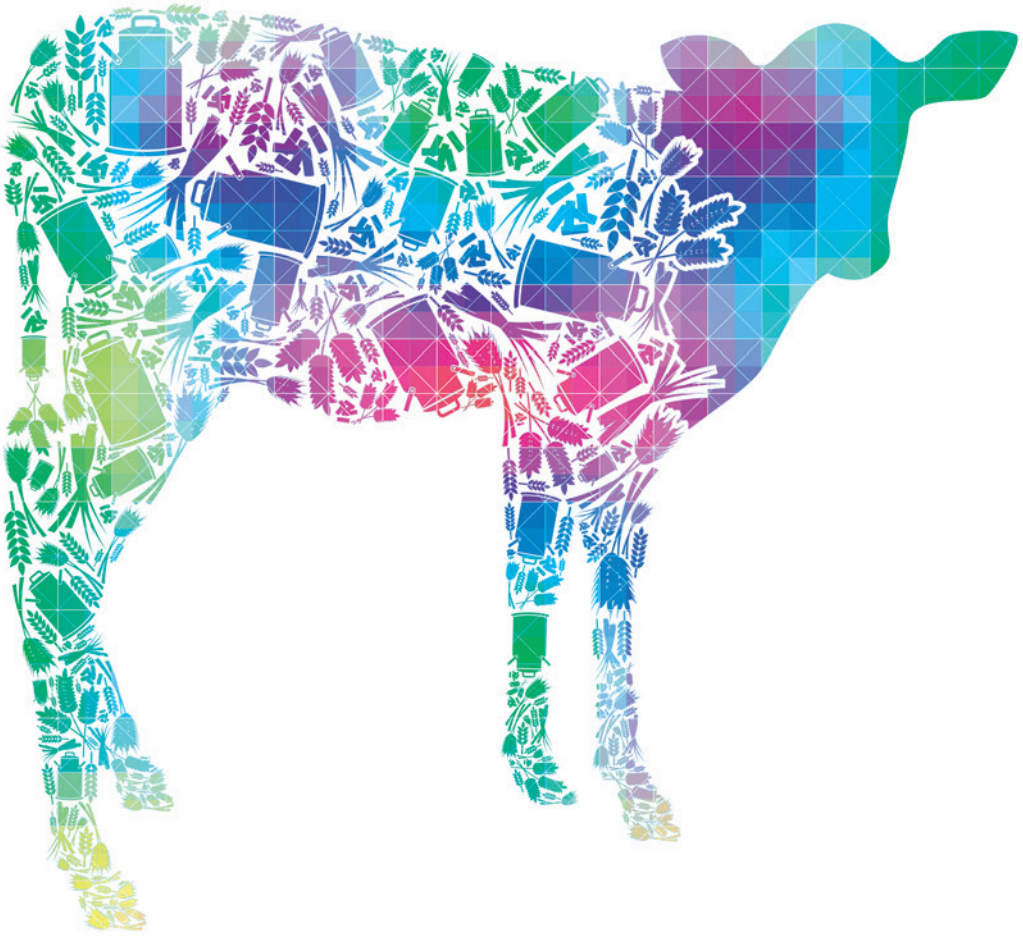
Nutrient utilization, dietary preferences, and gastrointestinal development in veal calves

Interactions between solid feed and milk replacer



Harma Berends

Thesis committee

Promotor
Prof Dr W.H. Hendriks
Professor of Animal Nutrition
Wageningen University

Co-promotors
Dr W.J.J. Gerrits
Associate professor, Animal Nutrition Group
Wageningen University

Dr J.J.G.C. van den Borne
Researcher, Animal Nutrition Group
Wageningen University

Other members
Prof. Dr A. Bach, Institut de Recerca i Tecnologia Agroalimenàries (IRTA), Barcelona, Spain
Prof. Dr I.J.M. de Boer, Wageningen University
Dr H. Everts, Utrecht University, the Netherlands
Prof. Dr C.K. Reynolds, University of Reading, United Kingdom

This research was conducted under the auspices of the Graduate School WIAS
(Wageningen Institute for Animal Sciences)

Nutrient utilization, dietary preferences, and gastrointestinal development in veal calves

Interactions between solid feed and milk replacer

Harma Berends

Thesis

submitted in fulfilment of the requirements for the degree of doctor
at Wageningen University
by the authority of the Rector Magnificus
Prof. Dr M.J. Kropff,
in the presence of the
Thesis Committee appointed by the Academic Board
to be defended in public
on Tuesday 2 September 2014
at 1.30 p.m. in the Aula.

Harma Berends
Nutrient utilization, dietary preferences, and gastrointestinal development in veal calves. Interactions between solid feed and milk replacer, 240 pages.

PhD thesis, Wageningen University, Wageningen, NL (2014)
With references, with summaries in English and Dutch

ISBN 978-94-6257-043-6

ABSTRACT

Solid feeds (SF), comprising roughages and concentrates, represent an increasingly important source of nutrients for veal calves. From a welfare and economic perspective, there is a strong incentive to replace a considerable portion of the milk replacer (MR) by SF in the diet. However, interactions between MR and SF complicate the prediction of the nutritional value of these ration components, and adverse effects on health may occur when combining MR and SF. To investigate these interactions, various combinations of MR, concentrates, and roughages were tested in a series of large-scale studies.

When provided with unrestricted access to MR, concentrates, maize silage, hay, and straw over a 6-month period, calves markedly changed their preferences over time, and individual differences appeared very large. However, the ratio between digestible crude protein and digestible energy in the diet of choice appeared remarkably constant between calves. Another set of studies aimed at defining age-related changes in utilization efficiency of SF. It was demonstrated that stimulating early rumen development (before 12 wk of age) improves the nutritional value of each kg of SF in later life. In another study, it was shown that the nutritional value of SF increases with age. This effect is likely related to improved fermentation of fibrous SF. Increasing SF intake lead to an increase in the passage rates of concentrates and straw through the rumen.

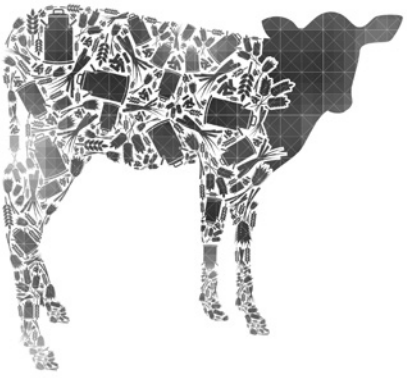
Compared to the feeding of MR alone, nitrogen (N) economy of veal calves can be improved by feeding a low-protein SF, creating a N shortage in the rumen. Urea-N, likely originating from the MR, was demonstrated to recycle back into the rumen for microbial protein production. In a subsequent study, it appeared that the feeding of a high-protein SF improved ruminal degradation of fibrous SF relative to a low-protein SF at equal protein intake, balanced via the MR. Urea recycling was demonstrated to be unable to completely compensate a N shortage in the rumen. An important interaction between MR and SF can be the influence of SF on the proportion of MR flowing in the rumen, where it is fermented and potentially causes health problems. The current standard to measure this so-called 'ruminal drinking' is the Co recovery method, which requires sacrificing the calves. Several non-terminal methods to quantify ruminal drinking were evaluated in three consecutive experiments. From a meta-analysis of Co recovery data, it was shown that on average 17% of the MR fed flows into the rumen instead of the abomasum. No associations with SF or MR intake related variables were found. Potential adverse effects of replacing MR by SF include abomasal damage, particularly in the pyloric area. This generally increases with the intake of SF, particularly in the presence of sharp, abrasive particles, and more so with a 20:80 than with a 50:50 mixture of roughage:concentrate. Results indicated that early rumen development can offer some protection in later life.

In conclusion, when taking interactions between MR and SF into account, it appeared possible to replace a considerable portion of MR by SF without compromising calf performance and health.

Table of Contents

Chapter 1

General Introduction



VEAL SECTOR

In Europe, 806 000 tons of veal is consumed annually. Veal originates from calves that are slaughtered before 12 months of age. Veal is consumed predominantly in Italy, France, and Germany. In Europe, veal production is concentrated in the Netherlands, France, Italy, and Belgium, and average production is approximately 6 million veal calves annually. In addition, veal is produced in North-America (Sans and De Fontguyon, 2009). The Netherlands is a large producer of veal globally, with more than 900 000 calves in 2013 (Figure 1.1). Approximately 90% of the veal produced in the Netherlands is exported (LEI, 2010; Sans and De Fontguyon, 2009). The dairy sector and the veal sector are historically linked because most veal calves were surplus dairy bull calves fed milk-based milk replacer (**MR**). At a national perspective, however, this traditional link in the Netherlands has become less strong, since large numbers of new-born calves are imported from Germany, Poland, and the Baltic States. Furthermore, dairy products as the main ingredients for MR are increasingly replaced by ingredients of vegetable origin.

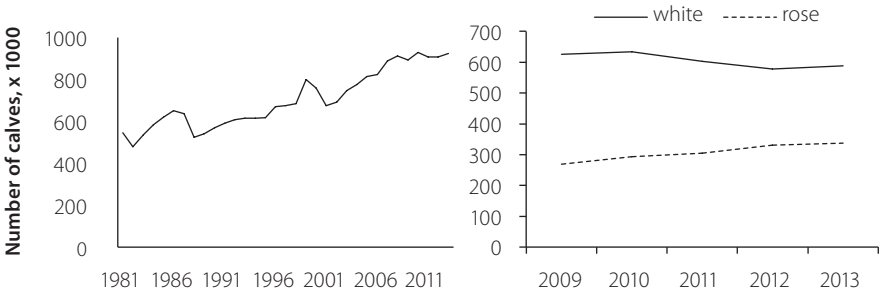


Figure 1.1 Development of the number of veal calves present at January 1st from 1981 to 2011 in the Netherlands (left), and a comparison between the number of white and rose veal calves over the last 5 years (right). Source: Statistics Netherlands.

Two types of veal are distinguished: rosé and white veal. These names refer to the color of the meat, influenced by differences in the diet composition. White veal originates from calves younger than 8 months of age that are fed a diet consisting of primarily MR, whereas rosé veal originates from calves younger than 12 months of age that are weaned at around 12 weeks of age and then fattened on solid feed (**SF**). Rosé veal is considerably cheaper than white veal, but the production costs are also lower because of the reduced feeding costs. Rosé veal production started in the 1990s and has evolved since then to a current market share of over 30% of the Dutch veal sector (Figure 1.1).

This thesis considers white veal production only. White veal is produced at specialized veal farms. The white veal sector is highly integrated, with processes such as production of MR, slaughtering, meat processing, and transport organized within one enterprise. Predominantly male calves are purchased from dairy farms at 2 weeks of age and reared at specialized veal farms for 19 to 27 weeks for the production of veal. Calves are purchased by companies, and farmers receive a fee for rearing the calves according to the company's guidelines on feeding and management. The most commonly used veal housing system globally is group housing on slatted floors in pens of 2 to 8 calves after a period (2 to 6 wk) of individual housing.

DIET

The paleness of white veal is achieved by feeding a diet consisting primarily of low-iron MR, which is typically provided twice daily. Veal calves are traditionally fattened on a diet consisting only of MR. In the past, MR was based on skim milk powder, a by-product of butter production, partly because the marketing of skim milk powder was subsidized to stimulate dairy production in Europe. From the 1970s onwards, there was a shift from the use of skim milk powders in MR towards whey powder, a by-product of cheese production, de-lactosed whey powder, and vegetable sources (mainly soluble or dispersible products originating from soy and wheat).
Today, SF represents an increasingly important part of the diet for white veal calves. This may be explained, first, by the increasing prices of MR ingredients, especially over the past 5 years. Figure 1.2 shows that prices for whey powder have doubled since 2009. For skim milk powder, prices have also increased to over €3 per kg.

A second reason is the introduction of a welfare-associated EU Directive introduced in 1997 that stimulated inclusion of SF in the ration of veal calves at the expense of MR. Veal calves only provided with MR often show non-nutritive and abnormal oral behaviors, including tongue playing and rolling, oral manipulation of inedible objects (trough, bucket, pen structure), and sham chewing (Kooijman et al., 1991, Van Putten, 1982, Veissier et al., 1998, Wiepkema et al., 1987). These behaviors indicate chronic stress and impaired welfare (Mason and Rushen, 2008). In veal calves, abnormal oral behaviors result from the absence of SF (Veissier et al., 1998). Indeed, providing SF to veal calves reduced abnormal oral behaviors (Kooijman et al., 1991, Mattiello et al., 2002, Veissier et al., 1998, Webb et al., 2012) and improved rumen development (Di Giancamillo et al., 2003). In 1995, the Scientific Veterinary Committee concluded that "the lack of SF led to impairments in rumen development in such a way that veal calves could not express their natural condition of ruminants" (Scientific Veterinary Committee, 1995). Therefore, a European Directive (Council Directive 97/2/EC, 1997) was adopted in 1997, which stipulated that veal calves

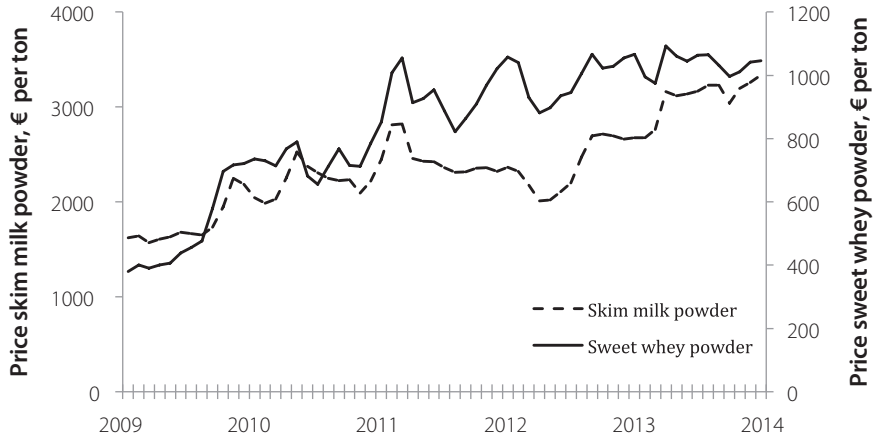


Figure 1.2 Development of world market prices of sweet whey powder (non-hygroscopic) and skim milk powder (1.25% butter fat) from 2009 till present. Source: USDA, 2014.

should be provided 'with a minimum daily amount of fibrous feed, ranging from 50 g/day at 8 weeks of age to 250 g/day at 20 weeks of age'.

A substantial increase of the SF component at the expense of MR in veal calf diets could improve calf welfare and be beneficial in terms of economic performance. However, optimal feeding strategies using MR combined with SF have not been established yet. No specifications were made in the Directive concerning the dry matter (**DM**) content, source, or particle size of SF. Fiber-rich sources are more effective in preventing abnormal oral behaviors when compared with concentrate sources of SF (Mattiello et al., 2002, Morisse et al., 2000, Webb et al., 2012) and increasing SF intake above 250 g/d decreases abnormal behaviors further (Webb et al., 2013). Observations in practice and under experimental conditions indicate that combining the feeding of SF and MR may induce a number of health and growth related issues which are caused by complex interactions between the MR and SF components of the diet. These partly interrelated issues, as further discussed in the next paragraphs, currently limit the increase of SF in veal calf diets.

HEALTH

Reported mortality rates for veal calves range between 2.5 and 5.3% (Bähler et al., 2012, Pardon et al., 2012a, Sargeant et al., 1994, Stull and McDonough, 1994, Wilson et al., 1994). The main causes of mortality in veal calves are pneumonia, ruminal disorders, idiopathic peritonitis, enterotoxaemia, and enteritis (Pardon et al., 2012b). Various factors may

contribute to the high incidence of intestinal and respiratory diseases in veal calves. First, after birth, some calves do not receive colostrum or a limited amount (Wilson et al., 1994). Colostrum intake is important for the transfer of passive immunity, but also supplies nutrients, hormones, growth factors, cytokines, enzymes, polyamines and nucleotides. This in turn can improve the development and function of the gastrointestinal tract, along with several other aspects (Blum and Hammon, 2000). Second, veal calves present at the same fattening unit usually originate from a multitude of different dairy farms and, therefore, carry a variety of infectious agents. Up until arrival at the veal farm, exposure to pathogens, starvation, transport, and dietary changes are major stress events. These factors challenge the calves' immune system and gut barrier function at an early stage of life. Antimicrobial drug use in the veal sector is highest of all food producing animal sectors (Pardon et al., 2012a). As a consequence, multidrug resistance is widely present in the veal industry (Catry et al., 2007, Cook et al., 2011, Graveland et al., 2010, van Cleef et al., 2011). Health aspects related to diet composition are discussed in the next paragraphs.

Rumen Development. At birth, the rumen of a calf has not yet developed (Figure 1.3). Milk bypasses the rumen by means of the esophageal groove. Papillary growth, rumen wall muscularization and vascularization, and reticulorumen volume are minimal (Tamate et al., 1962). When MR is the only source of nutrients, the rumen remains undeveloped (Suárez et al., 2006). Rumen development is initiated by the fermentation of SF, triggered by establishment of the anaerobic ruminal microbial ecosystem and absorption of fermentation end products (Baldwin VI et al., 2004). Ruminal papillae development is triggered by end-products of fermentation, volatile fatty acids (**VFA**), especially butyrate (Flatt et al., 1958, Sander et al., 1959), whereas the physical structure of roughages contributes to the muscular development and expansion of ruminal volume (Stobo et al., 1966). In veal calves, concentrate feeding in combination with MR has been shown to result in abnormal development and functioning of the rumen (Suárez et al., 2006). Poor rumen development and 'plaque formation' (coalescing rumen papillae with embedded hair, feed particles and cell debris) were found in 60% and 31% of all veal calves, respectively (Brscic et al., 2011).

