roughage contributed to improve well being of veal calves by promoting rumination, reducing the incidence of rumen hair balls and by reducing the incidence of abnormal oral behavior (Blokhuis et al., 2001). Moreover, these authors observed that, except straw, all roughage sources evaluated, stimulated to some extent the
development of the rumen. In addition, with straw as roughage source and undesirable effect was also observed as it stimulated the appearance of abomasal lesions (ulcers). Although this EU project yielded valuable information on these issues, it did not identify the type of roughage that was optimal for all aspects concerning to rumen development in veal calves. Moreover, more information was needed to design an optimal strategy of supplementation of a milk replacer based diet with a solid feed (i.e. distribution of solid feed intake throughout time). In addition, quantitative information on the contribution of the solid feeds (either concentrate or roughage) to the calves nutrient supply was still absent.

RUMEN DEVELOPMENT AS INFLUENCED BY NUTRITION

Rumen development (pre-ruminant or transition phase)

At birth the rumen of the calf is non-functional. Papillary growth, rumen wall muscularization and vascularization are minimal and the reticulorumen volume is small (Tamate et al., 1962). This transition phase (pre-ruminant) of the calf is critical, where the development of the rumen is characterized by the increase in the size of the organ (mostly rumen wall development) and the development of the rumen mucosa (papillae development) (see figure 1). Papillae and to a lesser extent rumen wall development can be influenced by feeding management.

In ruminant nutrition (especially in dairy cattle production), a smooth transition of the young calf from the stage of pre-ruminant to ruminant, reducing its nutritional dependence on milk and at the same time minimizing loss in growth, is always desired. Hence, to efficiently use concentrates and roughages, an adequate development of the rumen is necessary. Consequently, it is important to know and understand the factors responsible of initiating the cellular growth and maturation of the rumen tissues and how they can be influenced by the composition of the diet (concentrates and roughages). Moreover, specifically for veal calves, being raised at high intake levels of milk replacer, it is also important to identify areas of potential interactions between the milk replacer and rumen development.
Chapter 1

SOLIDS FEEDS, PHYSICAL STRUCTURE OF THE DIET AND RUMEN DEVELOPMENT

The intake of solid feed, unlike milk replacer, stimulates rumen microbial proliferation and volatile fatty acids (VFA) production, which have been shown to trigger rumen development (Church, 1988). Flatt et al. (1958) introduced sodium salts of the most important VFA (butyrate, propionate, acetate) directly into the rumen, monitoring rumen epithelial development. These authors found that sodium butyrate had the greatest influence on rumen epithelial development, followed by sodium propionate and sodium acetate. This order is the same in which those organic acids are known to be metabolized by the rumen epithelium (Bergman, 1990). Moreover, Menschel et al. (2001), clarified the effects of the different VFA on papillae growth. These authors demonstrated that the increased stimulatory effects of butyrate and propionate on the papillae length were explained by an increased mitotic rate. Butyrate and propionate led the rumen mucosa to a mitotic rate twice as high as in the control group. Differences between butyrate and propionate were explained by their different effects on apoptotic rates of epithelial cells which were only one third for butyrate compared to propionate. In conclusion, butyrate is a specific inhibitor of ruminal apoptosis in vivo.

It has well been established that concentration of VFA in rumen fluid can be influenced by the composition and intake of concentrates and roughages (Van Soest, 1994), and therefore may be used to manipulate rumen development. Nocek et al., (1984), showed that young calves fed with concentrates had increased rumen development when compared to roughage sources. These authors observed that rumen tissues were heavier and ruminal epithelium had more mucosa to muscle compared to the rumen tissues of calves fed ground or chopped hay. However, feeding a high proportion of concentrates may cause a shift in the rumen microbial population, increase butyrate and propionate production instead of acetate, with a concomitant production of stronger acids (lactate) and consequently decreasing pH. On the other hand, high intakes of roughages, increases ruminal pH, in dairy nutrition an important tool to balance dietary strategies when aiming for high rates of VFA production, while at the same time attempting to prevent ruminal acidosis (Mertens, 1997). In addition, when formulating rations for dairy cattle, differences in the amount and physical properties of the fiber can affect the diet utilization and animal performance. When too much fiber is included in the ration, intake and energy supply and hence milk production, will decrease. On the other hand, when the dietary fiber (total amount of NDF) or the
physical characteristics are not adequate, ruminal fermentation, animal metabolism and health can be negatively affected. In this area of research, numerous studies have shown the importance of an optimal dietary roughage to concentrate ratio (R: C) on animal productivity (Miller and O’Dell, 1975; Weiss and Shockey, 1991). Similarly, in rearing calves, the development and functioning of the rumen can also be modified by changing the R: C ratio of the diet or in pelleted diets by changing the physical structure of the feed (grinding or not the dietary ingredients). Large particle size and increased bulk of roughage sources increase physical stimulation of the rumen wall, rumen motility, muscularization, and volume (Vazquez-Anon et al., 1993; Zitnan et al., 1998). Coarsely or moderately ground concentrate diets have been shown to increase rumen volume and muscularization more than finely ground or pelleted concentrate diets, indicating that extent of processing and/or concentrate particle size affects the ability of concentrates to stimulate rumen capacity and muscularization (Beharka et al., 1998).

While much is known related to rumen development in rearing calves and the basics have been published in the literature, in veal production several areas of rumen development require additional study. The full understanding of the physiological changes that occur during rumen development and obtaining relevant information about digestion kinetics in veal calves can be future topics of research in this area.
Figure 1. Development stages in rumen epithelium. (With permission of Penn State University and Jud Heinrichs (Professor of Dairy and Animal Science; ajh@psu.edu). A) Exterior and interior appearance of a 4 week-old calf rumen. The calf was fed a diet of milk only. B) Exterior and interior appearance of a 8 week-old calf rumen. The calf was fed a diet of milk and then grain. The left side photo shows the excellent papillae development and healthy dark coloration associated with a proper feeding of the calves. C) Exterior and interior appearance of a 12 week-old calf rumen. The calf was fed a diet of milk, hay and grain. At this age the rumen should be developed in size, but also the interior papillae should be properly developed as those in the left side photo. The dark coloration is caused by an increase in tissue (mucosa) and vascularization.


**THESIS OBJECTIVES**

- To establish effects of two-phase feeding aimed to stimulate early rumen development to 1) optimize nutrient utilization from rumen fermentation and 2) prevent health problems in the lower gastrointestinal tract (e.g. ulcers in abomasum)

- To investigate these effects in veal calves because of potential interactions with milk replacer

- To develop techniques for estimating fermentation characteristics of different substrates, to facilitate the choice of feed ingredient to be included in a complete ration for veal calves.

**OUTLINE OF THE THESIS**

Figure 2 illustrates the outline of this thesis. To reach the objectives mentioned above, two experiments were conducted with veal calves from 2 to 14 weeks of age. In *Chapter 2*, the effects of feeding concentrates differing in carbohydrate source on growth performance and rumen fermentation characteristics of veal calves until 8 or 12 weeks of age is investigated. Effects of these dietary treatments on rumen development are described in *Chapter 3*. In addition, selected metabolites are evaluated as predictors of rumen development. Following the conclusions of this experiment, it was decided to conduct an experiment to evaluate the importance of roughage intake to obtain a desired rumen development in young veal calves. In this experiment, various roughage sources were provided at various intake levels in addition to a commercial milk replacer, subsequently measuring animal performance, rumen fermentation processes and development of the rumen wall. The results of this experiment are described in *Chapter 4*. In *Chapter 5*, fermentation characteristics of the main substrates in the diets used in experiments described in Chapters 1, 2 and 3, were determined by using the gas production technique (GPT). For this evaluation, inocula were obtained from rumen contents of calves either adapted or not adapted to the substrate in their diet. Finally, in *Chapter 6* the results of the experiments reported in chapters 2-4 and some additional data are integrated and discussed. In addition, selected new data are presented. Originally, results of one last experiment of this project, taking the veal calves fed various concentrates and roughages to slaughter weight, were also planned to be included in this thesis. The results of this experiment would have allowed conclusions on the objective of the thesis whether or not veal calves >12 weeks of age can benefit
from early rumen development. Unfortunately, results of this experiment only became available too late to be included in this thesis, but is currently being reported (Van Reenen et al., 2006).

Figure 2. A schematic representation of the outline of the thesis
REFERENCES


CHAPTER 2


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Chapter 2

ABSTRACT

The aim of this experiment was to examine the effects of concentrates feed, differing carbohydrate source, on growth performance and rumen fermentation characteristics of veal calves. For this purpose, 160 Holstein Friesian x Dutch Friesian crossbred male calves, were used in a complete randomized block design with a 5x2 factorial arrangement. Dietary treatments consisted of: 1) milk replacer control (CONTROL), 2) pectin-based concentrate (PECTIN), 3) neutral detergent fiber (NDF) based concentrate, 4) starch-based concentrate (STARCH) and 5) mixed concentrate (MIXED) (equal amounts of concentrates of treatments 2, 3, and 4). Concentrate diets were provided as pellets in addition to a commercial milk replacer. Calves were euthanized either at the end of 8 or 12 weeks of age. The overall dry matter intake (DMI) of the concentrate diets varied between 0.37 and 0.52 kg/d. Among concentrates diets the DMI was significantly lower in STARCH based diet (0.37 kg DM/d) and the difference between the NDF and PECTIN diet was also significant. The average daily gain (ADG) for all the dietary treatments varied between 0.70 and 0.78 kg/d. The MIXED and NDF fed calves had an increased ADG (0.78 and 0.77 kg/d, respectively) than STARCH and PECTIN fed calves (0.70 and 0.71 kg/d, respectively). In general the rumen fermentation in the calves fed concentrates was characterized by a low pH (4.9 – 5.2), VFA concentrations between 100 and 121 mmol/L and high concentrations of reducing sugars (33-66 g/kg DM). VFA concentrations of calves fed concentrates were higher than those of the CONTROL calves. All concentrate treatments showed a low acetate/propionate ratio in rumen fluid (between 1.3 and 1.9). Among concentrates, the NDF diet had the highest (55.5 %) and STARCH the lowest (45.5%) molar proportions of acetate. Calves fed MIXED, PECTIN and STARCH diets had significantly higher butyrate molar proportions (13.1 to 15.8 %) than NDF and CONTROL fed groups (9.9 and 9.6 %, respectively). Calves fed the CONTROL diet had higher lactate concentration (21 mmol/L) than concentrate fed calves (between 5 and 11 mmol/L). With the exception of the NDF diet, polysaccharides degrading enzymes (PDE) activities in rumen contents generally showed an adaptation of the microorganisms to the carbohydrate source in the diet. The MIXED diet exhibited the least variation in rumen PDE among the enzymes systems tested. Results indicate that the carbohydrate source can influence intake, growth rate and rumen fermentation in young veal calves.

Key Words: Veal Calf, Concentrate Feed Composition, Rumen Fermentation, Enzyme Activity.
INTRODUCTION

Traditionally, veal calves were fed until slaughter weight with milk replacer without the provision of concentrates. However, the absence of solid feed limits the physiological development of the forestomachs. Furthermore, provision of solid feed has reduced abnormal oral behaviors (Van Putten, 1982; Veissier et al., 1998). In 1997, a new EU legislation stipulated that a minimum amount of solid feed (fiber) has to be provided for the welfare of the calves; however, no specifications were made about the type and source of feed.

Rumen development is triggered by the production of VFA resulting from fermentation of OM in the rumen (Flatt et al., 1958). Butyrate, and to a lesser extent propionate stimulate the development of the rumen mucosa; mostly because of their use as energy sources by the rumen epithelium (Sander et al., 1959; Tamate et al., 1962). Since the early 70's extensive research has evaluated different protein and carbohydrate sources in calf starters. To date, only a few experiments have been conducted evaluating the effect of concentrate supplementation on performance and rumen development in veal calves. Concentrate feed, as opposed to roughages, are provided to young calves to obtain high DMI and subsequent rapid VFA production, promoting a fast papillae growth and rumen development. However, when compared with rearing calves, concentrates DMI in veal calves is hampered by high intakes of milk replacer. In addition, milk replacer intake may influence the fermentation process in the rumen. Consequently, the present study was conducted to evaluate the effects of concentrate intake, differing in carbohydrates composition in addition to a milk replacer, on performance and rumen fermentation characteristics in veal calves. In a companion paper, the effects of these diets on morphological rumen development are presented (Suárez et al., 2006).

MATERIALS AND METHODS

The present experiment was conducted at the experimental farm of Wageningen University & Research Centre, ID Lelystad; The Netherlands, complying with the Dutch law on experimental animals.

Calves and Diets Transport and Slaughter Procedure

One hundred and sixty male Holstein Friesian x Dutch Friesian calves, averaging 44.9 kg BW (SE= 2.8 kg) were purchased in two batches of 80 animals. On arrival, calves were weighed and
based on their BW assigned to one of eight blocks. Within blocks and batches, calves were randomly assigned to one of five dietary treatments and one of two slaughter ages. The dietary treatments included a milk fed control and four treatments in which a concentrate, varying in carbohydrate composition, was fed on top of the milk replacer: 1) Milk replacer (CONTROL); 2) Pectin-based concentrate (PECTIN); 3) Neutral detergent fiber-based concentrate (NDF); 4) Starch-based concentrate (STARCH); 5) Mixed- concentrate (MIXED: equal amounts of concentrates of treatments 2, 3, and 4). The detailed ingredient and nutrient composition of the concentrates are presented in Table 1. Animals were individually housed in open metal boxes (0.9 m²) with wooden slatted floor, without bedding material. Boxes were located in two stables, where the environmental temperature was maintained at least at 15ºC. In treatments 2 to 5, concentrates were provided as pellets, up to a maximum of 750 g DM/d. Milk replacer and concentrates were supplied in separate buckets and meals were supplied in equal amounts twice daily (0730 and 1830 h). For the pelleting process, special care was taken to minimize particle size reduction of the ingredients. Therefore, corn was only broken and barley was rolled prior to inclusion; soybean hulls (beans de-hulled after toasting) and corn grits were not further ground before pelleting. Sugar beet pulp (without molasses) was ground through a 2.75 mm screen on a hammer mill prior to pelleting. Average pellet was size 15 mm long and 6 mm in diameter.

The amount of milk replacer provided and hence consumed by the calves (g /calf/d) is shown in Table 2 and its nutrient and ingredient composition is presented in Table 3. For the CONTROL treatment, milk replacer was reconstituted in a ratio of ≈ 130 g/L of water and provided in two equal meals at 39º C. For treatments with additional solid feed (treatments 2 to 5), the amount of milk replacer was reduced to stimulate the intake of solid feed (see Table 2) and reconstituted in the same volume of water than CONTROL group. Calves were weighed every 4 wk and once a week, samples of milk replacer and concentrates were collected and pooled by month for further analysis.

Animals were sacrificed at the end of either an 8- or 12-wk feeding period, by a dose application of T61 (Embutramide, Intervet International, Germany). The slaughter activity took place in the laboratory of pathology of the research centre, located 5 min from the experimental farm. Before slaughter calves received only milk replacer and were euthanized within a range of 1 to 3 h after feeding. The sequence of slaughter was properly balanced across treatments. The reticulo-rumen (hereafter rumen) and the abomasum were tied at the end of the esophagus and
pylorus, respectively and removed. The rumen was dissected along the dorsal line, emptied and weighed. The rumen contents were weighed and representative samples of the contents were taken. A full description of the slaughter procedure is provided in the companion paper (Suárez et al., 2006). Samples of rumen contents were immediately cooled on ice and stored at -20°C until analysis. After centrifugation of the rumen contents (1000g for 10 min) pH was measured in the supernatant and a 5 ml aliquot was acidified with 1 ml of 5 % ortho-phosphoric acid and stored at -20°C until further analysis.

**Analytical Procedures**

Dry matter was determined by drying at 103 °C to a constant weight (ISO 6496) and ash by combustion at 550 °C (ISO 5948). Nitrogen was determined with the Kjeldahl method with CuSO₄ as the catalyst (ISO 5983). Total starch was analyzed by an enzymatic method according to Brunt (1993). Reducing sugars were extracted from the samples using 40% ethanol and subsequent hydrolysis in a weak acid environment. Protein was precipitated using a Carrez I and II solution. After the hydrolysis, copper (II) was reduced by oxidation of the monosaccharides to Copper (I). Copper I forms a yellow complex with neocuproïne (2,9-dimethyl-1,10-fenantroline hydrochloride). This complex can be measured in a spectrophotometer at 460 nm.

The NDF analysis was based on Van Soest et al. (1991) but including, after the neutral detergent treatment incubation, an enzyme cocktail containing termamyl (Novo Nordisk, Copenhagen, DK), amylase (Sigma 6814, Sigma-Aldrich, St. Louis, MO, USA) and protease (Novo Nordisk, Copenhagen, DK) in phosphate buffer (pH 7.0) to remove starch and proteins from the residue. The ADF analysis involved two steps. First, the sample was treated according to the described NDF method. In the second step, the neutral detergent residue was treated with the acid detergents as described by Van Soest (1973). The acid detergent lignin (ADL) analysis was based on the treatment of the sample with acid detergent followed by incubation with 72% sulphuric acid.

Volatile fatty acids concentrations and lactic acid were analyzed using HPLC with a Merck polyspher OA 51272 column as stationary phase (Merck & Co., Inc. Whitehouse Station, NJ) and 0.0025 M sulphuric acid as mobile phase (elution fluid). Detection was performed by using a refractive index detector. Calibration and quantification was done by using an external standard
solution. Ammonia concentrations were estimated according to the modified method of Berthelot (Schneider, 1976).

Isolation of rumen enzymes and subsequent measurement of their activities were performed as follows. Whole rumen contents of 8 of the 16 calves per treatment, slaughtered at 12 weeks of age, were used. The intra and extra-cellular microbial enzymes and those enzymes attached to rumen particles were extracted from whole rumen contents. A combination of methods was applied to maximize extraction of enzymes from the homogenized whole rumen contents, obtained at slaughter: freezing/thawing, sonication and osmotic shock. After thawing, samples were kept on ice and sonified (Branson Sonifier 250, Branson Ultrasonics Corporation, Danbury, USA), using a flat tip (1/2" diameter) during 2 min (at 40 % of duty cycle) in a 50 mM NaAc buffer (pH 5) including 2 M NaCl and 0.01% NaN3. Subsequently feed particles and lysed bacteria were removed (centrifugation: 22.5 min, 20000g, 4 °C) and the obtained enzyme cocktail dialyzed (dialysis tubing - size 5 inf. diam. 24/32’’-19.0 mm: 30 M aprox. 12-14000 Daltons Medicell International, Liverpool, UK) against a 50 mM NaAc buffer (pH 5.0, 18h; 4 °C) to remove dissolved sugars and NaCl. Changes in volume during dialysis were recorded. Enzyme activity was determined by the release of reducing sugars after 60 min incubation of 0.5 ml of the enzyme cocktail with 0.5 ml of 120 mM Sodium Acetate (NaAc) buffer (pH 5.0) and 0.5 ml of each of four substrate suspensions in an eppendorf mixer at 39 °C. The reaction was stopped heating at 100°C for 5 min. The substrate suspensions (sugar beet pulp, sugar beet pectin, soybean hulls and native corn starch, 2 % wt.vol¹) provided excess substrate for the incubations and were prepared in demineralised water containing 0.01% NaN3, and kept overnight at 39°C in a water bath before use. Incubates, substrate blanks and enzyme extract blanks were analyzed for reducing sugar end-groups according to Somogyi (1952). Enzyme activity was expressed as mmol reducing sugars (RS) released per minute per g of DM in the rumen.

Statistical Analysis

Data for dry matter intake, average daily gain and blood and rumen parameters were analyzed as a complete randomized block design, in a 5x2 factorial arrangement, with diets and length of the experimental period (i.e. 8 or 12 weeks of age at slaughter) as main factors. Continuous data were subjected to ANOVA, according to model [1]:

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¹ wt.vol = weight/volume
[1] \[ y = \mu + \text{Batch}_i + \text{Diet}_j + \text{Period}_k + (\text{Diets} \times \text{Period})_{jk} + \varepsilon_{ijkl} \]

Where:

\( y \) = Dependent variable;

\( \mu \) = Average experimental value;

\( \text{Batch}_i \) = Effect of batch \( i \); \( i = 1 \) of 2 (two batches of 80 calves);

\( \text{Diet}_j \) = Effect of dietary treatment \( j \); \( j = \text{CONTROL, PECTIN, NDF, STARCH, MIXED} \);

\( \text{Period}_k \) = Effect of period (age at slaughter) \( k \); \( k = 8 \) or 12 weeks;

\( (\text{Diet} \times \text{Period})_{jk} \) = Effect of interaction between diet and period;

\( \varepsilon_{ijkl} \) = Error term; \( l = 1, \ldots, 160 \).

Main effects and the interaction between diets and time of the experiment (age at slaughter) were evaluated using the F test, followed by post hoc pairwise comparisons (Fisher LSD method) when the F value showed significance \( (P < 0.05) \). Rumen parameters were also subjected to analysis of covariance. The average DMI during the last week prior to slaughter, expressed for each calf as the deviation from the overall mean, was included as a co-variable in model [1]. For calves in the \text{CONTROL} treatment, the co-variable was equal to 0. The statistical analyses were carried out using Genstat (Genstat Committee, 2000). The rumen enzyme activities were analyzed as a split-plot design using a GLM procedure of SAS (SAS Institute, 2002) according to model [2]:

[2] \[ y = \mu + \text{Diet}_i + (\text{Diet} \times \text{Calf})_{ij} + \text{Substrate}_k + (\text{Diet} \times \text{Substrate})_{ik} + \varepsilon_{ijkl} \]

\( y \) = Dependent variable;

\( \mu \) = Average experimental value;

\( \text{Diet}_i \) = Effect of dietary treatment \( i \), \( i = \text{CONTROL, PECTIN, NDF, STARCH, MIXED} \);

\( \text{Calf}_j \) = Effect of calf \( j \), \( j = 1, 2, 3, \ldots, 40 \);

\( \text{Substrate}_k \) = Effect of substrate \( k \), \( k = \text{soybean hulls, native corn starch, sugar beet pectin, sugar beet pulp} \);

\( \varepsilon_{ijkl} \) = Error term
Diet was considered as the main plot factor and substrate as the subplot. The term diet x calf was used as an error term to test the effect of the diet. Other effects were tested against the residual error term. The Tukey test was used to test for all pair wise comparisons among means when the F value was significant ($P < 0.05$).

**RESULTS**

**Feed Intake and Body Weight Gain**

All calves remained healthy throughout the experiment. Table 4 shows the effects of experimental treatments on concentrate DMI and on average daily gain. The averaged DMI of the concentrate diets varied between 0.37 and 0.52 kg DM/d. Calves receiving the STARCH based diet showed the lowest DMI (0.37 kg DM/d) throughout the trial. The DMI of the NDF diet was higher than that of the PECTIN diet ($P < 0.05$). A diet effect ($P < 0.001$) and diet by period interaction ($P < 0.01$) was observed at the last week of the experiment. Firstly, for calves in the last week of the 8 week period, the DMI of the STARCH diet was the lowest (0.40 kg DM/d) among the concentrate treatments. Calves fed the PECTIN diet had a lower DMI (0.71 kg DM/d) than those fed the MIXED diet (0.71 kg DM/d). Secondly, in the last week of the 12 week period, the diet by period interaction was due to a common decrease on DMI for the concentrate diets but a rise for the STARCH based diet.

The average daily gain for all the dietary treatments varied between 0.70 and 0.78 kg/d, being affected by diet and period ($P < 0.001$). The daily body weight gain of the calves fed the NDF (0.77 kg/d) and MIXED (0.78 kg/d) diets was higher ($P < 0.05$) than for those fed either PECTIN (0.71 kg/d) or STARCH diets (0.70 kg/d). Due to the different amounts of milk replacer supplied to the calves fed concentrated diets (Table 2), a direct comparison of the feed conversion of those treatments with the milk fed-only calves cannot be done. The analysis of the growth performance is presented in the discussion section.

**Rumen Fermentation Characteristics**

Table 5 shows the results of the analysis performed in the samples of rumen contents. The pH of rumen liquid varied between 4.9 and 5.6 and was affected by diet and period ($P < 0.001$). Also, an interaction between diet and period was observed ($P < 0.05$). This interaction was caused by the rise in pH of the CONTROL treatment from 5.2 to 5.6 from week 8 to 12.
respectively, while remaining constant in the calves fed concentrates. An exception was the STARCH based diet, for which was 4.9 at the end of the 8 week period and increased to 5.2 at the end of week 12.

The total VFA concentrations were affected by dietary treatments \( (P < 0.001) \), but not by period nor by the interactions between treatment and period. All concentrate treatments showed higher total VFA concentrations in rumen fluid (100 to 121 mmol/L) compared with CONTROL (36 mmol/L). In addition, the total VFA concentration on the STARCH based diet was lower (100 mmol/L; \( P < 0.05 \)) when compared with the other concentrate based treatments. The calves fed the NDF diet had the highest molar proportion of acetate (55.5%) among concentrates and STARCH the lowest (45.5%). Furthermore, STARCH fed calves showed the highest molar proportions of propionate (33.9%). Finally, the butyrate molar proportion was significantly higher for calves fed MIXED, PECTIN and STARCH diets (13.1 to 15.8%) compared with the NDF and CONTROL fed groups (9.9 and 9.6%, \( P < 0.01 \)). The molar proportion of isobutyrate in the rumen liquid was affected by an interaction between diet and period \( (P < 0.05) \). This interaction was caused by the rise in the molar proportions of isobutyrate of the CONTROL treatment from 0.3 to 1.3%, while a decrease in the molar proportion was observed in the calves fed concentrates from week 8 to 12 respectively, with the exception of the PECTIN based diet. With respect to the molar proportions of valerate and methyl-butyr ate in rumen liquid, no differences were found between the concentrate diets, but they were higher than CONTROL \( (P < 0.05) \). Moreover, for both acids a period effect was observed with decreased molar proportions for valerate \( (P < 0.05) \) and increased molar proportions for methyl-butyrate \( (P < 0.001) \) at 12 weeks respectively. Lactate concentrations in rumen fluid were significantly different between treatments \( (P < 0.05) \). CONTROL fed animals had elevated lactate concentration (21 mmol/L) when compared with concentrate fed animals (ranging between 5 and 11 mmol/L). Also, lactate concentration in rumen fluid of the calves fed the PECTIN diet was twice as high as in those fed the MIXED concentrate \( (P < 0.05) \). The amount of reducing sugars in rumen content varied between 45 and 24 g/kg DM. Control fed calves showed higher amount of reducing sugars than calves fed concentrate diets \( (P < 0.05) \).

The concentration of ammonia in rumen liquid varied between 14 and 21 mmol/L and no significant differences between the treatments were observed \( (P > 0.05) \). The ammonia
concentration in rumen fluid was significantly \((P < 0.001)\) higher at the end of the 12 weeks period (23 mmol/L) than at the end of the 8 weeks period (13 mmol/L).

Analysis of covariance revealed that variation in DMI did not significantly explain variation in total VFA or its molar proportions. For rumen fluid pH and concentrations of lactate and NH\(_3\), the inclusion of DMI as a co-variable appeared significant (estimates of regression coefficients \pm S.E. for DMI: 0.4\pm 0.1, -13.3 \pm 5.9, and -15.2 \pm 6.3, respectively; \(P < 0.05\)). Following analysis of covariance, differences in the rumen lactate concentrations between the CONTROL, the PECTIN and MIXED based diets remained as presented. The difference between the PECTIN and MIXED based diet, however, was decreased (predicted means 10 vs. 6 mmol/L, \(P = 0.20\)) after analysis of covariance. The effects on ammonia concentrations did not change. Likewise, for rumen pH, inclusion of the co-variable increased the \(P\) value for the interaction between diet and period (from \(P = 0.03\) to \(P = 0.06\)).

**Enzyme Activity in Whole Rumen Contents**

Table 6 shows the effects of experimental treatments on the polysaccharide degrading enzyme activity (PDE) extracted from rumen contents. Diet and substrate effects and a diet x substrate interaction \((P < 0.001)\) were observed. When averaged over substrates, PDE activities in the rumen content of calves fed concentrate diets varied between 3.9 and 6.0 mmol.min\(^{-1}\).g DM\(^{-1}\). Calves fed the PECTIN diet showed higher PDE than for those fed either STARCH or NDF diet (6.0 vs. 4.6 and 3.9 mmol.min\(^{-1}\).g DM\(^{-1}\)) respectively. In addition, the PDE activity of CONTROL fed calves was small (< 1 mmol.min\(^{-1}\).g DM\(^{-1}\)). When averaged over treatments, PDE activity ranged from 3.4 to 4.8 mmol.min\(^{-1}\).g DM\(^{-1}\), decreasing in the order native corn starch, sugar beet pectin, sugar beet pulp and soybean hulls as substrates (4.8, 4.5, 3.7 and 3.4 mmol.min\(^{-1}\).g DM\(^{-1}\), \(P < 0.05\)). Generally, the results in Table 6 illustrate an increased enzyme activity for substrates that were present in the diet, e.g. increased pectin and sugar beet pulp degrading (pectinases) activity at the PECTIN diet and increased STARCH degrading (amylase) activity at the STARCH diet. However, the NDF diet was an exception to this rule, as it showed higher pectin degrading activity (pectinase) when compared with degradation of soybean hulls (SBHase) \((P < 0.05)\). In addition, the highest soy bean hulls degrading (SBHase) activity was observed in calves fed the PECTIN, not the NDF diet. The MIXED diet induced fairly high activities of all enzymes tested and exhibited the least variation among treatments.
DISCUSSION

In rearing calves, intake of milk replacer is an important factor influencing DMI (Huber et al., 1984). In the present study, DMI of concentrate diets varied between 0.37 and 0.52 kg/d (Table 4) and calves did not consume the whole concentrate fed portion (maximized at 0.75 kg DM/d). The absence of increased DMI from concentrate with age was likely due to the increased supply of milk replacer at the end of the trial (Table 2). The increase of the STARCH diet with age was an exception (causing an interaction between treatment and period, Table 2). The low intake of the STARCH diet in the first period may have been related to the low ruminal pH observed, as acidosis generally depresses DM intake (Kellaway et al., 1977; Nocek, 1997; Owens et al., 1998).

Calves fed additional concentrates showed an ADG (700 to 780 g/d) close to the ADG of veal calves (Table 4). The higher intake of milk replacer in the CONTROL treatment, however, hampers a direct comparison of data performance (and efficiency) across treatments. To facilitate this comparison, growth rates on both levels of milk replacer intake were simulated using the veal calves growth model developed by Gerrits et al. (1997). Extra growth due to intake of concentrates could be calculated based on the simulated body weight gain on the low intake of milk replacer (Table 7). The simulated daily gain values on the high milk scheme were in line with the observed values (691 vs. 681 and 841 vs. 864 g/d at 8 and 12 weeks, respectively). Extra daily gain per kg of concentrate ranged from 0.82 to 1.04 kg and from 0.56 to 0.62 kg at 8 and 12 weeks of trial period, respectively. At 8 weeks, calves fed the STARCH diet showed the highest extra growth, whereas at 12 weeks calves fed STARCH and MIXED diets showed the highest extra growth. Compared with the 8 week period, at the 12 week period the extra growth achieved by all the concentrate treatments may be related with the decline in rate and growth efficiency accompanying increase in body weight present in farm animals.

The rumen pH observed in our experiment was quite low when compared with some studies with rearing calves. However, measurements of ruminal pH in some early studies with young calves may have been inaccurate due to the way of sampling (e.g. stomach tube) or housing conditions (e.g. straw bedding) (Murdock and Wallenius, 1980; Wheeler et al., 1980). Our results were more in line with later studies conducted by Anderson et al. (1987a,b), Beharka et al. (1998) and Lesmeister and Heinrichs (2004) in which rumen cannulated calves were used. These researchers observed a rumen pH of 5.4 to 5.6 in calves between 3 and 8 weeks of age (sampled 3-6 h after feeding) increasing above 5.6 to 5.8 as the calves reached 10 to 12 weeks of age.
Among the concentrates, calves fed the starch diet revealed a particular low rumen pH (4.9) at 8 weeks of age. At 12 weeks of age, the rumen pH was slightly higher than at 8 weeks, in particular for the starch diet. This rise in pH might indicate an increased absorption of VFA as consequence of a more developed and mature rumen.

The VFA concentrations on concentrate diets were close to the range normally observed in adult ruminants (120 to 160 mmol/L; Bergman, 1990). Similar results were obtained in rearing calves (Quigley et al., 1992a, b; Abdelgadir et al., 1996; Beharka et al., 1998; Lesmeister and Heinrichs, 2004). As expected, the rumen VFA concentrations observed in calves fed the concentrate diets were higher (100 to 121 mmol/L) than those observed in CONTROL fed calves (36 mmol/L). The VFA concentration found in the rumen of CONTROL fed calves suggests the presence of milk into the rumen.