Although the consequences for the calf's health and performance are still largely unknown, the absorptive capacity of the rumen papillae may be limited in the case of plaque formation, resulting in accumulation of VFA in the rumen. Adding roughage to the concentrate decreased the incidence of plaque formation as well as the incidence of a poorly developed rumen mucosa (Suárez et al., 2007), whereas type and level of roughage had a limited effect on rumen mucosa development (Suárez et al., 2007). Another factor associated to SF intake in veal calves is hyperkeratosis, or thickening of the stratum corneum, which is found in 6% of veal calves (Brscic et al., 2011). Hyperkeratosis creates a physical barrier, and may restrict VFA absorption and cause papillae degeneration and

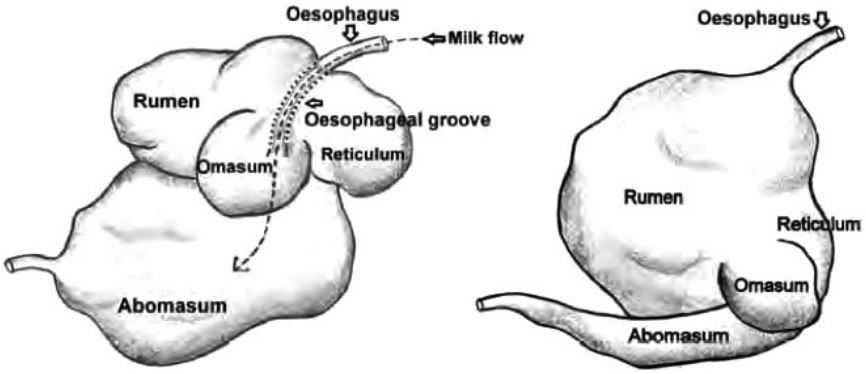


Figure 1.3 Comparative size of stomach compartments of a pre-ruminant calf (left) and of an adult ruminant (right).

sloughing (Beharka et al., 1998). Hyperkeratosis is associated with the abrasive value of the (concentrate) feed, and may decrease rumen pH as well (Greenwood et al., 1997). When the provision of SF in veal calf rations increases, adequate rumen development and fermentation becomes more important. The onset of rumen development is a process requiring time and energy, and potentially calves benefit from an early onset in the fattening period.

Ruminal Drinking. Ruminal drinking (**RD**), i.e. leakage of MR into the rumen, may result from failure of the esophageal groove reflex or backflow of MR from the abomasum. In a clinical case, RD can result in chronic maldigestion (Stocker et al., 1999), ruminal acidosis, lack of appetite, and recurrent bloat (van Weeren-Keverling Buisman et al., 1991), and consequently a reduced growth rate. Although clinical cases of RD have been described extensively (Gentile et al., 2004, Herrli-Gygi et al., 2006, Van Weeren-Keverling Buisman et al., 1990, van Weeren-Keverling Buisman et al., 1991), quantitative data on subclinical RD are scarce, partly due to methodological issues. Recent studies have shown that the incidence of subclinical RD can be substantial in veal calves. On average, between 21 and 35% of an orally supplied dose of CoEDTA was recovered in the rumen at slaughter (Suárez et al., 2007). In a subclinical case, nutrients from MR are subject to rumen fermentation which may reduce post-absorptive availability of nutrients. Ruminal drinking may not only decrease nutrient availability and the efficiency of nutrient utilization for protein and fat retention, the resulting fermentation of nutrients from MR in the rumen will also affect rumen functioning and fermentation of SF.

Addition of a SF component to the veal calf ration may reduce the incidence of acute clinical symptoms of RD such as recurrent bloat and ruminal acidosis, when the presence of SF in the rumen can prevent acute problems related to fermentation of MR. However, the effects of SF quantity and composition on the functioning of the reticular groove reflex have, to the best of our knowledge, not been reported, and the effects of SF provision and interactions with MR intake on ruminal drinking are, therefore, unknown. It has been suggested that the extent of ruminal drinking increases with SF provision and age of a calf (Guilhermet et al., 1975), although the number of calves tested was limited in this experiment. The volume of ruminal milk can be measured as the recovery of an indigestible dietary marker in the rumen (Suárez et al., 2007). However, measuring recovery at slaughter does not allow the repeated measurement of RD of one animal and it is unknown whether RD is constant over time (i.e. between meals or days). In order to study subclinical RD and related factors, there is a need for a method to quantify RD that does not require calves to be sacrificed.

Abomasal Lesions. Ulcers and erosions are commonly found in the abomasa of veal calves, with prevalence rates ranging from 32 to 76% (Brscic et al., 2011, Marshall, 2009). Abomasal lesions include erosions, ulcers, and scars. Erosions are defined as discrete mucosal defects that do not penetrate the muscularis mucosa; they are usually multiple, circular and appear as small hyperemic indentations in the mucosa (Marshall, 2009). Ulcers are defined as defects penetrating the entire thickness of the mucosa and extending through the submucosa, muscularis externa, and serosa; they occur both single and multiple and are variable in size (Marshall, 2009).

Multiple factors have been associated with abomasal ulcers in bovines such as abomasal hyperacidity (Ahmed et al., 2002), bacterial agents such as *Clostridium*, *Escherichia coli* and *Campylobacter* species (Jelinski et al., 1995), and stress (Braun et al., 1991, Wiepkema et al., 1987). In veal calves, the provision of SF has been shown to increase the prevalence of abomasal lesions (Brscic et al., 2011, Mattiello et al., 2002, Welchman and Baust, 1987), especially when the SF is rich in fiber (Webb et al., 2013). It is hypothesized that some roughage particles exert a mechanically abrasive effect on abomasal mucosa that is sensitive to ulceration due to overfilling, when large volumes of MR are consumed in one meal. Apart from reducing MR meal volume, abomasal ulcers may be reduced by stimulating early rumen development, thus reducing the occurrence of sharp particles before they enter the abomasum.

NUTRIENT UTILIZATION

Predicting growth performance becomes increasingly complex with the contribution of SF to the veal calves' diet. The growth performance of veal calves that are fed a combination of MR and SF cannot be predicted from feeding values derived from calves fed only SF or calves fed only MR, because interactions may occur between nutrients at the level of digestion (i.e. in the digestive tract), or at a post-absorptive (e.g. in the circulation) level. Some interactions may affect health or may be age-dependent, which further complicates an accurate prediction of growth performance. The most important interactions and complicating factors related to the prediction of growth performance are outlined below.

Interactions and Nitrogen and Energy Efficiency. In MR-fed calves, the efficiency of dietary nitrogen (**N**) utilization for growth is low, resulting in relatively high N emissions. Veal production, therefore, contributes to emissions of N in the form of ammonia (NH_3) and nitrous oxide (N_2O), with subsequent effects on eutrophication and global warming. The route of N excretion can be affected by the feeding strategy employed. As an example, a comparison between N utilization in milk-fed calves and SF-fed calves is made in Figure 1.4. In MR-fed calves, of every additional 100 g N ingested, 92 g of N is digested of which 30 g is retained for growth (Gerrits et al., 1996). In SF-fed calves, of every additional 100 g N ingested, 45 g of N is digested of which 25 g is retained for growth (Ortigues et al., 1990). This means that although digestibility of N is much greater in MR-fed calves than in SF-fed calves, digested N is retained more efficiently in SF-fed calves.

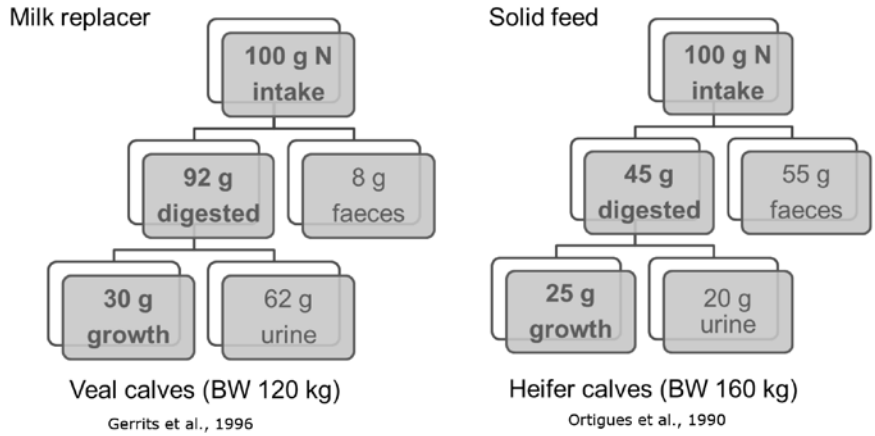


Figure 1.4 Examples of marginal response to N intake in milk-fed calves (left) and in SF-fed heifer calves (right).

The relatively low efficiency of N utilization in MR-fed calves could be explained by several factors, including a reduced post-absorptive amino acid utilization due to decreased insulin sensitivity, utilization of amino acids for gluconeogenesis, and an asynchronous nutrient supply (van den Borne et al., 2006a, van den Borne et al., 2012). In addition, the efficiency of post-absorptive N utilization in milk-fed calves is known to decrease with age (van den Borne et al., 2006b). In milk-fed calves, almost all of the urea produced by the liver is excreted in the urine. In ruminant animals, however, a major part of urea produced by the liver enters the gastrointestinal tract through either direct transfer from blood across the epithelial tissues or via saliva. In the gastrointestinal tract, urea is degraded to NH_3 through the action of microbial urease, and can be used for microbial protein synthesis. This recycling mechanism provides a source of N for microbial protein synthesis when N supply from the diet is insufficient (Lapierre and Lobley, 2001). Potentially, urea recycling could contribute quantitatively to improvement of N utilization efficiency when MR-fed calves are provided with low-N SF. A low-N SF is insufficient to meet N requirements for microbial digestion in the rumen and could stimulate the re-use of N from urea. This, in turn, may not only reduce the amount of N excreted but also the proportion excreted as volatile urinary urea N. In addition, the effect of route of protein administration (i.e. via SF or MR) is of interest, since N availability may limit microbial growth and fermentation capacity in the rumen when urea recycling is inadequate in quantity or timing. It is currently unknown how interactions between SF and MR affect protein deposition in calves. Also, the route of protein administration (SF vs. MR) may impact whole body protein metabolism.
Furthermore, energy obtained from SF versus MR may differently affect energy utilization. The main energy sources for milk-fed calves are lactose and fats, whereas the main energy sources for ruminants are VFA. When providing SF, 2 to 12% of gross energy from SF is lost as methane (Johnson and Johnson, 1995). Furthermore, energy required for maintenance may increase because of the maintenance requirements of increased rumen tissue in calves fed SF.

Passage Rates. The gastrointestinal passage rate of diet components, i.e. the rate at which digesta leave a compartment of the gut, largely determines both the site and extent of degradation of diet components, as well as the efficiency of microbial protein synthesis. Therefore, the development of a nutrient-based evaluation of veal calf rations requires knowledge about specific retention times of each dietary component. When increasing the dietary proportion of roughages and/or concentrates, gaining insight in rumen degradation kinetics becomes increasingly important. The passage rate of MR may be affected by roughage and concentrate intake. Furthermore, ruminal drinking may affect the passage rate of roughages and concentrates as it affects degradation processes in the rumen. As in fully ruminants (Colucci et al., 1990, Huhtanen and Kukkonen, 1995, Stensig and Robinson, 1997), passage kinetics are likely to be affected by roughage and concentrate

proportions and level of intake. Currently, there are no data on gastrointestinal passage kinetics in veal calves for rations including both SF and MR.

VOLUNTARY FEED INTAKE

When designing SF based rations for veal calves, both maximum growth performance and gastrointestinal development need to be achieved. Maximizing growth rate is realized through high MR whereas the development of a healthy rumen requires intake of both concentrates and roughages (Suárez et al., 2006, Suárez et al., 2007) which can only be realized at lower MR intakes. An early onset of rumen development may be beneficial to SF utilization at a later stage. On the other hand, nutrient utilization of MR is most efficient early in life (van den Borne et al., 2006a) and MR has a greater energy-density compared to SF sources. The combination of high amounts of MR and SF in a calf ration is associated to gastrointestinal problems such as abomasal lesions, plaque formation, and hyperkeratosis of the rumen wall (Prevedello et al., 2012). So far, studies on calf nutrition have primarily used experimental contrasts to study separate effects of dietary components. An alternative is to study the intake of dietary components in a free-choice setting; this can provide important information about feed preferences, as demonstrated in sheep (Cooper et al., 1996, Villalba and Provenza, 1996, Villalba and Provenza, 1999) and in weaned calves (Atwood et al., 2001). In free-choice experiments with calves, MR has never been a ration component despite its obvious potential contribution to energy intake. MR and SF may differentially affect satiety and voluntary feed intake. For example, SF intake could be restricted by digesta load in the rumen (Dado and Allen, 1995). In addition, calves may regulate feed intake based on specific nutrient intake. In weaned calves, large individual differences in feed preferences were observed but ratios of protein to energy intake are very similar for all calves (Atwood et al., 2001). In dairy cows, dietary inclusion of both unsaturated and saturated fatty acids has been shown to decrease DM intake (Allen, 2000, Benson and Reynolds, 2001, Harvatine and Allen, 2006), whereas intestinal and intravenous glucose infusions did not affect intake (Allen, 2000). Identification of cues driving the motivation for intake of roughage, concentrates and MR can contribute to fundamental understanding of (individual differences in) feed preferences in calves. In addition, there is a lack of information concerning the mechanisms that drive or limit voluntary feed intake in calves.

OBJECTIVE AND OUTLINE

The objective of this thesis was to provide a scientific basis for the development of novel feeding strategies combining MR and SF, optimizing the use of feed resources, and alleviating health problems related to interactions between SF and MR. To this end, effects of SF provision, when combined in a diet with MR, to protein and energy metabolism were quantified. Secondly, ruminal drinking was quantitatively assessed and an attempt was made to identify underlying mechanisms. Thirdly, dietary effects on abomasal damage, rumen development, and passage kinetics were studied. Finally, preferences for MR and SF sources were studied in calves to gain fundamental understanding of cues driving feed preferences.

In Chapter 2, an experiment revealing the effects of onset of rumen development on growth performance and abomasal lesions is described. In Chapter 3, effects of low-protein SF provided on top of a MR diet on energy and N utilization using indirect calorimetry are evaluated. Then, in Chapter 4, the contribution of urea kinetics and urea transporters to N utilization in calves fed MR and SF are evaluated. In Chapter 5, indirect and direct methods to quantify ruminal drinking are evaluated. In Chapter 6, an experiment evaluating feed preferences and their consequences for health and performance in calves from 2 wk to 6 months of age are presented. In Chapter 7, effects of SF level and composition on growth performance are evaluated in a large-scale paired-gain experiment. In Chapter 8, an isonitrogenous comparison was made to quantify the effect of level of SF intake and level of protein in SF and MR on urea recycling and N retention. In Chapter 9, effects of SF level and composition on ruminal drinking and passage kinetics of MR, concentrates and straw as well as organ characteristics were quantified. Chapter 10 includes a general discussion with emphasis on protein and energy metabolism, the quantitative assessment of ruminal drinking, and discussion of feed preferences and voluntary feed intake. Finally, general conclusions are drawn from this thesis.

REFERENCES

Ahmed, A. F., P. D. Constable, and N. A. Misk. 2002. Effect of feeding frequency and route of administration on abomasal luminal pH in dairy calves fed milk replacer. Journal of Dairy Science 85(6):1502-1508.

Allen, M. S. 2000. Effects of diet on short-term regulation of feed intake by lactating dairy cattle. Journal of Dairy Science 83(7):1598-1624.

Atwood, S. B., F. D. Provenza, R. D. Wiedmeier, and R. E. Banner. 2001. Influence of free-choice vs mixed-ration diets on food intake and performance of fattening calves. Journal of Animal Science 79(12):3034-3040.

Bähler, C., A. Steiner, A. Luginbühl, A. Ewy, H. Posthaus, D. Strabel, T. Kaufmann, and G. Regula. 2012. Risk factors for death and unwanted early slaughter in Swiss veal calves kept at a specific animal welfare standard. Research in Veterinary Science 92(1):162-168.

Baldwin VI, R. L., K. R. McLeod, J. L. Klotz, and R. N. Heitmann. 2004. Rumen development, intestinal growth and hepatic metabolism in the pre- and postweaning ruminant. Journal of Dairy Science 87:E55-E65.

Beharka, A. A., T. G. Nagaraja, J. L. Morrill, G. A. Kennedy, and R. D. Klemm. 1998. Effects of Form of the Diet on Anatomical, Microbial, and Fermentative Development of the Rumen of Neonatal Calves. Journal of Dairy Science 81(7):1946-1955.

Benson, J. and C. Reynolds. 2001. Effects of abomasal infusion of long-chain fatty acids on splanchnic metabolism of pancreatic and gut hormones in lactating dairy cows. Journal of Dairy Science 84(6):1488-1500.

Blum, J. W. and H. Hammon. 2000. Colostrum effects on the gastrointestinal tract, and on nutritional, endocrine and metabolic parameters in neonatal calves. Livestock Production Science 66(2):151-159.

Braun, U., R. Bretscher, and D. Gerber. 1991. Bleeding abomasal ulcers in dairy cows. Veterinary Record 129(13): 279-284.

Brscic, M., L. F. M. Heutinck, M. Wolthuis-Fillerup, N. Stockhofe-Zurwieden, B. Engel, E. K. Visser, F. Gottardo, E. A. M. Bokkers, B. J. Lensink, G. Cozzi, and C. G. Van Reenen. 2011. Prevalence of gastrointestinal disorders recorded at postmortem inspection in white veal calves and associated risk factors. Journal of Dairy Science 94(2):853-863.

Catry, B., J. Dewulf, T. Goffin, A. Decostere, F. Haesebrouck, and A. De Kruif. 2007. Short Communication: Antimicrobial Resistance Patterns of Escherichia coli Through the Digestive Tract of Veal Calves. Microbial Drug Resistance 13(2):147-150.

Colucci, P., G. Macleod, W. Grovum, I. McMillan, and D. Barney. 1990. Digesta kinetics in sheep and cattle fed diets with different forage to concentrate ratios at high and low intakes. Journal of Dairy Science 73(8):2143-2156.

Cook, A., R. J. Reid-Smith, R. J. Irwin, S. A. McEwen, V. Young, K. Butt, and C. Ribble. 2011. Antimicrobial resistance in Escherichia coli isolated from retail milk-fed veal meat from Southern Ontario, Canada. Journal of Food Protection 74(8):1328-1333.

Cooper, S. D. B., I. Kyriazakis, and J. D. Oldham. 1996. The effects of physical form of feed, carbohydrate source, and inclusion of sodium bicarbonate on the diet selections of sheep. Journal of Animal Science 74(6):1240-1251.

Dado, R. and M. Allen. 1995. Intake limitations, feeding behavior, and rumen function of cows challenged with rumen fill from dietary fiber or inert bulk. Journal of Dairy Science 78(1):118-133.

Di Giancamillo, A., G. Bosi, S. Arrighi, G. Savoini, and C. Domeneghini. 2003. The influence of different fibrous supplements in the diet on ruminal histology and histometry in veal calves. Histology and Histopathology 18:727-733.

Flatt, W. P., R. G. Warner, and J. K. Loosli. 1958. Influence of Purified Materials on the Development of the Ruminant Stomach. Journal of Dairy Science 41(11):1593-1600.

Gentile, A., S. Sconza, I. Lorenz, G. Otranto, G. Rademacher, P. Famigli-Bergamini, and W. Klee. 2004. D-lactic acidosis in calves as a consequence of experimentally induced ruminal acidosis. Journal of Veterinary Medicine Series a-Physiology Pathology Clinical Medicine 51(2):64-70.

Gerrits, W. J. J., G. H. Tolman, J. W. Schrama, S. Tamminga, M. W. Bosch, and M. W. A. Verstegen. 1996. Effect of Protein and Protein-Free Energy Intake on Protein and Fat Deposition Rates in Preruminant Calves of 80 to 240 kg Live Weight. Journal of Animal Science 74(9):2129-2139.

Graveland, H., J. A. Wagenaar, H. Heesterbeek, D. Mevius, E. van Duijkeren, and D. Heederik. 2010. Methicillin resistant Staphylococcus aureus ST398 in veal calf farming: human MRSA carriage related with animal antimicrobial usage and farm hygiene. PloS one 5(6):e10990.

Greenwood, R. H., J. L. Morrill, E. C. Titgemeyer, and G. A. Kennedy. 1997. A new method of measuring diet abrasion and its effect on the development of the forestomach. Journal of Dairy Science 80(10):2534-2541.

Guilhermet, R., C. Mathieu, and R. Toullec. 1975. Transit des aliments liquides au niveau de la gouttiere oesophagienne chez le veau preruminant et ruminant. Annales De Zootechnie 24:69-79.

Harvatine, K. and M. Allen. 2006. Effects of fatty acid supplements on feed intake, and feeding and chewing behavior of lactating dairy cows. Journal of Dairy Science 89(3):1104-1112.

Herrli-Gygi, M., H. M. Hammon, Y. Zbinden, A. Steiner, and J. W. Blum. 2006. Ruminal drinkers: Endocrine and metabolic status and effects of suckling from a nipple instead of drinking from a bucket. Journal of Veterinary Medicine Series A: Physiology Pathology Clinical Medicine 53(5):215-224.

Huhtanen, P. and U. Kukkonen. 1995. Comparison of methods, markers, sampling sites and models for estimating digesta passage kinetics in cattle fed at two levels of intake. Animal Feed Science and Technology 52(1):141-158.

Jelinski, M. D., C. S. Ribble, M. Chirino-Trejo, E. G. Clark, and E. D. Janzen. 1995. The relationship between the presence of *Helicobacter pylori, Clostridium perfringens* type A, *Campylobacter spp*, or fungi and fatal abomasal ulcers in unweaned beef calves. Canadian Veterinary Journal 36(6):379-382.

Johnson, K. A. and D. E. Johnson. 1995. Methane emissions from cattle. Journal of Animal Science 73(8):2483-2492.

Kooijman, J., H. K. Wierenga, and P. R. Wiepkema. 1991. Development of abnormal oral behavior in group-housed veal calves: Effects of roughage supply. New Trends in Veal Calf Production:54-58.

Lapierre, H. and G. Lobley. 2001. Nitrogen recycling in the ruminant: A review. Journal of Dairy Science 84:E223-E236.

Marshall, T. S. 2009. Abomasal ulceration and tympany of calves. Veterinary Clinics of North America: Food Animal Practice 25(1):209-220.

Mason, G. and J. Rushen. 2008. Stereotypic animal behavior: fundamentals and applications to welfare. CABI., Wallingford, UK

Mattiello, S., E. Canali, V. Ferrante, M. Caniatti, F. Gottardo, G. Cozzi, I. Andrighetto, and M. Verga. 2002. The provision of solid feeds to veal calves: II. Behavior, physiology, and abomasal damage. Journal of Animal Science 80(2):367-375.

Morisse, J. P., D. Huonnic, J. P. Cotte, and A. Martrenchar. 2000. The effect of four fibrous feed supplementations on different welfare traits in veal calves. Animal Feed Science and Technology 84(1-2):129-136.

Ortigues, I., T. Smith, M. Gill, S. B. Cammell, and N. W. Yarrow. 1990. The effect of fishmeal supplementation of a straw-based diet on growth and calorimetric efficiency of growth in heifers. British Journal of Nutrition 64(3):639-651.

Pardon, B., B. Catry, J. Dewulf, D. Persoons, M. Hostens, K. De Bleecker, and P. Deprez. 2012a. Prospective study on quantitative and qualitative antimicrobial and anti-inflammatory drug use in white veal calves. Journal of antimicrobial chemotherapy 67(4):1027-1038.

Pardon, B., K. De Bleecker, M. Hostens, J. Callens, J. Dewulf, and P. Deprez. 2012b. Longitudinal study on morbidity and mortality in white veal calves in Belgium. BMC veterinary research 8(1):26.

Prevedello, P., M. Brscic, E. Schiavon, G. Cozzi, and F. Gottardo. 2012. Effects of the provision of large amounts of solid feeds to veal calves on growth and slaughter performance and intravitam and postmortem welfare indicators. Journal of Animal Science 90(10):3538-3546.

Sander, E. G., R. G. Warner, H. N. Harrison, and J. K. Loosli. 1959. The stimulatory effect of sodium butyrate and sodium propionate on the development of rumen mucosa in the young calf. Journal of Dairy Science 42(9):1600-1605.

Sans, P. and G. de Fontguyon. 2009. Veal calf industry economics. Revue de Médecine Vétérinaire 160(8-9):420-424.

Sargeant, J. M., T. E. Blackwell, S. W. Martin, and R. Tremblay. 1994. Production practices, calf health and mortality on six white veal farms in Ontario. Canadian Journal of Veterinary Research 58(3):189.

Stensig, T. and P. Robinson. 1997. Digestion and passage kinetics of forage fiber in dairy cows as affected by fiber-free concentrate in the diet. Journal of Dairy Science 80(7):1339-1352.

Stobo, I. J., J. H. Roy, and H. J. Gaston. 1966. Rumen development in the calf. 1. The effect of diets containing different proportions of concentrates to hay on rumen development. British Journal of Nutrition 20(2):171-188.

Stocker, H., H. Lutz, and P. Rusch. 1999. Clinical, haematological and biochemical findings in milk-fed calves with chronic indigestion. Veterinary Record 145(11):307-311.

Stull, C. and S. McDonough. 1994. Multidisciplinary approach to evaluating welfare of veal calves in commercial facilities. Journal of Animal Science 72(9):2518-2524.

Suárez, B., C. Van Reenen, W. Gerrits, N. Stockhofe-Zurwieden, A. Van Vuuren, and J. Dijkstra. 2006. Effects of supplementing concentrates differing in carbohydrate composition in veal calf diets: II. Rumen development. Journal of Dairy Science 89(11):4376-4386.

Suárez, B. J., C. G. Van Reenen, N. Stockhofe-Zurwieden, J. Dijkstra, and W. J. J. Gerrits. 2007. Effect of roughage source and roughage to concentrate ratio on animal performance and rumen development in veal calves. Journal of Dairy Science 90(5):2390-2403.

Tamate, H., A. D. McGilliard, N. L. Jacobson, and R. Getty. 1962. Effect of various dietaries on the anatomical development of the stomach in the calf. Journal of Dairy Science 45(3):408-420.

Van Cleef, B. A., H. Graveland, A. P. Haenen, A. W. van de Giessen, D. Heederik, J. A. Wagenaar, and J. A. Kluytmans. 2011. Persistence of livestock-associated methicillin-resistant Staphylococcus aureus in field workers after short-term occupational exposure to pigs and veal calves. Journal of Clinical Microbiology 49(3):1030-1033.

Van den Borne, J., M. W. A. Verstegen, S. J. J. Alferink, R. M. M. Giebels, and W. J. J. Gerrits. 2006a. Effects of feeding frequency and feeding level on nutrient utilization in heavy preruminant calves. Journal of Dairy Science 89(9):3578-3586.

Van den Borne, J., J. Verdonk, J. W. Schrama, and W. J. J. Gerrits. 2006b. Reviewing the low efficiency of protein utilization in heavy preruminant calves - a reductionist approach. Reproduction Nutrition Development 46(2):121-137.

Van den Borne, J. J., S. J. Alferink, M. J. Heetkamp, A. A. Jacobs, M. W. Verstegen, and W. J. Gerrits. 2012. Asynchronous supply of indispensable amino acids reduces protein deposition in milk-fed calves. The Journal of nutrition 142(12):2075-2082.

Van Putten, G. 1982. Welfare in veal calf units. Veterinary Record 111(19):437-440.

Van Weeren-Keverling Buisman, A., J. M. V. M. Mouwen, T. Wensing, and H. J. Breukink. 1990. Intraruminal administration of milk in the calf as a model for ruminal drinking: Morphological and enzymatical changes in the jejunal mucosa. Veterinary Research Communications 14(2):129-140.

Van Weeren-Keverling Buisman, A., T. Wensing, H. J. Breukink, and J. M. V. M. Mouwen. 1991. Ruminal drinking in veal calves. New Trends in Veal Calf Production:113-117.

Veissier, I., A. R. Ramirez De La Fe, and P. Pradel. 1998. Nonnutritive oral activities and stress responses of veal calves in relation to feeding and housing conditions. Applied Animal Behavior Science 57(1-2):35-49.

Villalba, J. J. and F. D. Provenza. 1996. Preference for flavored wheat straw by lambs conditioned with intraruminal administrations of sodium propionate. Journal of Animal Science 74(10):2362-2368.

Villalba, J. J. and F. D. Provenza. 1999. Nutrient-specific preferences by lambs conditioned with intraruminal infusions of starch, casein, and water. Journal of Animal Science 77(2):378-387.

Webb, L., E. Bokkers, L. Heutinck, B. Engel, W. Buist, T. Rodenburg, N. Stockhofe-Zurwieden, and C. van Reenen. 2013. Effects of roughage source, amount, and particle size on behavior and gastrointestinal health of veal calves. Journal of Dairy Science 96(12):7765-7776.

Webb, L. E., E. A. M. Bokkers, B. Engel, W. J. J. Gerrits, H. Berends, and C. G. van Reenen. 2012. Behavior and welfare of veal calves fed different amounts of solid feed supplemented to a milk replacer ration adjusted for similar growth. Applied Animal Behavioral Sciences 136(2-4):108-116.

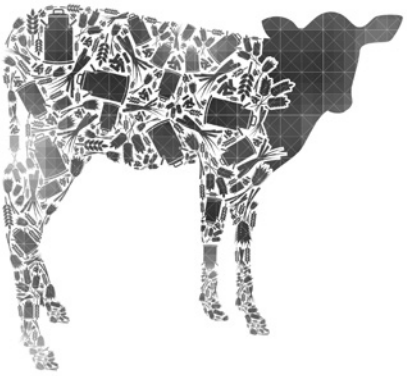
Welchman, D. D. and G. N. Baust. 1987. A survey of abomasal ulceration in veal calves. Veterinary Record 121(25-26):586-590.

Wiepkema, P. R., K. K. Van Hellemond, P. Roessingh, and H. Romberg. 1987. Behavior and abomasal damage in individual veal calves. Applied Animal Behavior Science 18(3-4):257-268.

Wilson, L., C. Egan, and T. Drake. 1994. Blood, Growth, and Other Characteristics of Special-Fed, Veal Calves in Private Cooperation Herds. Journal of Dairy Science 77(8):2477-2485.

Chapter 2

Effects of early rumen development and solid feed composition on growth performance and abomasal health in veal calves



H. Berends[1], C.G. van Reenen[2], N. Stockhofe-Zurwieden[3], and W.J.J. Gerrits[1]
Journal of Dairy Science 2012: 95 (6) 3190-3199

[1]Animal Nutrition Group, Wageningen University, the Netherlands; [2]Livestock Research, Wageningen University and Research Centre, the Netherlands; [3]Central Veterinary Institute, Wageningen University and Research Centre, the Netherlands

ABSTRACT

The experiment was designed to study the importance of early rumen development and of the composition of solid feed intake on growth performance and abomasal health in milk-fed veal calves. One hundred and six Holstein-Friesian male calves were included in the experiment, and studied during 2 successive 12-wk periods (period 1 and period 2). In a 2 x 2 factorial arrangement, effects of partially replacing milk replacer by solid feed during period 1 and partially replacing dry matter (DM) intake from maize silage and barley straw by concentrate during period 2 were tested. Solid feed during period 1 consisted of maize silage, barley straw, and concentrate (25:25:50 on a DM basis). Solid feed during period 2 consisted of maize silage and barley straw (50:50 ratio on DM basis) for the non-concentrate groups, and maize silage, barley straw and concentrates (25:25:50 on a DM basis) for the concentrate groups. At the end of period 1 (n = 16) and at the end of period 2 (n = 90), parameters of animal performance, rumen development, rumen fermentation, ruminal drinking, and abomasal damage were examined. Partially replacing milk replacer by solid feed during period 1 resulted in early rumen development (ERD) at the end of period 1, characterized by increased rumen weight, and an increased epithelial and absorptive surface area. Both ERD and partially replacing roughage by concentrates in period 2 increased the rumen development score at the end of period 2. Although ERD calves consumed more solid feed and less milk replacer during period 1 and 2 than non-ERD calves, carcass weight gains at 25 wk were identical, and utilization of the solid feed provided appeared similar to that of milk replacer. Partially replacing roughage by concentrates in period 2 increased dressing percentage and warm carcass weight.
Plaque formation at the rumen mucosa was unaffected by ERD or partially replacing roughage by concentrates and generally low in all calves. The prevalence of large scars in the abomasum in ERD calves was decreased compared with non-ERD calves. This may indicate that ERD provided protection against abomasal lesions. In conclusion, early compared with late rumen development improves feed utilization and may be beneficial for abomasal health.

Keywords: veal calf, roughage composition, rumen development, abomasum

INTRODUCTION

Since 1997, the European Union legislation stipulates provision of a minimum daily amount (50 to 250 g) of fibrous feed for veal calves on milk replacer (**MR**) diets in order to improve animal health and welfare. As no further specification was made, ingredients like maize silage and straw would both comply with legislation. With increasing prices of MR ingredients, an increasing economic incentive exists to replace MR by fibrous feed, hereafter referred to as solid feed (**SF**). SF provision has been shown to decrease oral stereotyped behaviors and improve rumen development and rumination (Kooijman et al., 1991; Veissier et al., 1998; Blokhuis et al., 2000), and can affect animal health and performance in both a positive and negative way.

When feeding concentrate feed as the only SF source to veal calves, Suárez et al. (2006b, 2007) observed poorly developed rumen mucosa and so-called plaque formation (i.e. patches of focal mucosa inflammation with coalescing and adhering papillae covered by feed particles, hair, and cell debris). In addition, they observed that 21 to 35% of the ingested milk enters the reticulorumen, rather than the abomasum (Suárez et al., 2007). Excessive ruminal drinking may lead to impaired digestion, metabolic acidosis (Gentile et al., 2004) or villus atrophy (van Weeren-Keverling Buisman et al., 1990). Furthermore, the provision of roughages to veal calves increased the prevalence of abomasal lesions (Welchman and Baust, 1987; Mattiello et al., 2002; Brscic et al., 2011). Their mechanically abrasive effect in combination with consumption of large volumes of milk may cause local ischaemia and subsequent focal necrosis (Welchman and Baust, 1987; Breukink et al., 1991). We hypothesized that poor rumen development deteriorates abomasal health and compromises digestive utilization of SF. Optimizing rumen development in veal calves requires information regarding the effects of dose, composition (specifically the concentrate-to-roughage ratio), and timing of SF provision. Whereas the separate effects of roughage and concentrate intake are well documented in replacement heifers and beef cattle (Zitnan et al., 1998; Coverdale et al., 2004; Hill et al., 2008), information for veal calves is limited to an age of 12 wk (Suárez et al., 2006a,b, 2007) or to selected sources and intake levels (Blokhuis et al., 2000; Cozzi et al., 2002; Labussière et al., 2009). Due to potential interactions between MR and SF, occurring either at the level of digestion or post-absorption, the contribution of SF to nutrient supply may differ from that in ruminants exclusively fed concentrates and roughage.