In agreement with expectations (Van Soest, 1994), the NDF based diet led to high molar proportions of acetate, the STARCH based diet led to high molar proportions of propionate and the PECTIN diet led to high molar proportions of butyrate. At a low pH, micro-organisms may shift fermentation pathways and increased proportions of propionate and lactate occur (review Dijkstra, 1994). Indeed, in the present experiment pH values of rumen fluid were low and molar proportions of propionate high (> 27 %).

Hristov et al. (2001) and Ghorbani et al. (2002) reported that in feedlot cattle perceived to have sub-clinical acidosis the lactate concentration in ruminal fluid rarely exceeds 10 mmol/L. Lactate concentrations observed in the present experiment (5 to 11 mmol/L), are higher than results reported in calves by Anderson et al. (1987a,b) Abdelgadir et al. (1996) and Beharka et al. (1998), but lower than those reported by Quigley et al. (1992c), ranging from 13 to 25 mmol/L (D (-) plus L (+) Lactate). Furthermore, the concentration of lactate present in the CONTROL fed diet was the highest (> 20 mmol/L). This information together with the high values of ammonia in rumen fluid (21 mmol/L) indicates fermentation of milk replacer in the rumen. The occurrence of ruminal drinking has been well documented (Toullec and Guilloteau, 1989; Dos Santos et al., 1986) but has rarely been quantified in experiments in which concentrates are fed. Therefore, the extent to which ruminal drinking contributes to the fermentation processes observed in this experiment is unknown.

Branched-chain volatile fatty acids (BCVFA) are mainly end products of protein fermentation and are considered a growth factor for fiber degrading microorganisms in the rumen.
Effects of Supplementing Concentrates Differing in Carbohydrate…

(Yang, 2002). Anderson et al. (1987a) showed that in rearing calves the molar proportions of isobutyrate declined with age as feed intake increased, indicating an increased cellulolytic activity as calves aged. They also reported that the molar proportion of valerate increased until 12 weeks of age, and then remaining constant thereafter. In the present trial, for the concentrate fed treatments, the molar proportions of iso-butryate ($P < 0.001$) and valerate ($P < 0.05$) decreased with age, but the ammonia concentration increased. Higher molar proportions of BCVFA were expected in the milk diet, in line with the observed high ammonia concentrations.

Enzyme Activities and Soluble Sugars in Rumen Content

Assessing the activity of the PDE of the microorganisms present in the rumen is a useful approach to obtain quantitative estimates of substrate degradation the rumen.

Provided that representative samples of whole rumen content are taken and that intra- and extra cellular enzymes are completely extracted and their activity is well preserved, the relationship between substrate degradation in vitro and their degradation in vivo should be good. Silva et al. (1987) reported a high correlation ($r = 0.98$) for in sacco DM degradation and cellulolytic (CMCase) activity after 24 hours of rumen incubation.

In contrast to other in vitro techniques regularly used to characterize substrate degradation in the rumen (e.g. Tilley and Terry, cumulative gas production technique) the enzyme assay does not have the disadvantage of isolation of the microflora and its adaptation to the incubation medium and substrate. Nevertheless, inter-assay variation of the analysis of enzyme is large, and procedural standardization among research groups applying this technique is lacking. Furthermore, the selection of substrates, as well as their pre-treatment (unpublished observations) is crucial when determining PDE activities. Between treatment variation, however, remains estimable using this technique.

The results from the present experiment illustrate adaptation of the rumen microflora to the diet. The effects of diet on PDE activities (high amylase and low SBH-ase) are in line with those reported for high grain / low roughage based diets in dairy cattle (Huhtanen and Khalili, 1992; Martin and Michalet-Doreau, 1995; Hristov et al., 1999). However the increased hydrolysis of SBH of the calves on the PECTIN treatments, compared with the NDF treatment, is difficult to explain.
Most techniques studying substrate degradation (e.g. enzyme assay) or substrate disappearance (e.g. the in sacco technique) assume that the end products of hydrolysis are rapidly utilized by the microbes (Dhanoa et al., 1999), resulting in concentrations of free RS in rumen fluid close to or below the detection level. An exception to these low levels are the high levels observed in rumen fluid immediately after a meal rich in easily degradable carbohydrates, since the supply of sugars may temporarily exceed the microbial capacity to utilize these sugars (Dijkstra et al., 2002). However, the high concentrations of RS in rumen contents found in the present experiment, suggest that the assumption of rapid utilization may not always be true.

In the present research, no big differences were observed in RS concentrations among treatments. Moreover, the concentration of RS varied between 45 and 61 g/kg DM, whereas in dairy cows reported concentrations ranged from 0.02 to 2.57 mmol/L of rumen fluid (approximately 0.04 and 5.55 g/kg DM, assuming an average of 120 g DM/kg in the rumen contents) (Hristov et al., 2000; Hristov et al., 2001; Hristov and Roop, 2003). The relatively high concentration of reducing sugars is likely related to relatively low bacterial growth rates while maintaining high rates of substrate hydrolysis. A reduced growth rate of bacteria may be related to the low rumen pH observed in this experiment. A low pH is known to increase the energy expenditure of rumen bacteria to maintain their intracellular pH (Russell and Wilson, 1996) and decrease the uptake of soluble carbohydrates by ruminal microorganisms (see e.g. Chow and Russell, 1992; Martin and Wani 2000; Moore and Martin, 1991; Martin, 1996). Maintaining a high rate of substrate hydrolysis could be related to the (extracellular) enzymes maintaining their activity while present in the rumen. This effect may be enlarged by low rumen motility, often observed in ruminants with low ruminal pH (Dirksen, 1989, cited by Nocek, 1997). Interestingly, it has been demonstrated that the PDE are still active far below the optimum pH at which the rumen bacteria produce them. For example, at pH 5.0, ruminal extracted cellulases still retained ≈ 65 % of their maximum activity (pH = 6) (Morgavi et al., 2000); whereas the activity of cellulolytic bacteria decreases rapidly at a pH below pH 5.6 (Russell and Wilson, 1996).
CONCLUSION

This trial illustrates that the carbohydrate source included in the concentrate feed affects intake, growth and parameters of the rumen fermentation in young veal calves. Concentrate intake was decreased when animals were fed with concentrate based on STARCH. Calves fed concentrates based on NDF had significantly higher DMI than those fed the PECTIN based concentrate. Although numerically higher, the concentrate DMI of NDF fed calves did not differ from MIXED fed calves. Accordingly, calves fed the MIXED and NDF concentrate based diets showed higher ADG than STARCH and PECTIN fed calves respectively. In general, the rumen metabolism of the calves fed concentrates was characterized by a low pH (4.9 – 5.2), VFA concentrations between 100 and 121 mmol/L, low acetate / propionate ratio and high concentrations of reducing sugars. The last observation may suggest an impaired growth of the microorganisms. With the exception of the NDF diet, results of the PDE activities showed adaptation of rumen microorganisms to the diet. The MIXED based diet showed the smallest variation in rumen PDE activity. Animal performance on all experimental treatments was good, despite the low rumen pH and very particular type of rumen fermentation observed on some of the treatments.
# Table 1. Ingredient and analyzed nutrient composition of the concentrates.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>PECTIN</th>
<th>NDF</th>
<th>STARCH</th>
<th>MIXED</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beet pulp (ground) (%)</td>
<td>4-00-672</td>
<td>91.3</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Soybean Hulls (%)</td>
<td>1-04-560</td>
<td>-</td>
<td>46.4</td>
<td>-</td>
</tr>
<tr>
<td>Corn Grits (%)</td>
<td>4-03-011</td>
<td>-</td>
<td>46.4</td>
<td>-</td>
</tr>
<tr>
<td>Corn (%)</td>
<td>4-20-689</td>
<td>-</td>
<td>-</td>
<td>46.4</td>
</tr>
<tr>
<td>Barley (crushed) (%)</td>
<td>4-00-549</td>
<td>-</td>
<td>-</td>
<td>46.4</td>
</tr>
<tr>
<td>Soybean Oil (%)</td>
<td>na</td>
<td>1.5</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Solpro 500 (%)</td>
<td>na</td>
<td>4.0</td>
<td>4.0</td>
<td>4.0</td>
</tr>
<tr>
<td>Urea (%)</td>
<td>na</td>
<td>1.2</td>
<td>1.2</td>
<td>1.2</td>
</tr>
<tr>
<td>Vinasse (%)</td>
<td>na</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
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</table>

<table>
<thead>
<tr>
<th>Analyzed nutrient composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry Matter (g/kg product)</td>
</tr>
<tr>
<td>Crude Protein (g/kg DM)</td>
</tr>
<tr>
<td>Crude Fat (g/kg DM)</td>
</tr>
<tr>
<td>Crude Ash (g/kg DM)</td>
</tr>
<tr>
<td>Starch (g/kg DM)</td>
</tr>
<tr>
<td>Sugars (Glucose g/kg DM)</td>
</tr>
<tr>
<td>NDF (g/kg DM)</td>
</tr>
<tr>
<td>ADF (g/kg DM)</td>
</tr>
<tr>
<td>ADL (g/kg DM)</td>
</tr>
<tr>
<td>Ca (g/kg DM)</td>
</tr>
<tr>
<td>P (g/kg DM)</td>
</tr>
<tr>
<td>Na (g/kg DM)</td>
</tr>
<tr>
<td>K (g/kg DM)</td>
</tr>
<tr>
<td>Mg (g/kg DM)</td>
</tr>
<tr>
<td>Fe (mg/kg DM)</td>
</tr>
</tbody>
</table>

IFN*: International Feed Number (obtained from NRC 1989). Na: Not available
Table 2. Milk replacer supplied and consumed throughout the experiment (g/calf/d)\(^*\).

<table>
<thead>
<tr>
<th>Scheme</th>
<th>Weeks of Trial</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Control</td>
<td>380</td>
</tr>
<tr>
<td>Concentrate</td>
<td>360</td>
</tr>
</tbody>
</table>

\(^*\) Since calves always consumed the whole portion of the supplied milk replacer.

Table 3. Ingredient and analyzed nutrient composition of milk replacer. (in product)

<table>
<thead>
<tr>
<th>Ingredient composition</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Whey protein concentration (%)</td>
<td>50</td>
</tr>
<tr>
<td>Whey powder (%)</td>
<td>27</td>
</tr>
<tr>
<td>Oil and Fat (%)(^2)</td>
<td>17</td>
</tr>
<tr>
<td>De-Lactosed Whey Powder (%)</td>
<td>5</td>
</tr>
<tr>
<td>Vitamin-mineral mix(^1) (%)</td>
<td>1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Nutrient Composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrogen (g/kg)</td>
</tr>
<tr>
<td>Crude Fat (g/kg)</td>
</tr>
<tr>
<td>Ash (g/kg)</td>
</tr>
<tr>
<td>Moisture (g/kg)</td>
</tr>
<tr>
<td>Ca (g/kg)</td>
</tr>
<tr>
<td>P (g/kg)</td>
</tr>
<tr>
<td>Na (g/kg)</td>
</tr>
<tr>
<td>K (g/kg)</td>
</tr>
<tr>
<td>Mg (g/kg)</td>
</tr>
</tbody>
</table>

\(^1\) Provided per kg of milk replacer (vitamin A: 25000 IU; vitamin D3: 2000 IU; vitamin E: 80 mg; vitamin C: 80 mg; Ca: 7.8 g; P: 6.5 mg; Mg: 1.4 g; Zn: 40 mg; Cu: 10 mg; Mn: 30 mg; Se: 0.15 mg; Fe: 33 mg).

\(^2\) Coconut oil and partly hydrogenated soybean oil.
Table 4. Effects of period (age at slaughter) and diets (supplemented concentrates differing in carbohydrate source) on concentrate dry matter intake and average daily gain.

| Variable                              | Dietary Treatments |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |
|---------------------------------------|--------------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
|                                      | CONTROL            | PECTIN| NDF   | STARCH| MIXED | SEM   | D     | P     | D x P | 8 weeks | 12 weeks | SEM |
| Dry Matter Intake (kg DM/d) \(^1\)   | -                  | 0.45b | 0.52a | 0.37c | 0.50ab| 0.024 | ***   | NS    | NS    | 0.44    | 0.48    | 0.017 |
| Dry Matter Intake in Last Week (kg DM/d) \(^1\) | -                  | 0.59b | 0.70ab| 0.40c | 0.71a | 0.042 |       |       | ***   | NS    | **     |
| 8 weeks                              |                    |       |       |       |       |       |       |       |       | 0.44    | 0.48    |       |
| 12 weeks                             |                    | 0.42b | 0.59a | 0.54a | 0.62a | 0.042 |       |       |       | 0.67    | 0.82    | 0.009 |
| Average Daily Gain (kg/d)            | 0.77a              | 0.71b | 0.77a | 0.70b | 0.78a | 0.015 | ***   | ***   | NS    | 0.67    | 0.82    |       |

\(^a,b,c\) Means in the same row with different superscript differ significantly (\(P < 0.05\)).

D= Diet; P= Period (age at slaughter); D x P = Interaction.

\(^1\) Excluding DMI from Milk.

* \(P \leq 0.05\).

** \(P \leq 0.01\).

*** \(P \leq 0.001\).
Table 5. Effects of period (age at slaughter) and diets (supplemented concentrates differing in carbohydrate source) on rumen fermentation characteristics

<table>
<thead>
<tr>
<th>Variable</th>
<th>Dietary Treatments</th>
<th>8 weeks</th>
<th>12 weeks</th>
<th>8 weeks</th>
<th>12 weeks</th>
<th>SEM</th>
<th>D</th>
<th>P</th>
<th>D x P</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>***</td>
<td>***</td>
<td>*</td>
</tr>
<tr>
<td>8 weeks</td>
<td>5.2\textsuperscript{a}</td>
<td>5.1\textsuperscript{a}</td>
<td>5.1\textsuperscript{a}</td>
<td>4.9\textsuperscript{b}</td>
<td>5.2\textsuperscript{a}</td>
<td>0.07</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>12 weeks</td>
<td>5.6\textsuperscript{a}</td>
<td>5.2\textsuperscript{b}</td>
<td>5.1\textsuperscript{b}</td>
<td>5.2\textsuperscript{b}</td>
<td>5.2\textsuperscript{b}</td>
<td>0.07</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Total VFA, mmol/L</td>
<td>36\textsuperscript{c}</td>
<td>114\textsuperscript{a}</td>
<td>121\textsuperscript{b}</td>
<td>100\textsuperscript{b}</td>
<td>120\textsuperscript{a}</td>
<td>4.9</td>
<td>***</td>
<td>NS</td>
<td>NS</td>
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<tr>
<td>Acetate %</td>
<td>59.1\textsuperscript{a}</td>
<td>52.0\textsuperscript{bc}</td>
<td>55.5\textsuperscript{ab}</td>
<td>45.5\textsuperscript{c}</td>
<td>49.0\textsuperscript{bc}</td>
<td>1.6</td>
<td>***</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Propionate %</td>
<td>25.8\textsuperscript{b}</td>
<td>27.6\textsuperscript{ab}</td>
<td>28.5\textsuperscript{ab}</td>
<td>33.9\textsuperscript{b}</td>
<td>27.6\textsuperscript{ab}</td>
<td>1.3</td>
<td>***</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Butyrate %</td>
<td>9.6\textsuperscript{b}</td>
<td>14.5\textsuperscript{a}</td>
<td>9.9\textsuperscript{b}</td>
<td>13.1\textsuperscript{a}</td>
<td>15.8\textsuperscript{a}</td>
<td>1.4</td>
<td>**</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Isobutyrate %</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8 weeks</td>
<td>0.3\textsuperscript{c}</td>
<td>0.6\textsuperscript{bc}</td>
<td>1.2\textsuperscript{ab}</td>
<td>1.7\textsuperscript{a}</td>
<td>1.4\textsuperscript{ab}</td>
<td>0.2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12 weeks</td>
<td>1.3\textsuperscript{a}</td>
<td>0.6\textsuperscript{b}</td>
<td>0.6\textsuperscript{b}</td>
<td>0.9\textsuperscript{b}</td>
<td>0.9\textsuperscript{b}</td>
<td>0.2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Valerate %</td>
<td>2.6\textsuperscript{b}</td>
<td>4.4\textsuperscript{a}</td>
<td>4.2\textsuperscript{a}</td>
<td>5.0\textsuperscript{a}</td>
<td>5.2\textsuperscript{a}</td>
<td>0.3</td>
<td>***</td>
<td>*</td>
<td>NS</td>
</tr>
<tr>
<td>Methyl butyrate %</td>
<td>1.7\textsuperscript{a}</td>
<td>0.7\textsuperscript{b}</td>
<td>0.6\textsuperscript{b}</td>
<td>0.9\textsuperscript{b}</td>
<td>0.9\textsuperscript{b}</td>
<td>0.1</td>
<td>***</td>
<td>***</td>
<td>NS</td>
</tr>
<tr>
<td>Lactate, mmol/L</td>
<td>21\textsuperscript{a}</td>
<td>11\textsuperscript{b}</td>
<td>6\textsuperscript{bc}</td>
<td>8\textsuperscript{bc}</td>
<td>5\textsuperscript{c}</td>
<td>2.0</td>
<td>*</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Ammonia, mmol/L</td>
<td>21</td>
<td>17</td>
<td>14</td>
<td>17</td>
<td>19</td>
<td>2.2</td>
<td>NS</td>
<td>***</td>
<td>NS</td>
</tr>
<tr>
<td>Reducing sugars, g/kg</td>
<td>124\textsuperscript{a}</td>
<td>61\textsuperscript{b}</td>
<td>45\textsuperscript{b}</td>
<td>59\textsuperscript{b}</td>
<td>45\textsuperscript{b}</td>
<td>18</td>
<td>*</td>
<td>§</td>
<td>§</td>
</tr>
<tr>
<td>Weight of fresh rumen contents, kg</td>
<td>3.6\textsuperscript{d}</td>
<td>6.7\textsuperscript{ab}</td>
<td>7.4\textsuperscript{a}</td>
<td>5.3\textsuperscript{c}</td>
<td>6.3\textsuperscript{bc}</td>
<td>0.3</td>
<td>***</td>
<td>***</td>
<td>NS</td>
</tr>
<tr>
<td>DM of fresh rumen contents, g/kg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8 weeks</td>
<td>79\textsuperscript{c}</td>
<td>99\textsuperscript{bc}</td>
<td>161\textsuperscript{a}</td>
<td>106\textsuperscript{b}</td>
<td>118\textsuperscript{b}</td>
<td>8.1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12 weeks</td>
<td>74\textsuperscript{c}</td>
<td>111\textsuperscript{b}</td>
<td>163\textsuperscript{a}</td>
<td>144\textsuperscript{b}</td>
<td>152\textsuperscript{b}</td>
<td>7.0</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 6. Effect of dietary treatment and substrate on enzymes activities (mmol.min\(^{-1}\).g DM\(^{-1}\)) of whole rumen content.

<table>
<thead>
<tr>
<th>Diet</th>
<th>Corn Native Starch</th>
<th>Sugar Beet Pulp</th>
<th>Soybean Hulls</th>
<th>Sugar Beet Pectin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.6(^{aw})</td>
<td>0.7(^{aw})</td>
<td>0.4(^{ay})</td>
<td>0.4(^{aw})</td>
</tr>
<tr>
<td>Pectin</td>
<td>4.4(^{abxy})</td>
<td>6.2(^{abz})</td>
<td>5.0(^{bz})</td>
<td>8.4(^{az})</td>
</tr>
<tr>
<td>NDF</td>
<td>2.5(^{bw})</td>
<td>3.6(^{abxy})</td>
<td>4.0(^{bza})</td>
<td>6.0(^{by})</td>
</tr>
<tr>
<td>Starch</td>
<td>9.9(^{az})</td>
<td>2.8(^{abw})</td>
<td>3.5(^{bza})</td>
<td>2.5(^{bzw})</td>
</tr>
<tr>
<td>Mixed</td>
<td>6.8(^{ay})</td>
<td>5.5(^{abzy})</td>
<td>4.2(^{bz})</td>
<td>5.3(^{bys})</td>
</tr>
<tr>
<td>SEM</td>
<td>0.47</td>
<td>0.47</td>
<td>0.47</td>
<td>0.47</td>
</tr>
</tbody>
</table>

\(^{a,b,c}\) Means in the same row with different superscript differ significantly \((P < 0.05)\).
\(^{w,x,y,z}\) Means in the same column with different superscript differ significantly \((P < 0.05)\).
\(^{***}\) \(P \leq 0.001\).
Table 7. Intake and simulated and observed daily gain on milk only or milk plus concentrate diets¹

<table>
<thead>
<tr>
<th>Variable</th>
<th>Dietary Treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MILK</td>
</tr>
<tr>
<td></td>
<td>High scheme, observed</td>
</tr>
<tr>
<td>Average daily gain (g/d)</td>
<td>691</td>
</tr>
<tr>
<td>Average concentrate intake (g/d)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>459</td>
</tr>
<tr>
<td>Cumulative milk powder intake (kg)</td>
<td>55.9</td>
</tr>
<tr>
<td>Extra growth per kg concentrate² (kg/kg)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.82</td>
</tr>
</tbody>
</table>

Week 12

| Average daily gain (g/d)                      | 841  | 864  | 539  | 778    | 840  | 808    | 847   |
| Average concentrate intake (kg)               |      |      |      |        |      |        |       |
|                                                 | 429  | 542  | 436  | 502    |      |        |       |
| Cumulative milk powder intake (kg)             | 105.7| 105.7| 77.1 | 75.9   | 75.9 | 75.9   | 75.9  |
| Extra growth per kg concentrate² (kg/kg)       |      |      |      |        |      |        |       |
|                                                 | 0.56 | 0.56 | 0.62 | 0.61   |      |        |       |

¹ Milk schemes presented in Table 2; diet composition and DMI in Table 1 and 4 respectively. Simulation carried out with model described by Gerrits et al. 1997.

² Calculated as (average growth – average simulated growth from the milk scheme)/average concentrate intake.
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CHAPTER 3

Effects of Supplementing Concentrates Differing in Carbohydrate Composition to Veal Calf Diets: II. Rumen Development

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ABSTRACT

The objective of this experiment was to examine the effects of concentrates feed, differing in carbohydrate source, on rumen development of veal calves. For this purpose, 160 male Holstein Friesian x Dutch Friesian crossbred calves were used in a complete randomized block design with a 5x2 factorial arrangement. Dietary treatments consisted of: 1) milk replacer control (CONTROL), 2) pectin-based concentrate (PECTIN), 3) neutral detergent fiber (NDF) based concentrate, 4) starch-based concentrate (STARCH) and 5) mixed concentrate (MIXED) (equal amounts of concentrates of treatments 2, 3, and 4). Concentrate diets were provided as pellets in addition to a commercial milk replacer. Calves were euthanized either at the end of 8 or 12 weeks of age. Plasma acetate and β-OH-butyrate (BHBA) were measured as indicators of rumen development. Empty rumen weight was determined, and wall samples were taken at slaughter. In most calves, a poorly developed rumen mucosa was observed. Coalescing rumen papillae with embedded hair, feed particles and cell debris were found in all calves fed concentrate diets. Calves fed concentrates had significantly heavier rumens than calves fed CONTROL. In the dorsal location of the rumen, calves fed concentrate diets showed an increased ratio of mucosa to serosa length (RMSL) than calves fed CONTROL, while in the ventral location only calves fed the PECTIN and MIXED diets showed larger RMSL. Mucosa thickness (MCT) and muscle thickness (MST) were bigger in the ventral and in the dorsal locations of the rumen, respectively. In both locations, the NDF diet resulted numerically in the lowest MCT and highest MSC amongst concentrate treatments. At 8 weeks, calves fed concentrate diets had higher plasma acetate concentrations than calves on the CONTROL treatment. However, at 12 weeks, only NDF fed calves showed significantly higher plasma acetate concentrations. The plasma BHBA concentration of calves at 8 weeks of age fed the PECTIN and MIXED diets was higher than those of the CONTROL fed calves. At 12 weeks, no differences in BHBA concentrations were observed among treatments. Results from a principal component analysis indicated that in addition to rumen volatile fatty acids (VFA) concentrations, other factors are likely to affect rumen development, and that the relationships between rumen development and individual types of VFA present in the rumen liquor are similar. Also, variation in rumen development coincides with variation in plasma acetate and BHBA concentrations.

Key Words: Veal Calf, Concentrate Feed Composition, Rumen Development and Rumen Development Predictors.
INTRODUCTION

The gastrointestinal tract of the newborn calf undergoes several anatomical and physiological changes during development, until the rumen is fully functional as in the adult ruminant. It has been demonstrated that the type of diet offered affects the rate of development of the ruminant forestomachs (Brownlee, 1956). In diets for rearing calves, forages are usually a minor proportion of the diet due to the low DMI and low rates of fermentation, whereas cereals in concentrate feed are widely used (Nocek and Kesler, 1980). Feeding calves with concentrate diets should result in high concentrations of VFA in the rumen, which are necessary to stimulate a fast rumen development (Sander et al., 1959; Tamate et al., 1962).

Carbohydrates are the main source of energy for rumen microorganisms, and feeding diets differing in carbohydrate composition may result in different patterns of rumen fermentation, subsequently resulting in different VFA profiles (e.g. Bannink et al., 2006). These differences, in turn, may differentially affect rumen development.

In 1997 a new legislation for the feeding of veal calves was set. Details of this new legislation were described in a companion paper (Suárez et al., 2006). Based on earlier research (Blokhuis et al., 2000) it was hypothesized that stimulating early rumen development in veal calves would be beneficial to their subsequent performance and health. In rearing calves, information on rumen fermentation of different sources of dietary carbohydrates is relatively well documented (see e.g. Maiga et al., 1994; Davis and Drackley, 1998; Lesmeister and Heinrichs, 2004) but little is known about their effects in veal calves. Mechanisms of rumen development are hypothesized to be similar to those in rearing calves, although the typical situation in veal calves (low DMI due to high intakes of milk replacer and potential direct interference of the milk replacer in the rumen) may complicate its predictability. Recent work of Dell’ Orto et al. (2002) even suggests a reduced rumen development following supplementation of a veal calf diet with concentrates or sugar beet pulp, compared with milk replacer alone. The objective of this study was to gain insight into the effects of age and concentrate supplementation, differing in carbohydrates composition, on rumen development in young veal calves. In addition, this study evaluated some selected metabolites as predictors of rumen development. Animal performance and parameters of rumen fermentation are presented in a companion paper (Suárez et al., 2006).
MATERIALS AND METHODS

Animals, feeding regimes, diets and general management have already been described (Suárez et al., 2006) and are summarized below. The ethical committee of the Dutch institute of animal health and welfare approved the experimental protocol.

One hundred and sixty Holstein Friesian x Dutch Friesian male calves, averaging 44.9 kg live weight (SE= 2.8 kg) were used in a complete randomized block design with a 5x2 factorial arrangement, with type of concentrate and age as the experimental factors. Dietary treatments consisted of: 1) milk replacer control (CONTROL), 2) pectin-based concentrate (PECTIN), 3) neutral detergent fiber (NDF) based concentrate, 4) starch-based concentrate (STARCH) and 5) mixed concentrate (MIXED) (equal amounts of concentrates of treatments 2, 3, and 4). Concentrate diets were provided as pellets, up to a maximum of 750 g of dry matter per day (DM/d) on top of a commercial milk replacer. Calves were individually housed in open metal boxes (0.9 m²). Concentrates contained either beet pulp (PECTIN diet), soybean hulls plus corn grits (NDF diet) or corn plus barley grits (STARCH diet) as the main source of pectin, neutral detergent fiber and starch, respectively. Milk replacer and concentrates were supplied in separate buckets and meals provided in equal amounts twice daily (0730 and 1830 h). At 8 or 12 weeks of the experimental period, animals were euthanatized by a T61 injection (Embutramide, Intervet International, Germany) and immediately after the slaughter the digestive tract was removed. The forestomachs and the abomasum were tied at the end of the esophagus and pylorus respectively, immediately removed and the weight of the reticulo-rumen was recorded with and without its contents. The rumen was dissected along the dorsal line and the mucosal surface was visually examined and qualitatively assessed according to the presence and density of rumen papillae as follows: 1= poor (few number of papillae or short papillae) and 2 = good (numerous papillae or long papillae). The vast majority of the calves showed focal or multi-focal patches of foci with coalescing and adhering papillae covered by a sticky mass of feed, hair and cell debris, which will be referred to in this paper as “plaque”. The presence or absence of plaque formation was visually assessed. For morphometrical analysis, one tissue specimen (ca. 2 x 2 cm) was taken from the saccus ruminis dorsalis (dorsal location) and one from the saccus ruminis ventralis cranial of the pilae ruminis (ventral location), attached to small dissection plates and fixed in 4% formaline. Each of the tissue specimens was embedded in paraffine wax and four tissue cross-sections were prepared with a distance of at least 100 µm and stained with hematoxylin-eosin. Because the rumen mucosa showed...
irregular shaped, branched and clumped papillae, it was impossible to consistently and properly measure the length and shape of individual papillae. Consequently, to avoid any bias of the observer due to non-random selection of suitable papillae, it was decided to measure the following rumen wall parameters within each cross-section: a) ratio of mucosa length to serosa length (RMSL); b) mucosa thickness (MCT); c) muscle thickness (MST). The latter two were scored at three randomly chosen sites within each cross-section. For each calf within each tissue specimen, RMSL, MCT and MST were calculated as the average for all 4 cross-sections. The morphometric analysis was performed at a magnification of 2.5 times (Olympus microscope) by using an image analysis software program (Image Pro Plus (Media Cybernetics)).

Blood samples were taken from the jugular vein at 8 and 12 weeks of age, between 3 and 5 hours after the milk feeding in heparinized vacutainers (Lithium Heparin tubes). Plasma was stored at -20 °C until analysis. Acetate in plasma was determined by ion exchange chromatography, by using Ion Pac ICE-AS1 (Dionex System) as column, Heptafluoricbutyric acid as eluent and suppressed conductivity as detection method. BHBA was determined by using an enzymatic method (3-hydroxybutyrate dehydrogenase). BHBA is transformed to acetoacetate by the dehydrogenase enzyme in the presence of NAD. During this reaction, NAD is transformed to NADH. The increase of the amount of NADH is measured at 340 nm and is proportional to the amount of BHBA. Quantification was done by using a chemical standard solution.

Statistical Analysis

Empty rumen weight, rumen wall and plasma parameters were analyzed as a complete randomized block design, in a 5x2 factorial arrangement, with diets and length of the experimental period (i.e. 8 or 12 weeks of age at slaughter) as main factors. Analysis of variance for the continuous data was performed according to the following model [1]:

\[ y = \mu + \text{Batch } i + \text{Diet } j + \text{Period } k + (\text{Diet } \times \text{Period}) jk + \varepsilon \ ijkl \]  

[1]

Where:
y = Dependent variable;
\( \mu \) = Average experimental value;
Batch \( _i \) = Effect of batch \( _i \); \( i = 1 \) or 2 (two batches of 80 calves)
Diet \( j \) = Effect of dietary treatment \( j \); \( j \) = CONTROL, PECTIN, NDF, STARCH, MIXED;
Period \( k \) = Effect of period (age at slaughter) \( k \); \( k \) = 8 or 12 weeks;
(Diet x Period) \( jk \) = Effect of interaction between diet and period;
\( \epsilon \) ijkl = Error term; \( l = 1, .. , 160 \).

Main effects and the interaction between Diet and Period (age at slaughter) were evaluated using the F test. For analysis of fractions (percentages) and binary variables (0-1) a logistic regression model was used with the same main effects described earlier. Inferences for fractions and binary variables were based on likelihood ratio tests. Dispersion parameters were estimated from Pearson’s generalized test (McCullagh and Nelder, 1989). Pair-wise comparisons were carried out using the Fisher LSD method (for fractions and binary variables on a logistic scale). Binary variables which were unevenly distributed over the sample groups (for example with only values 0 in one or more treatments) were analyzed parameter-free using the Fisher exact test.

In addition to the analysis described above, rumen weight and morphometric variables of rumen wall were analyzed by analysis of covariance. The average concentrate intake during the experimental period, expressed for each calf as the deviation from the overall mean, was included as a co-variable in the statistical model. Acetate and BHBA concentrations in plasma were also analyzed by analysis of covariance. For these variables the average concentrate intake in the last week before slaughter, likewise expressed for each calf as the deviation from the overall mean, was included in the model as co-variable. Significance was determined at \( P < 0.05 \) unless indicated otherwise.

**Principal Component Analysis.** Principal component analysis (PCA) was used to examine patterns of intercorrelations between multiple measures (Jolliffe, 1986), including the morphometric parameters of rumen development, pH, VFA and phosphorus in urine. Principal components produced by PCA are linear combinations of the original measures, reflecting independent characteristics (or dimensions) of the underlying correlation matrix. The loading of each measure on a principal component represents the correlation between the latent characteristic and the original measure, and thus indicates the importance of a measure for a principal component. Measures with high loadings on the same principal component of the same sign are positively, and of opposite sign are negatively correlated. The first component explains most of the variance (expressed in terms of
the first eigenvalue); the second component explains most of the remaining variation, and so forth. Measures were scaled prior to PCA, i.e., the analysis was performed on the Pearson correlation matrix. The first two principal components with eigenvalues equal to or larger than 1 were retained for further consideration.