Therefore, the aims of the present study were: 1) to investigate the influence of early compared with late rumen development on feed utilization and the incidence of abomasal lesions of veal calves during later life and 2) to investigate the difference between concentrate and roughages on animal performance and abomasal health in veal calves.

MATERIALS AND METHODS

The 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.

Animals, Diets, and Observations

Animals. One hundred and six male Holstein-Friesian veal calves were included in the experiment. Calves were purchased in 2 batches from commercial dairy farms to obtain a uniform group of calves of the same age (average age and BW upon arrival: 12 ± 0.3 d, and 46 ± 0.2 kg, respectively). Within each batch, calves were housed in groups of 5 calves, and groups were randomly assigned to dietary treatments. The experiment consisted of 2 successive 12-wk periods, period 1 (**P1**) and period 2 (**P2**) respectively. Calves were housed in group pens with wooden-slatted floors measuring 3 x 3 m, without bedding material. During P1, calves were kept individually in 0.9-m^2 temporary pens placed inside the group pen, which were removed at the start of P2. Environmental temperature in the stable was maintained at least at 15 °C. Animal health was checked daily by animal care takers. During the first 10 d after arrival, all calves were treated with oxytetracycline (broadspectrum antibiotic) and colistin (polypeptide antibiotic). Hemoglobin concentration in blood was monitored across the trial at wk 5, 10, 18, and 24 and corrected to comply with the minimum European Union level of 4.5 mmol/L at the end of the fattening period.

Dietary Treatments. Dietary treatments are presented in Table 2.1. In a 2 x 2 factorial arrangement, effects of a partial replacement of MR by SF during P1 (early rumen development, **ERD**), and a partial replacement of dry matter intake from maize silage and straw by concentrate in the diet during P2 (**CONC**), were tested. Additionally, a reference treatment was included, with partial replacement of MR by SF during P1 (ERD) and without any SF provision during P2. The reference treatment was included to generate reference values for rumen development, abomasal lesions, and performance at the end of P2, and was excluded from any statistical comparison. All diets were provided on top of an MR diet. During P1 and P2, SF intakes were maximized at 750 and 500 g of DM/d in P1 and P2, respectively. The amount and composition of SF, and the duration of P1 (12 wk) were based on previous results of Suárez et al. (2006b, 2007), who observed good rumen development in veal calves at 12 wk of age in a comparable setting. For treatments with SF supplementation in P1, milk schemes were reduced to stimulate the intake of SF (Table 2.1). In addition, 16 calves were included in the experiment during P1 and then slaughtered, to provide a reference value for comparison of rumen development at the end of P1 with other results. These 16 calves were assigned to either an MR diet (non-ERD; n=4) or an MR diet partially replaced by SF (ERD; n=12; Table 2.1).

Table 2.1 Experimental design, including milk replacer schemes and solid feed supply during period 1 and 2 for dietary treatments

	Treatment [1]				
	Non-ERD	Non-ERD	ERD	ERD	
Item	Non-CONC	CONC	Non-CONC	CONC	Reference[4]
n[2]	20	20	20	20	10
Period 1 (wk 1-12)					
MR[3]	A	A	B	B	A
Solid feed[4]	-	-	MSC 750	MSC 750	MSC 750
Period 2 (wk 13-25)					
MR[3]	B	B	B	B	B
Solid feed[4]	MS 500	MSC 500	MS 500	MSC 500	-

[1] ERD = early rumen development [partial replacement of milk replacer (MR) by solid feed during period 1 (P1, the first of two 12-wk periods)]; CONC= partial replacement of DM from maize silage and straw by concentrate in the diet during period 2 (P2). The reference treatment was excluded from any statistical comparison.

[2] This excludes the calves of the non-ERD non-CONC (n=4) and ERD non-CONC treatment (n=12) that were slaughtered at the end of P1 to determine rumen development.

[3] Milk replacer: A: milk replacer supply increased from 0.39, 0.51, 0.72, 0.94, 1.18, 1.40, 1.50, 1.54, 1.65, 1.75, 1.86 and 1.99 kg/d in wk 1 to 12, respectively; B: milk replacer supply increased from 0.36, 0.46, 0.48, 0.50, 0.59, 0.70, 0.75, 0.88, 1.01, 1.19, 1.40 and 1.60 kg/d in wk 1 to 12, respectively; and from 1.80, 1.80, 2.03, 2.32, 2.46, 2.58, 2.71, 2.85, 2.99, 3.13, 3.20, 3.20, 3.20, 3.20 kg/d in wk 13 to 24, respectively.

[4] Solid feed: MSC 750: 750 g DM/d MSC (maize silage, straw and concentrate mixture 25:25:50 on a DM basis); MS 500: 500 g DM/d MS (maize silage: straw 50:50 on a DM basis); MSC 500: 500 g DM/d MSC. To avoid selective intake of solid feed sources and to minimize orts, the expected DM intake was calculated weekly based on the realized DM intake in the previous wk and a projected weekly increase.

The diet was offered to the calves in 2 equal portions (at 0600 and 1600 h). Milk replacer was fed individually in buckets. Solid feed sources included maize silage, barley straw, concentrate or a mixture thereof. For the maize silage and straw mixture diets, maize silage and straw were mixed on a 50:50 DM basis. For the maize silage, straw and concentrate diets, maize silage, straw and concentrate were mixed on a 25:25:50 DM basis. Ingredient and nutrient compositions of the MR and SF sources are presented in Table 2.2. The concentrate was provided as pellets and the roughages were chopped. Solid feed was provided individually in buckets during P1. During P2, calves received roughage groupwise in a trough. Intake of SF was registered daily per calf during P1 and per group during P2. To avoid selection of dietary components and to minimize orts, the expected DM intake was calculated weekly based on the realized DM intake during the previous wk and a projected weekly increase. During the first 4 wk of P1, MR was reconstituted in the same volume of water for every treatment, and from wk 5 onwards calves were allowed ad libitum access to water for 2 h around noon.

Table 2.2 Analyzed nutrient composition of milk replacers, concentrates and roughages

	Milk replacer		Concentrate			
Nutrient[1]	**P1**[2]	**P2**[3]	**P1**[4]	**P2**[5]	**Straw**	**Maize silage**
CP	218	208	195	171	26	76
Crude fat	192	213	42	40	9	31
Crude ash	75	71	34	33	134	38
Starch and sugars	-	-	333	410	4	306
Neutral detergent fibre	-	-	250	217	755	408
Acid detergent fibre	-	-	108	87	462	228
Acid detergent lignin	-	-	11	10	54	20
Ca	10.2	8.6	1.4	1.9	3.4	2.2
P	7.4	6.8	4.4	4.7	0.4	1.9
Fe, mg/kg of DM	39	>15	121	132	124	144

[1] Presented as g/kg of DM unless specified otherwise.

[2] Ingredient composition of milk replacer in P1 (period 1): 50% whey protein concentrate, 26.6% whey powder, 17.1% oils and fats, 5% low lactose whey powder, 1.3% premix (provided per kilogram of milk replacer: lactose: 410 g; vitamin A: 25,000 IU; vitamin D3: 2,000 IU; vitamin E: 80 mg; vitamin C: 80 mg; Ca: 7.9 g; P: 6.3 g; Na: 6.1 g; K: 18.5 g; Mg: 1.4 g; Zn: 80 mg; Cu: 4 mg; Mn: 10 mg; Se: 0.1 mg; Fe: 2.8 mg).

[3] Ingredient composition of milk replacer in P2 (period 2): 19% whey protein concentrate, 36.8% whey powder, 20.5% oils and fats, 13.3% low lactose whey powder, 4% wheat protein, 2.5% soy protein concentrate, 2% starch, 0.3% L-lysine, 0.2% DL methionine, 1.5% premix (provided per kilogram of milk replacer: lactose: 410 g; vitamin A: 25,000 IU; vitamin D3: 2,000 IU; vitamin E: 80 mg; vitamin C: 80 mg; Ca: 7.9 g; P: 6.3 g; Na: 6.1 g; K: 18.5 g; Mg: 1.4 g; Zn: 80 mg; Cu: 4 mg; Mn: 10 mg; Se: 0.1 mg; Fe: 2.8 mg).

[4] Ingredient composition of concentrate in P1 (period 1): 10% maize, 20% maize feed meal, 20% barley, 5% oat husk meal, 20% lupines (CF < 70, CP < 335), 25% malt culms (CP < 200).

[5] Ingredient composition of concentrate in P2 (period 2): 30% maize, 10% maize feed meal, 20% barley, 20% lupines (CF < 70, CP < 335), 20% malt sprouts (CP < 200).

Slaughter Procedure. At the end of P1, 16 calves were slaughtered to provide a reference for the start of P2. The remaining calves (n = 90) were slaughtered at the end of P2. Calves were transported about 75 km (ca. 1 h) to a slaughter facility and euthanized by stunning (captive bolt pistol) and subsequent exsanguination. Carcasses were weighed. To quantify ruminal drinking (leakage of MR to the rumen), 3.0 g (P1) or 5.0 g (P2) of Co, complexed as CoEDTA (prepared according to Udén et al., 1980), was dissolved into the final MR meal, provided 30 min before transport. Time between final MR meal and slaughter averaged 4 h (2 to 6 h). To avoid reflux of MR containing CoEDTA from the abomasum into the forestomachs at slaughter, calves were lifted by the forelegs immediately following exsanguination. Subsequently, ligations were made at the level of the thoracic entrance, the omasal-cardia of the abomasum and the reticulo-omasal orifice. The weight of the reticulorumen was recorded with and without contents. Rumen contents were

quantitatively collected and solid and liquid phases were separated using a metal sieve (1.5 mm). A sample of the rumen liquid was used for pH measurement, acidified with H_3PO_4 (5%), and stored frozen for analysis of VFA, lactic acid, and ammonia. A reconstituted sample of rumen contents was prepared by proportional sampling of the liquid and solid phases, which was oven dried and stored for Co analysis. Blood samples were taken for analysis of hemoglobin levels. Measurement of carcass weight and visual classification of meat color was performed by a certified employee of the Central Office for Slaughter Livestock Services (BV CBS, Zeist, the Netherlands).

Analytical Procedures

Calves were weighed every 4 wk, and once-per-week samples of MR, diet ingredients, and orts were collected and pooled for analysis of chemical composition.

Ruminal Fluid. Concentrations of VFA and lactic acid were quantified by HPLC as described previously by Suárez et al. (2007). Ammonia concentrations were determined by a modified Berthelot method (Robinson et al., 1986). Rumen Co pool size (kg) was calculated from rumen DM pool (kg) and Co concentration (mg/kg of DM); Co recovery was expressed as a percentage of the Co pulsed dose. Cobalt concentration was determined in rumen contents by inductively coupled plasma atomic emission spectroscopy, as described by Suárez et al. (2007).

Rumen Wall Evaluation. The rumen was dissected along the dorsal line, emptied, and rinsed with cold water. The histological assessment and subsequent microscopic examination of 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 examined visually and the presence and density of rumen papillae were scored on a 5-point scale from poor to excellent: 1, 1.5, 2, 2.5, and 3. The prevalence of plaque formation was assessed visually. The morphometric analyses were conducted in 3 slides cut from a 2 x 2-cm section of rumen wall tissue from the saccus ruminis dorsalis, saccus ruminis ventralis and the atrium of the rumen. The slides were embedded in paraffin and subsequently stained with hematoxylin. Morphometric analyses were performed at a magnification of 2.5x (Olympus microscope; Olympus Corp., Tokyo, Japan) by using the image analysis software Image Pro Plus (Media Cybernetics Inc., Silver Spring, MD). The measurements for each slide comprised 1) the ratio of mucosa length to serosa length (**RMSL**) as a measure of the total absorptive surface, which was 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); 2) mucosa thickness, measured at 3 randomly chosen sites per slide; and 3) muscle layer thickness, measured at the same sites as for mucosa thickness.

Abomasal Lesions. The abomasal wall was visually inspected macroscopically for erosions, ulcers, and scars, each in the following size categories: small = modification ≤ 0.5 cm^2; medium = 0.5 cm^2 < modification < 1.0 cm^2; large = modification ≥ 1.0 cm^2.

Statistical Analysis

Group pens were considered the experimental units and means or percentages per pen were analyzed. The reference treatment was excluded from any statistical comparison. For continuous variables an ANOVA model was used. For percentages, a generalized linear model (**GLM**) was used, with a logit link and a multiplicative dispersion parameter with respect to the binomial variance function.

Analysis of variance comprised estimation by maximum likelihood and testing by the F-test, assuming normality and equal variances. Logistic regression comprised estimation by maximum quasi-likelihood and testing by the quasi-likelihood ratio test or the Wald test. Dispersion parameters were estimated from the Pearsons generalized chi-square statistic (McCullagh and Nelder, 1989). Analysis of variance or GLM included fixed effects for batches, ERD in P1, CONC, and the interaction between the latter 2 factors. In all analyses, nonsignificant interactions ($P > 0.05$) were excluded from the model. For several measures (including the prevalence of plaque and prevalence of medium and large abomasal erosions), GLM did not converge due to the presence of too many zeros in the data. In these cases, the Fisher exact test was used as an approximate test to examine differences between treatment groups. All calculations were performed with GenStat (Genstat Committee, 2000).

RESULTS

Characterization of Calves at 12 wk (P1)

The results on macroscopic and microscopic rumen development are presented in Table 2.3. Empty rumen weight was increased significantly in ERD calves ($P < 0.01$). The RMSL in both the rumen atrium and the dorsal location was increased in ERD calves ($P < 0.01$). Ruminal plaque formation was not observed at 12 wk (Table 2.3). During P1, the ADG of non-ERD calves was 12% higher than average daily gain (**ADG**) of calves supplemented with SF ($P < 0.001$, Table 2.4). Feed conversion ratio (**FCR**) was lower in non-ERD when compared with ERD calves ($P < 0.001$).

Feed Intake and Growth Performance

The least squares means of DM intake (**DMI**), ADG, and FCR are presented in Table 2.4. Compared with non-ERD calves, ERD calves showed an increase in ADG of 100 g/d ($P < 0.05$) during P2 and a tendency towards a lower FCR ($P < 0.10$). The CONC increased dressing percentage ($P < 0.01$) and carcass weight ($P < 0.05$). No effect of dietary treatment on carcass fat retention was observed (results not shown).

At the end of P2, hemoglobin levels were in the range of 5.0 to 5.4 mmol/L (SEM 0.11) for treatments with SF provision in P2, and the average hemoglobin level for reference calves was 4.9 mmol/L (SEM 0.20). Early rumen development coincided with an increase in

Table 2.3 Effects of early rumen development (ERD) in period 1 on macroscopic and microscopic parameters of the rumen mucosa in veal calves, determined at the end of period 1 (12 wk)

	Dietary treatment[1]		
Item	**Non-ERD**	**ERD**	**SEM**
N	4	12	
Macroscopic parameter			
Empty rumen weight, g	668[b]	1018[a]	52.2
Rumen development[2]	1.0	1.5	-
Rumen plaque, % of calves	0	0	-
Rumen Co recovery, %	22	10	4.3
Microscopic parameter			
Rumen dorsal location			
RMSL[3]	2.2[b]	3.9[a]	0.38
Mucosa thickness, µm	286[b]	898[a]	125.7
Muscle thickness, µm	1292	1700	253
Rumen ventral location			
RMSL[3]	3.9	5.4	0.56
Mucosa thickness, µm	1084	1210	153.0
Muscle thickness, µm	1408	1714	219.7
Rumen atrium			
RMSL[3]	4.7[b]	7.7[a]	0.71
Mucosa thickness, µm	1271	1786	195.3
Muscle thickness, µm	2276	2596	192.0

[a,b]Means in the same row with different superscripts differ ($P < 0.01$).

[1] Dietary treatments were as follows: Non-ERD = milk replacer only: milk replacer supply increased from 0.39, 0.51, 0.72, 0.94, 1.18, 1.40, 1.50, 1.54, 1.65, 1.75, 1.86 and 1.99 kg/d in wk 1 to 12, respectively; ERD = partial replacement of milk replacer by solid feed. Solid feed supply of 750 g DM/d of MSC (maize silage, straw and concentrate mixture 25:25:50 on a DM basis) on top of a milk replacer diet: milk replacer supply increased from 0.36, 0.46, 0.48, 0.50, 0.59, 0.70, 0.75, 0.88, 1.01, 1.19, 1.40 and 1.60 kg/d in wk 1 to 12, respectively.

[2] Average of a discrete examination scale of 5 scores from poor to excellent: 1,1.5,2,2.5, and 3.

[3] RMSL = ratio of mucosa to serosa length.

hemoglobin level (5.4 vs. 5.0 mmol/L for ERD vs. non-ERD; $P < 0.05$). Meat color was assessed visually on a discrete scale. Average meat color values ranged between 4.4 and 4.8 (SEM: 0.14; results not shown) with an average score for reference calves of 4.4 (SEM: 0.25). Meat color was not affected by ERD or CONC.

Table 2.4 Effects of early rumen development in period 1 (ERD) and inclusion of concentrate in the solid feed mixture in period 2 (CONC) on performance parameters in veal calves[1]

Item	Period 1		Period 2		SEM	ERD effect[2]	CONC effect[2]	Reference	SEM
	Non-ERD	ERD	Non-CONC	CONC		P-value	P-value		
Period 1 (wk 1-12)									
DMI MR, kg DM	107	69			0.3	***			
DMI SF, kg DM	0	44			1.6	***			
ADG, g/d	794	712			10.1	***			
FCR, kg/kg	1.62	1.92			0.029	***			
Period 2 (wk 13-24)									
DMI MR, kg DM	243	241	241	244	3.9	-	-	233	7.4
DMI SF, kg DM	42	45	43	44	1.2	-	-	0	-
ADG, g/d	1470	1572	1490	1552	33.2	*	NS	1284	63.7
FCR, kg/kg	2.09	1.97	2.06	1.99	0.047	†	NS	1.97	0.089
Dressing percentage, %	55	54	54	55	0.2	†	**	54	0.5
Warm carcass weight, kg	138	138	135	141	1.8	NS	*	123	3.5

[1] DMI, dry matter intake; DM, dry matter; MR, milk replacer; SF, solid feed; ADG, average daily gain; FCR, feed conversion ratio.

[2] No interactions between ERD and CONC were found ($P > 0.05$).

[2] Feed conversion ratio.

†$P \leq 0.10$; *$P \leq 0.05$; **$P \leq 0.01$;***$P \leq 0.001$.

Rumen Fermentation Parameters

The effects of dietary treatments on rumen fermentation parameters are shown in Table 2.5. The CONC increased molar proportions of butyrate, valerate, and methyl butyrate ($P < 0.001$) at the expense of acetate ($P < 0.01$). Lactate concentrations were unaffected by the dietary treatments. In comparison with roughage only, CONC significantly increased ammonia concentrations ($P < 0.01$), and decreased pH ($P < 0.01$). The weight of fresh reticulorumen contents at slaughter was affected by ERD, but not by CONC. The CONC increased Co recovery from 13.5 to 18.4% ($P < 0.05$). Early rumen development did not affect Co recovery at the end of P2.

Macroscopic and Microscopic Evaluation of the Rumen Wall

An overview of macroscopic and microscopic rumen wall parameters determined after P2 are shown in Table 2.6. The macroscopic rumen development score was higher in ERD compared with non-ERD calves ($P < 0.001$), and increased with CONC ($P < 0.05$). However, this did not coincide with significant changes in morphometric measures of rumen development, which exhibited large variation.

Empty rumen weight was unaffected by ERD or CONC. The prevalence of plaque formation numerically increased from 10 to 22% of the calves by CONC. Rumen plaque formation was not observed in calves fed the reference diet.

Abomasal Lesions

The effects of dietary treatments on abomasal lesions are shown in Table 2.7. In general, great variation existed in the prevalence of abomasal lesions. Early rumen development did not affect the percentage of calves with abomasal erosions or ulcers at the end of P2. However, ERD significantly decreased the prevalence of large scars at the end of P2 (6 vs. 20% for ERD vs. non-ERD, $P < 0.05$). Furthermore, CONC resulted in a higher percentage of calves with small scars ($P < 0.05$).

Table 2.5 Effects of early rumen development (ERD) and inclusion of concentrate in the solid feed mixture in period 2 (CONC) on rumen (fermentation) parameters in veal calves, determined at the end of period 2[1]

	Period 1		Period 2			ERD effect[1]	CONC effect[1]		
Item	**Non-ERD**	**ERD**	**Non-CONC**	**CONC**	**SEM**	**P-value**	**P-value**	**Reference**	**SEM**
Total VFA, mmol/L	78	77	73	81	4.3	NS	NS	38	7.5
Individual FA, mol/100 mol									
Acetate	65.2	65.4	67.1	63.2	0.85	NS	**	69.6	1.58
Butyrate	10.8	10.6	8.9	12.8	0.80	NS	***	6.3	1.13
Propionate	18.8	18.7	19.3	18.2	0.76	NS	NS	17.7	1.22
Isobutyrate	1.3	1.3	1.3	1.3	0.07	NS	NS	2.7	0.19
Methyl butyrate	2.3	2.6	2.2	2.8	0.10	NS	***	3.0	0.21
Valerate	1.6	1.4	1.3	1.8	0.09	NS	***	0.8	0.12
Lactate, mmol/L	3.3	2.4	1.8	3.9	2.9	NS	NS	7.6	5.2
Ammonia, mmol/L	12.8	14.7	11.0	16.5	1.36	NS	**	19.3	2.39
pH	5.9	5.9	6.1	5.7	0.08	NS	**	6.2	0.14
Weight of fresh rumen contents, kg	13.6	15.9	15.5	14.0	0.74	*	NS	12.3	1.30
DM of fresh rumen contents, g/kg	116	119	115	120	3.3	NS	NS	48	5.8
Rumen Co recovery, %	16.8	14.6	13.5	18.4	1.77	NS	*	17.9	3.92

[1] No interactions between ERD and CONC were found ($P > 0.05$).

†$P \leq 0.10$; *$P \leq 0.05$; **$P \leq 0.01$;***$P \leq 0.001$.

Table 2.6 Effects of early rumen development (ERD) and inclusion of concentrate in the solid feed mixture in period 2 (CONC) on macroscopic and microscopic parameters of the rumen mucosa in veal calves, at the end of period 2[1]

Item	Period 1		Period 2		SEM	ERD effect[1]	CONC effect[1]	Reference	SEM
	Non-ERD	ERD	Non-CONC	CONC		P-value	P-value		
Macroscopic parameter									
Empty rumen weight, g	2000[2]	2080	2011	2070	52.2	NS	NS	1685	91.7
Rumen development[3]	1.7	2.0	1.8	2.0	0.05	***	*	1.0	0.09
Plaque[4], % calves	15	17	10	22	-	NS	NS	0	0
Microscopic parameter									
Rumen dorsal location									
RMSL[5]	4.2	3.6	3.8	4.0	0.25	NS	NS	2.8	0.44
Mucosa thickness, µm	1065	920	1031	954	78.8	NS	NS	710	140.1
Muscle thickness, µm	1875	1656	1857	1673	85.5	NS	NS	1900	193.8
Rumen ventral location									
RMSL[5]	4.2	4.2	4.0	4.4	0.32	NS	NS	2.8	0.56
Mucosa thickness, µm	1169	1035	1061	1143	105.1	NS	NS	730	184.5
Muscle thickness, µm	1501	1484	1516	1469	155.4	NS	NS	1669	253.2
Rumen atrium									
RMSL[5]	7.1	7.1	7.2	7.0	0.35	NS	NS	3.9	0.61
Mucosa thickness, µm	1689	1645	1703	1632	115.9	NS	NS	986	203.3
Muscle thickness, µm	2092	1824	1993	1924	87.8	*	NS	2305	154.1

[1] No interactions between ERD and CONC were found ($P > 0.05$).

[2] Predicted means of ANOVA models are presented in the table, unless specified otherwise.

[3] Average of a discrete examination scale of 5 scores from poor to excellent: 1, 1.5, 2, 2.5, and 3.

[4] Actual percentages are given. Differences between treatment groups were examined with the Fisher exact test.

[5] RMSL: ratio of mucosa to serosa length.

†$P \leq 0.10$; *$P \leq 0.05$; **$P \leq 0.01$;***$P \leq 0.001$.

Table 2.7 Effects of early rumen development (ERD) and inclusion of concentrate in the solid feed mixture in period 2 (CONC) on the prevalence of abomasal lesions in veal calves at the end of period 2[1]

	Period 1		Period 2			ERD effect[1]	CONC effect[1]	
Item	Non-ERD	ERD	Non-CONC	CONC	SEM	P-value	P-value	Reference[2]
Erosions, % of calves	48[3]	38	50	35	6.5	NS	NS	40
Small (≤ 0.5 cm^2)	45	38	50	32	6.6	NS	†	40
Medium[4] (0.5 - 1 cm^2)	5	3	5	3	-	NS	NS	0
Large[4] (≥1.0 cm^2)	0	3	3	0	-	NS	NS	0
Ulcers, % of calves	43	50	40	53	7.0	NS	NS	20
Small (≤ 0.5 cm^2)	33	35	30	38	8.4	NS	NS	10
Medium (0.5 - 1 cm^2)	13	21	18	15	6.3	NS	NS	0
Large (≥1.0 cm^2)	10	11	13	9	4.5	NS	NS	10
Scars, % of calves	78	83	73	89	5.3	NS	NS	90
Small (≤ 0.5 cm^2)	63	74	60	77	4.4	†	*	70
Medium (0.5 - 1 cm^2)	20	27	18	30	6.9	NS	NS	30
Large (≥1.0 cm^2)	20	6	13	14	3.5	*	NS	10

[1] No interactions between ERD and CONC were found ($P > 0.05$).

[2] All data for the reference group are actual percentages (without SEM).

[3] Predicted means of generalized linear models are presented in the Table, unless specified otherwise.

[4] Actual percentages (without SEM) are given. Differences between treatment groups were examined with the Fisher exact test.

†$P \leq 0.10$; *$P \leq 0.05$; **$P \leq 0.01$;***$P \leq 0.001$.

DISCUSSION

Feed Intake and Growth Performance

The reduced ADG during P1 for ERD calves may be caused by the difference in digestibility between SF and MR (Labusièrre et al., 2009), and possibly by the energy required for rumen development. The initiation of SF intake and the subsequent ruminal fermentation resulted in an increase in rumen tissue mass and papillae growth, as was also shown previously (Sander et al., 1959; Baldwin et al., 2004, Suárez et al., 2006b, 2007). This process requires energy but enables the animal to cope with a different supply of dietary substrates. Our study shows that this investment in rumen development during the first wk is beneficial during the late fattening period, as demonstrated by the significant increase in ADG of 100 g/d and a tendency towards a lower FCR in ERD compared with non-ERD calves during P2. As the increased growth rate in P2 followed a period of decreased growth rate in P1, it may be referred to as compensatory gain, in part related to gastrointestinal contents, and increased mass of gastrointestinal tissues. However, as carcass gain over the entire 25-wk period was identical for ERD and non-ERD calves, it is clear that calf performance actually benefits from ERD. During the entire 25-wk period, a cumulative quantity of 42 kg MR DM was exchanged for 48 kg of SF DM (ERD vs. non-ERD). When assuming digestible energy (**DE**) values for MR (4.7 MCal/kg of DM, Van den Borne et al., 2006) and the SF mixture containing 50:25:25 of concentrate, maize silage and straw (3.4, 3.2, and 1.8 MCal/kg of DM, respectively, from beef cattle data, NRC, 2000), 196 MCal DE from MR was exchanged by 140 MCal of DE from SF, leading to similar carcass weight gain. It illustrates that important interactions may occur between MR and SF in veal calves, which warrants further investigation.

Rumen Development

The rumen development score at the end of P2 was increased by ERD and CONC. Our observations support earlier findings that enlargement of absorptive surface area is triggered by end products of microbial fermentation (Tamate et al., 1962). Confirming earlier observations by Harrison et al. (1960), when the SF supply was stopped during P2, both macroscopic and microscopic parameters were markedly decreased at the end of P2 to levels comparable to those observed in calves exclusively fed on MR and slaughtered after P1.

Furthermore, morphometric parameters were measured at 3 locations and considerable differences in muscle thickness, mucosa thickness, and RMSL between these locations were observed. Values found at dorsal and ventral locations are in close agreement with previous results of Suárez et al. (2006b; 2007) in veal calves of 8 to 12 wk. Values for RMSL and mucosa thickness were much higher in the rumen atrium, indicating that the rumen atrium is a major site of absorption. This is also supported by Kristensen et al. (2007), who observed the largest papillae length in the rumen atrium compared with saccus ruminis ventralis in dairy calves.

Except for the difference in rumen development between calves supplemented with SF compared with calves fed MR alone at the end of P1 and P2, differences in macroscopic rumen development did not match those in morphometric observations at the end of P2. Variation in morphometric parameters was similar to previous results of Suárez et al. (2006b, 2007). Their observations showed a higher response of morphometric parameters to concentrate composition than roughage-to-concentrate ratio.

Rumen and Abomasal Health

At the end of P1, plaque formation was not observed, in contrast to previous results of Suárez et al. (2006b), who observed plaque formation in 73 to 100% of the calves in their study. Their calves, however, were fed concentrate as the only SF source, and in subsequent studies, increasing the roughage proportion in the diet was shown to be effective in decreasing plaque formation (Suárez et al., 2007; Brscic et al., 2011). After P2, plaque formation was still generally low compared with previous results (Suárez et al., 2007; Brscic et al., 2011), but this can be explained by the high roughage-to-concentrate ratio of the SF mixture in the current study.

Suárez et al. (2007) were the first to gather quantitative information on ruminal drinking in calves fed concentrate or roughage, using CoEDTA as a marker. They observed that 3 h after feeding, 21 to 35% of the ingested milk was present in the reticulorumen, rather than the abomasum. The results of the current study showed large interindividual variation. In several calves slaughtered at the end of P1 (1 out of 4 non-ERD calves and 4 out of 12 ERD calves), no Co was recovered in the rumen contents. Higher Co recoveries observed by Suárez et al. (2007) may be caused by the higher proportion of concentrate feed in their study. Indeed, our results showed that CONC resulted in a significant increase in Co recovery. Furthermore, the increase in time between the last meal and slaughter and transport of the current study may have affected Co recovery as well.

Veal calves show a high prevalence of abomasal lesions, in the range of 48 to 74% of calves (Gottardo et al., 2002; Mattiëllo et al., 2002; Brscic et al., 2011). Multiple factors have been associated with abomasal ulcers in bovine, including abomasal hyperacidity (Ahmed et al., 2002) and the presence of *Clostridia* and *Campylobacter* species (Mills et al., 1990; Jelinski et al., 1995). In addition, the interaction between SF and a MR diet seems to be particularly relevant for milk-fed calves. In several studies, provision of SF to milk-fed veal calves was demonstrated to increase the prevalence of abomasal lesions, including pyloric ulcers, when compared with calves fed MR alone (Breukink et al., 1991; Mattiello et al., 2002; Brscic et al., 2011). These findings may be explained by SF particles exerting mechanically abrasive effects on the abomasal mucosa, which may be sensitized due to overfilling when large volumes of MR are provided. Promoting rumen development and, consequently, stimulating the fermentative degradation of potentially sharp particles would then reduce this problem. In the present study, ERD did not affect the prevalence of abomasal erosions and ulcers recorded at slaughter, but the prevalence of large scars in ERD calves was

significantly decreased compared with non-ERD calves (6% vs. 20%). This suggests that ERD provided some protection against abomasal lesions, probably at the time of transition between P1 and P2. The observed decrease in the prevalence of large scars in ERD calves supports the hypothesis that rumen development is positively related to abomasal health.

CONCLUSIONS

Both ERD and CONC increased rumen development score at the end of P2. At 25 wk, early rumen development (ERD, starting in wk 1) in veal calves was observed to yield comparable carcass weight to late rumen development (i.e., after 12 wk). Over the 25-wk period, the utilization of the SF provided appeared surprisingly similar to that of MR. Partially replacing roughage by concentrates in P2 increased dressing percentage and warm carcass weight. Plaque formation was generally low in all calves, which may be due to the relatively high roughage-to-concentrate ratio of the SF mixture. The prevalence of large scars in ERD calves was significantly decreased compared with non-ERD calves. This may indicate that ERD provided some protection against abomasal lesions. In conclusion, early compared with late rumen development resulted in improved feed utilization and may be beneficial for abomasal health.

ACKNOWLEDGEMENTS

This study was financially supported by the Product Board of Animal Feed (Den Haag, the Netherlands) and the Dutch Ministry of Economic Affairs, Agriculture and Innovation. Skilled technical assistance of Leonie Heutinck and Joyce van Delen (Livestock Research, Animal Sciences Group, Wageningen University and Research Centre, Lelystad, the Netherlands) is gratefully acknowledged.

REFERENCES

Ahmed, A. F., P. D. Constable, and N. A. Misk. 2002. Effect of feeding frequency and route of administration on abomasal luminal pH in dairy calves fed milk replacer. Journal of Dairy Science 85:1502-1508.

Baldwin, R. L. VI, K. R. McLeod, J. L. Klotz, and R. N. Heitmann. 2004. Rumen development, intestinal growth and hepatic metabolism in the pre- and postweaning ruminant. Journal of Dairy Science 87(E. Suppl.):E55-E65.

Blokhuis, H. J., C. G. Van Reenen, J. J. Heeres, L. F. M. Heutinck, A. M. Van Vuuren, N. Stockhofe-Zurwieden, A. E. van de Braak, J. J. de Groot, I. Veissier, B. J. Lensink, L. Florand, M. S. Cockram, G. Cozzi, F. Gottardo, M. Verga, S. Mattiello, E. Canali, S. Raussi, M. Pyykkönen, H. Saloniemi, H. Hepola, and L. Hänninen. 2000. Chain management of veal calf welfare. Final report. European Union project, Contract Number FAIR 3 PL96–2049.

Breukink, H. J., T. Wensing, and J. M. Mouwen. 1991. Abomasal ulcers in veal calves: Pathogenesis and prevention. Pages 118-122 in Proc. Int. Symp. Veal Calf Prod. Pudoc Wageningen, Wageningen, the Netherlands.

Brscic, M., L.F.M. Heutinck, M.Wolthuis-Fillerup , N. Stockhofe-Zurwieden, B Engel, E.K. Visser, F.Gottardo, E.A.M Bokkers, B.J Lensink, G. Cozzi , and C.G. Van Reenen. 2011. Prevalence of gastrointestinal disorders recorded at postmortem inspection in white veal calves and associated risk factors. Journal of Dairy Science 94 (2):853-863.

Coverdale, J. A., H. D. Tyler, J. D. Quigley Iii, and J. A. Brumm. 2004. Effect of various levels of forage and form of diet on rumen development and growth in calves. Journal of Dairy Science 87:2554-2562.

Cozzi, G., F. Gottardo, S. Mattiello, E. Canali, E. Scanziani, M. Verga, and I. Andrighetto. 2002. The provision of solid feeds to veal calves: I. Growth performance, forestomach development, and carcass and meat quality. Journal of Animal Science 80:357-366.