In the present study, the different measures included in a PCA were all hypothesized to be associated with the level of rumen development (see below). PCA would confirm this hypothesis if it would produce at least one component, putatively reflecting (mechanisms underlying) rumen development with high loadings from multiple measures. Two different principal component analyses were performed. In the first PCA, a data set containing the assessed morphometric parameters of the rumen wall along with ruminal pH, lactate and the VFA concentration (acetate, propionate and butyrate, data presented in the companion paper by Suárez et al., 2006) was used to perform the analysis. The second PCA was applied on a data set comprising the assessed morphometric parameters of the rumen wall, the concentration of acetate and BHBA in plasma and the ratio phosphorus / creatinine in urine. The reason of grouping variables in this way was to differentiate more efficiently the real causal factors, so-called stimulators, from the so-called predictors of rumen development. Individual VFA concentrations, rather than the total VFA were included as variables, since each individual VFA is absorbed and metabolized to a different extent (Bergman, 1990). The phosphorus / creatinine ratio was included based on the assumption that urinary excretion of phosphorus is negatively related with ruminating activity (data presented by Van Vuuren et al., 2004). Within both principal component analyses a separate PCA was carried out for the dorsal and ventral location of the rumen, as well as with and without inclusion of the CONTROL treatment. Excluding the CONTROL treatment from the analysis, i.e. excluding data from calves with the lowest levels of VFA and morphometric parameters of the rumen wall, reduced the extent of variation in putative measures of rumen development across treatments, and allowed us to examine whether patterns of intercorrelations demonstrated by PCA reflected interindividual differences within concentrate diets, or were a consequence primarily of differences between the CONTROL treatment and the other treatments. All the statistical analyses were carried out using Genstat (Genstat Committee, 2000).
RESULTS

Throughout the experimental period calves remained healthy. Animal performance and rumen fermentation characteristics have been described elsewhere (Suárez et al., 2006). Rumen contents of calves fed concentrates were pasty in texture, whereas contents of calves fed CONTROL were liquid. The mucosa of the calves fed concentrate diets was brown to dark, and was easily removed from the rumen wall. In addition, coalescing papillae with embedded hair and feed particles, defined as “plaque”, were observed particularly in the ventral location of the rumen.

Rumen Mucosa Development

In Table 1, the macroscopic evaluation of the rumen mucosa is presented. As expected, calves fed the CONTROL diet consistently had a poorly developed rumen mucosa. In calves fed concentrate diets, the incidence of animals with poor mucosal development varied between 10 and 44 %. The STARCH based diet had a higher \( P < 0.05 \) incidence (44 %) of poorly developed mucosa than the PECTIN and NDF diet (10 and 13 %, respectively).

The rumen of a large number of calves appeared to have plaques of feed and hair firmly attached to the mucosa. The size and distribution of plaques varied considerably between animals and ranged from a few local patches with a diameter of few centimeters to confluent patches covering larger areas of the rumen. Plaque formation was hardly present in calves fed the CONTROL diet only, but it appeared in all concentrate treatments, with incidences ranging from 6 to 100 %. In the 8 weeks period, calves fed the STARCH diet had the lowest incidence of plaque (6 %; \( P < 0.05 \)) among the concentrate fed calves, but this increased to 73 % at the end of the 12 weeks period, where differences between concentrate diets in the incidence of plaque formation were no longer present \((P > 0.05)\).

Rumen Weight and Morphometric Parameters

Empty rumen weights and rumen wall morphometric parameters are presented in Table 2. Empty rumen weights at slaughter were affected by diet and period \((P < 0.001)\). Also an interaction between diet and period was observed \((P < 0.01)\). Averaged by treatments, empty rumen weights varied between 563 and 1423 g and between 734 and 1788 g for the 8 and 12 week periods, respectively. Calves fed the concentrate based diets had higher empty rumen weights compared with calves fed the CONTROL diet \((P < 0.001)\). At the end of the 8 week period, calves fed the PECTIN
diet had the heaviest rumen (1423 g) and those fed the STARCH diet had the lowest rumen weight (894 g). The interaction between diet and period was mainly caused by the large increase in rumen weights of calves fed the STARCH based diet from 8 to 12 week period (894 vs. 1531 g, respectively).

The RMSL in the dorsal location of the rumen of concentrate fed calves was consistently higher compared with calves fed with milk replacer only \((P < 0.01)\), but among concentrate diets no significant differences were observed. The RMSL was smaller \((P < 0.001)\) at 12 weeks than at 8 weeks. In the ventral location of the rumen, the RMSL was higher compared with the dorsal rumen wall location and was not affected by period. Calves fed the MIXED and the PECTIN diet had a larger RMSL than calves fed the CONTROL diet \((P < 0.05)\).

In the dorsal location, MCT was decreased compared with that observed in ventral location of the rumen \((595 \text{ to } 853 \mu m \text{ for the dorsal location and } 863 \text{ to } 1399 \mu m \text{ for the ventral location, respectively})\). In the dorsal location of the rumen, the MCT did not differ among the concentrate treatments. However, calves fed the MIXED and the STARCH diet had a higher MCT than calves fed the CONTROL diet \((P < 0.05)\). In the ventral location, a diet effect was observed for MCT in the ventral location \((P < 0.001)\). Calves fed STARCH and PECTIN diets had increased MCT than calves on the NDF and CONTROL diets, respectively \((P < 0.05)\). Moreover, the MCT of calves fed the MIXED diet was larger than the MCT in calves fed the CONTROL diet \((P < 0.05)\).

Although not statically compared, the MST in the dorsal location of the rumen wall was increased compared with that observed in the ventral location \((\text{mean across treatments were } 1692 \text{ and } 1322 \mu m \text{ for the dorsal and ventral location, respectively)\). Furthermore, in the dorsal location only a period effect \((P < 0.05)\) was observed whereas in the ventral location both diet \((P < 0.01)\) and period effects were present \((P < 0.05)\). In both locations, the MST decreased when increasing length of the experimental period from 8 to 12 weeks, respectively. In the ventral location of the rumen, calves fed the NDF diet had a significant increased MST than calves fed the STARCH, MIXED and CONTROL diets, respectively.

The analysis of covariance of the rumen wall morphometric parameters demonstrated that a significant part of the variation in the RMSL and MCT in the dorsal location of the rumen, was explained by the within treatment variation in concentrate intake (estimates of regression coefficients: \(3.25 \pm 0.93 \text{ S.E., } P < 0.001 \) and \(454 \pm 239 \text{ S.E., } P < 0.06\), for RMSL and MCT, respectively).
**Beta-hydroxy Butyrate and Acetate in Plasma**

Plasma concentrations of BHBA and acetate are presented in Table 3. Results represent average values in the last week before slaughter (at 8 and 12 week period respectively). The plasma acetate concentration was affected by diet ($P < 0.001$), but not by period. Also, an interaction between diet and period ($P < 0.05$) was observed. In the 8 weeks period group, concentrations of acetate for calves fed concentrate diets ranged from 0.09 to 0.14 mmol/L, and were higher than those observed in calves fed the CONTROL diet (0.06 mmol/L; $P < 0.001$). In addition, plasma acetate concentration in calves fed the PECTIN based diet (0.14 mmol/L) was higher ($P < 0.05$) than in calves fed the NDF (0.10 mmol/L) or the STARCH (0.09 mmol/L) diet, respectively. The observed interaction between diet and period was due to a decrease in calves fed the PECTIN diet (0.14 vs. 0.11 mmol/L) and in calves fed the NDF diet (0.10 vs. 0.13 mmol/L) from week 8 to 12, respectively.

Plasma BHBA concentrations were also affected by diet ($P < 0.05$) and period ($P < 0.01$). Moreover, an interaction between diet and period was observed ($P < 0.01$). Plasma BHBA concentration varied between 0.09 and 0.14 mmol/L. In the 8 weeks period, plasma BHBA concentration of calves fed the PECTIN diet was higher than in calves fed other diets ($P < 0.05$). The observed diet by period interaction was caused by a decrease in calves fed PECTIN and MIXED diets (0.14 and 0.11 vs. 0.09 and 0.09) in week 8 and 12, respectively.

**Principal Component Analysis**

Results of PCA of pH, VFA and the morphometric variables are presented in Table 4. Eigenvalues of the first two components were always greater than 1 and they explained 53.0 and 52.7 % of the total variation when histological measures of the dorsal and ventral location of the rumen, respectively, were included in the analysis. Following PCA of the complete data set, the loading patterns on the first principal component were similar in both locations. The ruminal concentrations of acetate, propionate and butyrate, together with RMSL all loaded negatively, and the concentration of ruminal lactate loaded positively on the first principal component (Table 4). Thus, across all treatment groups high ruminal concentration of VFA, but not of lactate, coincided with high levels of RMSL and vice versa. The RMSL and MCT also carried high and same-sign loadings on the second principal component (Table 4), together with the level of ruminal lactate in ventral location.
Results of PCA of plasma acetate and BHBA, urine phosphorus/creatinine ratio and morphometric variables are presented in Table 5. Similar to the previous analysis, eigenvalues of the first two factors in the dorsal and ventral rumen location were greater than 1. The cumulative percentages of the total variation explained by the first two factors were 55.8 and 53.8% when histological measures of the dorsal and ventral location of the rumen, respectively, were included in the analysis (Table 5). In the first component produced by PCA of the complete data set, variables with high and same-sign loadings included the relative mucosa length and MCT together with acetate and BHBA in plasma. The first principal component also had high loadings for the ratio phosphorus/creatinine in urine, but with signs opposite to those for morphometric measures and acetate and BOHB in plasma. Thus, across treatment groups, high levels of morphometric measures of rumen development were associated with high concentration of acetate and BOHB in plasma but low ratios for phosphorus/creatinine in urine and vice versa. In particular MCT in particular exhibited cross loading between the first and second principal component when the complete data set was analyzed (Table 5).

In both principal component analyses, excluding the CONTROL treatment did not greatly change the loading patterns in the ventral location of the rumen. When morphometric data from the dorsal rumen were analyzed, however, loading patterns changed dramatically after exclusion of observations from the CONTROL treatment (Tables 4 and 5).

**DISCUSSION**

**Rumen Tissue Morphology**

Although not defined as plaque, other researchers reported observations similar to ours. Bull et al. (1962) and Nocek et al. (1984) found, in calves fed concentrate only, clumping of papillae with embedded hair and feed particles. Likewise, but in steers fed diets containing high levels of concentrate (> 90%), Haskins et al. (1969) described similar observations in the rumen mucosa. In the present study, the incidence of plaque formation in calves fed concentrate diets varied between 6 and 67% at 8 weeks of age, and between 73 and 100% at 12 weeks of age. The lower incidence of plaque at 8 weeks, and the subsequent rise at 12 weeks shown by calves fed the STARCH based diet, corresponds with the increase in concentrate intake from 8 to 12 weeks period in the STARCH group (Suárez et al., 2006).
To facilitate the comparison of empty rumen weights of concentrate fed calves with those found in literature and to avoid the confounding effect of different ages and concentrate intake, data of empty rumen weight gathered from literature were subsequently recalculated and expressed in g/kg of BW. Results are shown in Figure 1. In the present study, empty rumen weights (g/kg BW) of calves fed concentrate diets, ranged from 10.6 to 16.8 g/kg BW, and were in line with data reported by Klein (1987), Greenwood et al. (1997) and Anderson et al. (1987ab) for rearing calves of similar age, fed only with concentrate and with data reported by Cozzi et al. (2002) for veal calves fed coarse solids feed. However, empty rumen weights were slightly lower than those reported by Stobo et al. (1966) and Nocek et al. (1984), respectively. As already mentioned, dry feed intake stimulates rumen growth and dietary factors such as the level of milk or milk replacer feeding (Jenny et al., 1982; Huber et al., 1984), level of fiber (influenced by the source of NDF) and the physical nature of the dry feed (Morrill, 1992; Beharka et al., 1998) have been reported to affect dry feed intake and consequently rumen growth in young calves. Therefore correction for differences in dry feed intake should be implemented when comparing results of forestomachs growth.

In the present research, calves fed the STARCH based diet had lower empty rumen weights at 8 weeks period than those fed the other concentrate treatments, which may be the result of the lower concentrate intake of the calves (Suárez et al., 2006). However, the PECTIN treatment had the highest empty rumen weight at a low concentrate intake. Simple linear regressions were performed to clarify the relationship between the increase in empty rumen weight and the anatomical constituents of rumen wall in the dorsal and ventral rumen location. MCT and MST were considered as independent variables. Results from the linear regressions showed that albeit significant, only a small part of the variation in empty rumen weight was explained by MST in the dorsal and MCT in the ventral location of the rumen, accounting for 9.4 % \( (P < 0.01) \) and 3.0 % \( (P < 0.01) \) of the variation respectively. The estimate of the regression coefficient for MCT was positive (0.18 g/µm ± 0.07 S.E.), whereas the estimate of the regression coefficients for thickness of the muscle wall was negative (- 0.22 g/µm ± 0.09 S.E.). When comparing treatment effects on the incidence of plaque formation (Table 1) with those on empty rumen weights (Table 2), it is suggested that a considerable part in the variation in empty weights is due to variation in the extent of plaque formation.
Beta-Hydroxy Butyrate and Acetate in Plasma

Plasma BHBA and acetate concentrations were measured in view of the potential suitability as a quick, non-invasive indicator of rumen development. Plasma acetate concentrations were always higher for concentrate treatments than milk only. The concentration of BHBA in plasma significantly decreased as calves aged, and at the end of the 12 weeks period, plasma BHBA concentration in concentrate fed calves were not different from those fed CONTROL. Other researchers have observed an increase in plasma BHBA with age (Quigley et al., 1991; Quigley and Bernard, 1992; Greenwood et al., 1997). The increased supply of milk replacer and, except for the STARCH based diet, decreased intake of concentrate over time, (see Suárez et al, 2006) may explain the conflicting results. In addition, in the present study plasma BHBA was measured in peripheral blood samples. Thus values reported here also include any BHBA converted from butyrate and acetate in the liver (see Bergman, 1990). In order to better analyze the relationship between plasma acetate and BHBA concentrations, these latter variables (considered to be quick non-invasive predictors of ruminal activity) were included in a PCA together with those reflecting morphometric with rumen development.

Principal Component Analysis

First PCA: It has been demonstrated that at birth the rumen is undeveloped and its growth depends on the consumption of dry feed (Flatt et al., 1958; Huber, 1969). More specifically, the VFA (acetate, butyrate and propionate) resulting from fermentation of dietary carbohydrates, were identified as the main promoters of forestomachs development (Sutton et al., 1963a). Propionate and butyrate exert a larger stimulatory effect on the development of the rumen papillae than acetate (Sander et al., 1959; Tamate et al., 1962). Sutton et al. (1963b) observed that propionate and butyrate are the main promoters of the tissue growth in relation to the higher metabolisation of these acids upon absorption through the ruminal mucosa. In the present study, the loading patterns of the first principal component (PC 1) extracted by PCA of the complete data set (i.e., CONTROL treatment included) clearly demonstrate a positive relationship between the VFA concentrations in rumen liquid (acetate, propionate and butyrate) and morphometrical rumen development (RMSL and MCT, see Table 4). Moreover, this pattern of interrelationships was consistently maintained regardless of rumen location (dorsal or ventral). Results of this PCA did not support the notion that individual VFA may differ in the ability to stimulate mucosa.
development. Loadings of individual concentrations of VFA in rumen fluid on the first principal component were highly similar. This might be explained by assuming that either the variation in dietary carbohydrate composition was not large enough to elicit sufficient differences in mucosa growth, or that in the present experiment the response in mucosa development was mediated by others factors rather than VFA concentrations alone. Correspondingly, the fact that RMSL and MCT also had high loadings on the second principal component (see Table 4) strongly suggests that the development of the ruminal wall is indeed also mediated by factors other than VFA. Low ruminal pH, high lactate concentration, ruminitis and decreased rumen motility are generally associated with ruminal acidosis a fermentative disorder normally related to diets containing large amounts of highly digestible carbohydrates or lacking adequate level of effective fiber (Nocek, 1997; Owens et al., 1998). Results in the present study confirm these findings, as in component 1 lactate and pH were interrelated and negatively correlated with rumen mucosa development in both locations of the rumen. However, its must be pointed out that this negative correlation was no longer present when the CONTROL treatment was excluded from the analysis. This means that these patterns of intercorrelations apparently depended largely on the differences between the CONTROL treatment and the other treatments, rather than on differences between or interindividual differences within concentrate diets. In contrast to Dell’ Orto et al. (2002), who in an abstract reported reduced papillae length and epithelial thickness in the rumen of veal calves supplemented with corn silage or sugar-beet pulp compared with milk replacer alone, the present results clearly confirm that papillae length and epithelial thickness are larger upon supplementation with solid feed.

**Second PCA Study:** Quigley et al. (1991) and Quigley (1996) demonstrated that in young calves, the rumen epithelium has the capacity of absorbing and metabolizing VFA from an early age onwards. Consequently, in rearing calves, increased rumen VFA concentrations are associated with high plasma BHBA concentration. As a result BHBA has been widely used as a quick indicator of rumen development in rearing calves (Quigley et al., 1991; Quigley and Bernard, 1992). In addition, because of rumination activity adult ruminants normally excrete small amounts of phosphorus in the urine, being mostly eliminated through feces. Therefore, the ratio phosphorus / creatinine in urine has been recently proposed as a potential parameter to study rumen development in calves (Van Vuuren et al., 2004). The analysis of our findings confirmed
Effects of Supplementing Concentrates Differing…

the proposed relationships between rumen development and the physiological parameters used to evaluate this characteristic in the present study. Across all treatments, plasma acetate and BHBA were positively, and the ratio phosphorus / creatinine was negatively correlated with the morphometric parameters of rumen development (RMSL and MCT). Similar to the first PCA considered above, MCT had high loadings on both the first and the second principal component, which is consistent with the idea that mechanisms underlying rumen development in calves also involve factors other than VFA. Notably, in contrast to the PCA involving VFA in rumen fluid, the loading pattern linking plasma levels of acetate and BHBA and morphometric measures of rumen development in the ventral region to the same underlying characteristic was largely preserved after exclusion of the CONTROL treatment from the analysis (Table 5). This suggests that, within and/or between concentrate diets, there was a meaningful co-variation among these measures.

CONCLUSION

Feeding concentrates differing in carbohydrate composition to veal calves promoted rumen development compared with calves fed milk replacer only. Calves fed concentrate diets had heavier rumens than calves fed CONTROL; however a high incidence of coalescing rumen papillae with embedded hair and feed particles was observed in calves fed concentrate. In most calves, a poorly developed rumen mucosa was observed. In the dorsal location of the rumen, calves fed concentrate diets showed an increased RMSL when compared with calves fed CONTROL, while only calves fed the PECTIN and MIXED diets showed larger RMSL in the ventral location. Regardless of dietary treatments the MCT was bigger in the ventral than in the dorsal location of the rumen; in contrast, the MST was larger in the dorsal than in the ventral rumen location. In both locations, the NDF diet resulted numerically in the lowest MCT and highest MSC amongst concentrate treatments. In the companion paper (Suárez et al., 2006) it was demonstrated that variation in the carbohydrate composition of the concentrates led to variation in total VFA concentrations and its molar proportions. Although the variation in carbohydrate composition caused variation in rumen development, the latter was generally small. In addition, the common physical nature of the diets may have prevented the expression of gross anatomical differences in the rumen morphology. The PCA strongly suggested that variation in VFA concentration correlates to some extent with the
development of the rumen, and that individual VFA, including acetate, propionate and butyrate were similarly related to rumen development. The variation in rumen development also coincided with variation in plasma concentration of acetate and BHBA.
Table 1. Effects of period (age at slaughter) and diet (supplemented concentrates differing in carbohydrate source) on incidence of poor development of rumen mucosa and incidence of plaque formation (macroscopic)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Diets</th>
<th>Period</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CONTROL</td>
<td>PECTIN</td>
<td>NDF</td>
<td>STARCH</td>
</tr>
<tr>
<td>Poor development of mucosa (%)</td>
<td>100&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10&lt;sup&gt;c&lt;/sup&gt;</td>
<td>13&lt;sup&gt;c&lt;/sup&gt;</td>
<td>44&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Plaque (%)</td>
<td>8 weeks</td>
<td>6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>44&lt;sup&gt;a&lt;/sup&gt;</td>
<td>67&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>12 weeks</td>
<td>0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>80&lt;sup&gt;a&lt;/sup&gt;</td>
<td>94&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

abc Means in the same row with different superscript differ significantly (P < 0.05).
(Non-parametric analysis. Fisher’s exact tests).
Table 2. Effects of period (age at slaughter) and diet (supplemented concentrates differing in carbohydrate source) on rumen weight and morphometric variables of rumen wall, determined at slaughter.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Diets</th>
<th>SE</th>
<th>Diets</th>
<th>SEM</th>
<th>Period</th>
<th>8 weeks</th>
<th>12 weeks</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CONTROL PECTIN NDF STARCH MIXED</td>
<td></td>
<td>D P D x P</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Rumen weight (g)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8 week</td>
<td>563&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1234&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>894&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1117&lt;sup&gt;b&lt;/sup&gt;</td>
<td>76.3</td>
<td>*</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>12 week</td>
<td>734&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1788&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1531&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1726&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>76.3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Rumen dorsal location</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ratio of mucosa length to serosa length (RMSL)</td>
<td>3.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.2</td>
<td>***</td>
<td>*** NS</td>
<td></td>
</tr>
<tr>
<td>Mucosa thickness (MCT, μm)</td>
<td>595&lt;sup&gt;b&lt;/sup&gt;</td>
<td>691&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>799&lt;sup&gt;a&lt;/sup&gt;</td>
<td>853&lt;sup&gt;a&lt;/sup&gt;</td>
<td>61.5</td>
<td>*</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Muscle thickness (MST, μm)</td>
<td>1797</td>
<td>1851</td>
<td>1621</td>
<td>1610</td>
<td>86.1</td>
<td>NS</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td><strong>Rumen ventral location</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ratio of mucosa length to serosa length (RMSL)</td>
<td>4.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.7&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>6.2&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>8.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.0</td>
<td>*</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Mucosa thickness (MCT, μm)</td>
<td>863&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1320&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1177&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>94.1</td>
<td>*** NS</td>
<td>NS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Muscle thickness (MST, μm)</td>
<td>1294&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1292&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1181&lt;sup&gt;b&lt;/sup&gt;</td>
<td>66.8</td>
<td>** NS</td>
<td>*</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a,b</sup> Means in the same row with different superscript differ significantly (*P < 0.05)*.

D= Diet; P= Period; D x P= Interaction.

* *P < 0.05

** P < 0.01

*** P < 0.001.
Table 3. Effects of period (age at slaughter) and diet (supplemented concentrates differing in carbohydrate source) on rumen weight and Morphometric variables of rumen wall on plasma acetate and β-hydroxy butyrate concentrations in the last week before slaughter.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Dietary treatments</th>
<th>SEM</th>
<th>P</th>
<th>Period</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CONTROL</td>
<td>PECTIN</td>
<td>NDF</td>
<td>STARCH</td>
</tr>
<tr>
<td>Acetate (mmol/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8 weeks</td>
<td>0.06&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.10&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.09&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>12 weeks</td>
<td>0.09&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.11&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.11&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>β-hydroxy butyrate (mmol/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8 weeks</td>
<td>0.09&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.09&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>0.09&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>12 weeks</td>
<td>0.09</td>
<td>0.09</td>
<td>0.08</td>
<td>0.09</td>
</tr>
</tbody>
</table>

<sup>abc</sup> Means in the same row with different superscript differ significantly (P < 0.05).

D = Diet; P = Period; D x P = Interaction.

* P < 0.05
** P < 0.01
*** P < 0.001.
Table 4. Loadings\(^1\) on the first (PC 1) and second (PC 2) principal component extracted by principal component analysis (PCA) of histological measurements of dorsal and ventral rumen development, pH and VFA in ruminal fluid recorded in calves fed concentrate diets differing in carbohydrate source. Results are presented of analyses with (Complete data set) and without the CONTROL treatment (Milk replacer only) included (Without CONTROL).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Dorsal Rumen Location</th>
<th>Ventral Rumen Location</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Complete data set</td>
<td>Without CONTROL</td>
</tr>
<tr>
<td></td>
<td>PC 1</td>
<td>PC 2</td>
</tr>
<tr>
<td>Ratio of mucosa length to serosa length (RMSL)</td>
<td>-0.59</td>
<td>-0.53</td>
</tr>
<tr>
<td>Mucosa Thickness (MCT, (\mu m))</td>
<td>-0.25</td>
<td>-0.85</td>
</tr>
<tr>
<td>Muscle Thickness (MST, (\mu m))</td>
<td>0.23</td>
<td>-0.39</td>
</tr>
<tr>
<td>Rumen pH</td>
<td>0.44</td>
<td>0.28</td>
</tr>
<tr>
<td>Acetate in rumen fluid, mmol/L</td>
<td>-0.80</td>
<td>0.31</td>
</tr>
<tr>
<td>Propionate in rumen fluid, mmol/L</td>
<td>-0.82</td>
<td>0.06</td>
</tr>
<tr>
<td>Butyrate in rumen fluid, mmol/L</td>
<td>-0.69</td>
<td>0.13</td>
</tr>
<tr>
<td>Lactate in rumen fluid, mmol/L</td>
<td>0.61</td>
<td>-0.27</td>
</tr>
<tr>
<td>Eigenvalue</td>
<td>2.82</td>
<td>1.42</td>
</tr>
<tr>
<td>Variance explained (%)</td>
<td>35.25</td>
<td>17.77</td>
</tr>
</tbody>
</table>

\(^1\)Loadings greater than 0.50 are indicated in bold
Table 5. Loadings on the first (PC 1) and second (PC 2) principal component extracted by principal component analysis (PCA) of histological measurements of dorsal and ventral rumen development, acetate and ß-OH-Butyrate in plasma and phosphorus /creatinine ratio in urine recorded in calves fed concentrate diets differing in carbohydrate source. Results are presented of analyses with (Complete data set) and without the CONTROL treatment (Milk replacer only) included (Without CONTROL).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Dorsal Rumen Location</th>
<th>Ventral Rumen Location</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Complete data set</td>
<td>Without CONTROL</td>
</tr>
<tr>
<td>Ratio of mucosa length to serosa length (RMSL)</td>
<td>-0.80 -0.24</td>
<td>-0.59 -0.46</td>
</tr>
<tr>
<td>Mucosa Thickness (MCT, µm)</td>
<td>-0.52 -0.75</td>
<td>-0.87 -0.22</td>
</tr>
<tr>
<td>Muscle Thickness (MST, µm)</td>
<td>0.24 -0.73</td>
<td>-0.53 0.10</td>
</tr>
<tr>
<td>Plasma acetate concentration</td>
<td>-0.61 0.05</td>
<td>-0.31 0.07</td>
</tr>
<tr>
<td>Plasma ß-OH-Butyrate concentration</td>
<td>-0.53 0.21</td>
<td>0.09 -0.70</td>
</tr>
<tr>
<td>Ratio Phosphorus /Creatinine in urine</td>
<td>0.54 -0.48</td>
<td>-0.43 0.74</td>
</tr>
<tr>
<td>Eigenvalues</td>
<td>1.92 1.43</td>
<td>1.68 1.30</td>
</tr>
<tr>
<td>Variance explained (%)</td>
<td>32.03 23.79</td>
<td>28.00 21.71</td>
</tr>
</tbody>
</table>

1Loadings greater than 0.50 are indicated in bold
Klein et al. 1987 (3-6 wk)
Greenwood et al. 1997 (6wk)
Anderson et al. 1987ab (8wk)
Suárez et al. 2006 (8 wk)
Suárez. et al 2006 (12 wk)
Stobo et al. 1966 (12 wk)

Figure 1. Relationship between dry matter intake and empty rumen weight (g/kg BW)
REFERENCES


CHAPTER 4


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ABSTRACT

Sixty four male Holstein Friesian x Dutch Friesian veal calves (46 kg ± 3.0 kg), were used to evaluate the effect of the inclusion of different levels and sources of dietary roughage on animal performance and rumen development. Treatments consisted of: 1) C100= concentrate only, 2) C70-S30= concentrate (70%) with straw (30%), 3) C70-G30= concentrate (70%) with dried grass (30%), 4) C70-G15-S15= concentrate (70%) with dried grass (15%) and straw (15%), 5) C70-CS30= concentrate (70%) with corn silage (30%), 6) C40-CS60= concentrate (40%) with corn silage (60%), 7) C70-CS30-AL= concentrate (70%) with corn silage (30%) ad libitum, 8) C70-G15-S15-AL= concentrate (70%) with dried grass (15%) and straw (15%) ad libitum. All dietary treatments were provided in addition to a commercial milk replacer. Concentrate was provided as pellets and roughage was chopped. The dietary treatments 1 to 6 were supplied restrictedly to a maximum of 750 g of dry matter per day (DM/d), whereas treatments 7 and 8 were offered ad libitum in combination with a reduced amount of milk replacer. Calves were euthanized after 10 wk. Straw supplementation (C70-S30 vs. C70-G30 and C70-CS30) reduced dry matter intake (DMI) and ad libitum supply of concentrate and roughage increased DMI. Roughage addition did not affect growth performance. Rumen fermentation was characterized by low pH and high total volatile fatty acids and reducing sugars concentrations. Ad libitum fed calves showed lower ruminal lactate concentrations than calves fed restrictedly. Ammonia concentrations were highest in calves fed C-100 and lowest in ad libitum fed calves. The Co-EDTA recovery (added to milk replacer) varied between 20.5 and 34.9 %, indicating that significant amounts of milk entered the rumen. Roughage addition decreased the incidence of plaque formation (rumen mucosa containing focal or multi-focal patches of foci with coalescing and adhering papillae covered by a sticky mass of feed, hair and cell debris) and the incidence of calves with poorly developed rumen mucosa. However, morphometric parameters of the rumen wall were hardly influenced by the type and level of roughage.

Ruminal polysaccharide degrading enzyme activities reflected the adaptation of the microorganisms to the dietary concentrate/roughage source. Results indicated that in veal calves, the addition of roughage to concentrate diets did not affect growth performance and positively influenced the macroscopic appearance of the rumen wall.

Key Words: Veal Calf, Concentrate and Roughage Composition, Rumen Fermentation, Rumen Development.
INTRODUCTION

Ingestion of solid feed is necessary to stimulate rumen development in the young calf. Concentrate diets are generally provided because DMI and VFA production is higher than with diets based only on roughage. Volatile fatty acids are required for a fast development of the rumen papillae (Flatt et al., 1958). On the other hand, concentrate diets may cause a rapid accumulation of the fermentation end products accompanied by a decrease in pH (Beharka et al., 1998), decreased rumen motility (Nocek, 1997; Owens et al., 1998), overgrowth and keratinization of the rumen papillae (Nocek and Kesler, 1980) and consequently a decrease in VFA absorption (Hinders and Owen, 1965). In previous research with concentrate-fed veal calves a particular rumen environment was observed, characterized by a low pH (<5.2), relatively low VFA concentrations (100-120 mmol/L), high concentrations of reducing sugars (45 – 124 g/kg DM) and high activity of microbial polysaccharide degrading enzymes (PDE) (Suárez et al., 2006a). In addition, and depending on concentrate composition, poorly shaped papillae and a high incidence of foci with feed and cell debris sticking to the rumen wall (in this paper referred to as plaque formation) were observed (Suárez et al., 2006b).

In veal calves, typically fed with milk replacer and supplemented with relatively small quantities of concentrates, information concerning the effects of roughage intake on performance and rumen development is scarce (Blokhuis et al., 2000; Cozzi et al., 2002). Feeding roughage without concentrates to young calves generally does not promote rapid papillae development, probably due to insufficient VFA production rates (Nocek and Kesler, 1980). Roughage consumption, however, stimulates muscle development of the rumen wall (Tamate et al., 1962) and promotes rumination (Hodgson, 1971). Furthermore, the physical characteristics present in the roughages, such as coarseness, bulkiness and abrasiveness have been reported as necessary to maintain the integrity and healthiness of the rumen wall (Haskins et al., 1969). Therefore, it was hypothesized that adding roughage to a concentrate diet reduces the incidence of poorly developed mucosa and plaque formation observed in concentrate fed veal calves (Suárez et al., 2006a, b), and stimulates the development of the rumen wall, without negative effects on performance.

The objective of the present experiment was to determine the effect of various roughage sources, concentrate to roughage ratios, and intake levels of concentrate and roughage fed in addition to a milk replacer diet, on rumen development and growth performance of veal calves.
MATERIALS AND METHODS

The present experiment was conducted at the experimental farm of Wageningen University & Research Centre in Lelystad, the Netherlands. Experimental procedures complied with the Dutch law on experimental animals.

Calves, Diets, Transport and Slaughter Procedure

Sixty four Holstein Friesian x Dutch Friesian male calves of approximately 10 days of age (46 kg ± 3.0 kg) were allotted to one of eight blocks and within blocks, randomly assigned to one of eight dietary treatments. Calves were housed in individual mesh-bottomed pens of 0.9 m² without bedding material. Ambient temperature was maintained at least at 15° C.