Genstat Committee. 2000. Reference Manual. Procedure Library PL12. R.G. Payne and G.M. Arnold, ed. VSN International, Oxford, UK.

Gentile, A., S. Sconza, I. Lorenz, G. Otranto, G. Rademacher, P. Famigli-Bergamini and W. Klee. 2004. D-Lactic acidosis in calves as a consequence of experimentally induced ruminal acidosis. Journal of Veterinary Medicine Series A 51:64–70.

Gottardo, F., S. Mattiello, G. Cozzi, E. Canali, E. Scanziani, L. Ravarotto, V. Ferrante, M. Verga, and I. Andrighetto. 2002. The provision of drinking water to veal calves for welfare purposes. Journal of Animal Science 80:2362-2372.

Harrison, H. N., R. G. Warner, E. G. Sander, and J. K. Loosli. 1960. Changes in the tissue and volume of the stomachs of calves following the removal of dry feed or consumption of inert bulk. Journal of Dairy Science 43:1301-1312.

Hill, T. M., H. G. Bateman Ii, J. M. Aldrich, and R. L. Schlotterbeck. 2008. Effects of feeding different carbohydrate sources and amounts to young calves. Journal of Dairy Science 91:3128-3137.

Jelinski, M. D., C. S. Ribble, M. Chirino-Trejo, E. G. Clark, and E. D. Janzen. 1995. The relationship between the presence of *Helicobacter pylori, Clostridium perfringens* type A, *Campylobacter* spp, or fungi and fatal abomasal ulcers in unweaned beef calves. Canadian Veterinary Journal 36:379-382.

Kooijman, J., H. K. Wierenga, and P. R. Wiepkema. 1991. Development of abnormal oral behavior in group-housed veal calves: Effects of roughage supply. Page 54-58 in New Trends in Veal Calf Production. Proc. Int. Symp. Veal Calf Prod., Wageningen, the Netherlands. EAAP Publ. No. 52. Pudoc, Wageningen, the Netherlands.

Kristensen, N. B., J. Sehested, S. K. Jensen, and M. Vestergaard. 2007. Effect of milk allowance on concentrate intake, ruminal environment, and ruminal development in milk-fed Holstein calves. Journal of Dairy Science 90:4346-4355.

Labussière, E., S. Dubois, J. van Milgen, G.Bertrand, and J. Noblet. 2009. Effect of solid feed on energy and protein utilization in milk-fed veal calves. Journal of Animal Science 87:1106-1119.

Mattiello, S., E. Canali, V. Ferrante, M. Caniatti, F. Gottardo, G. Cozzi, I. Andrighetto, and M. Verga. 2002. The provision of solid feeds to veal calves: II. Behavior, physiology, and abomasal damage. Journal of Animal Science 80:367-375.

McCullagh, P. and J. A. Nelder. 1989. Generalized Linear Models. 2nd ed. Chapman & Hall, London, UK.

Mills, K.W., J.L .Johnson, R.L. Jensen, L.F.Woodard, and A.R. Doster. 1990. Laboratory findings associated with abomasal ulcers/tympany in range calves. Journal of Veterinary Diagnostics and Investigation 2:208-212.

NRC. 2000. Nutrient Requirements of Beef Cattle. 7th revised edition. National Academy of Sciences, Washington, DC.

Robinson, P. H., S. Tamminga, and A. M. van Vuuren. 1986. Influence of declining level of feed intake and varying the proportion of starch in the concentrate on rumen fermentation in dairy cows. Livestock Production Science 15:173-189.

Sander, E. G., R. G. Warner, H. N. Harrison, and J. K. Loosli. 1959. The stimulatory effect of sodium butyrate and sodium propionate on the development of rumen mucosa in the young calf. Journal of Dairy Science 42:1600-1605.

Suárez, B. J., C. G. Van Reenen, G. Beldman, J. Van Delen, J. Dijkstra, and W. J. J. Gerrits. 2006a. Effects of supplementing concentrates differing in carbohydrate composition in veal calf diets: I. Animal performance and rumen fermentation characteristics. Journal of Dairy Science 89:4365-4375.

Suárez, B. J., C. G. Van Reenen, W. J. J. Gerrits, N. Stockhofe-Zurwieden, A. M. Van Vuuren, and J. Dijkstra. 2006b. Effects of supplementing concentrates differing in carbohydrate composition in veal calf diets: II. Rumen development. Journal of Dairy Science 89:4376-4386.

Suárez, B. J., C. G. Van Reenen, N. Stockhofe-Zurwieden, J. Dijkstra, and W. J. J. Gerrits. 2007. Effect of roughage source and roughage to concentrate ratio on animal performance and rumen development in veal calves. Journal of Dairy Science 90:2390-2403.

Tamate, H., A.D. McGilliard, N.L. Jacobson, and R. Getty. 1962. Effect of various dietaries on the anatomical development of the stomach in the calf. Journal of Dairy Science 45:408-420.

Udén, P., P. E. Colucci, and P. J. Van Soest. 1980. Investigation of chromium, cerium and cobalt as markers in digesta. Rate of passage studies. Journal of Science in Food and Agriculture 31:625-632.

Van den Borne, J.J.G.C., M. W. A. Verstegen, S. J. J. Alferink, R. M. M. Giebels, and W. J. J. Gerrits. 2006. Effects of feeding frequency and feeding level on nutrient utilization in heavy preruminant calves. Journal of Dairy Science 89:3578–3586.

Van Weeren-Keverling Buisman, A., J. M. V. M. Mouwen, T. Wensing, and H. J. Breukink. 1990. Intraruminal administration of milk in the calf as a model for ruminal drinking: Morphological and enzymatical changes in the jejunal mucosa. Veterinary Research Communications 14:129-140.

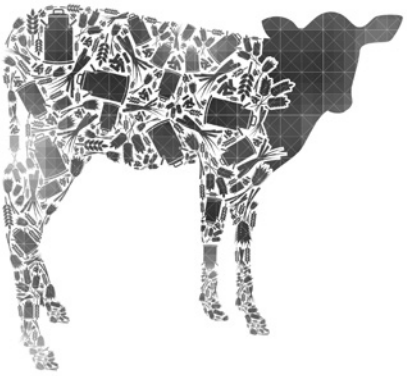
Veissier, I., A. R. Ramirez De La Fe, and P. Pradel. 1998. Nonnutritive oral activities and stress responses of veal calves in relation to feeding and housing conditions. Applied Animal Behavioral Sciences 57:35-49.

Welchman, D. D. and G. N. Baust. 1987. A survey of abomasal ulceration in veal calves. Veterinary Record 121:586-590.

Zitnan, R., J. Voigt, U. Schönhusen, J. Wegner, M. Kokardová, H. Hagemeister, M. Levkut, S. Kuhla, and A. Sommer. 1998. Influence of dietary concentrate to forage ratio on the development of rumen mucosa in calves. Archives of Animal Nutrition 51:279-291.

Chapter 3

Low-protein solid feed improves the utilization of milk replacer for protein gain in veal calves



H. Berends[1], J.J.G.C. van den Borne[1], S.J.J. Alferink[1], C.G. van Reenen[2], E.A.M. Bokkers[3], and W.J.J. Gerrits[1]

Journal of Dairy Science 2012: 95 (11) 6654-6664

[1]Animal Nutrition Group, Wageningen University, the Netherlands; [2]Livestock Research, Wageningen University and Research Centre, the Netherlands; [3]Animal Production Systems Group, Wageningen University, the Netherlands

ABSTRACT

This study was designed to quantify the contribution of low-protein solid feed (**SF**) intake, in addition to milk replacer, to protein and energy retention in veal calves. Because of potential interactions between milk replacer and SF, occurring at either the level of digestion or postabsorption, this contribution might differ from that in calves fed either SF or milk replacer alone. Forty-eight Holstein Friesian male calves, 55 ± 0.3 kg of bodyweight (BW), were divided across 16 groups of 3 calves each. Groups were assigned randomly to 1 of 4 incremental levels of SF intake: 0, 9, 18, or 27 g DM of SF·kg $BW^{-0.75}$·d^{-1}. The SF mixture consisted of 25% chopped wheat straw, 25% chopped corn silage, and 50% nonpelleted concentrate (on a DM basis). Each group was housed in a respiration chamber for quantification of energy and N balance at each of 2 BW: at 108 ± 1.1 kg and at 164 ± 1.6 kg. The milk replacer supply was 37.3 g DM of MR·kg $BW^{-0.75}$·d^{-1} at 108 kg of BW and 40.7 g DM of MR·kg $BW^{-0.75}$·d^{-1} at 164 kg of BW, irrespective of SF intake. Within a chamber, each calf was housed in a metabolic cage to allow separate collection of feces and urine. Indirect calorimetry and N balance data were analyzed by using regression procedures with SF intake-related variables. Nitrogen excretion shifted from urine to feces with increasing SF intake. This indicates a higher gut entry rate of urea and may explain the improved N utilization through urea recycling, particularly at 164 kg of BW. At 108 kg of BW, the gross efficiency of N retention was 61% for calves without SF, and it increased with SF intake by 6.7% / kg DM of SF per day. At 164 kg of BW, this efficiency was 49% for calves without SF, and it increased by 12.3% / kg DM of SF per day. The incremental efficiency of energy retention, representing the increase in energy retained per kilojoule of extra digestible energy intake from SF, was 41% at 108 kg of BW and 54% at 164 kg of BW. Accordingly, the apparent total tract digestibility of NDF increased with BW, from 46% at 108 kg of BW to 56% at 164 kg of BW. On average, 5.5% of gross energy from SF was released as methane in veal calves, which is similar to reported values in cattle fed only SF. In conclusion, the provision of low-protein SF resulted in improved N utilization for protein gain, particularly toward the end of the fattening period. In heavy calves, recycling of urea originating from amino acids in milk replacer potentially contributes substantially to the N retention of veal calves fed SF.

Keywords: digestibility, energy and nitrogen utilization, methane, roughage

INTRODUCTION

Optimizing solid feed (**SF**) strategies for veal calves has gained interest since the approval of European guidelines (97/2/EC Directive by the EU Council) on compulsory provision of SF in addition to the traditional milk replacer (**MR**) diet. Solid feed increasingly represents an important portion of the diet for veal calves, affecting gastrointestinal development, product quality, and nutrient utilization. Although the effects of SF (Vermorel et al., 1980; Ortigues et al., 1990) and MR (Gerrits et al., 1996; Diaz et al., 2001; van den Borne et al., 2006b) on nutrient utilization are well documented, little information is available on the effects of interactions between MR and SF on energy and protein utilization in calves.

Studies available on the effect of SF and MR on nutrient utilization (e.g., Vermorel et al., 1980; Labussière et al., 2009a), have not been designed to measure incremental responses of energy and protein deposition to SF intake. Interactions between MR and SF may affect nutrient utilization. Such potential interactions include urea recycling and leakage of significant (21 to 35%) amounts of MR to the rumen (Suárez et al., 2007; Berends et al., 2012). In addition, "plaque" (e.g., rumen mucosa containing focal or multifocal patches with coalescing and adhering papillae covered by a sticky mass of feed, hair and cell debris) formation on the rumen wall is related to the roughage-to-concentrate ratio in the diet (Suárez et al., 2007; Brscic et al., 2011) and could inhibit nutrient uptake from SF. Toward the end of the fattening period, veal calves become less efficient with N from MR protein (Gerrits et al., 1996), with excessive urea-N excretion in urine as a result. The reuse of urea-N through recycling by microbes in the gastrointestinal tract is low in calves fed exclusively MR (Gerrits et al., 1999) because of a lack of microbial activity in the rumen. Provision of SF to veal calves could result in increased microbial activity and subsequent incorporation of urea-N into microbial protein, which may contribute to the N supply. As reported by Reynolds and Kristensen (2008), urea recycling into the rumen is stimulated by a low protein-to-energy ratio in rumen contents and by high plasma urea concentrations. The latter condition is encountered in veal calves, as illustrated by the low efficiency of utilization of digested proteins from MR (Van den Borne et al., 2006b). To quantify the specific contribution of SF to protein and energy metabolism, responses to incremental quantities of SF should be measured, keeping the MR intake constant.

The aim of this study was to quantify the contribution of a low-protein SF to protein and energy retention in veal calves at 2 stages in the fattening period.

MATERIALS AND METHODS

This study was conducted at the research facilities of Wageningen University (the Netherlands). Procedures complied with the Dutch Law on Experimental Animals, which complies with ETS123 (Council of Europe 1985 and the 86/609/EEC Directive) and were approved by the Animal Care and Use Committee of Wageningen University.

Animals and Experimental Design

Forty-eight male Holstein-Friesian calves were used. Calves were raised on a commercial veal farm and were selected based on age, BW, uniformity, and clinical health. Mean age and BW upon arrival were 53 ± 0.7 d and 55 ± 0.3 kg, respectively. Calves arrived in 1 batch at the research facilities, and measurement periods were staggered. Therefore, calves were divided across 4 blocks. Within blocks, calves were assigned randomly to groups of 3 calves (4 groups per block). Groups were assigned randomly to 1 of 4 dietary treatments and exposed to their diet for at least 6 wk before the first measurement period. The first measurement period was at 108 ± 1.1 kg of BW and lasted 4 d. After the first measurement period, calves were housed in group pens for 7 wk. The second measurement period was at 164 ± 1.6 kg of BW and lasted 4 d.

Housing

For at least 6 wk, groups were housed in pens (2.35 x 2.45 m) equipped with wooden-slatted floors and open fences, and without bedding material. During the last 5 d before each measurement period, calves were adapted to individual housing in metabolic cages (0.79 x 1.85 m) equipped with wooden-slatted floors and open fences, and without bedding material. Cages enabled audiovisual contact between group mates. During the measurement periods, each group was housed in 1 of 2 identical 80 m^3 indirect calorimetric chambers. Within each chamber, calves were housed individually in metabolic cages. Temperature was maintained at 18°C, relative humidity at 65%, and air velocity at < 0.2 m/s. Calves were exposed to light (420 lx) from 0545 to 2300 h and to darkness (3.5 lx) during the remainder of the day.

Table 3.1 Experimental treatments for veal calves (n = 48) fed incremental amounts of solid feed (SF; 50% concentrate, 25% corn silage, 25% straw, DM basis) in addition to a milk replacer diet

Treatment	No. of groups[1]	Milk replacer, g of DM·kg $BW^{-0.75}$·d^{-1}		Solid feed, g of DM·kg $BW^{-0.75}$·d^{-1}
		108 kg BW	164 kg BW	
SF0	4	37.3	40.7	0
SF9	4	37.3	40.7	9
SF18	4	37.3	40.7	18
SF27	4	37.3	40.7	27

[1] Three calves per group

Diets and Feeding

Groups were assigned randomly to 1 of 4 incremental levels of SF intake: 0, 9, 18, or 27 g DM of SF·kg $BW^{-0.75}$·d^{-1} (Table 3.1), referred to as **SF0**, **SF9**, **SF18**, and **SF27**, respectively. Provision of SF was adjusted weekly based on the mean BW per group. Dietary treatments were imposed 7 d after arrival of the calves at the experimental facilities, and the quantity of MR fed was calculated to allow calves to reach a similar BW and growth rate at 2 wk before the start of each of the 2 measurement periods. From 2 wk before the start of the measurement periods onward, MR intake was fixed at 37.3 g DM of MR·kg $BW^{-0.75}$·d^{-1}at the first measurement period and 40.7 g DM of MR·kg $BW^{-0.75}$·d^{-1}at the second measurement period, irrespective of SF intake. Ingredient and nutrient composition of SF components and MR are shown in Table 3.2. The SF consisted (on a DM basis) of 25% chopped wheat straw, 25% corn silage, and 50% concentrate. The concentrate composition (Table 3.2) was designed to meet the mineral and vitamin requirements for beef cattle (NRC, 2000).

Table 3.2 Analyzed nutrient composition of milk replacer and solid feed components

Nutrient[1]	Milk replacer[2]	Concentrate[3]	Corn silage	Straw
DM, g/kg of product	968	883	497	923
CP[4]	212	128	77	22
Crude fat	192	36	28	9
Crude ash	76	66	36	66
Starch	19	450	356	11
Neutral detergent fibre	7	151	400	814
Gross energy, MJ/kg of DM	21.7	17.7	18.7	18.0

[1] Expressed in grams per kilogram of DM unless specified otherwise.

[2] Milk replacer composition: 20.0% whey powder concentrate, 13.3% delactosed whey, 35.7% whey powder, 2.0% starch, 4.0% wheat protein, 2.5% soy concentrate, 6.0% lard, 4.6% coconut oil, 6.0% tallow, 2.0% palm oil, 1.4% soy lecithin and 2.5% premix (provided per kilogram of milk replacer: 3.5 g of lysine and 2 g of methionine).

[3] Concentrate composition: 36.1% barley, 12.5% carob, 15% lupines (CP < 335 g/kg), 30% corn, 1% premix (lactose carrier, provided per kilogram of concentrate: vitamin A, 4000 IU; vitamin D, 500 IU; vitamin E, 100 IU; zinc, 25 mg; manganese, 20 mg; iodine, 0.8 mg; selenium, 0.15 mg; copper, 15 mg; cobal,: 0.1 mg), 1.15% $MgSO_4.7H_2O$, 0.77% NaCl, 2.15% $CaCO_3$, 1.35% KH_2PO_4.

[4] N × 6.25.

Milk replacer was reconstituted with water and supplied in a bucket at 40 to 41°C. Milk replacer concentration was 125 g/L during the first measurement period and 143 g/L during the second measurement period. Milk replacer was provided daily in 2 equally sized meals at 0700 and 1600 h, respectively. Calves were allowed 15 min to consume the milk; refusals were weighed twice daily. The SF was supplied directly after each milk meal in the same bucket; refusals were weighed once daily at 0645 h. Calves had free access to water via drinking nipples.

Measurements

Gas exchange was measured in 9-min intervals by measuring the exchange of O_2, CO_2, and CH_4, as described by Verstegen et al. (1987). Each calf was weighed before and after each measurement period. Feces were collected in plastic bags that were attached to the calves. Samples were stored at -20°C. Urine was collected in buckets and acidified to a pH ≤ 2 with H_2SO_4 to prevent microbial activity and NH_3 volatilization. Aerial $NH_3$was collected from a quantified sample of the outgoing air in H_2SO_4, and NH_4^+ in water that condensed on the heat exchanger was collected quantitatively. Milk replacer and SF components were sampled during each measurement period.

Chemical Analyses

Samples of MR, concentrate, and straw were pooled by block and BW. Feed components (MR, corn silage, concentrate, straw) were analyzed for DM, ash, N, starch, NDF (except for MR), crude fat, and gross energy content. Fecal samples were pooled by measurement period for each animal and analyzed for DM, ash, N, starch, NDF, crude fat, and gross energy content. Feed refusals were analyzed for DM, N, and gross energy content. Urine was pooled by measurement period for each animal and analyzed for N and gross energy content. In addition, N content was determined in the acid solution containing aerial NH_3 and in water that condensed on the heat exchanger.

For determination of DM content, feed refusals, corn silage, and feces were freeze-dried. Feces, feed components, and feed refusals were ground to pass a 1-mm screen. Dry matter content was determined by drying to a constant weight according to ISO standard 6496 (ISO, 1998b). Crude ash content was determined by incineration in a muffle furnace by combustion at 550°C according to ISO standard 5984 (ISO, 2002). Kjeldahl N content was determined according to ISO standard 5983 (ISO, 1997). Starch content was determined enzymatically as described by Rijnen et al. (2001). Neutral detergent fiber content was analyzed according to the method of Van Soest et al. (1991), with prior amylase treatment, and Na_2SO_3 was added to the neutral detergent solution to remove keratinaceous residues of animal origin (hair). Crude fat content was determined after acid hydrolysis according to ISO standard 6496 (ISO, 1999). Gross energy content was analyzed using an adiabatic bomb calorimeter (model C7000 calorimeter; IKA Werke GmbH & Co. KG, Staufen, Germany) according to ISO standard 9831 (ISO, 1998a). For urine, gross energy content was obtained after freeze-drying approximately 8 to 12 mL in polyethylene bags. All analyses were carried out in duplicate, except for starch content in feces (single) and crude fat content (triplicate).

Calculations

Intake of gross energy was calculated as feed intake (feed supply minus refusals) multiplied by gross energy content of each feed component. Intake of metabolizable energy (**ME**) was determined by subtracting energy excretion in feces, urine, and CH_4 from gross

energy intake. Heat production (**HP**) was calculated according to the equation of Brouwer (Brouwer, 1965) from gas exchange during the last 3 d of the measurement period. Energy retention was determined by the difference between ME intake and HP. Nitrogen retention was determined as N intake minus N losses in feces, urine, aerial NH_3, and NH_4^+ in water that condensed on the heat exchanger. Protein retained (N retention × 6.25) and energy retained as protein (protein retained × 23.7 kJ/g) were determined from N retention. Energy retained as fat was determined by the difference between energy retained and energy retained as protein. The incremental efficiency of energy utilization for growth is expressed as a percentage of extra energy retained (kJ·kg $BW^{-0.75}$·d^{-1}) per extra gross energy, digestible energy, or ME intake (kJ·kg $BW^{-0.75}$·d^{-1}), and was determined by regression of energy retention against gross energy, digestible energy, or ME intake. The incremental efficiency of N utilization for growth is expressed as a percentage of extra N retained (g N·kg $BW^{-0.75}$·d^{-1}) per extra N intake (g N·kg $BW^{-0.75}$·d^{-1}) and was determined by regression of N retention against (digested) N intake. Apparent total tract digestibility coefficients were determined by regression of the digested nutrient intake against gross nutrient intake.

Statistical Analysis

The group was the experimental unit, and both the dependent variables and covariables were expressed as averages per group. Dependent variables (i.e., energy and N balance traits) and apparent total tract digestibility were analyzed by mixed model analysis (PROC MIXED in SAS 9.20; SAS Institute, 2000), including the fixed effects of block and BW, and SF intake-related variables as covariables, with a random group term to account for repeated observations of the same group. The interaction between SF intake-related variables and BW was included in all models.

Variance-covariance structures were selected based on the Bayesian information criterion. Because the repeated statement consisted of only 2 levels, variance-covariance structures tested were limited to unstructured and compound symmetry. When model residuals were not normally distributed, data were transformed (log, quadratic, or inverse) to obtain homogeneity of variance. Data are presented as nontransformed means with their SEM. Regression coefficients are presented with their calculated intercepts for the SF0 treatment. Circadian patterns of heat and CH_4 production are presented as means per hour with their standard errors of the mean. Differences were considered significant at $P < 0.05$. Figures showing circadian patterns of CH_4 and heat production are descriptive and are excluded from any statistical analysis.

RESULTS

In measurement period 1, 4 calves (2 SF0, 2 SF27) were excluded based on exclusion criteria for health or feed refusals. In measurement period 2, 8 calves (3 SF0, 2 SF9, 3 SF27) were excluded based on exclusion criteria for health or feed refusals, or because they were excluded in the first measurement period. When a calf was excluded from measurements, a group consisted of 2 instead of 3 calves. Results were not affected by block, unless specified. Realized intakes of MR and SF were similar to targeted intakes as a result of negligible feed refusals. The feeding schedule aimed at similar BW. Average BW was 108 ± 1.7 kg in the first measurement period and 164 ± 2.1 kg in the second measurement period, and was not affected ($P > 0.05$) by SF level.

Digestibility of SF

Apparent total tract digestibility coefficients of SF in the first (108 kg of BW) and second (164 kg of BW) measurement period are presented in Table 3.3. Apparent total tract digestibility coefficients of DM, crude ash, crude fat, gross energy, and NDF from SF were in the range from 46 to 66%. The apparent total tract digestibility of CP from SF averaged 35% at 108 kg of BW and 39% at 164 kg of BW. The apparent total tract digestibility of starch from SF exceeded 97% at 108 and 164 kg of BW. The apparent total tract digestibility of NDF from SF was greater ($P < 0.01$) at 164 kg of BW (56%) than at 108 kg of BW (46%).

Table 3.3 Apparent total tract digestibility coefficients of solid feed in veal calves at 108 and 164 kg of BW

Item	Apparent total tract digestibility coefficient[1]		Effect of BW[2]
	108 kg of BW	164 kg of BW	
Dry matter	0.62 ± 0.022	0.66 ± 0.013	NS
Protein[3]	0.35 ± 0.057	0.39 ± 0.059	NS
Ash	0.51 ± 0.035	0.51 ± 0.028	NS
Fat[4]	0.55 ± 0.113	0.54 ± 0.077	NS
Energy	0.58 ± 0.028	0.62 ± 0.016	NS
Starch[5]	0.97 ± 0.004	0.98 ± 0.004	NS
Neutral detergent fibre	0.46 ± 0.027	0.56 ± 0.025	**

[1] Calculated by regression coefficient β, ($y = a + \beta \cdot x$), representing the change in apparent total tract digested intake per increase in gross intake, where intercept a represents the digested nutrient intake at SF0 (calves fed no solid feed).

[2] The *P*-value represents the effect of BW (i.e., measurement period) on the apparent total tract digestibility coefficient.

[3] Data were transformed (quadratic) to obtain homogeneity of variance.

[4] The effect of block was significant ($P < 0.05$).

[5] Data were transformed (inverse) to obtain homogeneity of variance.

**$P < 0.01$.

N Balance

Nitrogen intake increased with increasing SF intake, from 1.26 to 1.65 g N·kg $BW^{-0.75}$·d^{-1} at 108 kg of BW and from 1.38 to 1.76 g N·kg $BW^{-0.75}$·d^{-1} at 164 kg of BW (Table 3.4). The incremental efficiency with which ingested N was retained tended to increase ($P < 0.10$) from 43% at 108 kg of BW to 77% at 164 kg of BW (Table 3.5; estimates for ß). The incremental efficiency with which digested N was retained exceeded 100%, and it increased ($P < 0.05$) from 124% at 108 kg of BW to 187% at 164 kg of BW (Table 3.5; estimates for ß). Fecal N excretion increased with N intake from SF ($P < 0.001$) and was unaffected by BW. Urinary N excretion did not change with increasing N intake at 108 kg of BW, but it did decrease with increasing N intake at 164 kg of BW ($P < 0.001$). The efficiency of N retention, expressed as a percentage of N intake, increased with increasing N intake from SF ($P < 0.05$; Table 3.5).

Energy Balance

The effects of SF intake on N and energy balance parameters at 108 and 164 kg of BW are shown in Table 3.5. Table 3.4 includes descriptive energy and N balance data for each treatment separately. The incremental efficiency with which digestible energy from SF was retained was 41% at 108 kg of BW and 54% at 164 kg of BW (Table 3.5; estimates for ß). Calves in the SF0 group retained 106 kJ·kg $BW^{-0.75}$·d^{-1} of energy as protein at a digestible energy intake of 770 kJ·kg $BW^{-0.75}$·d^{-1}at 108 kg of BW, and 97 kJ·kg $BW^{-0.75}$·d^{-1} of energy as protein at a digestible energy intake of 845 kJ·kg $BW^{-0.75}$·d^{-1} at 164 kg of BW (Table 3.5; SF0 level). Protein retention increased ($P < 0.001$) with increasing digestible energy intake from SF, with incremental efficiencies of 9% at 108 kg of BW and 14% at 164 kg of BW. Fat retention increased ($P < 0.001$) with increasing digestible energy intake from SF, with incremental efficiencies of 33% (108 kg of BW) and 40% (164 kg of BW).

The influence of SF intake on the circadian patterns of HP is shown in Figure 3.1. Differences between treatments (i.e., greater HP with increasing SF intake) seemed more pronounced during the postprandial period than during the pre-prandial period at 108 kg of BW. At 164 kg of BW, treatment differences were more constant during the day. The proportion of ME intake spent on HP decreased ($P < 0.05$) with BW (Table 3.5). Methane production was 1.2 and 1.6 kJ·kg $BW^{-0.75}$·d^{-1} in SF0 calves of 108 and 164 kg of BW, respectively. Methane production increased (0.09 kJ of CH_4 per kJ of digestible energy; $P < 0.001$) with increasing digestible energy from SF. The influence of SF intake on the circadian pattern of CH_4 production is shown in Figure 3.2 for 108 kg and 164 kg of BW. Effects of SF intake were more pronounced during the early postprandial period at 108 kg of BW than at 164 kg of BW.

Table 3.4 Nitrogen and energy balances (mean ± SEM) in veal calves (108 and 164 kg of BW) fed incremental amounts of solid feed (SF) in addition to a milk replacer (MR)[1]

	108 kg of BW			
Treatment[2]	SF0	SF9	SF18	SF27
No. of groups, no. of animals	4, 10	4, 12	4, 12	4, 10
Metabolic BW, $kg^{0.75}$ / animal	33.3 ± 0.82	33.4 ± 0.29	33.1 ± 0.62	34.0 ± 1.28
N balance, g of N·kg $BW^{-0.75}$·d^{-1}				
Total N intake	1.26 ± 0.004	1.39 ± 0.005	1.53 ± 0.005	1.65 ± 0.006
N from MR	1.26 ± 0.004	1.26 ± 0.006	1.26 ± 0.003	1.26 ± 0.002
N from SF	0 ± -	0.13 ± 0.001	0.26 ± 0.002	0.39 ± 0.004
Fecal N	0.09 ± 0.006	0.20 ± 0.014	0.31 ± 0.024	0.34 ± 0.023
Digestible N intake	1.17 ± 0.009	1.20 ± 0.013	1.21 ± 0.027	1.32 ± 0.022
Urinary N	0.43 ± 0.038	0.45 ± 0.031	0.44 ± 0.019	0.40 ± 0.002
N retention	0.73 ± 0.04	0.73 ± 0.041	0.76 ± 0.010	0.91 ± 0.026
Energy balance, kJ·kg $BW^{-0.75}$·d^{-1}				
Total GE intake	804 ± 2.4	973 ± 2.9	1144 ± 2.1	1305 ± 3.0
GE from MR	804 ± 2.4	802 ± 3.2	806 ± 0.6	803 ± 1.0
GE from SF	0 ± -	171 ± 0.6	339 ± 2.4	502 ± 2.6
Fecal energy	34 ± 4.8	95 ± 7.1	191 ± 13.3	234 ± 11.5
DE intake	770 ± 5.8	878 ± 5.8	954 ± 14.3	1072 ± 9.0
Methane	1.2 ± 0.49	11.7 ± 1.65	19.7 ± 0.84	28.9 ± 3.34
Urinary energy	20 ± 2.1	26 ± 1.3	30 ± 1.5	28 ± 1.2
ME intake	749 ± 4.8	839 ± 5.8	904 ± 14.4	1015 ± 9.9
Heat production	577 ± 17.2	603 ± 3.1	660 ± 2.1	721 ± 14.5
Energy retention	172 ± 20.0	236 ± 6.6	244 ± 13.4	294 ± 20.1
As protein	108 ± 5.9	108 ± 6.0	112 ± 1.4	135 ± 3.9
As fat	64 ± 19.4	128 ± 6.2	132 ± 13.1	159 ± 18.2

[1] GE = gross energy; DE = digestible energy. [2] Treatments correspond to SF intake levels: 0 (SF0), 9 (SF9), 18 (SF18) or 27 (SF27) g of DM /kg $BW^{-0.75}$per day. The SF (50% concentrate, 25% straw, 25% corn silage, DM basis) was fed in addition to an MR diet.

	164 kg of BW			
Treatment[2]	SF0	SF9	SF18	SF27
No. of groups, no. of animals	4, 9	4, 10	4, 12	4, 9
Metabolic BW, $kg^{0.75}$ / animal	45.4 ± 0.99	46.2 ± 0.26	45.4 ± 0.34	46.4 ± 1.55
N balance, g of N·kg $BW^{-0.75}$·d^{-1}				
Total N intake	1.38 ± 0.005	1.51 ± 0.007	1.64 ± 0.006	1.76 ± 0.021
N from MR	1.38 ± 0.005	1.37 ± 0.006	1.37 ± 0.007	1.37 ± 0.006
N from SF	0 ± -	0.14 ± 0.001	0.27 ± 0.003	0.39 ± 0.017
Fecal N	0.10 ± 0.007	0.18 ± 0.001	0.25 ± 0.010	0.33 ± 0.020
Digestible N intake	1.28 ± 0.003	1.33 ± 0.007	1.39 ± 0.013	1.42 ± 0.019
Urinary N	0.64 ± 0.021	0.58 ± 0.023	0.55 ± 0.023	0.50 ± 0.026
N retention	0.62 ± 0.02	0.73 ± 0.029	0.82 ± 0.035	0.91 ± 0.037
Energy balance, kJ·kg $BW^{-0.75}$·d^{-1}				
Total GE intake	881 ± 5.1	1054 ± 4.3	1218 ± 8.5	1373 ± 26.6
GE from MR	881 ± 5.1	877 ± 3.4	876 ± 4.4	874 ± 3.9
GE from SF	0 ± -	176 ± 1.7	342 ± 7.4	500 ± 26.9
Fecal energy	36 ± 2.4	94 ± 1.2	147 ± 4.1	225 ± 11.9
DE intake	845 ± 7.2	960 ± 5.2	1070 ± 12.3	1148 ± 15.5
Methane	1.6 ± 0.38	15.4 ± 1.03	22.9 ± 1.51	29.8 ± 2.63
Urinary energy	31 ± 1.3	34 ± 2.3	37 ± 4.7	41 ± 4.1
ME intake	812 ± 7.4	911 ± 7.1	1011 ± 9.7	1075 ± 21.0
Heat production	576 ± 17.4	616 ± 5.6	655 ±13.6	682 ± 10.6
Energy retention	236 ± 21.6	295 ± 3.7	356 ±15.4	393 ± 18.6
As protein	92 ± 2.9	108 ± 4.3	122 ± 5.2	134 ± 5.5
As fat	144 ± 20	186 ± 3.4	234 ±11.9	259 ± 16.1

Table 3.5 Effects of incremental solid feed (SF) intake, fed in addition to a milk replacer (MR) diet, on N and energy balance parameters in veal calves at 108 and 164 kg of BW[1]

Response parameters				β[4]			P-value[6]	
	BW	Covariable[2]	SF0 level[3]	Estimate	SE	P-value[5]	BW	Covariable.BW
N balance, g of N·kg $BW^{-0.75}$·d^{-1}								
N retention	108	N intake	0.70	0.43	0.124	**	*	†
	164	N intake	0.62	0.77	0.093	***		
N retention	108	Digested N	0.72	1.24	0.200	***	*	*
	164	Digested N	0.87	1.87	0.204	***		
Fecal N[7]	108	N intake	0.10	0.65	0.057	***	NS	NS
	164	N intake	0.10	0.61	0.059	***		
Urinary N[8]	108	N intake	0.45	- 0.08	0.092	NS	**	*
	164	N intake	0.64	- 0.38	0.072	***		
N retention efficiency, %	108	N intake	61	16	7.1	*	*	*
	164	N intake	49	40	7.3	***		
Energy balance, kJ·kg $BW^{-0.75}$·d^{-1}								
Energy retention	108	GE	180	0.22	0.039	***	NS	†
	164	GE	237	0.33	0.039	***		
Energy retention	108	DE	175	0.41	0.057	***	NS	†
	164	DE	233	0.54	0.054	***		
Fat retention	108	DE	83	0.33	0.054	***	NS	NS
	164	DE	155	0.40	0.052	***		
Protein retention	108	DE	106	0.09	0.020	***	†	NS
	164	DE	97	0.14	0.019	***		
CH_4 production	108	DE	5	0.09	0.009	***	NS	NS
	164	DE	7	0.09	0.008	***		
Heat production	108	ME	573	0.53	0.059	***	NS	*
	164	ME	581	0.37	0.057	***		