Dietary treatments (Table 1) consisted of a 100% concentrate control treatment and seven treatments in which a variety of roughage sources were supplied in various quantities in addition to a commercial milk replacer (970.9 g/kg DM; 36.8 g/kg nitrogen; 181 g/kg crude fat; 73.5 g/kg ash). Milk replacer and dietary treatments were offered to the calves twice daily (0600 and 1600h). The roughage sources used were either corn silage, barley straw or dried grass. Acronyms for the dietary treatments are presented in Table 1 and will be used accordingly in the text. Ingredient and nutrient composition of the concentrate as well as the nutrient composition of roughages is presented in Table 2. The concentrate was provided as pellets and the roughages were chopped. Except for the ad libitum treatments (see Table 1), the supplied DMI from concentrates and roughage was maximized at 750 g/d and for these restrictedly fed diets the daily supply of milk replacer was gradually increased and amounted to 360, 480, 530, 570, 620, 700, 810, 980, 1170 and 1360 g/d in week 1 to 10, respectively. For the ad libitum treatments the supply of milk replacer was identical to the restricted fed treatments up to week 6, but remained constant at 700 g/d thereafter. Concentrates and roughages portions were weighed and mixed separately (50% of the expected daily intake per meal), and supplied in a different bucket immediately after the calves had finished the milk replacer. Buckets were weighed and emptied prior to the next milk replacer meal, and pooled samples of concentrates, roughage sources and orts were obtained for chemical analysis. To avoid selective intake of concentrates or roughages, the daily supply was based on minimization of orts. Therefore, the expected DMI of a calf was calculated weekly, based on the realized DMI of that calf in the previous week, and a projected weekly increase.
Calves were euthanized after 10 weeks by an intravenous administration of T61 (Embutramide, Intervet International, Germany). To quantify ruminal drinking, 3.0 g of Co-EDTA (Uden et al., 1980) was dissolved into the last milk replacer meal. Calves were assigned to one of four consecutive slaughtering days (balanced across dietary treatments), and to one of four fixed intervals of 1.5, 2.5, 3.5 and 4.5 h between the last milk feeding and time of slaughter. Prior to slaughter and after calves finished the milk replacer, the concentrates and roughages portions were allowed to be consumed for 30 min. Then, calves were transported from the experimental farm to the pathology lab (5 min), and euthanized. To avoid reflux of milk replacer containing Co-EDTA from the abomasum into the forestomachs, the calves were lifted by the forelegs, the stomach was tied caudal of the pylorus and caudal of the esophagus and removed. The rumen weight was recorded with and without its contents. Cobalt recovery (%) was determined by calculating ruminal DM pool size (kg), cobalt concentration (mg/kg DM), ruminal cobalt pool size and expressed as the percentage of cobalt intake (initial pulse dose).

The rumen was dissected along the dorsal line, emptied and rinsed with cold water. The examination and assessment of the rumen mucosa and sites of sampling of the rumen wall for further morphometric analysis were carried out as described by Suárez et al. (2006b). Briefly, the mucosal surface was visually examined and qualitatively assessed according to the presence and density of rumen papillae as follows: 1 = poor (few number of papillae or short papillae) and 2 = good (numerous papillae or long papillae). The incidence of plaques (rumen mucosa containing focal or multi-focal patches of foci with coalescing and adhering papillae covered by a sticky mass of feed, hair and cell debris) was visually assessed. The morphometric analyses were conducted in four slides (embedded in paraffin, stained with hematoxylin-eosin) cut from a 2 x 2 cm section of rumen wall tissue, taken either from a dorsal or a ventral location in the rumen. The measurements comprised a) Ratio of mucosa length to serosa length (RMSL), as a measure of absorptive area and determined as the length of the mucosal surface within a slide, divided by the length of the corresponding serosa (the latter being about 2 cm); b) Mucosa thickness (MCT), measured at 3 randomly chosen sites within each slide; c) Muscle thickness (MST), measured at the same sites as under b). The measurement of MCT was chosen to avoid bias of the observer by defining suitable, measurable papillae in a slide, as irregular shapes of papillae frequently occurred. Morphometrical analyses were performed at a magnification of 2.5 x (Olympus microscope) by using the image analysis software Image Pro Plus (Media Cybernetics).
By using a metal sieve (1.5 mm) the solid and liquid phases of the rumen contents were separated and weighed. The separation of both phases was done by gently squeezing the rumen contents until no more leakage of liquid was observed. A representative sample of rumen contents was reconstituted from the solid and liquid phases. Rumen pH was measured in the rumen liquid. Rumen liquid was centrifuged (1000 g, 10 min), sampled, acidified with orthophosphoric acid and stored at -20º C until analysis. The fractional rumen liquid passage rate ($K_{pl}$) was calculated as the slope of the regression of the natural logarithm of cobalt pool size against the time interval between milk feeding and slaughter.

**Analytical Procedures**

Dry matter (DM) was determined by drying at 103 ºC and ash by combustion at 550 ºC according to ISO 6496 and ISO 5948, respectively. Kjeldahl-N and fat content were determined based on ISO 5983 and 6492, respectively and total starch was analyzed according to Brunt (1993). Reducing sugars (RS) were extracted from the dried rumen samples using 40% ethanol and determined as described by Suárez et al. (2006a). Neutral detergent fiber, ADF and acid detergent lignin (ADL) were analyzed according to a method adapted from Van Soest et al. (1991). Briefly, after the neutral detergent treatment incubation, an enzyme cocktail containing termamyl (Novo Nordisk, Copenhagen, DK), amylase (Sigma 6814, Sigma-Aldrich, St. Louis, MO, USA) and protease (Novo Nordisk, Copenhagen, DK) in phosphate buffer (pH 7.0) was added to remove starch and proteins from the residue. For ADF determination, first the samples were treated as in the NDF methodology and afterwards the residues incubated with acid detergent as described by Van Soest and McQueen (1973). The ADL analysis was based on the treatment of the sample with acid detergent followed by incubation with 72% sulphuric acid.

The VFA and lactic acid concentrations were analyzed using HPLC, with a Merck polyspher OA 51272 column as stationary phase (Merck & Co., Inc. NJ,USA) and 0.0025 M sulphuric acid as mobile phase (elution fluid) and a refractive index detector. Calibration and quantification was done by using an external standard solution. Ammonia concentrations were estimated according to the modified method of Berthelot (Schneider, 1976).

For the Co-EDTA determination, samples of whole rumen contents were freeze dried, ground and subsequently incinerated at 250ºC and 550ºC. The remaining ash was destructed with concentrated nitric acid during 2 hours at 100ºC. The supernatant was diluted with a solution
containing lithium-chloride and yttrium (as internal standard). The content of cobalt was measured by ICP-AES at 228.616 nm.

Isolation of rumen enzymes and subsequent measurement of their activities were carried out as described by Suárez et al. (2006a). The substrates incubated with the enzyme cocktails were suspensions (2 % wt.vol⁻¹) of sugar beet pulp, soybean hulls, native corn starch and straw. Incubates, substrate blanks and enzyme extract blanks were analyzed for RS end-groups according to Somogyi (1952). After correcting by substrate and enzyme extracts blanks, the enzyme activity was expressed as mmol of RS released per minute per g of dry matter in the rumen.

Statistical analysis

Data rumen fermentation parameters, empty rumen weight and rumen wall parameters were analyzed as a complete randomized block design, with dietary treatments as main factor. Continuous data were subjected to ANOVA according to model [1]:

\[
y = \mu + \text{Diet}_i + \text{Slaughter day}_j + \varepsilon_{ijk}
\]

Where:
\(y\) = Dependent variable;
\(\mu\) = Average experimental value;
\(\text{Diet}_i\) = Effect of dietary treatment \(i\); \(i = \text{diet 1 to 8}\);
\(\text{Slaughter day}_j\) = Effect of Slaughter day \(j\); \(j = 1\ldots 4\);
\(\varepsilon_{ijk}\) = Error term; \(k = 1, \ldots, 64\) (i.e. calf).

The same model, but excluding the effect of slaughter day, was used to analyze data of DMI and average daily gain (ADG). The parameters where the post feeding slaughter time was expected to have an effect (rumen pH, ruminal VFA concentrations and Co-EDTA recovery) were analyzed using the following model [2]:

\[
y = \mu + \text{Diet}_i + \text{Slaughter day}_j + \text{Interval}_k + (\text{Diet} \times \text{Interval})_{ik} + \varepsilon_{ijkl}
\]
Where:

\[ y = \text{Dependent variable}; \]
\[ \mu = \text{Average experimental value}; \]
\[ \text{Diet}_i = \text{Effect of dietary treatment } i; \ i = \text{diet 1 to 8}; \]
\[ \text{Slaughter day}_j = \text{Effect of slaughter day } j; \ j = 1... 4; \]
\[ \text{Interval}_k = \text{Effect of interval feeding- slaughter}; \ k = 1.5, 2.5, 3.5, 4.5 \text{ h}; \]
\[ (\text{Diet x Interval})_{ik} = \text{Effect of interaction between dietary treatment and interval feeding- slaughter}\]
\[ \varepsilon_{ijkl} = \text{Error term}; \ l = 1, \ldots, 64 \text{ (i.e calf)}. \]

The F-test was used to evaluate the effect of dietary treatment. For the analysis of fractions (percentages), a logistic regression model was used with the same main effects as described earlier. Inferences for fractions were based on likelihood ratio tests. Dispersion parameters were estimated from Pearson’s generalized test (McCullagh and Nelder, 1989). Post-hoc pair-wise comparisons between diets were carried out using the Fisher LSD method (for fractions on a logistic scale), employing the pooled error variance from the analysis of variance. In addition, predetermined contrasts between specific (combinations of) feed rations were examined, including: A) roughage supplementation: C100 vs. all roughage containing diets, except the two ad libitum diets; B) the effect of source of roughage supplementation: C70-S30 vs. C70-G30 vs. C70-CS30; C) the effect of supplementing a single or a combined source of roughage: C70-G15-S15 vs. (C70-S30 and C70-G30); D) the effect of different levels of roughage supplementation: C70-CS30 vs. C40-CS60; E) the effect of restricted vs. ad libitum roughage supplementation: (C70-G15-S15 and C70-CS30) vs. (C70-G15-S15-AL and C70-CS30-AL). Each contrast was tested for significance with a t-test, again using the pooled error variance from the analysis of variance. Measures of rumen fermentation (pH, total VFA, molar proportions, lactate and ammonia), empty rumen weight, and morphometric variables of the rumen wall were also subjected to analyses of covariance, introducing DMI as a covariable in the appropriate analysis of variance model (see above). For measures of rumen fermentation, the average DMI during the last week prior to slaughter was used as a covariable, and expressed as the deviation from the overall mean. For the empty rumen weight, and morphometric variables of the rumen wall, the average DMI during the entire experimental period was used as a covariable, and likewise expressed as the deviation from the overall mean. In this manner, least
square means of dependent variables following analyses of covariance were appropriately corrected for differences in DMI. Binary variables were analyzed parameter-free using the Fisher exact test. Significance was determined at P<0.05 unless indicated otherwise. All calculations were performed with Genstat (Genstat Committee, 2000).

The rumen enzyme activities were analyzed as a complete randomized block design with dietary treatments and substrates as main factors; by using the GLM procedure of SAS (SAS Institute, 2002) according to model [3]:

\[ y = \mu + \text{Diet}_i + \text{Substrate}_j + (\text{Diet} \times \text{Substrate})_{ij} + \epsilon_{ijk} \]

Where:
- \( y \) = Dependent variable;
- \( \mu \) = Average experimental value;
- \( \text{Diet}_i \) = Effect of dietary treatment \( i \); \( i = \) diet 1 to 8;
- \( \text{Substrate}_j \) = Effect of substrate \( j \), \( j = \) soybean hulls, native corn starch, sugar beet pulp, straw;
- \( \epsilon_{ijk} \) = Error term

Main effects and the interaction between diets and substrate were evaluated. Moreover, predetermined contrasts between diets (mentioned above) were performed by substrate.

**RESULTS**

**Feed Intake and Body Weight Gain**

Throughout the experiment, no signs of illness were observed. The least square means for milk replacer intake, non milk replacer DMI as well as for final BW, ADG and feed conversion (FC) are presented in Table 3. The average DMI from concentrate and roughage of the restrictedly fed calves ranged from 0.43 to 0.59 kg/d, and for the ad libitum fed groups from 0.78 to 0.81 kg/d. Calves fed the C70-G30 and C70-CS30 had a higher average DMI (0.59 and 0.55 kg/d, respectively) when compared with calves fed the C70-S30 diet (0.43 kg/d; contrast B; \( P < 0.05 \)). In week 5 and 6 this contrast was also significant, but not in week 1 and week 10. In
addition, calves fed ad libitum showed a higher overall DMI and higher DMI in week 6 and 10 than restricted fed calves (contrast E; \( P < 0.001 \)).

The weight at slaughter was not affected by the diets. Average daily gain ranged from 688 to 779 g/d. Calves fed the C70-G30 and C70-CS30 diets had increased ADG (779 and 774 g/d, respectively) compared with those on the C70-S30 treatment (668 g/d; contrast B; \( P < 0.05 \)). Feed conversion was not affected by dietary treatments among restrictedly fed calves (\( P > 0.05 \)), but it was increased in the calves of the ad libitum fed groups (\( P < 0.001 \); contrast E).

**Rumen Fermentation Parameters**

The effects of dietary treatments on rumen weight and fermentation parameters are shown in table 4. The pH in rumen liquid varied between 5.1 and 5.3, and was higher for calves fed the C70-G15-S15 diet than for calves on the C70-S30 and C70-G30 treatments (contrast C; \( P < 0.05 \)).

The total VFA concentration in rumen fluid ranged from 108 to 150 mmol/L and was slightly lower in calves fed the C40-CS60 than in those fed the C70-CS30 diet (contrast D; \( P < 0.10 \)). Substituting part of the concentrate by roughage increased acetate and decreased propionate molar proportions (contrast A; \( P < 0.001 \)). The molar proportion of acetate increased and that of butyrate decreased when straw was the single source of roughage (contrast B; \( P < 0.001 \) and \( P < 0.05 \), respectively). Furthermore, the molar proportion of propionate decreased from 27.3 to 21.6 % by increasing the corn silage to concentrate ratio (contrast D; \( P < 0.01 \)). Finally, calves fed a roughage combination of dried grass and straw (C70-G15-S15) tended to have a lower molar proportion of propionate in comparison with calves fed either only dried grass or only straw as source of roughage (contrast C; \( P < 0.1 \)).

The molar proportion of isobutyrate in the rumen liquid was lower in C70-G30 fed calves than in those fed either the C70-S30 or C70-CS30 diets (contrast B; \( P < 0.05 \)). In addition, lower molar proportions of isobutyrate and of methyl-butyrate were observed in ad libitum fed calves (contrast E; \( P < 0.05 \) and \( P < 0.01 \), respectively). The molar proportion of methyl- butyrate was higher in calves fed a roughage combination of dried grass and straw (C70-G15-S15) (contrast C; \( P < 0.001 \)). A tendency to a higher molar proportion of methyl-butyrate was present in calves fed a higher corn silage to concentrate ratio (contrast D; \( P < 0.1 \)). Finally, lower molar proportions of valerate were observed in calves fed straw compared with calves fed either dried grass or corn silage as additional source of roughage (contrast B; \( P < 0.001 \)).
Lactate concentrations varied between 2.0 and 11.8 mmol/L and were lower in calves fed ad libitum (contrast E; \( P < 0.05 \)). Furthermore, rumen lactate concentrations were slightly lower in calves fed C70-CS30 than in calves fed C70-G30 (4.6 vs. 10.9 mmol/L; contrast B; \( P < 0.1 \)).

Ruminal concentrations of ammonia ranged from 8.2 to 25.8 mmol/L, and were lower in ad libitum fed calves (contrast E; \( P < 0.01 \)), and higher in calves fed concentrates only (contrast A; \( P < 0.001 \)). Finally, calves fed a mixture of grass and straw as source of roughage had higher concentrations of ammonia than calves fed either grass or straw as a roughage source (contrast C; \( P < 0.05 \)).

The concentration of RS varied between 47.67 and 75.1 g/kg DM of whole rumen content. Calves fed the C70-CS30 diet had a lower RS concentration (47.7 g/kg DM) than calves fed the C70-G30 diet (75.1 g/kg DM; contrast B; \( P < 0.05 \)). Ad libitum feeding did not have an effect on the rumen RS concentration (contrast E, \( P > 0.05 \)).

The recovery of cobalt ranged from 21.8 to 34.9% and was highly variable between calves within treatments. Calves fed the C70-G15-S15 diet tended to have lower cobalt recoveries than calves fed either C70-G30 or C70-S30 diets (\( P < 0.1 \); contrast C).

Results from the analysis of covariance showed that variation in DMI (average during the last week prior to slaughter) significantly explained variation in total VFA, the molar proportion of butyrate and the concentration of ammonia (estimates of regression coefficients ± S.E. for DMI: 77.1 ± 22.4, 0.53 ± 0.22, and -11.5 ± 4.7, respectively; \( P < 0.05 \)). Since DMI in ad libitum fed calves was much higher than in calves fed restricted diets (Table 3), but dietary treatments did not significantly affect total VFA concentrations (Table 4), the contrast between restricted and ad libitum fed diets (Contrast E) in total VFA concentrations became significant (\( P = 0.01 \)) after analysis of covariance. Significant differences in the molar proportion of butyrate between dietary treatments C70-S30, C70-G30 and C70-CS30 (contrast B), consistently coincided with differences in DMI (Tables 3 and 4). Thus, following analysis of covariance, contrast B in the molar proportion of butyrate was no longer significant (\( P > 0.05 \)). Likewise, the significant contrast between restricted and ad libitum diets in the concentration of ammonia (Table 4, contrast E) became non-significant after analysis of covariance because of accompanying differences in DMI (Table 3).

Effects of time between last feeding and slaughter on rumen pH, total VFA and lactate concentrations, and cobalt recovery are shown in table 5. Rumen pH was not significantly influenced by the time of slaughter (\( P > 0.05 \)). However, the total VFA concentrations in rumen
fluid rose from 108.3 mmol/L 1.5 h post feeding to 143.8 mmol/L 3.5 h post feeding, and decreased to 134.6 mmol/L 4.5 h post feeding ($P < 0.05$). Lactate concentration and cobalt recovery declined with time post feeding ($P < 0.001$).

**Macroscopic and Microscopic Evaluation of the Rumen Wall**

Results from the macroscopic evaluation of the rumen mucosa are shown in Table 6. The incidence of plaque formation was affected by dietary treatments and varied between 0 and 100%. The rumen mucosa of calves fed the C100, C70-CS30 and C40-CS60 diets showed higher incidence of plaque formation (63 to 100%) than those fed either C70-S30, C70-G30 or ad libitum diets (0 to 13%; $P < 0.05$). Although in some dietary treatments the incidence of calves with poor developed mucosa was zero (0 %), no significant differences ($P > 0.05$) were observed.

Empty rumen weights and the morphometric parameters of the rumen wall are presented in Table 7. The empty rumen weights of the calves varied between 953 and 1448 g. Calves in the ad libitum fed groups had heavier empty rumen weight ($P < 0.001$; contrast E). Likewise, among restrictedly fed treatments, calves on the all concentrate diet showed the highest averaged empty rumen weight ($P < 0.001$; contrast A).

In the dorsal rumen location, calves fed dried grass as additional source of roughage showed a higher mucosa to serosa length ratio (RSML) than those fed with either straw or corn silage ($P < 0.05$; contrast B). Furthermore, ad libitum fed calves tended to have ($P < 0.1$) an increased thickness of the mucosa (MCT) compared with restrictedly fed calves (contrast E; $P < 0.1$). Finally, increasing the corn silage to concentrate ratio tended to increase the thickness of the muscle wall (MST) (contrast D; $P < 0.1$). In the ventral location of the rumen, except for a decrease in the MST of the rumen wall in calves fed all concentrate diet (contrast A, $P < 0.05$), no other treatment effects were observed.

The analysis of covariance of rumen weight and the rumen wall morphometric parameters revealed that variation in empty rumen weight was significantly explained by variation in DMI (average during the entire experimental period) (estimate of regression coefficient ± S.E. for DMI: $651 ± 145$, $P < 0.001$). Variation in DMI tended to explain variation in the ratio of mucosa to serosa length (RMSL) and mucosa thickness (MCT) in the ventral rumen (estimates of regression coefficients ± S.E. for DMI: $2.91 ± 1.52$, and $695 ± 370$, respectively; $P < 0.07$).
**Enzyme Activity in Whole Rumen Contents**

The effects of experimental treatments on PDE activities extracted from the whole rumen contents are presented in Table 8. With soybean hulls as substrate, PDE activities varied between 1.8 and 4.1 mmol.min\(^{-1}\).g DM\(^{-1}\). Calves fed C70-G30 showed higher PDE activities than those fed C70-S30 or C70-CS30, respectively (contrast B; \(P < 0.001\)). Furthermore, ad libitum fed calves had decreased PDE activities when compared to restrictedly fed calves (contrast E; \(P < 0.001\)). With sugar beet pulp as substrate, the PDE activities varied between 3.1 and 6.5 mmol.min\(^{-1}\).g DM\(^{-1}\). Among restrictedly fed treatments, the calves fed concentrate showed higher PDE activities than those fed additional roughage (contrast A; \(P < 0.001\)). With native corn starch as substrate, the PDE activities varied between 2.2 and 9.4 mmol.min\(^{-1}\).g DM\(^{-1}\). Calves fed C70-S30 showed lower PDE activities than those fed C70-CS30 or C70-G30, respectively (contrast B; \(P < 0.001\)). Ad libitum fed calves had lower PDE activities than those fed restrictedly (contrast E; \(P < 0.001\)). Finally, PDE activities in calves fed a combination of straw and grass (C70-G15-S15) were lower than in calves fed either straw or dried grass (contrast C; \(P < 0.001\)). With straw as substrate, the PDE activities varied between 0.4 and 0.7 mmol.min\(^{-1}\).g DM\(^{-1}\). Ad libitum fed calves had lower PDE activities than restrictedly fed calves (contrast E; \(P < 0.001\)). Moreover, calves fed concentrates only tended to have lower PDE activity (contrast A; \(P < 0.1\)).

**DISCUSSION**

**Feed Intake and Body Weight Gain**

In the present experiment, DMI from concentrate and roughage was restricted to 750 g/d in all except the ad libitum fed treatments. Treatment effects on DMI of roughage and concentrates in this experiment were in line with effects on feed intake found previously in literature (Mertens, 1997, compare contrast B, Table 3), where increased fiber content in the negatively affected DMI (Mertens, 1997, compare contrast B, Table 3). Substituting a part of the concentrate with roughage, however, did not affect DMI and ADG (contrast A).

The lower intake of milk replacer in the ad libitum fed calves (see description in material and methods) does not allow a direct comparison of calf performance across treatments. Therefore, growth rates on both intake levels of milk replacer were simulated using the growth model of Gerrits et al. (1997). Additional growth (AG) from roughage and concentrate was calculated by the difference between the observed ADG and the simulated ADG on milk replacer only, and
expressed per unit DMI from concentrate and roughage. Additional growth in the various treatments ranged from 0.49 to 0.55 and from 0.46 to 0.50 kg/kg feed for restrictedly and ad libitum fed calves, respectively. For restrictedly fed calves, AG for calves fed the C-100 diet was higher (0.55 kg/kg) than for calves fed C70-S30 (0.49 kg/kg). This may be related to the increased yield of absorbed nutrients of concentrates when compared with straw. Moreover, among ad libitum fed groups a lower AG was obtained in calves fed the C70-G15-S15-AL diet than in those fed the C70-CS30-AL diet. Finally, restrictedly fed calves showed increased averaged AG (0.52 kg/kg) when compared with ad libitum fed calves (0.48 kg/kg).

In the present experiment, the observed AG in the restricted treatments was not within the AG range observed in a previous experiment (Suárez et al., 2006a). Moreover, the AG on the all concentrate diet was slightly lower than the AG obtained in a previous experiment where a similar diet was fed to calves until 8 or 12 weeks of age (0.86 and 0.61 kg/kg, respectively; Suárez et al., 2006a).

**Ruminal Fermentation Parameters**

In the present experiment an unexpected small range of low rumen pH was observed (5.1 - 5.3) and roughage did not significantly increase rumen pH. The rumen pH value of the calves fed the concentrate only diet is in line with the value of 5.2 reported previously in calves aged 8 and 12 weeks fed a similar diet (Suárez et al., 2006a). In rearing calves maximum VFA concentrations have been reported 3-4 h post feeding (= 100-110 mmol/L; Stobo et al., 1966). In line with these findings, in the present experiment maximum VFA concentrations were observed 3.5 h after feeding. In this experiment the total VFA concentrations were close to those observed in adult ruminants (120 to 160 mmol/L; Bergman, 1990) and to those observed in roughage supplemented rearing calves (= 120 mmol/L; Vázquez-Anon et al., 1993; = 150 mmol/L; Zitnan et al., 1998). Total VFA concentrations of calves fed C-100 observed in this study were higher than those previously recorded in calves fed a similar diet and slaughtered at a similar age (Suárez et al. 2006a), mostly likely reflecting differences in time of sampling post-feeding (1 to 3 h vs. 1.5 to 4.5 h, respectively). Ruminant diets based on roughage, especially those rich in structural carbohydrates, are known to produce high molar proportions of acetate. Furthermore, as the roughage to concentrate ratio decreases, the acetate to propionate ratio also decreases (Bannink et al., 2006). In line with these observations, in the current trial the molar proportion of
acetate increased when straw was present in the diet (see table 4, contrast B). Also, molar proportions of acetate and propionate were lowest and highest in calves fed the all concentrate diet, respectively (contrast A), in line with observations in rearing calves (Bull et al., 1965; Stobo et al., 1966; Anderson et al., 1982; Zitnan et al., 1998). The effect of diet on the molar proportion of butyrate seemed to be mediated by DMI, since the contrast between C70-S30, C70-G30 and C70-CS30 (contrast B) was no longer significant after analysis of covariance with the average DMI during the last week prior to slaughter acting as a covariable.

Lactate concentration in rumen fluid was reduced in the ad libitum fed groups (≈ 3.1 vs. ≈ 8.3 mmol/L). In addition, lactate concentrations in the restrictedly-fed groups were close to concentrations reported in adult ruminants facing sub-clinical acidosis (≈ 10 mmol/L; Hristov et al., 2001). The lower concentrations in the ad libitum fed groups may reflect an improved capability of rumen microorganisms to utilize lactate (Counotte et al., 1981; Williams and Martin, 1990; Van Soest, 1994). The rapid decline in lactate concentrations after 1.5 h after feeding is in line with observations reported in dairy cattle (Counotte et al., 1983; Chamberlain et al., 1983). Finally, the lactate concentration of the control group (C-100) observed in this study (9.4 mmol/L) was higher than that previously recorded in calves fed a similar diet (5 mmol/L, Suárez et al., 2006a).

The ammonia concentrations reported in this experiment are within the range reported by Zitnan et al. (1998) (6 - 21 mmol/L) in rearing calves fed concentrate and roughage (alfalfa) supplemented. Ammonia concentrations were clearly associated with DMI; correcting for differences in DMI removed significance of the contrast in ammonia between restricted and ad libitum diets. The reduced ammonia concentrations in calves fed ad libitum may indicate an improved utilization (higher microbial efficiency) by the rumen microorganisms related to increased fractional passage rates that usually occur upon higher DM intake levels (Dijkstra et al., 2002), or increased absorption across a more mature rumen wall. The implication of a higher microbial efficiency is supported by a decrease in the proportion of branched –chain volatile fatty acids observed in the ad libitum fed groups. Branched-chain volatile fatty acids are mainly end products of protein fermentation and are, along with ammonia, often used as quick indicators of protein fermentation in the rumen (Yang, 2002).

The etiology of ruminal drinking (ruminal drinkers refers to calves that develop chronic indigestion because milk is deposited into the rumen as a result of failure of the reticular groove
reflex during drinking) has been well described (Pochón, 2002). The pathology with the clinical symptoms was experimentally reproduced by Van Weeren-Keverling Buisman et al. (1990a, b) and the metabolic and endocrine profiles were characterized by Herrli-Gygi et al. (2006). Quantitative information on ruminal drinking, however, is scarce and absent in calves fed concentrates or roughage in addition to milk replacer. Small amounts of milk leakage have been observed in normal veal calves (≈ 3%) but quite considerable amounts have been reported in veal calves considered clinically sick (up to 50%; Dos Santos et al., 1986). In the present study, although the presence of milk replacer in rumen was considerable (≈ 25%), the concentrations of lactate were lower than results reported by Gentile et al. (2004) in clinically sick animals (15-30 mmol/L). Moreover, despite the on average high recovery of Co in the rumen, none of the calves showed signs of illness, like reduced appetite or bloat which indicates that in veal calves fed additional solid feeds (either concentrate or concentrate with roughage) a substantial amount of milk can flow into the rumen without inducing symptoms of the syndrome. The marker technique used does not allow discriminating between the functioning of the esophageal groove reflex and potential reflux of milk replacer from the abomasum, and the technique may suffer from some limitations. Potential influences of transport and slaughter procedures on Co recovery cannot be excluded, although extreme caution was taken to avoid any such bias. The fractional passage rate of rumen liquid ($K_{pl}$) was estimated at 46%/h, which is extremely high when compared with adult cows (≈15%/h; Offer and Dixon, 2000). Applying this fractional passage rate to estimate the Co pool size at time = 0 h indicates that all milk replacer entered the rumen, suggesting either methodological problems or that variable fractional passage rates apply. Nonetheless, the high recovery of Co in the rumen illustrates a potentially large problem, worthwhile investigating.

**Macroscopic and Microscopic Evaluation of the Rumen Wall**

Previously, Suárez et al. (2006b) observed in concentrate-only supplemented calves a high incidence of plaque formation (see picture 1) and poor rumen development when judged macroscopically. The occurrence of plaque has been related to the absence of coarse material in the rumen (Nocek et al., 1984). Similar observations were described by Di Giancamillo et al. (2003) in veal calves fed either pelleted or extruded diets. In the present trial, the macroscopic evaluation of the rumen clearly showed the beneficial effects of roughage (except corn silage treatments fed restrictedly) to reduce incidence of plaque and of poorly developed mucosa (see
The increased empty rumen weight found in calves fed only concentrate may have been influenced by plaque formation (see table 6). The ad libitum treatments resulted in significantly higher empty rumen weights related to the higher intake whereas no plaque was observed in these calves.

Harrison et al. (1960) already suggested that adequate physical stimulation (“roughage scratch factor”) is required to maintain a healthy rumen mucosa, to stimulate rumen motility and consequently the proper development of the muscular wall. Despite the effect of roughage intake on the macroscopic evaluation of rumen mucosa, the addition of roughage hardly affected the microscopic morphology of the rumen wall. Roughage addition did significantly increase muscle thickness in the ventral rumen indicating enhanced physical stimulation and rumen motility.

**Enzyme Activity and Soluble Sugars in Rumen Contents**

Quantification of the activity of various enzyme systems in whole rumen contents is a way to evaluate the capability of the ruminal microflora to degrade the dietary substrates (Silva et al., 1987; Suárez et al., 2006a). Treatment effects on PDE (a consortium of enzyme activities incubated with various natural substrates) observed in this experiment generally indicated an adaptation of the rumen microorganism to the diets, similar to effects observed by Suárez et al. (2006a). In the present experiment the activities of most PDE (except when using sugar beet pulp as a substrate, Table 8) were reduced in the ad libitum-fed groups, indicating a reduced fractional substrate hydrolysis rate at high intake levels. The increased enzyme activity in restricted fed calves may be the result of different patterns of feed intake and consequently of microbial activity. Ad libitum fed calves consumed the concentrate/roughage portions throughout the day, whereas restricted fed calves consumed the feed rapidly after supply. Therefore, at time of slaughter relatively more freshly entered substrate is available for microorganisms in the restricted fed calves, which may have stimulated the enzymatic activity.

In adult ruminants it is assumed that soluble carbohydrates are rapidly taken up by rumen microorganisms. Consequently, concentrations of RS are usually low (≈ 0.04 and 5.55 g/kg DM; Hristov et al., 2000; Hristov et al., 2001; Hristov and Ropp, 2003). The results of the present experiment confirm earlier observations of high concentrations of RS in rumen contents (Suárez et al., 2006a). It was suggested that at low pH, high concentrations of RS in rumen contents may result from reduced uptake of RS by the microorganisms to regulate their intracellular pH, whilst
the PDE were still active. The additional supply of roughage in the present experiment did not improve the RS utilization by the rumen microorganisms (see table 4): concentrations ranged from 47.7 to 75.1 g RS/kg DM in the present experiment, and between 45 and 61 g RS/kg DM in the concentrate-fed veal calves in an earlier experiment (Suárez et al., 2006a).

CONCLUSIONS

The results of the present experiment show substitution of part of the concentrate by roughage did not affect DMI and ADG, but among roughage sources feeding straw reduced DMI and ADG. The addition of roughage did not affect rumen pH, total VFA concentrations and RS levels, but increased acetate to propionate ratio. Cobalt recovery, as an indication of milk leakage, was high but not affected by dietary treatments. Ad libitum fed calves had lower rumen lactate and ammonia levels and a higher empty rumen weight and fresh rumen contents. Results indicated that in veal calves, roughage addition to concentrate diets did not affect growth performance and, by decreasing the incidence of plaque formation, positively influenced the macroscopic appearance of the rumen wall. However, morphometric parameters of the rumen wall were hardly influenced by the type and level of roughage.
Table 1 Milk replacer scheme, concentrate and roughage source, amount of DM supplied and acronyms of diets.

<table>
<thead>
<tr>
<th>Dietary Treatment</th>
<th>Milk replacer scheme</th>
<th>Concentrate supply</th>
<th>Straw supply</th>
<th>Grass supply</th>
<th>Corn silage supply</th>
<th>Maximum supplied (g DM/d)</th>
<th>Acronyms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentrate</td>
<td>1</td>
<td>100</td>
<td></td>
<td></td>
<td></td>
<td>750</td>
<td>C100</td>
</tr>
<tr>
<td>Concentrate/ Corn Silage</td>
<td>1</td>
<td>40</td>
<td>60</td>
<td></td>
<td></td>
<td>750</td>
<td>C40-CS60</td>
</tr>
<tr>
<td>Concentrate/Straw</td>
<td>1</td>
<td>70</td>
<td>30</td>
<td></td>
<td></td>
<td>750</td>
<td>C70-S30</td>
</tr>
<tr>
<td>Concentrate / Grass</td>
<td>1</td>
<td>70</td>
<td>30</td>
<td></td>
<td></td>
<td>750</td>
<td>C70-G30</td>
</tr>
<tr>
<td>Concentrate / Corn Silage</td>
<td>1</td>
<td>70</td>
<td>30</td>
<td></td>
<td></td>
<td>750</td>
<td>C70-CS30</td>
</tr>
<tr>
<td>Concentrate / Grass / Straw</td>
<td>1</td>
<td>70</td>
<td>15</td>
<td>15</td>
<td></td>
<td>750</td>
<td>C70-G15-S15</td>
</tr>
<tr>
<td>Concentrate/ Corn Silage</td>
<td>2</td>
<td>70</td>
<td></td>
<td>30</td>
<td>Ad lib</td>
<td></td>
<td>C70-CS30-AL</td>
</tr>
<tr>
<td>Concentrate/ Grass / Straw</td>
<td>2</td>
<td>70</td>
<td>15</td>
<td>15</td>
<td>Ad lib</td>
<td></td>
<td>C70-G15-S15-AL</td>
</tr>
</tbody>
</table>

1 Milk replacer scheme in material and methods. 2 Concentrate composition in Table 2. 3 Chopped barley straw. 4 Chopped dried grass. 5 Corn Silage: maximal 120 ppm of iron on DM basis.

Table 2. Ingredient and analyzed nutrient composition of the concentrates and roughages.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>IFN1</th>
<th>Concentrate</th>
<th>Corn Silage</th>
<th>Straw</th>
<th>Grass</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beet pulp (ground) (%)</td>
<td>4-00-672</td>
<td>30.3</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Soybean Hulls (%)</td>
<td>1-04-560</td>
<td>15.5</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Corn Grits (%)</td>
<td>4-03-011</td>
<td>15.5</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Corn (broken) (%)</td>
<td>4-20-689</td>
<td>15.5</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Barley (crushed) (%)</td>
<td>4-00-549</td>
<td>15.5</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Soybean Oil (%)</td>
<td>na</td>
<td>0.5</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Soluble wheat protein (%)</td>
<td>na</td>
<td>4.0</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Urea (%)</td>
<td>na</td>
<td>1.2</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Vinasses (%)</td>
<td>na</td>
<td>2.0</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Analyzed nutrient composition

<table>
<thead>
<tr>
<th></th>
<th>Concentrate</th>
<th>Corn Silage</th>
<th>Straw</th>
<th>Grass</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry Matter (g/kg product)</td>
<td>878</td>
<td>360</td>
<td>899</td>
<td>881</td>
</tr>
<tr>
<td>Crude Protein (g/kg DM)</td>
<td>193</td>
<td>68</td>
<td>BD</td>
<td>227</td>
</tr>
<tr>
<td>Crude Fat (g/kg DM)</td>
<td>41</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Crude Ash (g/kg DM)</td>
<td>48</td>
<td>46</td>
<td>101</td>
<td>122</td>
</tr>
<tr>
<td>Starch (g/kg DM)</td>
<td>250</td>
<td>375</td>
<td>BD</td>
<td>6</td>
</tr>
<tr>
<td>Sugars (g of glucose/kg DM)</td>
<td>47</td>
<td>7</td>
<td>BD</td>
<td>84</td>
</tr>
<tr>
<td>NDF (g/kg DM)</td>
<td>292</td>
<td>343</td>
<td>805</td>
<td>433</td>
</tr>
<tr>
<td>ADF (g/kg DM)</td>
<td>158</td>
<td>193</td>
<td>525</td>
<td>233</td>
</tr>
<tr>
<td>ADL (g/kg DM)</td>
<td>8</td>
<td>13</td>
<td>63</td>
<td>22</td>
</tr>
<tr>
<td>Ca (g/kg DM)</td>
<td>4.7</td>
<td>1.6</td>
<td>3.2</td>
<td>7.3</td>
</tr>
<tr>
<td>P (g/kg DM)</td>
<td>2.3</td>
<td>2.0</td>
<td>0.3</td>
<td>3.9</td>
</tr>
</tbody>
</table>

BD = Below detection levels (= 7.5 mg Cu/kg DM; 0.075 g Na/kg DM; 6 g sugars/kg DM; 3 g starch/kg; 19 g crude protein/kg DM; 3 g N/kg*6.25). IFN1: International Feed Number (obtained from NRC 1989). Na: Not available
Table 3. Effect of dietary treatments on milk replacer intake, dry matter intake (DMI), final body weight, average daily gain (ADG) and feed conversion ratio.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Dietary Treatments</th>
<th>Contrast\textsuperscript{1,2}</th>
<th>SEM</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk Replacer Intake (kg)</td>
<td>C100</td>
<td>C40-CS60</td>
<td>C70-S30</td>
<td>C70-G30</td>
<td>C70-CS30</td>
<td>C70-G15-S15</td>
<td>C70-CS30-AL</td>
<td>C70-G15-S15-AL</td>
</tr>
<tr>
<td>(Week 1-10)</td>
<td>53.1</td>
<td>53.1</td>
<td>53.0</td>
<td>53.1</td>
<td>53.1</td>
<td>42.4</td>
<td>42.2</td>
<td>0.07</td>
</tr>
<tr>
<td>Average DMI (kg/day) (Week 1-10)</td>
<td>0.50</td>
<td>0.49</td>
<td>0.43\textsuperscript{b}</td>
<td>0.59\textsuperscript{a}</td>
<td>0.55\textsuperscript{a}</td>
<td>0.54</td>
<td>0.81</td>
<td>0.78</td>
</tr>
<tr>
<td>Wk 1</td>
<td>0.12</td>
<td>0.07</td>
<td>0.07</td>
<td>0.09</td>
<td>0.09</td>
<td>0.06</td>
<td>0.12</td>
<td>0.09</td>
</tr>
<tr>
<td>Wk 5</td>
<td>0.51</td>
<td>0.53</td>
<td>0.47\textsuperscript{b}</td>
<td>0.69\textsuperscript{a}</td>
<td>0.65\textsuperscript{a}</td>
<td>0.58</td>
<td>0.69</td>
<td>0.61</td>
</tr>
<tr>
<td>Wk 6</td>
<td>0.63</td>
<td>0.65</td>
<td>0.54\textsuperscript{b}</td>
<td>0.73\textsuperscript{a}</td>
<td>0.70\textsuperscript{ab}</td>
<td>0.66</td>
<td>0.94</td>
<td>0.82</td>
</tr>
<tr>
<td>Wk 10</td>
<td>0.50</td>
<td>0.60</td>
<td>0.53</td>
<td>0.72</td>
<td>0.67</td>
<td>0.69</td>
<td>1.26</td>
<td>1.47</td>
</tr>
<tr>
<td>Final Body Weight (kg)</td>
<td>99.5</td>
<td>97.8</td>
<td>95.6</td>
<td>102.3</td>
<td>101.4</td>
<td>99.8</td>
<td>99.0</td>
<td>96.1</td>
</tr>
<tr>
<td>ADG (g/d)</td>
<td>754</td>
<td>726</td>
<td>688\textsuperscript{b}</td>
<td>779\textsuperscript{a}</td>
<td>774\textsuperscript{a}</td>
<td>753</td>
<td>742</td>
<td>696</td>
</tr>
<tr>
<td>Feed Conversion (Total kg DMI/ kg Growth) \textsuperscript{4}</td>
<td>1.64</td>
<td>1.70</td>
<td>1.69</td>
<td>1.69</td>
<td>1.66</td>
<td>1.69</td>
<td>1.88</td>
<td>1.96</td>
</tr>
</tbody>
</table>

\textsuperscript{a,b} Means in the same row, within columns C70-S30, C70-G30 and C70-CS30 with different superscript differ significantly ($P < 0.05$).

\textsuperscript{1} Contrast: A= C100 vs. (all diets with additional roughage, except the two ad libitum); B= C70-S30 vs. C70-G30 vs. C70-CS30 (mutually); C= C70-G15-S15 vs. (C70-S30 and C70-G30); D= C70-CS30 vs. C40-CS60; E= (C70-G15-S15 and C70-CS30) vs. (ad libitum diets).

\textsuperscript{2} Statistical significance + $P < 0.10$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

\textsuperscript{3} DMI from concentrate and roughage, excluding DMI from milk.

\textsuperscript{4} Total dry matter intake (including milk replacer)/body weight gain.
Table 4. Effect of dietary treatments on rumen fermentation characteristics, weight of rumen contents and cobalt recovery.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Dietary Treatments</th>
<th>SEM A</th>
<th>SEM B</th>
<th>SEM C</th>
<th>SEM D</th>
<th>SEM E</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total VFA, mmol/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetate %</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Propionate %</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Butyrate %</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Isobutyrate %</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methyl butyrate %</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Valerate %</td>
<td></td>
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<td>Lactate, mmol/L</td>
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<td>Ammonia, mmol/L</td>
<td></td>
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<tr>
<td>Reducing Sugars, g/kg DM</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Cobalt recovery %</td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>Weight of fresh rumen contents, kg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DM of fresh rumen contents, g/kg</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

Contrast 1^2:
- A = C100 vs. (all diets with additional roughage, except the two ad libitum)
- B = C70-S30 vs. C70-G30 vs. C70-CS30 (mutually)
- C = C70-G15-S15 vs. (C70-S30 and C70-G30)
- D = C70-CS30 vs. C40-CS60
- E = (C70-G15-S15 and C70-CS30) vs. (ad libitum diets)

Statistical significance + P<0.10; * P≤0.05; ** P≤0.01; *** P<0.001.

---

^a,b, Means in the same row, within columns C70-S30, C70-G30 and C70-CS30 with different superscript differ significantly (P<0.05).

1 Contrast: A= C100 vs. (all diets with additional roughage, except the two ad libitum); B= C70-S30 vs. C70-G30 vs. C70-CS30 (mutually); C= C70-G15-S15 vs. (C70-S30 and C70-G30); D= C70-CS30 vs. C40-CS60; E= (C70-G15-S15 and C70-CS30) vs. (ad libitum diets).

2 Statistical significance + P<0.10; * P≤0.05; ** P≤0.01; *** P<0.001.
Table 5. Effect of time of sampling after feeding on rumen pH, rumen VFA, rumen lactate and cobalt recovery.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Time after feeding (h)</th>
<th>SEM</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1.5</td>
<td>2.5</td>
<td>3.5</td>
</tr>
<tr>
<td>pH</td>
<td>5.20</td>
<td>5.22</td>
<td>5.15</td>
</tr>
<tr>
<td>Total VFA, mmol/L</td>
<td>108.3b</td>
<td>134.8a</td>
<td>143.8a</td>
</tr>
<tr>
<td>Lactate, mmol/L</td>
<td>12.4a</td>
<td>7.8b</td>
<td>5.4bc</td>
</tr>
<tr>
<td>Cobalt recovery %</td>
<td>48.0a</td>
<td>26.8b</td>
<td>18.3bc</td>
</tr>
</tbody>
</table>

\[ab\] Means in the same row with different superscript differ significantly (P< 0.05).

Table 6. Effect of dietary treatments on the incidence of poorly developed rumen mucosa and the incidence of plaque Formation (macroscopic).

<table>
<thead>
<tr>
<th>Diets</th>
<th>Plaque (% Calves)</th>
<th>Poor development of mucosa (% Calves)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C100</td>
<td>C40-CS60</td>
</tr>
<tr>
<td></td>
<td>C70-S30</td>
<td>C70-G30</td>
</tr>
<tr>
<td></td>
<td>C70-CS30</td>
<td>C70-G15-S15</td>
</tr>
<tr>
<td></td>
<td>C70-CS30-AL</td>
<td>C70-G15-S15-AL</td>
</tr>
<tr>
<td>Plaque (% Calves)</td>
<td>100a</td>
<td>63b</td>
</tr>
<tr>
<td>Poor development of mucosa (%</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>C100</td>
<td>38</td>
<td></td>
</tr>
</tbody>
</table>

\[ab\] Means in the same row with different superscript differ significantly (P< 0.05).

(Non-parametric analysis. Fisher’s exact tests).
### Table 7. Effect of dietary treatments on rumen weight and morphometric variables of rumen wall.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Diet</th>
<th>SEM</th>
<th>Contrast¹²</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C100</td>
<td>C40-C60</td>
<td>C70-S30</td>
</tr>
<tr>
<td>Rumen weight (g)</td>
<td>1448</td>
<td>1079</td>
<td>953</td>
</tr>
<tr>
<td>Rumen dorsal location</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ratio of mucosa length to serosa length</td>
<td>5.0</td>
<td>4.2</td>
<td>4.0 b</td>
</tr>
<tr>
<td>Mucosa Thickness (MCT, μm)</td>
<td>702</td>
<td>681</td>
<td>506</td>
</tr>
<tr>
<td>Muscle Thickness (MST, μm)</td>
<td>1285</td>
<td>1678</td>
<td>1484</td>
</tr>
<tr>
<td>Rumen ventral location</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ratio of mucosa length to serosa length</td>
<td>4.3</td>
<td>4.0</td>
<td>4.2</td>
</tr>
<tr>
<td>Mucosa Thickness (MCT, μm)</td>
<td>595</td>
<td>572</td>
<td>593</td>
</tr>
<tr>
<td>Muscle Thickness (MST, μm)</td>
<td>815</td>
<td>1204</td>
<td>1198</td>
</tr>
</tbody>
</table>

¹ Contrast: A= C100 vs. (all diets with additional roughage, except the two ad libitum); B= C70-S30 vs. C70-G30 vs. C70-CS30 (mutually); C= C70-G15-S15 vs. (C70-S30 and C70-G30); D= C70-CS30 vs.C40-CS60); E= (C70-G15-S15 and C70-CS30) vs. (ad libitum diets).

² Statistical significance: + P<0.10; * P≤ 0.05; ** P≤ 0.01; *** P< 0.001.
**Table 8.** Effects of dietary treatments on enzyme activity of whole rumen contents (mmol reducing sugars released min\(^{-1}\)g DM\(^{-1}\))

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Diets</th>
<th>Contrast 1,2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C100</td>
<td>C40-CS60</td>
</tr>
<tr>
<td>Soybean hulls</td>
<td>3.6</td>
<td>3.7</td>
</tr>
<tr>
<td>Sugar beet pulp</td>
<td>6.5</td>
<td>3.4</td>
</tr>
<tr>
<td>Native corn starch</td>
<td>6.4</td>
<td>9.4</td>
</tr>
<tr>
<td>Straw</td>
<td>0.4</td>
<td>0.7</td>
</tr>
</tbody>
</table>

1. Contrast: A= C100 vs. (all diets with additional roughage, except the two ad libitum); B= C70-S30 vs. C70-G30 vs. C70-CS30 (mutually); C= C70-G15-S15 vs. (C70-S30 and C70-G30); D= C70-CS30 vs. C40-CS60); E= (C70-G15-S15 and C70-CS30) vs. (ad libitum diets).

2. Statistical significance + P<0.10; * P ≤ 0.05; ** P ≤ 0.01; *** P < 0.001;
Picture 1. a) This photo shows a poor development of the rumen mucosa, with little papillae and a lack of healthy dark coloration associated with microbial fermentation in the rumen. b) This photo shows excellent mucosa development, with a healthy dark coloration associated with proper feeding of veal calves (up to 10 wks of age). The dark/brown colour is due to a microbial fermentation and increased mucosa development. c) This photo shows the plaque formations in the rumen mucosa. Plaque= (rumen mucosa containing focal or multi-focal patches of foci with coalescing and adhering papillae covered by a sticky mass of feed, hair and cell debris).
REFERENCES


CHAPTER 5

The Importance of Using Adapted Microflora as Inoculum for Testing Fermentation Characteristics of Carbohydrate Sources for Veal Calves, Using the In Vitro Cumulative Gas Production Technique.

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Chapter 5

ABSTRACT

Two experiments were conducted to estimate the fermentation kinetics of various solid feeds fed to veal calves, and to study the effect of adaptation of the rumen microflora to these solid feeds on their subsequent in vitro fermentation patterns.

In the first experiment, three out of five dietary treatments were selected as inoculum sources (each inoculum consisted of pooled rumen contents from three crossbred calves, slaughtered at 12 weeks of age). Briefly, the selected dietary treatments were concentrates based on pectin (PECTIN), neutral detergent fiber (NDF), and starch (STARCH). Sugar beet pulp (SBP), sugar beet pectin (SBPec), native corn starch (NCS), soy bean hulls (SBH) and crystalline cellulose (AVICEL) were selected as in vitro substrates to examine the in vitro degradation of the main sources of dietary carbohydrate and characterization of the cell wall fraction.

In the second experiment, three out of eight dietary treatments were selected as inocula (each comprising mixed rumen contents from two crossbred calves, slaughtered at 10 weeks of age). The selected diet treatments were: C100 = concentrate only, C70-S30 = concentrate (70%) with straw (30%) and C70-CS30 = concentrate (70%) with corn silage (30%). For this gas production experiment, straw (STRAW), soy bean hulls (SBH), native corn starch (NCS) and sugar beet pectin (SBPec) were chosen as in vitro substrates. Soy bean hulls with STRAW were selected to characterize the dietary cell wall fraction degradation, whereas the last two substrates were selected to characterize dietary pectin and starch fraction degradations.

In both experiments, cumulative gas production was measured over time (72 h) as an indicator of the kinetics of fermentation. Fermentation end-products, including volatile fatty acids and ammonia, and organic matter loss, were also measured.

In both experiments significant differences between the inoculum sources, in terms of both fermentation kinetics characteristics and end-products of fermentation were observed. Similarly, significant effects were also observed for substrate compositions. Differences between the fermentation characteristics of NCS, SBPec and SBH, were consistent for both experiments. The total VFA production was not different among these substrates in both experiments. Finally, for both experiments, there was a significant inocula and substrate interaction which may indicate differences in the microbial activity occurring between the calves. Therefore, it was concluded that rumen inoculum from adapted animals should be used to obtain a more accurate assessment of feed ingredients in veal calf diets.

Keywords: Cumulative gas production; fermentable carbohydrate; in vitro fermentation; microbial activity; veal calves.
INTRODUCTION

Traditionally, veal calves have been fed until slaughter weight with milk replacer without the provision of some fiber containing material (solid feeds). However, since 1997, supplementation of veal calf diets with solid feeds is obligatory in EU countries. This supplementation is expected to stimulate rumen development and consequently reduce abnormal oral behavior. Rumen development is triggered by the production of volatile fatty acids (VFA) resulting from fermentation of organic matter in the rumen (Flatt et al., 1958).

Several methodologies have been developed to characterize feedstuffs in terms of digestibility and degradability, comprising in vivo, in situ and in vitro methods. While in vivo measurements are the standard measure of total tract digestibility, there is significant animal variation and high costs involved, so interest is increasingly focused on the use of in vitro and in situ methods (i.e. batch culture digestibility analysis, nylon bag technique, gas production and analysis of enzyme activities (Tilley & Terry 1963, Mehrez and Orskov, 1977; Theodourou et al., 1994; Hristov et al., 1999; Suárez et al., 2006a, b) to estimate digestibility and the extent of substrate degradation in the rumen.

The gas production technique (GPT) provides gas production profiles that give an indication of the fermentative characteristics of the feed. New automated equipment (Automated Pressure Evaluation System (APES); Davies et al., 2000) has made the GPT a useful technique to characterize and improve the current knowledge in the rumen fermentation kinetics of different feedstuffs. However, the GPT can be affected by a number of factors including inoculum source as affected by animal, diet and time effects, inoculum size, and composition and buffering of the incubation medium (Mould et al., 2005). The choice of non-linear models to estimate the fermentation rate is also important as it will affect the calculated rate and extent of degradation (France et al., 2005).

The objective of this paper was to estimate fermentation kinetic parameters of various solid feeds supplied to veal calves using the GPT, and to study the effect of adaptation of the rumen microflora to these solid feeds on their subsequent fermentation patterns.
MATERIALS AND METHODS

Experimental Design, Calves and Diets

Two in vivo experiments were carried out to examine the effect of dietary carbohydrates sources on veal calves performance, rumen fermentation and rumen development (Suárez et al., 2006a,b). As part of each in vivo experiment, an in vitro gas production fermentation run was carried out with various substrates, using the APES (Davies et al., 2000).

In vivo experiments were conducted at the experimental farm “Waiboerhoeve” of Wageningen University & Research Centre in Lelystad, The Netherlands. The in vitro gas productions were carried out at Wageningen University, in Wageningen.

Experiment 1: In this experiment, 160 Holstein Friesian x Dutch Friesian crossbred male calves (averaging 45 kg live weight) were used in a complete randomized block design with a 5x2 factorial arrangement, with carbohydrate source and age as the experimental factors (Suárez et al. 2006a). Three calves from each of three treatments were selected to provide inoculum for the GPT. Briefly, these treatments consisted of: 1) pectin-based concentrate (PECTIN), 2) neutral detergent fiber (NDF) based concentrate, and 3) starch-based concentrate (STARCH). All concentrate diets were provided as pellets, up to a maximum of 750 g DM/d in addition to a commercial milk replacer. Calves were euthanized at 12 weeks of age.

Experiment 2: Sixty-four Holstein Friesian x Dutch Friesian crossbred male calves (averaging 46 kg live weight) were assigned to one of eight treatments in a completely randomized block design. The treatments were designed to evaluate various roughage sources in variables quantities (Suárez et al. 2006b). Two calves from each of three treatments were selected to provide inoculum for the GPT. Briefly, these treatments consisted of (on a DM basis): 1) C100 = concentrate only, 2) C70-S30 = concentrate (70%) with straw (30%), 3) C70-CS30 = concentrate (70%) with corn silage (30%). Concentrate was provided as pellets and roughage was chopped. The dietary treatments were supplied up to a maximum of 750 g DM/d, in addition to a commercial milk replacer. Milk replacer and dietary treatments were provided in separate buckets twice daily (0600 and 1600h). Calves were euthanized at 10 weeks of age. The ingredients and chemical composition are presented in Table 1.
The Importance of Using Adapted Microflora as Inoculum...

Substrates

**Experiment 1:** Five substrates were selected to obtain information on the degradation of the main carbohydrate sources used: Sugar beet pulp (SBP) and sugar beet pectin (SBPec; Genu® Pectin, CP Kelco. DK) were chosen for the characterization of the pectin fraction of the diet; Native corn starch (NCS) was chosen for the characterization of the starch fraction of the diet and soy bean hulls (SBH) and crystalline cellulose (AVICEL, Sigma) were chosen for the characterization of the cell wall fraction.

**Experiment 2:** For this gas production experiment straw (STRAW), soy bean hulls (SBH), native corn starch (NCS) and sugar beet pectin (SBPec) were chosen as substrate. As in the previous experiment SBPec and NCS were selected to characterize dietary pectin and starch fractions respectively, and SBH and STRAW for characterization of the dietary cell wall fraction degradation. The substrates used in both experiments (SBPec, SBH and NCS) belonged to the same original batches and were stored at 4 °C. All substrates were analyzed for their dry matter (DM), ash, nitrogen (N) and acid detergent fiber (ADF). The analyzed composition of all substrates is presented in Table 2.

Inocula

**Experiment 1:** For this study, rumen fluid from three (NDF, PECTIN and STARCH) were used as sources of inoculum. Rumen samples were collected from calves sacrificed at 12 weeks of age.

**Experiment 2:** For this study, rumen fluid from three treatments were used as inocula: C-100, C70-S30 and C70-CS30. Samples were collected from calves sacrificed at 10 weeks of age.

Rumen contents were obtained from calves (n= 3 and n= 2 calves per inoculum for experiments 1 and 2, respectively) euthanased approximately 2-3 hours after the morning feeding. The rumen was opened and samples were collected under a continuous stream of CO₂, and collected in vacuum flasks. These flasks were pre-warmed and also flushed with CO₂. Rumen contents of three calves on the same dietary treatment were pooled on a per weight basis (about 300 g wet wait per calf). The inocula sources were then homogenized using a hand mixer for 60s, strained through a double layer of cheesecloth and prepared as described by Theodorou et al. (1994),
comprising mixing 2.5 ml of rumen fluid and 2.5 ml of 0.9% saline solution. In each experiment, for all inoculum sources, sub-samples were collected and kept at – 20 °C for DM, ash, ammonia and volatile fatty acid (VFA) analysis. Methodologies of each analysis are described in the analysis section.

**Incubations:**

Approximately 500 mg of ground substrates (1 mm sieve) were fermented in 140 ml Duran bottles containing 81 ml of medium (medium B, Williams et al., 2005) and 5 ml inoculum. All samples were incubated in triplicate for each inoculum/substrate combination; bottles containing no substrate were included as blanks.

Patterns of gas production in these samples were studied over a period of 72 hours incubation using an automated in vitro gas production system (Davies et al., 2000- APES-IGER, Aberystwyth, UK). In order to stop the fermentation, bottles were immediately autoclaved at 130 °C (2.0 kg cm⁻² steam pressure) for 30 min. A sample of the fermentation fluid was collected for DM, ash, VFA, and ammonia analysis.

**Analytical Procedures**

Dry matter (DM) of all substrates was determined by drying to a constant weight at 103°C (ISO 6496) and ash by combustion at 550°C (ISO 5984). Nitrogen concentration was determined by the Kjeldahl method with CuSO₄ as the catalyst (ISO 5983). Concentration of ADF was determined according to Van Soest et al. (1991).

The DM content of all the inocula sources was determined by freeze-drying to a constant weight. The OM loss from the bottles was estimated by filtering the residue remaining in the bottles using pre-weighed sintered crucibles (Schott-Duran, porosity #2, Mainz, Germany) under vacuum and incinerating at 550 °C for 4 h. The proportion of OM fermented was calculated as the OM loss divided by OM incubated.

After 72 hours of fermentation, samples from the fermentation fluid were collected and analyzed for volatile fatty acids (VFA) and ammonia concentrations. For VFA analysis, 5 ml of the buffered rumen liquid was added to a glass bottle that contained 0.5 ml of 85 % phosphoric acid (Merck Art. 573, Amsterdam, The Netherlands), samples were centrifuged at 12000 rpm for 10 min. After centrifugation 0.5 ml of the supernatant, 0.2 ml of destilled water and 0.3 ml of iso-
caproic acid were transferred to an auto-sampler glass vial for VFA analysis. The VFA concentrations (in the inoculum sources and in the post-fermentation samples) were analyzed by gas chromatography (Fisons, HRGC Mega 2, CE instruments, Milan, Italy), using a glass column fitted with Chromosorb 101, as carrier gas N\textsubscript{2} saturated with methanoic acid, at 190 °C and using iso-caproic acid as internal standard. The branched and higher chain molar proportion of VFA (BCP) was calculated as the sum of iso-butyric acid, iso-valeric acid and valeric acid divided by the total amount of VFA. Ammonia was determined according to the Berthelot method (Schneider, 1976).

**Curve fitting**

The gas production profiles (mL g\textsuperscript{-1} OM incubated) over 72 hours were fitted to a generalized mono-phasic Michaelis-Menten equation (equation 1; France et al., 2000), by using the non linear regression (NLIN) of the Statistical Analysis Institute Systems package (Marquardt method; SAS Institute Inc., 2003). Briefly, this model allows the estimation of a lag-phase, i.e. the period between the start of the incubation and the start of gas production, and is derived from compartmental analysis. The model allows the fractional degradation rate to decrease continually or to increase to reach a maximum and then decrease again. The criterion for goodness-of-fit was the residual sum of squares (RSS). For SBH substrate, using a di-phasic model provided a better fit (P<0.05); however, in order to be able to compare the SBH degradation profile with the others substrates, the mono-phasic fit was chosen.

\[
Y = \frac{A (t - L)^B}{(t - L)^B + C^B} \\
\text{for } t \geq L 
\]

\(Y\) = Cumulative gas production (mL gas g\textsuperscript{-1} OM incubated);  
\(A\) = Asymptotic gas production (mL gas g\textsuperscript{-1} OM incubated);  
\(B\) = Indicator of the curve slope;  
\(C\) = Time after lag-phase at which 50% of the asymptotic gas production is reached (h);  
\(t\) = Time (h);  
\(L\) = Lag phase before gas production commences (h).
In the aforementioned model, the constraints $L \geq 0$, $B > 0$ and $C > 0$ have to be satisfied. The maximum rate of gas production ($R_{\text{max}}$; ml/g OM incubated/h) and the time at which it occurs ($T_{\text{max}}$; h), were calculated according to the following equation (France et al. 2000)

$$R_{\text{max}} = A C^B B (T_{\text{max}} - L)^{(-B-1)} / (1 + C^B (T_{\text{max}} - L)^{(-B)})^2$$

$$T_{\text{max}} = C [(B-1) / (B+1)]^{(1/B)} + L$$

**Statistical Analysis**

For the two gas production data set, the results for percentage of OM fermented, cumulative gas production and the fitted parameters were subjected to analysis of variance (ANOVA) using the GLM procedure of SAS (2003) with substrate and inoculum as main factors. The statistical model was as follows (2):

$$Y = \mu + I_i + S_j + (I*S)_{ij} + \epsilon_{ijk}$$

(2)

Where:

- $Y$ = Dependent variable to be tested;
- $\mu$ = Experimental mean of the variable;
- $I_i$ = Effect of inoculum; $i$ = Exp 1: NDF, PECTIN, STARCH and Exp 2: C-100, C70-S30, C70-CS30;
- $S_j$ = Effect of substrate; $j$ = Exp 1: Avicel, SBP, SBPec, NCS, SBH and Exp 2: SBPec, SBH, STRAW, NCS;
- $(I*S)_{ij}$ = Effect of inoculum by substrate interaction
- $\epsilon_{ijk}$ = Error term; $k = 1, \ldots, 3$ (3 - replicated bottles).

Differences between treatments means were evaluated using Tukey’s Studentized range test of multiple comparisons. All statistical analyses were performed using the SAS GLM procedure (SAS Institute Inc., 2003).
RESULTS

Representative examples of gas production kinetics (ml g\(^{-1}\) OM), during 72 hrs of fermentation, for all substrate-inoculum combinations, are shown in figure 1 to 5 (Experiment 1) and figure 6 to 9 (Experiment 2).

Experiment 1

Results of the gas production analysis and fermentation end-products of the substrates NCS, SBP, SBPec and SBH used in Experiment 1, are presented in Tables 3 and 4. Using crystalline cellulose as a substrate, no gas was produced (<10 mL/g OM), except when incubated with NDF inoculum (230 mL/g OM). Both the organic matter disappearance (53% for NDF inoculum, 0% for the other inocula) and VFA concentrations at the end-point of 72 hours (7.3 mmol/g OM for the NDF inoculum and close to the blank for the other inocula) were in agreement with this observation. The inclusion of crystalline cellulose in the data set, meant that all treatment effects, including interactions, reached statistical significance. Therefore, it was decided to omit the cellulose data from the statistical analyses.

Fermentation of SBH led to a bi-phasic pattern of gas production (all inocula, see Figure 4), which therefore had a much better fit using a biphasic model (Groot et al., 1996) (P<0.05). However, this complicated the comparison between substrates and inocula, so it was decided to use the parameters of the mono-phasic curves (equation 1), as shown in Figure 4 for the statistical analysis. This was also evident from the large difference between the estimated asymptotic gas production and the measured cumulative gas production for SBH as a substrate (Table 3). Nonetheless, the rate parameters (C, Rmax and Tmax) of these curves were good approximations of the averages of the two phases, and are therefore included.

Significant effects of substrate, inocula, and their interactions, were observed for most parameters. The most important effects are summarized below. Native cornstarch was well fermented with all inocula used (>96%), with Rmax values varying between 33.0 and 39.0 mL/g OM/h. The time at which this maximum was reached (Tmax) was earlier for the STARCH inoculum, when compared with the NDF and PECTIN inocula (4.6 vs. 8.8 and 9.1 h respectively). Fermentation of SBP was less than starch (91- 93%), but reached its maximum quite fast (Tmax of 3.2 or 3.5 h). Sugar beet pectin was almost completely fermented (98- 99%). The Rmax was reached as soon as with SBP, but was higher compared with SBP (61.2 and 52.4
vs. 30.6 and 31.1 mL/g OM/h for NDF and PECTIN inocula, respectively). Fermentation of SBH was more complete for the NDF compared with the PECTIN and STARCH inocula (91 vs. 79 and 80%). For all inocula, SBH had a latest C compared with the other substrates.

Total VFA concentrations were not affected by substrate but differed between inocula (P<0.01). The PECTIN inoculum led to lower VFA concentrations compared with STARCH and NDF inoculum for all substrates. VFA molar proportions were significantly affected by substrate and inoculum and (with one exception) the interaction between substrate and inoculum was also significant. The propionate proportion was higher for the STARCH inoculum for all substrates, though most significantly for NCS. Conversely, NCS also resulted in lower proportions of acetate when compared with other substrates. The SBPec always resulted in the highest molar proportions of acetate, though this was not significant in all cases. The acetate proportion was always higher for the NDF inoculum, compared with the other inocula. The highest butyrate proportion was obtained for NCS as substrate, while SBPEC had the lowest proportion of butyric acid. Ammonia concentrations were lowest for NCS as substrate, and was highest for SBH, particularly when the NDF inoculum was used.

Experiment 2

Results of the gas production analysis and fermentation end-products of Expt. 2 are shown in Tables 5 and 6. As for Expt. 1, biphasic patterns in the fermentation of SBH were observed, leading to a considerable overestimate of the asymptotic gas production. Hence the parameters from the mono-phasic curve were again used for the statistical analysis.

Most of the cumulative gas production parameters were affected by substrate and inoculum (P<0.05). In addition, there was often a significant substrate by inocula interaction for some of these parameters. The total gas production for substrate decreased in the order SBPEC>NCS>SBH>straw. Fermentation of organic matter was highest for NCS and SBPEC (>99%), lower in SBH (>88%), but lowest for straw (~40%). Native cornstarch was fermented most rapidly, with Rmax values of 29.6-52.9 mL/g OM/h, reached in 7.1 to 16.0 h (Tmax). Sugar beet pectin was also rapidly (Rmax 42.6-71.2 mL/g OM/h) fermented, with maximum rates occurring at about 5h after the start of fermentation. The maximum rate of gas production of SBH was slower compared with NCS and SBPEC (8.9 – 12.5 ml/g OM/h), and was reached between 14.1 and 18.7 h after the start of fermentation. Fermentation of straw was characterized by a low
Rmax (< 5 mL/h), reached at ~20h after the start of fermentation. Cumulative gas production was highest for the C100, and lowest for the C70-CS70 inoculum (P<0.001).

Differences in VFA concentrations between substrates were large, in part reflecting the extent of fermentation (i.e. low VFA concentrations for straw, and high concentrations for SBPec). A reduced extent of fermentation of SBH when compared with NCS and SBPec was suggested by a lower cumulative gas production, though not in reduced VFA concentrations at the end of fermentation. The proportion of acetate decreased in the order SBPec, SBH, Straw and NCS. The propionate molar proportion was lower for NCS and SBPec as substrates. The butyrate molar proportion was substantially higher for NCS when compared with the other substrates. The BCP was increased when using NCS and straw when compared with SBPec and SBH as substrates. Inocula effects on VFA production were significant (P<0.01). Interactions between substrate and inocula were observed for the composition of the VFA (P<0.01), but not for total VFA nor ammonia concentrations (P>0.05).

DISCUSSION

The results of the VFA analyses generally confirm those of the gas production analysis. Crystalline cellulose was poorly fermented in the GPT system. This may be related to the crystalline structure, being difficult to access for microbial enzymes (Nozière and Michalet-Doreau, 1994). The only inoculum capable to some extent of fermenting crystalline cellulose was the NDF inoculum, i.e. obtained from the calves with the highest cellulose intake (Suárez et al., 2006a). Fermentation of straw was poor and slow, but better than that of crystalline cellulose. Differences between the fermentation characteristics of NCS, SBPec and SBH were consistent across experiments. The total VFA production was also consistent for these substrates in both experiments. However, cumulative rates of gas production were consistently lower for SBH. Fermentation of NCS and SBPec was characterized by high maximal rates of gas production (30-70 mL/h), which is consistent with observations of Bauer et al. (2001).

In both experiments, the inocula used affected both VFA production and gas characteristics (P < 0.05). The highest cumulative gas production rates were observed for inocula obtained from calves fed the NDF diet (Expt. 1) and the C-100 diet (Expt. 2). In Expt. 2, however, the lowest VFA production rates were observed for the inoculum obtained from calves fed the C-100 diet. The high molar proportion of propionate produced with the STARCH inoculum (Expt. 1) was
remarkable, and is a clear example of a substrate x inoculum interaction, as this increase was quite specific for the NCS substrate/STARCH inoculum combination in Expt. 1. It also confirms the VFA pattern observed in the rumen fluid analyzed in the in vivo trial (Suárez et al., 2006a). The increased molar proportion of acetate for the NDF inoculum when compared with the other inocula, also corresponded with the increased molar proportion of acetate of calves on the NDF diet in the in vivo trial (Suárez et al., 2006a).

Another example of a substrate x inoculum interaction was the poor fermentation of SBH on the PECTIN inoculum in Expt. 1 (low cumulative gas production and VFA production). Unexpectedly, the molar proportion of butyrate was particularly reduced when using SBP or SBPec as substrates, and was markedly increased when using NCS as a substrate in both experiments. This did not correspond with the effect of a starch-rich concentrate on rumen butyrate concentrations (Suárez et al., 2006a). Such an increase in butyrate from fermented starch has been observed before, using the same in vitro gas production technique (Bauer et al., 2001), and has also been described in the human colon (Brouns et al., 2002). Nonetheless, in ruminants, fermentation of starch is commonly accepted to preferentially increase the propionate molar proportion (Van Soest, 1994).

The ammonia concentrations were high for SBH, and low for NCS as substrates. This may relate to the lower crude protein content of NCS (Table 1), or alternatively, by a reduction of protein fermentation. The increased branched-chain proportion observed when using SBH as substrate corresponds with this observation, but this proportion was also increased when NCS was used as substrate.

In general, the results from these experiments indicate differences between various substrates and they are being fermented in relation to a “pre-conditioned” inoculum. The differences observed between inocula have shown that an adapted microflora of calves fed on diets containing different carbohydrates for several weeks, leads to different fermentation patterns. A clear example of this, was the STARCH inoculum, which led to a faster fermentation of NCS (low Tmax), and a strongly increased propionate proportion. Another example is the increased molar proportion of acetate for the NDF inoculum. The interaction between substrate and inoculum, observed for various parameters indicates that for feed evaluation purposes, using inoculum from a non-adapted source may lead to misleading conclusions and mistaken ranking of feedstuffs in terms of their fermentation characteristics.
CONCLUSION

It was concluded that both the kinetics and end products of fermentation of the selected feed ingredients varied with the diet fed to calves the inocula was obtained from. Presence of interactions between substrates and inocula on gas production kinetics and VFA characteristics illustrate the importance of using inocula from calves adapted to the substrate of interest.

ACKNOWLEDGEMENTS

The authors would like to thank Cira de Luca, Emanuela Parlato, Allesandra Nasti for their skilful assistance and Seerp Tamminga for his valuable comments on the manuscript.
### Table 1. Ingredient and analyzed nutrient composition of the concentrate and roughage source for experiment 1 and 2.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>PECTIN</th>
<th>Experiment 1</th>
<th>Experiment 2</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Concentrate</td>
<td>Corn Silage</td>
<td>Straw</td>
<td>Grass</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beet pulp (ground) (%)</td>
<td>91.3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Soya Hulls (%)</td>
<td>-</td>
<td>46.4</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Maize Grits (%)</td>
<td>-</td>
<td>46.4</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Maize (broken) (%)</td>
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<td>-</td>
<td>46.4</td>
<td>15.5</td>
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<td>-</td>
<td>-</td>
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<tr>
<td>Barley (crushed) (%)</td>
<td>-</td>
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<td>46.4</td>
<td>15.5</td>
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<td>-</td>
<td>-</td>
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<tr>
<td>Soya Oil (%)</td>
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<td>0.5</td>
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<td>-</td>
<td>-</td>
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<td>Soluble wheat protein (%)</td>
<td>4.0</td>
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<td>4.0</td>
<td>4.0</td>
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<td>-</td>
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<td>Urea (%)</td>
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<td>-</td>
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<tr>
<td>Vinasses (%)</td>
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</table>

#### Analyzed nutrient composition

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Dry Matter (g/kg product)</th>
<th>Crude Protein (g/kg DM)</th>
<th>Crude Fat (g/kg DM)</th>
<th>Starch (g/kg DM)</th>
<th>Sugars (Glucose g/kg DM)</th>
<th>NDF (g/kg DM)</th>
<th>ADF (g/kg DM)</th>
<th>ADL (g/kg DM)</th>
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<td>22</td>
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<td>340</td>
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<tr>
<td>Corn Silage</td>
<td>902</td>
<td>194</td>
<td>31</td>
<td>108</td>
<td>26</td>
<td>493</td>
<td>265</td>
<td>9</td>
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<tr>
<td>Straw</td>
<td>888</td>
<td>181</td>
<td>32</td>
<td>593</td>
<td>28</td>
<td>94</td>
<td>32</td>
<td>6</td>
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<td>Grass</td>
<td>878</td>
<td>193</td>
<td>41</td>
<td>250</td>
<td>47</td>
<td>292</td>
<td>158</td>
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<tr>
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<td>375</td>
<td>7</td>
<td>343</td>
<td>193</td>
<td>13</td>
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<tr>
<td>Grass</td>
<td>899</td>
<td>BD</td>
<td>-</td>
<td>BD</td>
<td>BD</td>
<td>805</td>
<td>525</td>
<td>63</td>
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<tr>
<td>BD</td>
<td>881</td>
<td>227</td>
<td>-</td>
<td>6</td>
<td>84</td>
<td>433</td>
<td>233</td>
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</table>

BD = Below detection levels (=6 g sugars/kg DM; 3 g starch/kg; 19 g crude protein/kg DM; 3 g N/kg*6.25).  
1 Chopped dried grass
Table 2. Analyzed chemical composition of the selected substrates.

<table>
<thead>
<tr>
<th>Incubated Substrates</th>
<th>DM (g/kg)</th>
<th>Ash (g/kg DM)</th>
<th>Nitrogen (g/kg DM)</th>
<th>NDF (g/kg DM)</th>
<th>ADF (g/kg DM)</th>
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</thead>
<tbody>
<tr>
<td>Sugar beet pectin</td>
<td>915.0</td>
<td>29.1</td>
<td>6.7</td>
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<td>-</td>
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<tr>
<td>Sugar beet pulp</td>
<td>895.3</td>
<td>44.1</td>
<td>13.9</td>
<td>380.3</td>
<td>244.6</td>
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<td>Crystalline cellulose (Avicel)</td>
<td>961.9</td>
<td>0.3</td>
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<td>935.9</td>
<td>881.4</td>
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<tr>
<td>Soy bean hulls^1</td>
<td>904.5</td>
<td>45.5</td>
<td>20.5</td>
<td>530</td>
<td>409.0</td>
</tr>
<tr>
<td>Native corn starch (Meritena, Amylum)</td>
<td>893.4</td>
<td>1.5</td>
<td>&lt;3</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Straw</td>
<td>899</td>
<td>93.6</td>
<td>&lt;3</td>
<td>805</td>
<td>525</td>
</tr>
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</table>

(<3 = 19 g crude protein/kg DM: 3 g N/kg*6.25). ^1 Soy bean hulls were toasted before being de-hulled.
Table 3. Experiment 1: Effect of inoculum (rumen content from calves fed NDF, PECTIN and STARCH) and substrate on cumulative gas production and fermentation kinetics

<table>
<thead>
<tr>
<th>Inoculum</th>
<th>Substrate</th>
<th>Cumulative Gas Production (mL g OM⁻¹)</th>
<th>Organic matter Fermented (%)</th>
<th>Fermentation Kinetics Parameters&lt;sup&gt;2&lt;/sup&gt;</th>
<th>L (h)</th>
<th>A (mL g OM⁻¹)</th>
<th>C (h)</th>
<th>Tmax (h)</th>
<th>Rmax (mL g OM⁻¹ h⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NDF</td>
<td>Native corn starch</td>
<td>367&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>96&lt;sup&gt;abcd&lt;/sup&gt;</td>
<td>1.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>370&lt;sup&gt;de&lt;/sup&gt;</td>
<td>8.4&lt;sup&gt;de&lt;/sup&gt;</td>
<td>8.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>39.0&lt;sup&gt;ab&lt;/sup&gt;</td>
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<tr>
<td></td>
<td>Sugar beet pulp</td>
<td>347&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>93&lt;sup&gt;bcd&lt;/sup&gt;</td>
<td>0.6&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>394&lt;sup&gt;bcd&lt;/sup&gt;</td>
<td>8.0&lt;sup&gt;de&lt;/sup&gt;</td>
<td>3.5&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>30.6&lt;sup&gt;ab&lt;/sup&gt;</td>
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<tr>
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<td>Sugar beet pectin</td>
<td>393&lt;sup&gt;a&lt;/sup&gt;</td>
<td>99&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.6&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>375&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>5.0&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>61.2&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td></td>
<td>Soy bean hulls</td>
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<td>0&lt;sup&gt;c&lt;/sup&gt;</td>
<td>432&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>19.7&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>99&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>5.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>339&lt;sup&gt;de&lt;/sup&gt;</td>
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<td>Sugar beet pulp</td>
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<td>72.4&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>1.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>373&lt;sup&gt;de&lt;/sup&gt;</td>
<td>6.3&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>36.7&lt;sup&gt;ab&lt;/sup&gt;</td>
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<td></td>
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<tr>
<td></td>
<td>Sugar beet pectin</td>
<td>329&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>98&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>1.2&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>327&lt;sup&gt;c&lt;/sup&gt;</td>
<td>5.6&lt;sup&gt;c&lt;/sup&gt;</td>
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</table>

<sup>1</sup> Cumulative gas production after 72 h fermentation, in mL g OM⁻¹ incubated; <sup>2</sup> Percentage of OM incubated after 72 hours of incubation; <sup>3</sup> L = Lag phase before gas production commences; A = Asymptote of gas production; C = Time after incubation at which half of the maximal gas production is reached. Tmax = Time after incubation at which the gas production rate is maximal; Rmax = Maximal gas production rate. ND:
Inoculum: □ NDF; △ Starch; * Pectin
Figure 4. Soy bean hulls as substrate

Inoculum: □ NDF; Δ Starch; * Pectin

Figure 5. Crystalline cellulose (AVICEL) as substrate

Inoculum: □ NDF; Δ Starch (no gas produced); * Pectin (no gas produced)
Table 4. Experiment 1: Effect of inoculum (rumen content from calves fed NDF, PECTIN and STARCH) and substrate on VFA concentrations, VFA branched-chain ratio (BCP) and ammonia (NH$_3$), after 72 hours of fermentation.

<table>
<thead>
<tr>
<th>Inoculum</th>
<th>Substrate</th>
<th>NH$_3$ (mmol/g OM$^{-1}$)</th>
<th>VFA (mmol/g OM$^{-1}$)</th>
<th>Acetate$^3$</th>
<th>Propionate$^3$</th>
<th>Butyrate$^3$</th>
<th>BCP$^3$</th>
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<tr>
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<tr>
<td>Native corn starch</td>
<td>2.8$^e$</td>
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<td>49.7$^e$</td>
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<td>23.9$^a$</td>
<td>8.6$^{bc}$</td>
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<td>3.8$^{bc}$</td>
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<td>62.2$^{bc}$</td>
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<td>9.0$^{de}$</td>
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<tr>
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<td>3.4$^{cd}$</td>
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<td>67.1$^a$</td>
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<td>Soy bean hulls</td>
<td>4.7$^a$</td>
<td>9.5</td>
<td>65.1$^{ab}$</td>
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<td>8.2$^{ef}$</td>
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<td>Native corn starch</td>
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<td>8.2</td>
<td>64.0$^{ab}$</td>
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<td>59.4$^c$</td>
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<td>8.9$^{de}$</td>
<td>8.4$^c$</td>
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<tr>
<td><strong>Blank$^2$</strong></td>
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<td>50.0</td>
<td>18.2</td>
<td>9.1</td>
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<tr>
<td><strong>STARCH</strong></td>
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<td>Native corn starch</td>
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<td>39.1$^e$</td>
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<td>Sugar beet pulp</td>
<td>3.4$^{cd}$</td>
<td>9.1</td>
<td>53.4$^d$</td>
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<tr>
<td>Sugar beet pectin</td>
<td>3.4$^{cd}$</td>
<td>9.6</td>
<td>61.8$^{bc}$</td>
<td>26.8$^{bc}$</td>
<td>6.5$^{ef}$</td>
<td>5.5$^{ef}$</td>
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</tr>
<tr>
<td>Soy bean hulls</td>
<td>4.0$^b$</td>
<td>9.1</td>
<td>59.4$^c$</td>
<td>22.8$^d$</td>
<td>9.2$^{de}$</td>
<td>8.4$^c$</td>
<td></td>
</tr>
<tr>
<td><strong>Blank$^2$</strong></td>
<td>5.8</td>
<td>2.7</td>
<td>44.4</td>
<td>22.2</td>
<td>11.1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

SEM 0.09 0.60 0.64 0.30 0.66 0.3
P-value
Inoculum <0.01 <0.01 <0.01 <0.01 <0.01 <0.01
Substrate <0.01 <0.01 <0.01 <0.01 <0.01 <0.01
Subs * inoculum <0.01 0.48 <0.07 <0.01 <0.01 <0.01

$^1$Expressed in mmol/gram OM$^{-1}$ incubated. $^2$No corrected for blank. The blanks are expressed in mmol per 170 mL, where 1 gram OM$^{-1}$ was weighed. Therefore the blanks are comparable with the observations. $^3$Molar proportions.
Table 5. Experiment 2: Effect of inoculum (rumen content from calves fed C70-S30, C70-CS30, C-100\textsuperscript{**}) and substrate on cumulative gas production and fermentation kinetics

<table>
<thead>
<tr>
<th>Inoculum</th>
<th>Substrate</th>
<th>Cumulative Gas production (mL g OM\textsuperscript{-1})</th>
<th>Organic matter Fermented \textsuperscript{2} (%)</th>
<th>Fermentation Kinetics Parameters\textsuperscript{3}</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>L (h)</td>
<td>A (mL g OM\textsuperscript{-1})</td>
<td>C (h)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C70-S30</td>
<td>Native corn starch</td>
<td>319\textsuperscript{abc}</td>
<td>100\textsuperscript{a}</td>
<td>5.5\textsuperscript{bc}</td>
<td>324\textsuperscript{bcd}</td>
</tr>
<tr>
<td></td>
<td>Sugar beet pectin</td>
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<td>100\textsuperscript{a}</td>
<td>4.1\textsuperscript{bc}</td>
<td>339\textsuperscript{cd}</td>
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<tr>
<td></td>
<td>Soy bean hulls Straw</td>
<td>236\textsuperscript{d}</td>
<td>89\textsuperscript{bc}</td>
<td>0\textsuperscript{c}</td>
<td>263\textsuperscript{b}</td>
</tr>
<tr>
<td></td>
<td></td>
<td>73\textsuperscript{e}</td>
<td>39\textsuperscript{e}</td>
<td>13.7\textsuperscript{ab}</td>
<td>92\textsuperscript{a}</td>
</tr>
<tr>
<td>C70-CS30</td>
<td>Native corn starch</td>
<td>290\textsuperscript{bc}</td>
<td>100\textsuperscript{a}</td>
<td>4.7\textsuperscript{bc}</td>
<td>303\textsuperscript{bc}</td>
</tr>
<tr>
<td></td>
<td>Sugar beet pectin</td>
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<td>100\textsuperscript{a}</td>
<td>3.0\textsuperscript{b}</td>
<td>322\textsuperscript{bcd}</td>
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<tr>
<td></td>
<td>Soy bean hulls Straw</td>
<td>240\textsuperscript{d}</td>
<td>88\textsuperscript{c}</td>
<td>0\textsuperscript{c}</td>
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<tr>
<td></td>
<td></td>
<td>39\textsuperscript{e}</td>
<td>42\textsuperscript{d}</td>
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<td>45\textsuperscript{a}</td>
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<td>C-100</td>
<td>Native corn starch</td>
<td>338\textsuperscript{ab}</td>
<td>99\textsuperscript{a}</td>
<td>3.0\textsuperscript{bc}</td>
<td>341\textsuperscript{cd}</td>
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<tr>
<td></td>
<td>Sugar beet pectin</td>
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<td>100\textsuperscript{a}</td>
<td>2.0\textsuperscript{bc}</td>
<td>347\textsuperscript{cd}</td>
</tr>
<tr>
<td></td>
<td>Soy bean hulls Straw</td>
<td>275\textsuperscript{cd}</td>
<td>90\textsuperscript{b}</td>
<td>0\textsuperscript{c}</td>
<td>314\textsuperscript{bcd}</td>
</tr>
<tr>
<td></td>
<td></td>
<td>275\textsuperscript{de}</td>
<td>41\textsuperscript{de}</td>
<td>6.5\textsuperscript{abc}</td>
<td>56\textsuperscript{b}</td>
</tr>
<tr>
<td>SEM</td>
<td></td>
<td>9.7</td>
<td>0.4</td>
<td>2.3</td>
<td>13.1</td>
</tr>
<tr>
<td>Substrate</td>
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<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
</tr>
<tr>
<td>Inoculum</td>
<td></td>
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<td>0.33</td>
<td>0.11</td>
<td>0.01</td>
</tr>
<tr>
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<td>0.12</td>
<td>0.001</td>
<td>0.40</td>
<td>0.14</td>
</tr>
</tbody>
</table>

\textsuperscript{1} Cumulative gas production after 72 h fermentation, in mL g OM\textsuperscript{-1} incubated; \textsuperscript{2} Percentage of OM incubated after 72 hours of incubation; \textsuperscript{3} L = Lag phase before gas production commences; A = Asymptote of gas production; C = Time after incubation at which half of the maximal gas production is reached; Tmax = Time after incubation at which the gas production rate is maximal; Rmax = Maximal gas production rate. ** For acronyms descriptions see materials and methods.
Table 6. Experiment 2: Effect of inoculum (rumen content from calves fed C70-S30, C70-CS30, C-100) and substrate on VFA concentrations, VFA branched-chain ratio (BCP) and ammonia (NH₃), after 72 hours of fermentation.

<table>
<thead>
<tr>
<th>Inoculum</th>
<th>Substrate</th>
<th>NH₃ (mmol/g OM¹)</th>
<th>TOT VFA (mmol/g OM¹)</th>
<th>Acetate³</th>
<th>Propionate³</th>
<th>Butyrate³</th>
<th>BCP³</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C70-S30</td>
<td>Native corn starch</td>
<td>2.5</td>
<td>10.0 ab</td>
<td>44.9 d</td>
<td>19.4 f</td>
<td>25.6 a</td>
<td>10.0ab</td>
</tr>
<tr>
<td></td>
<td>Sugar beet pectin</td>
<td>3.4</td>
<td>11.3 ab</td>
<td>70.1 a</td>
<td>20.2 fg</td>
<td>5.6 c</td>
<td>4.0 f</td>
</tr>
<tr>
<td></td>
<td>Soy bean hulls Straw</td>
<td>3.9</td>
<td>10.2 ab</td>
<td>60.6 b</td>
<td>25.0 bde</td>
<td>7.9 c</td>
<td>6.4 cd</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.1</td>
<td>7.0 cd</td>
<td>56.6 b</td>
<td>25.2 bcd</td>
<td>8.1 c</td>
<td>10.0 ab</td>
</tr>
<tr>
<td>Blank²</td>
<td></td>
<td>6.0</td>
<td>3.0</td>
<td>53.3</td>
<td>16.7</td>
<td>10.0</td>
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</tr>
<tr>
<td>C70-</td>
<td>Native corn starch</td>
<td>2.8</td>
<td>9.8 ab</td>
<td>46.1 d</td>
<td>21.9 efg</td>
<td>20.7 b</td>
<td>11.2 a</td>
</tr>
<tr>
<td>CS30</td>
<td>Sugar beet pectin</td>
<td>3.1</td>
<td>11.6 a</td>
<td>67.2 a</td>
<td>22.4 def</td>
<td>5.5 c</td>
<td>4.7 de</td>
</tr>
<tr>
<td></td>
<td>Soy bean hulls Straw</td>
<td>3.8</td>
<td>10.8 ab</td>
<td>57.0 b</td>
<td>26.6 ab</td>
<td>8.3 c</td>
<td>8.0 bc</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.5</td>
<td>6.3 d</td>
<td>53.1 c</td>
<td>29.0 a</td>
<td>7.8 c</td>
<td>10.0 ab</td>
</tr>
<tr>
<td>Blank²</td>
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<td>5.7</td>
<td>2.6</td>
<td>50.0</td>
<td>15.4</td>
<td>11.5</td>
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</tr>
<tr>
<td>C-100</td>
<td>Native corn starch</td>
<td>2.7</td>
<td>9.3 b</td>
<td>44.8 d</td>
<td>21.4 fg</td>
<td>23.2 ab</td>
<td>10.5 a</td>
</tr>
<tr>
<td></td>
<td>Sugar beet pectin</td>
<td>2.9</td>
<td>10.5 ab</td>
<td>67.5 a</td>
<td>20.1 fg</td>
<td>6.9 c</td>
<td>5.4 de</td>
</tr>
<tr>
<td></td>
<td>Soy bean hulls Straw</td>
<td>3.7</td>
<td>9.1 bc</td>
<td>60.3 b</td>
<td>22.5 cdef</td>
<td>9.0 e</td>
<td>8.1 bc</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.5</td>
<td>6.1 d</td>
<td>56.7 b</td>
<td>25.0 bde</td>
<td>7.8 c</td>
<td>10.4 a</td>
</tr>
<tr>
<td>Blank²</td>
<td></td>
<td>5.7</td>
<td>2.7</td>
<td>51.9</td>
<td>14.8</td>
<td>11.1</td>
<td></td>
</tr>
</tbody>
</table>

SEM 0.3 0.8 0.8 0.6 0.9 0.4
Substrate 0.001 0.001 0.001 0.001 0.001 0.001
P-value 0.50 0.02 0.003 0.001 0.13 0.002
Subs * inoculum 0.85 0.65 0.02 0.01 0.10 0.24

1 Expressed in mmol/gram OM⁻¹ incubated.
2 No corrected for blank. The blanks are expressed in mmol per 170 mL, where 1 gram OM⁻¹ was weighed. Therefore the blank’s are comparable with the observations.
3 Molar proportions
## For acronyms descriptions see materials and methods.
Figure 6. Native corn starch as substrate

Cumulative gas production (ml/g OM) vs. Incubation time (h)

Inoculum: △ C70-S30; O C70-CS30; □ C-100

Figure 7. Sugar beet pectin as substrate

Cumulative gas production (ml/g OM) vs. Incubation time (h)

Inoculum: △ C70-S30; O C70-CS30; □ C-100
Figure 8. Soy bean hulls as substrate

Cumulative gas production (ml/g OM)

Incubation time (h)

Figure 9. Straw as substrate

Cumulative gas production (ml/g OM)

Incubation time (h)

Inoculum: ▲ C70-S30; O C70-CS30; □ C-100

123
REFERENCES


CHAPTER 6

General Discussion
General Discussion

The main objective of this thesis (see introduction) was to gather information about the effect of different solid feeds on the development of the rumen in veal calves and its potential interactions with a milk replacer basal diet. Results of in vivo experiments were coupled with information obtained from in vitro experiments (Gas Production Technique (GPT) and Polysaccharides Degrading Enzymes (PDE) activities technique). However, the general discussion of this thesis focuses not only on the results obtained in the previous chapters but also presents and discusses some extra information (assessment of whole rumen content buffering capacity) that was not included in the previous chapters. Therefore, this general discussion consists of four parts:

- Factors influencing rumen development in rearing and veal calves.
- The importance of ruminal drinking in veal calves fed solids feeds.
- Effects of feeding strategies on ruminal pH and buffering capacity of rumen contents in veal calves.
- A comparative analysis of the results obtained from the GPT (results presented in Chapter 5) and the PDE activities (results presented in Chapter 2 and 4).
1- Rumen development

The development of a functional rumen involves several processes to be set in motion in the early calf’s life. These processes start with the physical and physiological changes of the rumen tissues, which are triggered by the consumption of dry feed and the resulting end products of fermentation (VFA) (Flatt et al., 1958). However, as mentioned in the introduction of this thesis, not all VFA have the same effect. Butyrate and propionate are generally considered to have the greatest stimulatory effect on growth and development of the rumen mucosa (Sakata and Tamate, 1978; Mentschel et al., 2001). In agreement with literature, results from the experiments described in Chapters 2 and 3, showed that intake of solid feed and the end products of its fermentation (VFA) stimulated rumen development. In Chapter 3, results from the principal component analysis (PCA) supported these observations, demonstrating a positive relationship between the concentrations of VFA in rumen liquid and the morphometrical parameters of rumen development. However, results of this PCA study did not support the in vivo finding of Sakata and Tamate, (1978) and Mentschel et al. (2001) that butyrate, propionate and acetate differ in their ability to stimulate mucosa development. Possible explanations for this discrepancy include the following.

Firstly, the variation in the dietary carbohydrate composition in the experiment described in Chapters 2 and 3 was not large enough to produce a large contrast in VFA’s molar proportions and consequently in the rumen mucosa. Secondly, the lack of response of rumen development to high butyrate proportions may be related to methodological differences, notably differences between in vitro (Baldwin, 1999; Baldwin and McLeod, 2000) and in vivo approaches (Chapter 2-4). In vitro, cells are mostly kept at a constant pH (pH= 7) in a buffered system, whereas in vivo, the pH of the rumen may vary greatly depending on the VFA production. In ruminants, it has been reported that the rate of VFA absorption varies according to the ruminal pH, the absorption being increased as pH decreases (Dijkstra et al., 1993). Therefore, it is not surprising that butyrate could have different effects in vitro than in vivo. Thirdly, factors other than VFA concentrations mediate the response in mucosa development. Raab et al. (1998) showed that in pigs the energy content of the diet determined the formation of insulin like growth factor-I (IGF-I), which is known to stimulate mitosis in body tissues. On the other hand, Gerrits et al., (1998), observed in pre-ruminant calves increased IGF-I levels in plasma with increased protein intake, whereas no effect on IGF-I levels was found with increased energy intake.
In adult ruminants, although the possible interactions between energy and protein levels and IGF-I levels and its effect on mucosa development has not been completely elucidated, research done by Shen et al. (2004) showed increased rumen papillae size in goats consuming an energy-rich diet (containing high levels of rumen-soluble carbohydrates), which was associated not only with increased butyrate concentrations in the rumen but also with increased high IGF-I concentrations in blood and increased IGF-I receptors in rumen papillae. Accordingly, in our experiments the increased supply of milk replacer (increased energy and protein intake) and its potential effect on IGF-I levels could have played an endocrinal effect on rumen development. A final endocrine explanation for the disparate actions of butyrate in vivo and in vitro are due to a mechanism involving the metabolism of butyrate in vivo and consequently, a hormonal response to the metabolites of butyrate (Baldwin, 1999). Several hormones (insulin, pentagastrin, glucagon, and cortisol) have been mentioned as possible mediators of the butyrate induced stimulation of ruminal epithelial proliferation in vivo. From those, only insulin has been shown to stimulate cell growth in vitro (Neogrady et al., 1989) and also to stimulate the cell mitotic indices of ruminal cells when administered in vivo via intravenous infusion (Sakata et al., 1980). Increased postprandial insulin levels have been reported in veal calves (> 130 µg/L; Van den Borne, 2006) when compared with suckling (Egli and Blum, 1998) and weaned calves (Hugi and Blum, 1998), in which mean postprandial insulin concentrations ranged from 0.8 to 1.5 µg/L. Therefore and in addition to IGF-I, the insulin levels may also endocrinally have affected rumen development.

It has been reported that some VFA produced in the rumen are metabolized to ketones by the rumen epithelium during their absorption (Bergman, 1990). As a consequence, plasma concentrations of butyrate’s ketone, β-OH-Butyrate (BHBA), and acetate have been used as a non invasive indicator of ruminal activity and rumen development (Quigley et al., 1991; Quigley and Bernard, 1992). In Chapter 3, the second PCA study confirmed the proposed relationships. Across all treatments, plasma BHBA and acetate were positively correlated with the morphometric parameters of rumen development.

**The importance of particle size**

In experiment 1 (Chapter 2), although special care was taken to minimize particle size reduction while making the pellets (see materials and methods Chapter 2), yet these precautions
were not sufficient to avoid feed particles to become impacted in between the ruminal papillae, which was subsequently defined as plaque formation. The incidence of plaque formation was high in the experiment in which concentrates were the only source of solid feed (Chapter 3). Supplementation of roughage was demonstrated to be successful in reducing the incidence of plaque formation and it improved the macroscopic appearance of the rumen wall (Chapter 4), stressing the importance of particle size in rumen development. Effects of roughage supplementation on morphometric parameters were, however, quite small. Consequently, the role of particle size in rumen development is not fully understood. In dairy cattle, the concept of particle size was previously developed. Initially this was an attempt to formulate diets based on the amount of dietary NDF required to maintain milk fat percentages (3.5%; Grant et al., 1990a, b). Further improvements of this concept were carried out by Mertens (1997), who proposed that a distinction should be made between the effectiveness of fiber in maintaining milk fat percentages and in stimulating chewing activity. Accordingly Mertens, (1997) proposed the physical effective fiber (peNDF) and effective fiber (eNDF) concepts. The peNDF is related to the physical characteristics of the fiber that influence chewing activity (particle size reduction), maintaining the biphasic structure of the rumen content (floating mat of large particles on a pool of liquid and small particles). The eNDF is only related to the total ability of a feed to replace roughage in a ration so that the percentage of fat in milk is effectively maintained.

In dairy cattle different practical systems have been proposed to measure peNDF (Mertens, 1997; Lammers, et al., 1996). The most widely used method is to determine the proportion of DM (or NDF) retained on a sieve with an aperture of 1.18 mm as a simple laboratory method that might be applicable to the routine analysis of peNDF in feeds (Mertens, 1997). Similarly, in rearing calves attempts to elucidate the right size of fiber in concentrate feeds have also been made. Based on the results of a series of studies carried out earlier by Warner et al. (1973), Davies and Drackley, (1998) recommend that in concentrate diets for young calves, at least 50% of the feed particles (based on weight), should be larger than 1.19 mm (pore size of a 16-mesh screen). More recent studies carried out by Greenwood et al. (1997) and Beharka et al. (1998) reached slightly different conclusions. Greenwood et al. (1997) developed a method to measure the abrasiveness of a calf’s diet in the rumen wall. These authors defined dietary abrasive value (DAV) as the feed efficiency in physically removing keratin or dead epithelial cells from the rumen wall. They concluded that a DAV of 20 is a reasonable value to control parakeratosis in
the rumen epithelium of young calves fed ad libitum with a fully concentrate diet. A DAV value of 20 corresponds to a diet with intermediate particle size and is equal to 65% of the concentrate particles retained on a Tayler sieve size of 1.18 to 1.25 mm (ruminal VFA concentrations of ≈ 87 mmol/L; pH = 5.4 and rumen papillae lengths of ≈ 1.6 mm). Beharka et al. (1998) observed in rearing calves (10 weeks of age) fed ad libitum with a concentrate/roughage based diet (75% and 25% respectively; roughage was chopped at 0.6 cm), that approximately 30% of the concentrate grain and 55% of the roughage has to be retained on a 1.0 mm sieve to avoid branching of the rumen papillae (ruminal VFA concentrations of 140 to 150 mmol/L; pH = 5.5 to 5.8, lactate < 2.0 mmol/L and rumen papillae lengths of ≈ 2.5 mm). Papillae branching has been defined as an adaptation of rumen mucosa to overcome reduced absorption caused by parakeratosis.

In young calves, high roughage intakes have not been recommended because of their low rate of fermentation and high molar proportions of acetate, when compared to concentrates. However, roughage or coarse particle size consumption is needed to promote muscular development of the rumen (Tamate et al., 1962; Zitnan et al., 1998), to maintain papillae healthiness and, by physically removing dead cells and keratin deposition, to maintain the absorptive capacity of rumen mucosa (Bull et al., 1965; Nocek et al., 1984). Therefore and based on the macro- and microscopic development of the rumen achieved in experiment 1 (Chapter 2), the dietary inclusion of roughage (30% of the dietary DM) was chosen in the design of experiment 2 (Chapter 4). Results presented in Chapter 4 show that although differences in the microscopic development were not observed, the addition of roughage decreased the incidence of plaque formation and poorly developed mucosa. Therefore, it can be advisable, especially if the particle size distribution of the concentrate is unknown, to supply some roughage to maintain mucosa healthiness and absorptive capability.

At the present time, although a standard validated method to measure effective fiber of feeds for calves is missing, the concepts of Mertens (1997) for dairy cattle and the approaches of Greenwood et al. (1997) and Beharka et al. (1998) are the steps towards the quantification of the chemical and physical attributes of fiber into a single measurement. More research is needed to identify other chemical and physical characteristics of feeds that influence their ability to maintain optimal rumen function and animal health in calves, before specific values for effectiveness of various roughage and non fiber sources can be determined as is applied in dairy cattle with eNDF and peNDF concentrations.
2- Ruminal Drinking

Hristov et al. (2001) and Ghorbani et al. (2002) reported that in feedlot cattle perceived to have sub-clinical acidosis the lactate concentration in ruminal fluid rarely exceeds 10 mmol/L.

The first documented observation that milk bypassed the reticulo-rumen in the young calf was made by Wise (1939). In later studies, Wise and Anderson (1939) and Wise et al., (1942) established that the closure of the esophageal groove was a neural reflex involving excitatory areas located in the mouth and pharynx and when a failure of this reflex occurs, milk, instead of being delivered into the abomasum, enters the reticulo-rumen. This situation is known as “ruminal drinking” and as a consequence lactose and easily degradable proteins contained in the milk are fermented by the ruminal microflora. Clinical signs of ruminal drinkers have been characterized by decreased growth rate of the animal, bloat, hyperkeratosis and parakeratosis of the rumen wall, clay-like faeces and ruminal acidosis characterized by low pH (pH= 3.8 to 5.0) and high lactate concentrations (from 15 up to 45 mmol/L) (Van Weeren-Keverling Buisman et al., 1990a; Gentile et al., 2004). Furthermore, feed intake depression and the absence of a sucking reflex in the young calf are considered as signs of systemic acidosis (D-lactate concentrations between 3 and 8 mmol/L; Gentile et al., 2004).

In the first experiment (Chapter 2), ruminal drinking was not quantified because its occurrence was not expected to be of quantitative importance. However, the finding of condensed fat in the extracts of rumen contents when performing the PDE activities analysis (rumen extracts were centrifuged at 4 °C; see Chapter 2), the presence of high concentrations of lactate (5 to 21 mmol/L) and ammonia (14 to 21 mmol/L) in rumen liquid across dietary treatments led us to suspect significant amounts of milk replacer entering the rumen. This situation was confirmed in the second trial (Chapter 4) where a considerable amount of Co-EDTA, provided with the milk replacer meal was recovered from the rumen (≈ 25 %). In both experiments no clinical signs of ruminal drinking or illness were observed in the calves.

The lack of roughage or adequate particle size of the fiber fraction in diets of adult ruminants has been reported to result in digestive abnormalities (rumen acidosis, decreased rumination and rumen motility). However, Colvin and Daniels (1965), demonstrated in concentrate (pellets) fed steers that although the amplitude of the ruminal contraction decreased, the frequency and eructation were not affected. In both in vivo experiments, the addition of solid feed (either
concentrate or roughage) with its stimulatory effect on mucosa development and rumen motility, could have contributed to make the episode of ruminal drinking clinically unnoticed. A more developed mucosa has the capability to absorb more VFA (Bull et al., 1962; Sutton et al., 1963) and as long as rumen motility and eructation are maintained, the milk replacer entering the rumen can quickly disappear. This would allow the rumen to regain physiological pH values (5.6 to 6.8). On the other hand, in absence of solid feeds in the rumen, the milk replacer can remain in the lumen if milk persistently enters the rumen the acidification increases. Finally, whether the absence of clinical signs was due just to the single effect of a healthier fermentation or to differences in ruminal pool sizes can not be completely clarified.

As mentioned in Chapter 4, although the marker technique used to quantify milk recovery in the rumen (Co-EDTA pulse dose) does not allow discriminating between the proper functioning of the esophageal groove and a potential backflow of milk replacer from the abomasum, it highlights a problem that is currently occurring in veal calf systems even if it is not clinically manifested. Although the high recovery of cobalt from milk replacer in the rumen of young veal calves fed concentrates and roughage (Chapter 4) is alarming, some methodological issues need to be discussed. For example, the stress induced to the calves during transport (calves were transported from the experimental farm to the pathology laboratory (5 min; see methodology Chapter 3) and the slaughter methodology (calves were lifted by the forelegs, stomach was tied at the end of the esophagus and pylorus and immediately emptied) both may have affected the recovery of cobalt and their effects cannot be completely excluded. Finally, although a soluble marker should be ideally unabsorbable, up to 4 to 5 % of absorption has been described in ruminants and excreted in urine (Van Soest, 1994), which in condition of very low pH and high osmotic pressure, obtained when feeding rapidly fermented matter, has been reported to increase up to 10 % (Dobson et al., 197?; cited by Udên et al., 1980). Consequently, this potential effect of pH on the marker absorption may have contributed to overestimate cobalt recovery and to explain the high fractional passage rate of the rumen liquid ($K_{pl} = 46\%$/h), reported in Chapter 4. This fractional passage rate of rumen fluid was estimated by regression of the natural logarithm of the Co-pool size, measured at slaughter against the time interval between feeding and slaughter. The estimate obtained is at least twice as high than usually observed in dairy cattle ($\approx 15 \%/h$; Offer and Dixon, 2000) and rearing calves ($\approx 11 \%/h$; Beharka et al., 1998; Vasquez-Anon, 1993).
In veal calves, the pressure exerted by large amounts of milk replacer in the abomasum has been described as one of the etiologies causing abomasum ulcers in veal calves (Welchman and Baust, 1987). Assuming that large amounts of milk replacer indeed may exceed the abomasal capacity, physical effects associated with a developing rumen (increase in size and ruminal motility; Tamate et al., 1962) on the abdominal cavity and consequently on the abomasum, may also contribute to an increased incidence of ruminal drinking and make this situation even more complex. Although reduced provision of milk and weaning on hay and concentrates have been reported to reduce clinical symptoms, allowing recovery within days (Radostits et al., 2000), such feeding strategies are not in the presently applied management of veal systems. Von Rademacher et al. (2003) mentioned that a practical solution to decrease ruminal drinking in calves is the supply of a less diluted milk replacer. Van Reenen et al. (2006) (unpublished data) observed Co recoveries of 10-22% in veal calves at 12 weeks of age, and between 13 and 23% in veal calves slaughtered at 26 weeks of age. The lower Co recovery in the study of Van Reenen et al. (2006) when compared with the study presented in Chapter 4 may be related to the feeding of a less diluted milk replacer (120 and 50 g/L in the restricted and ad libitum fed calves in Chapter 4 vs. 178 g/L in the study of Van Reenen et al. (2006). Furthermore, it was remarkable that in the study of Van Reenen et al (2006), both at 12 and 26 weeks of age, Co-recovery was, at least numerically, lowest at the highest level of solid feed intake (13.3% at 1 kg DM/d vs. 19.1 % at 0.5 kg DM/d). It should be noted that these effects not necessarily result from a difference in ruminal drinking. It may also be the consequence of increased passage rates of fluid and liquid through the rumen, commonly observed to increase with dry matter intake (Hartnell and Satter, 1979). In addition, exchanging concentrates for a mixture of straw and corn silage (on a DM basis) reduced Co recovery from 18.4 to 13.5% (P<0.05).

Furthermore, as shown by Herrli-Gygi et al. (2006) switching to a system of nipple feeding (instead of open buckets) also has a positive effect on decreasing ruminal drinking in veal calves by decreasing the rate of milk consumption and perhaps by stimulating the closure of the esophageal groove when sucking from the nipple.

3-Ruminal pH and Ruminal Buffering Capacity

In ruminant nutrition, especially in systems of dairy and beef cattle production, a fast degradation of feed DM in the rumen may be desired to maximize energy intake and synthesis of microbial protein. As a result of the fast DM degradation, a rise in the fermentation end products
(VFA) and a drop in rumen pH is normally observed a few hours after feeding (as an example see figure 1; rearing calves were fed 4.5 kg/d concentrate and ad libitum hay; steers were ad libitum fed (twice daily) with a diet based on 90 % concentrate and 10 % hay; veal calves consumed 0.7 kg DM/d (70 % concentrate and 30 % roughage). However, if rumen pH continually decreases (pH < 5.6), appetite and rumen motility decreases (Nocek, 1997; Owens et al., 1997) and microbial yield can be reduced (Hoover, 1986). When rumen pH severely decreases (pH < 5.0), health problems such as laminitis, liver abscess, and even death could be the result (Allen, 1997; Nocek, 1997).

Figure 1 shows that in veal calves, although VFA concentrations rise up to 3.5 h after feeding, rumen fluid pH does not follow the abovementioned pronounced pattern of pH decline observed in adult ruminants. The reason for this discrepancy following solid feed consumption may be partly caused by the earlier supply of milk replacer, which to some extent had entered the rumen (see cobalt recovery; Chapter 4) with the consequent effects on ruminal lactate concentrations (see ruminal drinking discussion). More lactic acid (from milk replacer) will decrease pH drastically since lactate (pK= 3.8) is a much stronger acid than VFA. Then at the moment solid feeds enter the rumen more VFA are produced but less lactate, hence pH may remain constant.
In rearing calves, during the transition period from a pre-ruminant state to a full ruminant condition, establishing a mature like fermentation (pH usually ranging from 5.8 to 6.8 and VFA > 100 mm/L) is a critical factor. Although numerous ruminal pH measurements were made in earlier studies with calves, in most of them the samples were collected by stomach tube, with the potential contamination with saliva and consequently reduced accuracy of the pH values reported. However, Anderson et al. (1987a,b) and Beharka et al. (1998) observed in rumen cannulated rearing calves during the first 7 to 8 weeks of age, when the establishment of the microbial population is taking place, a relatively low ruminal pH (≈ 5.4 to 5.6). Although DM intake level and DM chemical composition, and in this particular situation the effect of milk being present in the rumen, have to be considered when comparing profiles of rumen fermentation across literature, in this thesis the observed pH values (although slightly lower) were in line with those mentioned above in rearing calves (Anderson et al., 1987a; Beharka et al., 1998).

Figure 1 Rumen pH and VFA concentrations at different time points after feeding in steers, rearing calves and veal calves
The rumen pH is a balance between the rate of feed fermentation (rate of VFA production, absorption and passage) and the buffering capacity (BC) of the rumen contents. In a review, Tamminga and Van Vuuren (1988) reported a strong negative relationship ($r^2 = 0.71; n=244; \text{pH} = 7.73 - 0.014[VFA]$; pH range 5.2 to 7.0) between VFA concentration and rumen pH. However, Allen (1997) reported that although rumen VFA concentrations were negatively related ($P<0.001$) to rumen pH, the observed correlation was low ($r^2 = 0.13; \text{RMSE} = 0.23; n=99; \text{pH} = 6.56 - 0.0049[VFA]$, pH range 5.5 to 6.6). Allen (1997) explained the low correlation coefficient from variation in buffering capacities in the rumen.

The VFA values measured in the in vivo experiments presented in this thesis (Chapters 2 and 4) were regressed against the measured values of pH in rumen fluid. The line of best fit when VFA was the only explaining factor was $\text{pH} = 5.41 - 0.0027[VFA]$ ($P<0.001; r^2 = 0.12; n=224$; data from all dietary treatments were included). Application of the equation reported by Tamminga and Van Vuuren (1988) to predict pH values with the VFA concentrations reported in the in vivo trials (Chapter 2 and 4) resulted in predicted pH values between 5.6 and 7.2, and when based on the equation of Allen (1997) between 5.8 and 6.4, for the VFA concentrations in the range 36 to 150 mmol/L. These predicted values are much higher than the observed ones, which show that equations originally developed for dairy cattle may not be a useful tool to accurately predict pH in veal calves. The predicted higher pH values could be partly caused by the relatively high presence of lactic acid (pKa= 3.1) in veal calves, which was not included by Tamminga and Van Vuuren (1988) and Allen (1997) when developing their equations for dairy cows. In veal calves, lactic acid concentrations in various treatments varied between 5 and 21 mmol/l (Chapter 2) or between 2 and 12 mmol/l (Chapter 4). In dairy cattle, lactate is usually only a minor intermediate in ruminal metabolism (Allen, 1997) and concentrations are typically lower than those observed for veal calves.

As mentioned earlier, the magnitude of a decrease in pH following an increase in the fermentation rate most likely depends on the BC of the rumen. In the adult ruminant, bicarbonate (pK= 6.4) and VFA (pK ≈ 4.8) in saliva are the main components of the buffering systems present in the rumen fluid. Phosphate from saliva also contributes to neutralize acids and regulate rumen pH, but because its concentration in saliva is much lower than bicarbonate (20 vs. 80 meq/L, respectively) it plays a minor role (Counotte et al., 1979; Van Soest, 1994). Dietary fiber may also help to buffer the rumen environment due to an inherent capacity of plant cell walls to
bind H\(^+\) ions as well as due to the stimulatory effect of fiber on rumination and consequently on saliva production (Mc Burney et al., 1983).

The main objective of the experiment in Chapter 4 was to evaluate the effect of the inclusion of different levels and sources of dietary roughage on animal performance and rumen development. It was hypothesized that, in comparison to concentrate fed veal calves, roughage addition would stimulate salivation and reduce the incidence of poorly developed mucosa and plaque formation in the rumen. In addition to an evaluation of rumen fermentation parameters (pH, VFA’s, ammonia, reducing sugars and lactate), additional analyses concerning the fermentative status of the rumen (buffering capacity) were carried out. The methodology of the BC analysis was as follows. Buffering capacity of the whole rumen contents as well as of the feeds was estimated by an adapted procedure of Counotte et al. (1979). Briefly, 100 g of demineralized water was added to 100 g of whole rumen contents or feeds, mixed and subsamples of 50 g each were subsequently titrated under continuous stirring to pH=7 by adding small amounts of 0.1 M Sodium Hydroxide (NaOH) and then titrated back to pH=4 by adding 0.1 M Hydrochloric Acid (HCl). Titration curves were plotted with pH (from 7 to 4) on the Y-axis and the cumulative amount of added H\(^+\) (mmol / 100 g fresh rumen contents or feeds) on the X-axis. Buffering capacity was subsequently estimated as the slope of the titration curve at: a) the pH measured in the rumen liquid after slaughter, b) at the pK of bicarbonate (pH≈ 6.4) and c) at the averaged pK of acetate, propionate and butyrate (pH≈ 4.8). To determine the slope of the titration curve, the four measurement points closest to the pH of interest were taken and BC expressed as Δ pH/mmol H\(^+\)/100 g fresh sample (see figure 2 for an example of titration curves with feeds.
Results of the BC of the feed ingredients are shown in Table 1. Except for concentrate the estimated BC of the roughages was higher around the pK point of the average VFA (pK\textsubscript{VFA} ≈ 4.8), and ranged from -0.28 to -0.66 ΔpH/mmol H:\textsuperscript{+}/100 g sample. For the pK of the ruminal bicarbonate buffer (pK\textsubscript{HCO3}) the estimated BC of roughages ranged from -0.38 to -1.06 ΔpH/mmol H:\textsuperscript{+}/100 g sample. Concentrate showed increased buffer capacity at the pK of the bicarbonate buffer (pK\textsubscript{HCO3}) than at pK point of the average VFA (-0.93 vs. -1.53; respectively). Among roughages dried grass showed the higher BC either at he pK of the bicarbonate buffer (pK\textsubscript{HCO3}) or at pK point of the average VFA; probably caused by its increased protein content (see table feed ingredient chapter 4).

Results of the rumen contents BC are shown in Table 2. For diet abbreviations and preplanned contrast see experiment 2 (Chapter 4). The estimated BC of the whole rumen contents was generally higher around the pK point of the average VFA (pK\textsubscript{VFA} ≈ 4.8), and ranged from -0.15 to -0.07 ΔpH/mmol H:\textsuperscript{+}/100 g fresh sample. For the pK point corresponding with the pH
recorded at slaughter (pK_{pH-slaughter}), the BC varied between -0.22 and -0.11 ΔpH/mmol H⁺/100 g fresh sample. Finally, for the pK of the ruminal bicarbonate buffer (pK_{HCO₃}) the estimated BC ranged from -0.44 to -0.25 ΔpH/mmol H⁺/100 g fresh sample. Pre-planned contrasts showed that at the pK_{VFA}, rumen contents from calves fed concentrate only and ad libitum fed calves had increased and decreased BC, respectively (contrast A; P ≤ 0.01 and contrast E; P ≤ 0.001, respectively). At the pK_{HCO₃}, the ad libitum fed calves had the lowest BC (contrast E; P ≤ 0.05). No significant effect of treatment was observed in the whole rumen BC at the pK_{pH-slaughter}.

In vitro research carried out by Jasaitis et al. (1987) showed that the BC of feedstuffs varies considerably (cereal grains to have low BC, grass roughages and feeds with low protein content have intermediate BC and roughages of legumes and feeds with high protein content have high BC). However, and despite the variation in the feed’s inherent BC, research carried out by Wohlt, et al. (1987) demonstrated that feed per se when in the buffering range considered as optimum for a healthy rumen fermentation (pH 5.6 to 6.8) has little buffering effect and showed that most of the BC of feeds occurs below pH 5.0. Except for the concentrate, the results roughage’s BC (see table 1) are in line with the reported observations.

In the present experiment (Chapter 4), although the different roughages supplied to the calves differed in fiber and protein content (see diets composition in Chapter 4) and theoretically in the capacity of cation exchange, even at pH values close to optimal BC of feeds (pH ≈ 5), feed did not exert a major role in the rumen buffering environment. In addition, Allen, (1997) mentioned that when compared with the buffering effect of saliva, the direct buffering by the diet is less important. However, in our experiment the lowest BC of the rumen contents was observed at pK_{HCO₃} indicating that in young veal calves saliva poorly contributed to the buffering system present in the rumen fluid. Furthermore, at pK_{HCO₃}, ad libitum fed calves showed significantly decreased BC of the rumen contents than restricted fed calves (see table 1; contrast E; * P≤ 0.05).

Salivary production rates of more than 15 L/day have been reported in grazing sheep (Kay, 1960), whereas secretion rates ranging from 180 L/day (Van Soest, 1994) up to 270 L/d (DMI = 20 kg/d; Allen, 1997) have been reported in dairy cattle. Ternouth et al. (1985) observed in young calves (≈ 100 kg BW; DMI= 4.5 kg/d; 3.0 kg of concentrate and 1.5 kg straw) saliva productions of 2-6 L/d, which when compared with data reported by Allen, (1997) seems to be a low amount (dairy cattle 13.5 L/kg DMI vs. young calves 1.3 L/kg DMI; calculated data correspond to total
saliva production which is the sum of resting, ruminating and eating flows). Furthermore, low BC of rumen contents as response to DMI and physical form of the diet has been reported in rearing calves (Beharka et al., 1998). These authors reported that in rearing calves (10 wk of age) the physical form of the diet (coarse concentrate vs. pellets) did not affect the BC of rumen fluid; indicating that in young rearing calves the potential effect of the physical form of the diet on rumination and saliva production was not reflected in the BC of rumen contents. Moreover, although BC numerically increased as calves aged (2 to 6 wk; corresponding to increased DMI) the differences where not significant ($P > 0.05$). This information shows that in young calves apparently a combination of a low volume of saliva production (perhaps because the salivary glands are not yet fully developed) with the presence of milk replacer in the rumen, hence increased lactate, led to low rumen pH in veal calves fed solids feed (concentrate/roughage). However, the fact that those rumen contents of calves fed only concentrate were the ones that showed the highest BC, can not be easily explained. A potential explanation can be related to the protein content of the diets. Giger-Reverdin et al. (2002), showed that protein content of feedstuffs was the chemical factor which best explained the variation on feedstuffs BC. In Chapter 4, calves receiving only concentrate (which contained higher amounts of CP than roughages; see ingredient and nutrient composition) could have obtained a dietary BC benefit, which may explain the small but significant increased BC of rumen content obtained from those animals.

Furthermore, although the VFA buffering is less relevant in an adult ruminant, mostly because the buffering occurs at a pH too low in physiological conditions, in the present study the pH of rumen contents was much lower than in adult ruminants and the neutralization of $H^+$ ions through the removal of VFA played the most important role in buffering the rumen (see Table 1). Controlling the rate of fermentation in the rumen through diet formulation can be one of the areas that need more attention to obtain healthier (smoother peaks of VFA increase and pH decrease, respectively) and rumen fermentation in young veal calves feed solids feeds.
Table 2. Effect of dietary treatments on rumen buffering capacity (ΔpH/ mmol H\\(^+\)/ 100 g fresh sample) at the fluid pH at the pK point of averaged VFA (pK\(_{VFA}\) ≈ 4.8) and of bicarbonate (pK\(_{HCO₃}\) ≈ 6.4) and at pH registered at slaughter (pK\(_{pH/\text{slaughter}}\) ) levels.

<table>
<thead>
<tr>
<th>Buffering Capacity</th>
<th>Dietary Treatments</th>
<th>Contrast</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C100</td>
<td>C40-CS60</td>
</tr>
<tr>
<td>pK(_{VFA})</td>
<td>-0.07</td>
<td>-0.11</td>
</tr>
<tr>
<td>pK(_{pH/\text{slaughter}})</td>
<td>-0.11</td>
<td>-0.14</td>
</tr>
<tr>
<td>pK(_{HCO₃})</td>
<td>-0.25</td>
<td>-0.34</td>
</tr>
</tbody>
</table>

1Contrast: A= C100 vs. (all diets with additional roughage, except the two ad libitum diets); B= C70-S30 vs. C70-G30 vs. C70-CS30 (mutually); C= C70-G15-S15 vs. (C70-S30 and C70-G30); D= C70-CS30 vs.C40-CS60; E= (C70-G15-S15 and C70-CS30) vs. (ad libitum diets). For diet abbreviations see Chapter 4.

2Statistical significance + P<0.10; * P ≤ 0.05; ** P ≤ 0.01; *** P < 0.001

3Buffering capacity was estimated at different pH by linear regression from titration curves (cumulative mmol H\\(^+\) titrated against pH) over the pH range 4.0 – 7.0.
Gas Production Technique and Polysaccharides Degrading Enzymes Activities

As discussed in Chapter 3, development of techniques to estimate substrate degradation in the rumen is of great interest to ruminant nutritionists. Currently, the fermentation characteristics of feeds in the rumen can be studied in vivo, in situ and in vitro. In vivo determinations use large number of animals, are laborious and expensive. The in situ technique (Mehrez and Orskov, 1977; also called in sacco) is the basis of several systems of feed evaluation for ruminants (e.g. Tamminga et al., 1994) but also has the disadvantage of being time consuming and expensive. On the other hand, in vitro approaches like that of Tilley & Terry (1963), the gas production technique (GPT) (Theodourou et al., 1994) and recently, polysaccharide degrading enzyme activity (PDE) (Martin and Michalet-Doreau, 1995; Hristov et al., 1999; Suárez et al., 2006), have reduced the dependence on animals, creating more flexibility in feed evaluation. The GPT has attracted the attention of researchers from different disciplines worldwide because it allows in a non invasive way to screen a large number of substrates. It also provides valuable information about the kinetics of degradation and last but not least it is relatively cheap. Assessing the activity of polysaccharide degrading enzymes of the microorganisms present in the rumen is also a useful approach to obtain quantitative information about the activity of the microbiota in the rumen in degrading substrates (Silva et al., 1987; Suárez et al., 2006). The objective of applying the PDE test in Chapter 2 and 4 was to assess the capability of the microbiota in the rumen of the calves at slaughter to degrade feedstuffs. In Chapter 5, the GPT was applied to estimate the kinetics of degradation of the main feed ingredients. In both chapters, the main interest was in the comparison of the inocula (enzyme extracts of calves fed different diets in PDE; inoculum effects in the GPT), rather than in the comparison of substrates, but differences between the degradation of substrates could be shown by both techniques and can consequently be compared. Therefore, the aim of this last section of general discussion was to compare the results obtained from the GPT (results presented in Chapter 5) and the PDE (results presented in Chapter 4), focusing on comparison of substrates and on comparison of inocula. Initially in the experiment described in Chapter 4, four sources of inoculum (two calves per inoculum) were incubated with each of four different substrates; however, during the slaughtering process (inocula sampling) one of the dietary treatments was mistaken, hence it was not included in the results presented in Chapter 5, but can be used for the comparative purposes. Accordingly, selected GPT parameters were compared with PDE activities of the same calves using the same substrates. From the GPT, 2
parameters were selected: $R_{\text{max}} =$ Maximal gas production rate and $\text{OMCV} =$ Cumulative gas production after 72 h fermentation, in mL g OM incubated$^{-1}$, for regression against enzyme activity (mmol.min$^{-1}$.g DM$^{-1}$) of whole rumen content as X variables. Results are presented in table 3.

The results of the regression analysis indicate that both GPT and PDE analyses reflect to some extent the large differences between substrates in a similar way (results pooled by inoculum). However, variation between inocula (results pooled by inoculum), obtained with the GPT did not correlate that obtained with the PDE method.

Earlier attempts to compare organic matter degradation from feedstuffs in the rumen with GPT incubations have led to variable results depending on the gas production parameters evaluated and the models used to fit their equations. Cone et al. (2002) reported a moderate relationship for extent of OM degradation (values ranging from 29.0 up to 76.4 %) in feedstuffs ($R^2$ 0.50 to 0.70) determined using an in situ (in sacco) and the gas production technique. Gosselink et al. (2004) observed that in vivo fermentable OM degradation (with values ranging from 50 to 70 %) was best correlated ($R^2 = 0.74$ to 0.90; $n = 12$) with gas production after 20 hours of incubation. Earlier research carried out by Silva et al. (1987) observed a high correlation ($r = 0.98$ and $r = 0.94$) for in sacco DM degradation and cellulytic (CMCase) activity after 24 and 48 hours of rumen incubation, respectively (DM degraded varied between 25 to 38 % and between 30 to 55 % after 24 and 48 h of incubation, respectively). Therefore, based on the assumption that the enzymes extracted from the rumen contents were a good representation of the calves’ ruminal microflora activity at the time of slaughter, it was thought that the relationship between substrate degradation in vitro (GPT) and in vivo (PDE) would be high. Although, the results from the PDE in both experiments (Chapter 2 and 4) illustrate adaptation of the rumen microflora to the diet and similar adaptations of rumen microflora have been reported (Martin and Michalet-Doreau, 1995; Hristov et al., 1999); results of the regression analysis did not support the hypothesis that the GPT technique and measurements of PDE obtained from rumen contents of calves fed a similar diet could be used as a reflection of the degradation that would be observed in vitro. Although treatment variation, can be estimated using the PDE technique, the variation of the analysis of enzyme is still large.

Apart from methodological differences, explanations for this lack of correlation between GPT and PDE include the following: The GPT measures the appearance of end products, with still
intact rumen microorganisms, which after few hours of being incubated will definitively adapt to the substrate.

The PDE activities techniques estimate the capability of the extracted enzymes to degrade or hydrolyze carbohydrates whereas the GPT measure the whole process of fermentation (the monomers of glucose are not the end product, whereas they are going to used by the bacteria), and assuming that en PDE, hydrolysis is not limiting step (as shown by the high amount of reducing sugars presents in the rumens contents in chapter 4) these two methodologies may measure different rate limiting steps.
Table 2. Parameters of linear regression equations comparing the results obtained with the gas production test (as dependent variables) with the polysaccharide degrading enzyme activity (as regressor), either by substrate (= pooled over inocula) and by inoculum (= pooled over substrates) respectively.

<table>
<thead>
<tr>
<th>Pooled by substrate</th>
<th>Parameter</th>
<th>Intercept (slope)</th>
<th>SE (slope)</th>
<th>se (slope)</th>
<th>r²</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Native corn starch</td>
<td>Rmax (mL g OM⁻¹ h⁻¹)</td>
<td>38.9 (0.6)</td>
<td>10.2 (1.7)</td>
<td>0.6 (2.7)</td>
<td>0.06</td>
<td>0.75</td>
</tr>
<tr>
<td></td>
<td>Cumulative Gas production (mL g OM⁻¹)</td>
<td>347.8 (20.8)</td>
<td>16.0 (6.4)</td>
<td>-5.0 (2.7)</td>
<td>0.64</td>
<td>0.19</td>
</tr>
<tr>
<td>Soy Hulls</td>
<td>Rmax (mL g OM⁻¹ h⁻¹)</td>
<td>10.7 (2.7)</td>
<td>3.5 (2.7)</td>
<td>0.1 (1.3)</td>
<td>0.00</td>
<td>0.92</td>
</tr>
<tr>
<td></td>
<td>Cumulative Gas production (mL g OM⁻¹)</td>
<td>287.6 (20.8)</td>
<td>56.4 (13.6)</td>
<td>-9.7 (2.7)</td>
<td>0.09</td>
<td>0.68</td>
</tr>
<tr>
<td>Sugar beet pectin</td>
<td>Rmax (mL g OM⁻¹ h⁻¹)</td>
<td>78.0 (7.5)</td>
<td>15.0 (4.7)</td>
<td>-7.5 (4.7)</td>
<td>0.55</td>
<td>0.25</td>
</tr>
<tr>
<td></td>
<td>Cumulative Gas production (mL g OM⁻¹)</td>
<td>354.5 (12.6)</td>
<td>39.8 (20.8)</td>
<td>-1.6 (12.6)</td>
<td>0.00</td>
<td>0.91</td>
</tr>
<tr>
<td>Straw</td>
<td>Rmax (mL g OM⁻¹ h⁻¹)</td>
<td>4.5 (1.1)</td>
<td>1.1 (1.3)</td>
<td>-2.1 (1.3)</td>
<td>0.39</td>
<td>0.37</td>
</tr>
<tr>
<td></td>
<td>Cumulative Gas production (mL g OM⁻¹)</td>
<td>83.4 (12.6)</td>
<td>11.9 (20.8)</td>
<td>-48.2 (21.1)</td>
<td>0.72</td>
<td>0.15</td>
</tr>
<tr>
<td>Pooled by Inoculum</td>
<td>C70-S30</td>
<td>Rmax (mL g OM⁻¹ h⁻¹)</td>
<td>5.6 (3.1)</td>
<td>39.0 (21.2)</td>
<td>13.7</td>
<td>21.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cumulative Gas production (mL g OM⁻¹)</td>
<td>50.7 (43.7)</td>
<td>80.6 (78.8)</td>
<td>116.9</td>
<td>43.7</td>
</tr>
<tr>
<td></td>
<td>C70-CS30</td>
<td>Rmax (mL g OM⁻¹ h⁻¹)</td>
<td>12.2 (3.1)</td>
<td>17.2 (21.2)</td>
<td>2.9</td>
<td>3.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cumulative Gas production (mL g OM⁻¹)</td>
<td>152.1 (43.7)</td>
<td>120.7 (78.8)</td>
<td>16.5</td>
<td>18.5</td>
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<tr>
<td></td>
<td>C-100</td>
<td>Rmax (mL g OM⁻¹ h⁻¹)</td>
<td>-4.48 (2.9)</td>
<td>11.9 (2.9)</td>
<td>9.2</td>
<td>9.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cumulative Gas production (mL g OM⁻¹)</td>
<td>46.0 (8.2)</td>
<td>33.7 (8.2)</td>
<td>58.1</td>
<td>8.2</td>
</tr>
<tr>
<td></td>
<td>C70-G15-S15-AL</td>
<td>Rmax (mL g OM⁻¹ h⁻¹)</td>
<td>-14.8 (13.2)</td>
<td>21.9 (13.2)</td>
<td>30.6</td>
<td>13.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cumulative Gas production (mL g OM⁻¹)</td>
<td>16.6 (24.8)</td>
<td>41.3 (24.8)</td>
<td>170.1</td>
<td>24.8</td>
</tr>
</tbody>
</table>

¹ Cumulative gas production after 72 h fermentation, in mL g OM⁻¹ incubated; Rmax = Maximal gas production rate. For inoculums acronyms see descriptions see materials and methods chapter 2.
Rmax pooled by Substrate

OM Cumulative Gas Production pooled by Substrate
REFERENCES


Summary

Historically, veal calves were fed until slaughter weight with only milk replacer and, in absence of solid feed the physiological development of the forestomachs was limited. In 1997, a new EU legislation stipulated that a minimum amount of solid feed (fiber) has to be provided for the welfare of the calves (solid feed supply reduce abnormal oral behaviors in veal calves; Van Putten, 1982; Veissier et al., 1998); however, no specifications were made about the type and source of feed.

Rumen development is triggered by the production of VFA resulting from fermentation of OM in the rumen (Flatt et al., 1958). Butyrate, and to a lesser extent propionate stimulate the development of the rumen mucosa; mostly because of their use as energy sources by the rumen epithelium (Sander et al., 1959; Tamate et al., 1962). In rearing calves, information on rumen fermentation of different sources of dietary carbohydrates is relatively well documented (Davis and Drackley, 1998; Lesmeister and Heinrichs, 2004) but only a few experiments have been conducted in veal calves.

Based on earlier research (Blokhuis et al., 2000) it was hypothesized that stimulating early rumen development in veal calves would be beneficial to their subsequent performance and health. Therefore in vivo experiments were designed to establish the effects of stimulating an early rumen development in veal calves, aiming to optimize nutrient utilization from rumen fermentation and to prevent health problems in the lower gastrointestinal tract (e.g. ulcers in abomasum). In addition the potential interactions of feeding solid feed with a milk replacer based diet were investigated. Finally but not least, the development and evaluation (comparison) of techniques for estimating fermentation characteristics of different substrates, to facilitate the choice of feed ingredient for veal calves diets was also carried out.

Chapter 2: This study was conducted to evaluate the effects of concentrate intake, differing in carbohydrates composition in addition to a milk replacer, on growth performance and rumen fermentation characteristics in veal calves. Accordingly, 160 Holstein Friesian x Dutch Friesian male calves, were fed with one of the following treatments: 1) milk replacer control (CONTROL), 2) pectin-based concentrate (PECTIN), 3) neutral detergent fiber (NDF) based concentrate, 4) starch-based concentrate (STARCH) and 5) mixed concentrate (MIXED) (equal
amounts of concentrates of treatments 2, 3, and 4). Concentrate diets were provided in addition to a commercial milk replacer. **Results:** Calves showed an ADG varying between 0.70 and 0.78 kg/d, with a rumen fermentation in concentrates fed calves characterized by a low pH (4.9 – 5.2), relatively low VFA concentrations between 100 and 121 mmol/L and high concentrations of reducing sugars (33-66 g/kg DM). Calves fed the CONTROL diet had higher lactate concentration (21mmol/L) than concentrate fed calves (between 5 and 11 mmol/L). Results indicated that the carbohydrate source can influence intake, growth rate and rumen fermentation in young veal calves.

**Chapter 3:** This experiment aimed to gain an insight into the effects of age (calves were euthanized either at the end of 8 or 12 weeks of age) and concentrate supplementation, differing in carbohydrates composition, on rumen development in young veal calves. Moreover, some selected plasma metabolites as predictors of rumen development were evaluated. Diets treatments correspond to those described in Chapter 2. **Results:** Feeding concentrates differing in carbohydrate composition to veal calves promoted rumen development compared with calves fed milk replacer only. In most calves, a poorly developed rumen mucosa was observed. Coalescing rumen papillae with embedded hair, feed particles and cell debris were found in all calves fed concentrate diets. Calves fed concentrates had significantly heavier rumens than calves fed CONTROL. Although the variation in carbohydrate composition caused variation in rumen development, the latter was generally small. In the dorsal location of the rumen, calves fed concentrate diets showed an increased ratio of mucosa to serosa length (RMSL) than calves fed CONTROL. Mucosa thickness (MCT) and muscle thickness (MST) were bigger in the ventral and in the dorsal locations of the rumen, respectively.

At 8 weeks, calves fed concentrate diets had higher plasma acetate concentrations than calves on the CONTROL treatment. However, at 12 weeks, only NDF fed calves showed significantly higher plasma acetate concentrations. For plasma BHBA concentration no differences were observed among treatments at 12 weeks. Results from a principal component analysis indicated that veal calves, in addition to rumen volatile fatty acids (VFA) concentrations, other factors are likely to affect rumen development.

**Chapter 4:** This experiment was designed based on the results obtained in experiment 1 (Chapter 2 and 3) where in concentrate-fed veal calves a rumen environment, characterized by a
sub clinical acidosis (pH< 5.2), relatively low VFA concentrations (100-120 mmol/L) and a rumen mucosa characterized by poorly shaped papillae with feed and cell debris embedded between them (referred as plaque formation) were observed. Feeding only roughage to young calves generally does not promote rapid papillae development (Nocek and Kesler, 1980); however, roughage consumption and its inherent coarseness stimulate the development of the rumen wall (Tamate et al., 1962) and rumination (Hodgson, 1971) and the healthiness of the rumen mucosa (Haskins et al., 1969). Information concerning the effects of roughage intake on veal calves performance and rumen development is scarce (Blokhuis et al., 2000; Cozzi et al., 2002). Therefore, it was hypothesized that adding small amount of roughage to a concentrate diet will improve and stimulates the development of the rumen wall, without having negative effects on calf’s performance. Sixty four male Holstein Friesian x Dutch Friesian veal calves (46 kg ± 3.0 kg), were fed on of the following diets: 1) C100= concentrate only, 2) C70-S30= concentrate (70%) with straw (30%), 3) C70-G30= concentrate (70%) with dried grass (30%), 4) C70-G15-S15= concentrate (70%) with dried grass (15%) and straw (15%), 5) C70-CS30= concentrate (70%) with corn silage (30%), 6) C40-CS60= concentrate (40%) with corn silage (60%), 7) C70-CS30-AL= concentrate (70%) with corn silage (30%) ad libitum, 8) C70-G15-S15-AL= concentrate (70%) with dried grass (15%) and straw (15%) ad libitum. All dietary treatments were provided in addition to a commercial milk replacer. Concentrate was provided as pellets and roughage was chopped. Results: Roughage and intake level affects rumen fermentation and rumen development of veal calves. Substitution of part of the concentrate by roughage did not affect DMI and ADG, but among roughage sources feeding straw reduced DMI and ADG. The addition of roughage did not affect rumen pH (pH >5.3). Rumen fermentation was characterized by high total volatile fatty acids (VFA) and reducing sugars (RS) concentrations. Cobalt recovery, as an indication of milk leakage was found in the rumen, varying between 20.5 and 34.9 %, but it was not affected by dietary treatments. Roughage addition decreased the incidence of plaque formation and the incidence of calves with poorly developed rumen mucosa. However, morphometric parameters of the rumen wall were hardly influenced by the type and level of roughage. Results indicated that in veal calves, the addition of roughage to concentrate diets did not affect growth performance and positively influenced the macroscopic appearance of the rumen wall.
Summary

Chapter 5: Several methodologies have been developed to characterize feedstuffs in terms of digestibility and degradability, comprising in vivo, in situ and in vitro methods.

The gas production technique (GPT) provides gas production profiles that give an indication of the fermentative characteristics of the feed. The objective of this experiment was to estimate fermentation kinetic parameters of various solid feeds supplied to veal calves using the GPT, and to study the effect of adaptation of the rumen microflora to these solid feeds on their subsequent fermentation patterns. Thus, from the in vivo experiment described in Chapter 2 and 3; three out of five dietary treatments were selected as inoculum sources: pectin (PECTIN), neutral detergent fiber (NDF), and starch (STARCH). Sugar beet pulp (SBP), sugar beet pectin (SBPec), native corn starch (NCS), soy bean hulls (SBH) and crystalline cellulose (AVICEL) were selected as substrates. For the second in vitro experiment, three out of eight dietary treatments (from the in vivo experiment described in Chapter 4) were selected as inocula. The selected diet treatments were: C100= concentrate only, C70-S30= concentrate (70%) with straw (30%) and C70-CS30= concentrate (70%) with corn silage (30%). For this gas production experiment, straw (STRAW), soy bean hulls (SBH), native corn starch (NCS) and sugar beet pectin (SBPec) were chosen as in vitro substrates.

For both in vivo experiments, cumulative gas production was measured over time (72 h) as an indicator of the kinetics of fermentation. Fermentation end-products, including volatile fatty acids and ammonia, and organic matter loss, were also measured. Results: In both experiments significant differences between the inoculum sources, in terms of both fermentation kinetics characteristics and end-products of fermentation were observed. Similarly, significant effects were also observed for substrate compositions. Differences between the fermentation characteristics of NCS, SBPec and SBH, were consistent for both experiments. The total VFA production was not different among these substrates in both experiments. Finally, for both experiments, there was a significant inocula and substrate interaction which may indicate differences in the microbial activity occurring between the calves. Therefore, it was concluded that rumen inoculum from adapted animals should be used to obtain a more accurate assessment of feed ingredients in veal calf diets.

The Chapter 6 (General discussion) focused in four points: a) Factors influencing rumen development in rearing and veal calves; b) The importance of ruminal drinking in veal calves fed
solids feeds; c) Effects of feeding strategies on ruminal pH and buffering capacity of rumen contents in veal calves; d) comparative analysis of the results obtained from the GPT (results presented in Chapter 5) and the PDE activities (results presented in Chapter 2 and 4).
SAMENVATTING
Samenvatting

Voor de productie van wit- en rosévlees werd traditioneel aan vleeskalveren enkel kalvermelk verstrekt en geen ruw- of krachtvoer. Het achterwege laten van deze voercomponent vormde een beperking in de fysiologische ontwikkeling van de voormagen. In 1997 werd in de EG-wetgeving vastgelegd dat in de rantsoenen van vleeskalveren een minimum vastgesteld aandeel “vezelhoudend voer” moest worden opgenomen om een verbeterd welzijn van de dieren te bewerkstelligen (het verstrekken van ruwvoer verlaagt het tot expressie komen van afwijkend oraal gedrag bij vleeskalveren; Van Putten, 1982; Veissier et al., 1998). Er werden echter geen voorwaarden gesteld aan het type voer of waarvan het afkomstig is.

De ontwikkeling van de pens bij kalveren wordt gestuurd door vluchtige vetzuren (VFA) die geproduceerd worden tijdens de fermentatieve afbraak van organische stof in de pens (Flatt et al., 1958). Boterzuur, en in mindere mate propionzuur, hebben een stimulerend effect op de ontwikkeling van de penswand doordat de pensepitheelcellen ze gebruikt als energiebron (Sander et al., 1959; Tamate et al., 1962).

Hoewel de fermentatieve afbraak van verschillende koolhydratfracties in de pens van opfokkalveren relatief uitgebreid gedocumenteerd is (Davis en Drackley, 1998; Lesmeister en Heinrichs, 2004), zijn er slechts enkele studies uitgevoerd met vleeskalveren.

Op basis van een door Blokhuis en co-auteurs (2000) uitgevoerde studie, is de hypothese opgesteld dat het stimuleren van een vroege pensontwikkeling bij vleeskalveren een positief effect kan hebben op de gezondheid en productie van de dieren. Aan de hand van deze hypothese zijn een aantal in vivo proeven opgezet om de effecten van een vroege pensontwikkeling bij vleeskalveren te onderzoeken. Hierbij werd als doel gesteld om een optimale benutting van nutriënten afkomstig van fermentatie in de pens te behalen en om gezondheidsproblemen in het achterliggende maagdarmkanaal te voorkomen (bijv. vorming van lasies in de lebmaag).

Daarnaast zijn de interacties bestudeerd die mogelijk plaatsvinden indien een combinatie van ruwvoer en kalvermelk aan de dieren wordt verstrekt. Tevens zijn technieken ontwikkeld om de fermentatiekarakteristieken van verschillende substraten te bepalen, en daarnaast zijn deze geëvalueerd. Deze technieken kunnen worden gebruikt om een betere afweging te maken bij de grondstoffkeuze voor de samenstelling van het rantsoen.
Hoofdstuk 2: In de proef die in hoofdstuk 2 is beschreven is gekeken naar het effect van de opname van krachtvoeders van verschillende koolhydraatsamenstelling in combinatie met kalvermelmelkgift op de groei en pensfermentatie bij vleeskalveren. Honderdzestig stierkalveren (HF x DF) werden toegekend aan een van de volgende rantsoenbehandelingen: 1) kalvermelk (CONTROLE), 2) een pectinerijk krachtvoer (PECTINE), 3) een celwandrijk krachtvoer (NDF), 4) een zetmeelrijk krachtvoer (ZETMEEL) en 5) een krachtvoermengsel (MIX) waarin de krachtvoeders van behandelingen 2, 3 en 4 proportioneel vertegenwoordigd zijn. De kalveren van behandeling 2-4 kregen maximaal 750 g DS/d van het krachtvoer naast hun dagelijkse portie kalvermelmelk. Van elke behandeling werden kalveren geslacht na 8 of 12 weken. Resultaten: De gemiddelde groeisnelheid per dier per dag varieerde tussen de 0,70 en 0,78 kg/dag. Bij kalveren met krachtvoer in hun rantsoen werd fermentatie in de pens waargenomen. De inhoud van de pens bij deze dieren werd gekarakteriseerd door een lage pH (4,9 – 5,2), een relatief lage concentratie aan VFA (100 and 121 mmol/L) en een hoge concentratie aan reducerende suikers (33-66 g/kg DM). Kalveren uit de CONTROLE behandeling hadden een hoger melkzuurgehalte (21 mmol/L) in de pens in vergelijking met de dieren met een krachtvoerbehandeling (5 tot 11 mmol/L). De resultaten gaven een indicatie dat het type koolhydraat van invloed kan zijn op de opname, de groeisnelheid en de mate van pensfermentatie in jonge vleeskalveren.

Hoofdstuk 3: Deze proef had tot doel om inzicht te verschaffen naar de effecten van leeftijd en krachtvoer suppletie op de pens ontwikkeling bij vleeskalveren op jonge leeftijd. De kalveren ontvingen krachtvoerbehandelingen zoals beschreven in hoofdstuk 2, waarbij de krachtvoeders verschilden in koolhydraatsamenstelling. De ontwikkeling van de penswand werd op 8 of op 12 weken leeftijd beoordeeld. Tevens werd het bloedplasma op een aantal metabolieten geanalyseerd en is onderzocht of deze als verklarende variabelen golden ten aanzien van de ontwikkeling van de pens. Resultaten: Het verstrekken van krachtvoeders naast de kalvermelmelk had een positief effect op de ontwikkeling van de penswand in vergelijking met kalveren die enkel kalvermelmelk ontvingen. Ten aanzien van deze laatste groep (CONTROLE) werd bij de meeste dieren een matig ontwikkelde penswand gevonden. In alle krachtvoer gesuppleerde kalveren werden verklevingen van penswandpapillen, haar, voerdeeltjes en celwandmateriaal waargenomen. Krachtvoer gesuppleerde dieren hadden een significant zwaardere pens in vergelijking met de CONTROLE dieren. Binnen de gesuppleerde dieren lieten de verschillende
koolhydraatbehandelingen een verschil in pensontwikkeling zien. De behandelings-effecten waren echter klein. In het dorsale deel van de pens werd een hogere ratio voor de mucosa: serosa lengte (RMSL) gevonden bij krachtvoer gesuppleerde kalveren ten opzichte van de dieren op een CONTROLE rantsoen. De dikte van de mucosa (MCT) nam toe in het ventrale deel van de penswand terwijl de spierdikte (MST) toenam in het dorsale deel van de pens.

Op een leeftijd van acht weken gaven de met krachtvoer gesupplementeerde kalveren hogere plasma azijnzuur concentraties in vergelijking met de CONTROLE dieren. Echter, na 12 weken gaven alleen de dieren uit de NDF-behandeling significant hogere azijnzuur concentraties in het plasma. Ten aanzien van bèta-hydroxyboterzuur (BHBA) werden geen verschillen waargenomen tussen de behandelingen in week 12. Analyse door middel van ‘principal component analyses’ gaf aan dat naast de VFA concentraties op pensniveau andere factoren van invloed zijn op het ontwikkelen van de pens.

**Hoofdstuk 4:** De opzet van de hier beschreven proef is gebaseerd op de resultaten van de eerste proef (beschreven in hoofdstuk 2 en 3), waar bij krachtvoer gesuppleerde kalveren een pens milieu werd aangetroffen met indicaties van een subklinische acidose (pH<5.2) die optrad bij relatief lage concentraties aan VFA (110-120 mmol/L), en een slecht ontwikkelde penswand die gekarakteriseerd wordt door matig ontwikkelde papillen die tezamen met voerdeeltjes en celwandmateriaal verklevingen vormen (zgn. plaque formatie). Het enkel verstrekken van ruwvoer aan jonge kalveren draagt niet bij een versnelde ontwikkeling van de papillen (Nocek en Kesler, 1980). Echter, ruwvoer als zodanig (en dan met name de structuur die met het ruwvoer wordt opgenomen) stimuleert de ontwikkeling van de penswand (Tamate et al., 1962) en het proces van herkauwen (Hodgson, 1971), en draagt bij aan een gezonde pens mucosa (Haskins et al., 1969). Informatie over de invloed van ruwvoeropname in relatie tot productieparameters van vleeskalveren en de ontwikkeling van de pens zijn beperkt voor handen (Blokhuis et al., 2000; Cozzi et al., 2002). Op basis van de eerdergenoemde literatuur en eigen observaties werd als hypothese gesteld dat het opnemen van een kleine hoeveelheid ruwvoer in een op krachtvoer gebaseerd rantsoen stimulerend werkt op de ontwikkeling van de penswand, zonder dat de productieparameters negatief beïnvloed worden. Vierenzestig stierkalveren (46 kg ± 3.0 kg; HF x DF) ontvingen de volgende rantsoenen: 1) C100= alleen krachtvoer, 2) C70-S30= krachtvoer (70%) met stro (30%), 3) C70-G30= krachtvoer (70%) met gedroogd gras (30%), 4) C70-G15-
S15= krachtvoer (70%) met gedroogd gras (15%) en stro (15%), 5) C70-CS30= krachtvoer (70%) met maïßilage (30%), 6) C40-CS60= krachtvoer (40%) met maïssilage (60%), 7) C70-CS30-AL= krachtvoer (70%) met maïssilage (30%) ad libitum verstrekt, 8) C70-G15-S15-AL= krachtvoer (70%) met gedroogd gras (15%) en stro (15%) ad libitum verstrekt. Ruw en krachtvoer werd bij de kalveren van behandelingen 1-6 tot een maximum van 750 g DS/d verstrekt naast een gewone portie kalvermelk. Bij behandeling 7 en 8 werd de kalvermelkgift verminderd om een goede opname aan ruw- en krachtvoer mogelijk te maken. Het krachtvoer werd verstrekt in de vorm van een brok en het ruwvoer was gehakseld. Resultaten: Verstrekking van ruwvoer en het niveau van voeropname beïnvloedde de pensfermentatie en de ontwikkeling van de pens bij vleeskalveren. Een gedeeltelijke vervanging van het krachtvoer door ruwvoer had geen effect op de drogestof opname (DMI) en gemiddelde dagelijkse groeisnelheid (ADG) van dieren, maar een vergelijking tussen ruwvoercomponenten liet zien dat stro een negatief effect had op de DMI en ADG. Het verstrekken van kracht- en ruwvoer had geen effect op de pH in de pens (pH >5,3), maar gaf een hoge concentratie aan vluchtige vetzuren (VFA) en reducerende suikers (RS). De recovery van cobalt, dat door de laatste melkvoerbeurt werd gemengd, in de pens, is een maat voor de hoeveelheid melk die langs de slokdarmsleuf in de pens lekt, ofwel terugvloeit vanuit de lebmaag naar de pens. Deze varieerde tussen 20 en 35% maar werd niet beïnvloed door de rantsoenbehandelingen. De verstrekking van ruwvoer gaf een vermindering in de zogenaamde plaque formatie (het verkleven van papillen onderling en met voerdeeltjes en celwandmaterialen) en het aantal kalveren met een matig ontwikkelde pensmucosa (macroscopisch beoordeeld) nam af. Echter, de morfometrische parameters van de penswand (mucosadikte, spierlaagdikte, ratio mucosa:serosa lengte) werden nagenoeg niet beïnvloed door het type ruwvoer en het niveau van opname. De resultaten lieten zien dat het verstrekken van ruwvoer aan vleeskalveren geen effect had op de groei van de dieren maar wel een positief effect had op de penswand.

**Hoofdstuk 5:** In de loop der tijd zijn verschillende methoden ontwikkeld om grondstoffen te karakteriseren voor hun mate van verteerbaarheid en afbraak. Deze omvatten *in vivo*, *in situ* en *in vitro* technieken.

De *in vitro* gasproductie techniek (GPT) genereert gasproductie profielen die een indicatie geven van de fermentatie karakteristieken van een voer of een grondstof. Het doel van de in dit
Samenvatting

Hoofdstuk beschreven proeven was het vaststellen van de fermentatiekinetiek van verschillende grondstoffen van voeders die verstrekt waren aan vleeskalveren. Daarnaast werd gekeken in hoeverre de fermentatiekinetiek van de verschillende voeders beïnvloed werd door deze te incuberen met microflora uit de pens van dieren die aan deze voeders geadapeerd zijn. Vanuit de in vivo proeven zoals eerder beschreven in hoofdstuk 2 en 3 werden de dieren uit drie van de vijf rantsoen behandelingen geselecteerd als inoculum donor, te weten: pectine (PECTINE), celwanden (NDF) en zetmeel (STARCH). Suikerbietenpulp (SBP), suikerbieten pectine (SBPec), natief maïszetmeel (NCS), sojahullen (SBH) en een kristallijn cellulose product (AVICEL) werden geselecteerd als substraat.

Voor de tweede in vitro proef werden inoculum gebruikt van drie van de acht rantsoenbehandelingen (zoals beschreven in de in vivo proef onder hoofdstuk 4), te weten: C100= alleen krachtvoer, C70-S30= krachtvoer (70%) met stro (30%) en C70-CS30= krachtvoer (70%) met maïssilage (30%). Voor deze gasproductie proef werden stro (STRO), sojahullen (SBH), natief maïszetmeel (NCS) en suikerbieten pectine (SBPec) als substraten gebruikt. Voor beide gasproductie proeven is de cumulatieve gasproductie gemeten gedurende een periode van 72 uur. Fermentatie eind-producten (vluchtige vetzuren en ammoniak) en organische stof verlies werden tevens bepaald. Resultaat: Beide proeven lieten significante verschillen tussen de inocula zien met betrekking tot de fermentatie kinetiek parameters en de fermentatie eind-producten. Overeenkomstige significante effecten werden waargenomen voor de verschillende substraten. De verschillen in fermentatiekarakteristieken tussen NCS, SBPec en SBH waren consistent tijdens beide proeven. De totale VFA productie verschilde niet tussen de substraten in beide proeven. Voor beide proeven bleek er wel een significant inoculum-substraat interactie te zijn. Dit kan indicatief zijn voor een verschil in microbiële activiteit van de inocula tussen kalveren. Hieruit werd geconcludeerd dat indien men een correctere schatting van de voercomponent wil behalen inoculum zal moeten worden gebruikt van dieren die aan de voercomponent zijn geadapeerd.

Hoofdstuk 6: In dit hoofdstuk (General Discussion) wordt verder ingegaan op een viertal punten: a) Factoren die van invloed zijn op de ontwikkeling van de pens bij opfok- en vleeskalveren; b) De betekenis van het pensdranken door vleeskalveren die naast de kalvermelk bijgevoerd worden; c) De invloed van voederstrategieën op de pH en de buffercapaciteit van de
pensinhoud van vleeskalfen; d) Een vergelijkende analyse van resultaten verkregen via de *in vitro* gasproductie test (resultaten gepresenteerd in hoofdstuk 5) met die van activiteit van uit de pens geïsoleerde microbiële enzymen (resultaten gepresenteerd in hoofdstuk 2 en 4).
PUBLICATIONS AND ORAL PRESENTATIONS


**Reports**


In Memory of My Grandmother “Josefa”

Dedicated to those whom I care for
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Bernardo Jorge Suárez
ABOUT THE AUTHOR

Bernardo Jorge Suárez was born on the 12th of November 1971, in La Carlota, Córdoba, Argentina. In 1989 he graduated from high school education and in 1990 entered the faculty of Veterinary Sciences, Río Cuarto University in Río Cuarto, Córdoba, Argentina. At the beginning of 1997 he obtained his degree in Veterinary Science and until 1999 he worked as a private consultant veterinarian. At the end of this year he was awarded with a BID scholarship to do a Master in animal Nutrition and Physiology at the Universidad Pontificia Católica de Chile (Santiago de Chile), where at the end of 2001 he obtained his diploma in with two votes of distinction.

In 2002 he was appointed at the Wageningen University as an AIO/ PhD student in the project ‘Modelling the quantitative impact of solid feeds to the protein and energy metabolism of veal calves’. Some of the he results obtained from this research are shown in this book.
# TRAINING AND SUPERVISION PLAN

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<tr>
<td><strong>Project title</strong></td>
<td>Modelling the quantitative impact of solids feeds to the protein and energy metabolism of veal calves</td>
</tr>
<tr>
<td><strong>Group</strong></td>
<td>Animal Nutrition Group</td>
</tr>
<tr>
<td><strong>Daily supervisor(s)</strong></td>
<td>dr ir Walter Gerrits</td>
</tr>
<tr>
<td><strong>Supervisor(s)</strong></td>
<td>dr ir Walter Gerrits, dr ir Jan Dijkstra</td>
</tr>
<tr>
<td><strong>Project term</strong></td>
<td>from 01/03/2002 until 01/03/2006</td>
</tr>
<tr>
<td><strong>Submitted date</strong></td>
<td>28/08/2006</td>
</tr>
<tr>
<td><strong>certificate</strong></td>
<td>first plan / midterm / certificate</td>
</tr>
</tbody>
</table>

## EDUCATION AND TRAINING (minimum 21 cp, maximum 42 cp)

<table>
<thead>
<tr>
<th>The Basic Package (minimum 2 cp)</th>
<th>year</th>
<th>cp</th>
</tr>
</thead>
<tbody>
<tr>
<td>Course on Philosophy of Science and Ethics (mandatory)</td>
<td>2003</td>
<td>1.0</td>
</tr>
<tr>
<td>WIAS Introduction Course (mandatory)</td>
<td>2004</td>
<td>1.0</td>
</tr>
</tbody>
</table>

**Subtotal Basic Package**: 2.0

<table>
<thead>
<tr>
<th>Scientific Exposure (conferences, seminars and presentations, minimum 5 cp)</th>
<th>year</th>
<th>cp</th>
</tr>
</thead>
<tbody>
<tr>
<td>International conferences (minimum 2 cp)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Annual Meeting ADSA, ASAS, PSA, St Louis Missouri, USA</td>
<td>2004</td>
<td>1.0</td>
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</table>

**Subtotal International Exposure**: 1.7

<table>
<thead>
<tr>
<th>In-Depth Studies (minimum 4 cp)</th>
<th>year</th>
<th>cp</th>
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</thead>
<tbody>
<tr>
<td>Disciplinary and interdisciplinary courses</td>
<td></td>
<td></td>
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<tr>
<td>WIAS Advanced statistics course: Design of Animal Experiments</td>
<td>2002</td>
<td>0.6</td>
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<tr>
<td>VLAG Advanced Course on Ecophysiology of the Gastrointestinal Tract</td>
<td>2003</td>
<td>1.0</td>
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<tr>
<td>WIAS Debating Course &quot;Broaden your Horizon&quot;</td>
<td>2003</td>
<td>1.0</td>
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<tr>
<td>Advances in Feed Evaluation Science</td>
<td>2004</td>
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**Subtotal In-Depth Studies**: 11.6

<table>
<thead>
<tr>
<th>Professional Skills Support Courses (minimum 2 cp)</th>
<th>year</th>
<th>cp</th>
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</thead>
<tbody>
<tr>
<td>Use of Laboratory Animals (mandatory when working with animals)</td>
<td>2002</td>
<td>3.0</td>
</tr>
<tr>
<td>Wageningen University Language Center Course: English Pre-Intermediate Level</td>
<td>2002</td>
<td>1.2</td>
</tr>
<tr>
<td>Wageningen University Language Center: Course Intermediate/Fluency Level</td>
<td>2002</td>
<td>1.2</td>
</tr>
<tr>
<td>Wageningen University Language Center Course: Upper Intermediate Level</td>
<td>2003</td>
<td>1.2</td>
</tr>
<tr>
<td>WGS Course Career Orientation</td>
<td>2003</td>
<td>1.0</td>
</tr>
<tr>
<td>WIAS Course Techniques for Scientific Writing (advised)</td>
<td>2004</td>
<td>0.8</td>
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</table>

**Subtotal Professional Skills Support Courses**: 8.4

<table>
<thead>
<tr>
<th>Didactic Skills Training (optional)</th>
<th>year</th>
<th>cp</th>
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</thead>
<tbody>
<tr>
<td>Supervising MSc theses (maximum 1 cp per MSc student)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MSc-Thesis S. Fiardo</td>
<td>2002</td>
<td>1.0</td>
</tr>
<tr>
<td>PhD Student Raffaela Tuddisco (Italian Marie Curie student)</td>
<td>2003</td>
<td>1.0</td>
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</table>

**Subtotal Didactic Skills Training**: 2.0

<table>
<thead>
<tr>
<th>Education and Training Total (minimum 21 cp, maximum 40 cp)</th>
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<th></th>
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</thead>
<tbody>
<tr>
<td><em>One credit point (cp) equals a study load of approximately 40 hours</em></td>
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