[1] GE = gross energy; DE = digestible energy.
[2] Covariable represents the N intake, digested N intake, GE or DE.
[3] The SF0 level (in kJ or g of N·kg $BW^{-0.75}$·d^{-1} or %) represents an estimate of the response parameter in calves fed no SF.
[4] Regression coefficient β, dimensionless ($y = a + \beta \cdot x$), represents the change in the response parameter per increase in the covariable; it represents the incremental efficiency of utilization of energy or N from SF.
[5] Probability for the test if the regression coefficient (β) equals 0. The effect of block was not significant and was therefore excluded from the model.
[6] The *P*-value for BW represents the statistical significance of the effect of BW on the response parameter; the P-value for covariable BW represents the significance of the interaction between the covariable and the main effect of BW. The interpretation of a significant interaction between the covariable and BW is that the regression coefficient differs between levels of BW.
[7] Data were transformed (log) to obtain homogeneity of variance.
[8] Data were transformed (quadratic) to obtain homogeneity of variance.
†$P < 0.10$; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

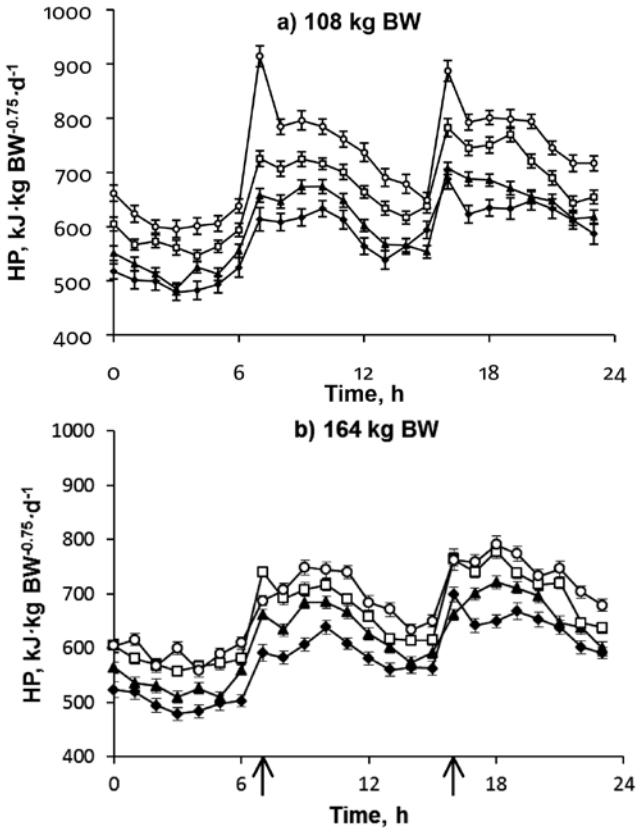


Figure 3.1 Influence of solid feed intake on the circadian patterns of heat production (HP) in veal calves of 108 kg of BW (a) and 164 kg of BW (b). Solid feed intake was 0 (♦), 9 (▲), 18 (□) or 27 (○) g of DM·kg $BW^{-0.75}$·d^{-1}. Solid feed (50% concentrate, 25% straw, 25% corn silage; DM basis) was fed in addition to a milk replacer diet. Results are expressed as means ± SEM, n=4 for each treatment. Arrows represent feeding times.

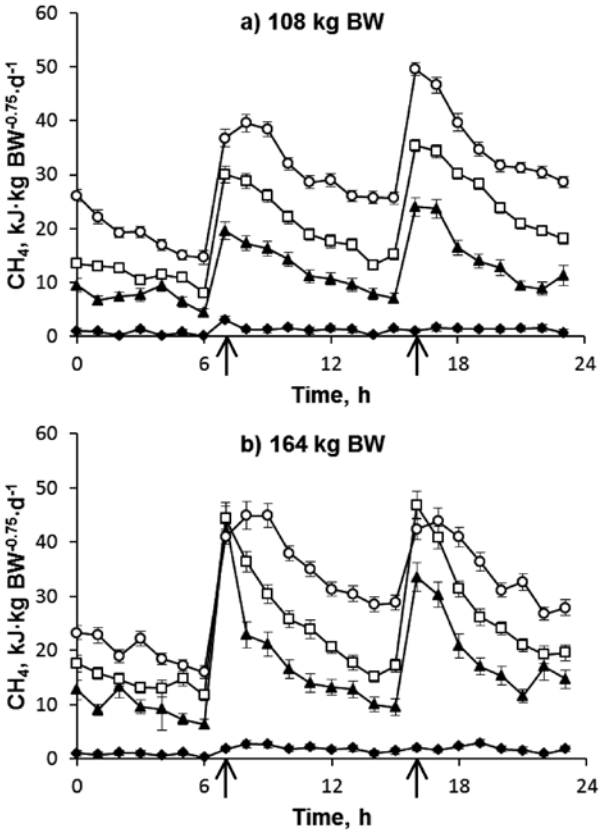


Figure 3.2 Influence of solid feed intake on the circadian patterns of CH_4 production in veal calves of 108 kg of BW (a) and 164 kg of BW (b). Solid feed intake was 0 (♦), 9 (▲), 18 (□) or 27 (○) g of DM·kg $BW^{-0.75}$·d^{-1}. Solid feed (50% concentrate, 25% straw, 25% corn silage; DM basis) was fed in addition to of a milk replacer diet. Results are expressed as means ± SEM, n=4 for each treatment. Arrows represent feeding times.

DISCUSSION

Digestibility and CH_4

Apparent total tract digestibility coefficients for SF0 calves were in the range of 91 to 99% for DM, N, crude fat, gross energy, and starch (results not shown) and are in close accordance with values for calves fed exclusively milk from 65 to 200 kg of BW (Gerrits et al., 1996; Diaz et al., 2001; van den Borne et al., 2006b). Apparent total tract digestibility coefficients of DM (64%) and gross energy (60%) from SF in our calves were in close agreement with those in heifer calves (67%; Ortigues et al., 1990) fed SF with a similar composition (roughage-to-concentrate ratio of 55:45 and CP content of 10.5%) as the SF provided to our calves. However, apparent total tract digestibility of CP was lower in our study (37%) compared with the study of Ortigues et al. (1990), who found a CP digestibility of 45%. Apparent total tract digestibility of NDF increased from 46% at 108 kg of BW to 56% at 164 kg of BW. This suggests that rumen or hindgut fermentation, or both, are further optimized with time, although rumen development is assumed to require less than 7 or 8 wk of SF exposure (Zitnan, 1998; Suárez et al., 2006). This increase could also point to a decreased rumen passage rate at 108 kg of BW relative to 164 kg of BW.

The digestibility coefficients of SF are calculated as the incremental response of digested nutrient intake to nutrient intake; therefore, it is assumed that digestion of nutrients from MR is not affected by SF intake. Although passage rate may be decreased by SF intake, the increase in apparent digestibility of MR associated with this decrease is expected to be small because of a generally high level of DM digestibility (94 to 96%; Van den Borne et al., 2006a; Labussière et al., 2009b).

Methane production from calves in the SF0 group was negligible, as shown previously in calves fed milk (van den Borne et al., 2006a; Labussière et al., 2009a). Methane production increased with SF intake and with BW from 5.4% of gross energy from SF at 108 kg to 5.5% of gross energy from SF at 164 kg of BW. These values are close to the range of 6.0 to 6.6% of gross energy reported for adult dairy cows (Mills et al., 2001). In veal calves, SF supplementation with very high (> 90%) levels of concentrates resulted in CH_4 yields ranging from 3.2 to 9.1% of gross energy from SF (Labussière et al., 2009a). The latter values are remarkably high because it has been demonstrated in beef cattle that high-concentrate diets typically produce CH_4 at levels between 2 and 3% of gross energy (Johnson and Johnson, 1995). It is possible that ruminal drinking (i.e., leakage of MR to the rumen) contributed to enhanced CH_4 production because of anaerobic fermentation of MR (see Van den Borne et al., 2004). Observations of ruminal drinking in future studies on CH_4 production in veal calves would be needed to further substantiate this idea. In the present study, the apparent total tract digestibility of NDF increased by 10% when BW increased from 108 kg to 164 kg, and this increase was partly accompanied by an increase in proportional CH_4 loss, from 5.4% to 5.5% of gross energy from SF.

The circadian pattern of CH_4 production shown in Figure 3.2 can be explained by the positive relationship between DMI and CH_4 production (Dijkstra et al., 2011). Time between feeding (indicated with arrows) and the maximum rate of CH_4 production of SF27 were within the range of 47 to 141 min reported by Crompton et al. (2011) for dairy cows, although calves in the SF9 and SF18 groups reached their maximum rate of CH_4 production earlier than 47 min.

N Utilization

For calves in the SF0 group, the gross efficiency of N retention (61 ± 3.0% at 108 kg of BW and 49 ± 1.6% at 164 kg of BW) is well in line with measurements performed on calves fed exclusively on MR (van den Borne et al., 2006b; Labussière et al., 2009b), decreasing with age or BW (Gerrits et al., 1996; Labussière et al., 2009b). The gross efficiency of N retention increased with SF intake, with 6.7% / kg of DM of SF per day at 108 kg of BW and with 12.3% / kg of DM of SF per day at 164 kg of BW. When expressed per g of N intake, the increase in gross efficiency of N retention was 0.5% / g of N from SF per day at 108 kg of BW and 0.9% / g of N from SF per day at 164 kg of BW. The incremental efficiency with which total protein and all essential amino acids are used for growth is typically low (30 to 40%) in calves exclusively fed MR (Gerrits et al., 1996, 1998). In our study, the incremental efficiency of N utilization from SF was substantially greater (43 and 77% at 108 and 164 kg of BW, respectively), which is remarkable, especially when considering the poor N digestibility of the SF sources used (92 to 95% for MR vs. 55% for SF, respectively; Van den Borne et al., 2006b; CVB, 2007; Labussière et al., 2009a). It is hypothesized that the high incremental efficiency of N utilization in our calves is caused by recycling of urea-N. In this way, urea-N can increase the microbial protein supply to the animal, which can subsequently be used for protein synthesis. This corresponds with the observed shift in N excretion from urine to feces, particularly at 164 kg of BW, leading to low estimates of apparent N digestibility of SF (35 to 39%) when compared with studies in heifers (45%; Ortigues et al., 1990). Moreover, it explains the observation that the incremental N retained exceeded the incremental apparently digested N from SF (0.43 vs. 0.35 at 108 kg of BW and 0.77 vs. 0.39 at 164 kg of BW). These data indicate that urea recycling contributes at least to 19% of the extra N retention at 108 kg of BW (difference between incremental N retained and the incremental apparently digested N from SF, as a percentage of incremental N retained) and to at least 49% at 164 kg of BW. This substantial urea recycling may be particularly stimulated by the low protein content of the SF. It has been demonstrated that urea recycling is stimulated by a low protein-to-energy ratio in rumen contents, but also by high blood urea concentrations (Reynolds and Kristensen, 2008).

In contrast to our findings, Labussière et al. (2009a) found that the provision of SF in addition to MR was associated with a reduced efficiency of N retention in calves. Several explanations are conceivable for the differences between their study and the present experiment. First, to stimulate urea recycling, the CP content in the SF was deliberately

reduced to 88 g of CP/kg of DM compared with approximately 163 g of CP/kg of DM in the diets of Labussière et al. (2009a). The incorporation of blood urea-N into microbial protein through urea recycling is inversely related to the level of protein intake in heifer calves (Bunting et al., 1989; Marini and Van Amburgh, 2003). Therefore, the reduced CP in our experiment compared with that in the study of Labussière et al. (2009a) could have increased urea recycling and contributed to the greater incremental efficiency of N retention. Second, high concentrate proportions (> 70%) in SF provided to veal calves next to MR have been shown to lead to poor rumen development and plaque formation on the rumen wall (Suárez et al., 2006, 2007; Brscic et al., 2011). When SF consists of only concentrates, plaque is present in almost all calves (Suárez et al., 2006, 2007). Plaque formation might inhibit not only nutrient uptake by the rumen wall, but also urea-N entry into the rumen. The proportion of concentrate in the SF mixture fed by Labussière et al. (2009a) exceeded 90%, whereas our SF consisted of 50% concentrate. Therefore, plaque formation may have inhibited nutrient transport through the rumen wall in the study by Labussière et al. (2009a).

The effects of SF intake on N efficiency are more pronounced at 164 kg of BW than at 108 kg of BW. The greater incremental N efficiency at 164 kg of BW could be explained by the lower efficiency (45 vs. 58%) with which dietary N was retained for calves in the SF0 group at 164 kg compared with those at 108 kg of BW. This corresponds with the general decrease in efficiency of N utilization with age in calves fed exclusively on MR (Gerrits et al., 1996; van den Borne et al., 2006a,b) and allows more room for improving N utilization in heavier calves. In addition, the observed increase in NDF digestibility with BW indicates improved fermentation and therefore a greater demand for urea-N for microbial protein synthesis.

Energy Utilization

The incremental efficiency of energy retention (energy retained per kJ of digestible energy from SF) was 41% at 108 kg of BW and increased to 54% at 164 kg of BW. When expressed per kilojoule of ME, the incremental efficiency of energy retention was 47% at 108 kg of BW, and 63% at 164 kg of BW. The efficiency of energy retention at 164 kg of BW was greater than that in weaned calves of 200 (49%) and 286 (55%) kg of BW fed SF ad libitum (Vermorel et al., 1980). In addition, the incremental efficiency of energy retention calculated from the data of Ortigues et al. (1990) was 41% in calves of similar BW (161 kg) fed exclusively SF of a similar composition (roughage-to-concentrate ratio of 55:45 and CP content of 10.5%) as provided to our calves.

As discussed by Labussière et al. (2009a, 2011), any increase in ME intake (from SF or MR) would typically increase fasting HP, likely related to an increased contribution of metabolically very active tissues. For the range in ME intake from SF in our experiment, the fasting HP could be increased by 0.1 kJ per kJ·kg $BW^{-0.75}$·d^{-1}. This increase, however, is already accounted for in the energetic efficiencies described above. It would imply,

however, that the extra energy retained with more SF intake is not completely reflected in increased carcass weights. Increasing the intake of SF may increase weights of the gastrointestinal tissues, such as the rumen (Berends et al., 2012). Current data showed an increase in energetic efficiency from SF with BW. Apart from the more efficient utilization of protein for growth, this increase in efficiency reflects preferential fat retention with increasing age or BW (Gerrits et al., 1996).

CONCLUSIONS

The provision of low-protein SF enhanced N utilization for protein gain in veal calves, particularly toward the end of the fattening period. At 108 kg of BW, the gross efficiency of N retention was 61% for calves without SF, and it increased with SF intake by 6.7% / kg of DM of SF per day. At 164 kg of BW, this efficiency was 49% for calves without SF, and increased by 12.3% / kg of DM of SF per day. Recycling of urea (mainly originating from milk amino acids) potentially contributed substantially to incremental N retention in heavy veal calves when SF was fed.

The incremental efficiency of energy retention, representing the increase in energy retained per kilojoule of extra digestible energy intake from SF, was greater at 164 kg of BW (54%) than at 108 kg of BW (41%). Similarly, the apparent total tract digestibility of NDF increased with BW, from 46% at 108 kg of BW to 56% at 164 kg of BW, which may also have increased the demand for urea-N for microbial protein synthesis and thus stimulated urea recycling. Methane production from SF in veal calves was similar to reported values in cattle fed only SF.

ACKNOWLEDGEMENTS

The authors thank Carène Bulte, Maike Pegge, Sabrina Podesta, Janneke Snijders, Laura Webb, Tamme Zandstra, and the personnel of the De Haar research facilities for their technical assistance. Financial support of the Netherlands Organization for Scientific Research (The Hague, the Netherlands) and the Product Board Animal Feed (The Hague, the Netherlands) is gratefully acknowledged.

REFERENCES

Berends, H., C. G. Van Reenen, N. Stockhofe-Zurwieden, and W. J. J. Gerrits. 2012. Effects of early rumen development and solid feed composition on growth performance and abomasal health in veal calves. Journal of Dairy Science 95: 3190-3199.

Brouwer, E. 1965. Report of sub-committee on constants and factors. Pages 441–443 in Energy Metabolism. K. L. Blaxter, ed. Academic Press, London, UK.

Brscic, M., L.F.M. Heutinck, M. Wolthuis-Fillerup, N. Stockhofe-Zurwieden, B. Engel, E.K.Visser, F. Gottardo, E.A.M. Bokkers, B. J. Lensink, G. Cozzi, and C. G. van Reenen. 2011. Prevalence of gastrointestinal disorders recorded at postmortem inspection in white veal calves and associated risk factors. Journal of Dairy Science 94: 853-863.

Bunting, L. D., J. A. Boling, and C. T. MacKown. 1989. Effect of dietary protein level on nitrogen metabolism in the growing bovine: I. Nitrogen recycling and intestinal protein supply in calves. Journal of Animal Science 67:810-819.

Crompton, L.A., J.A.N. Mills, C.K. Reynolds, and J. France. 2011. Fluctuations in methane emission in response to feeding pattern in lactating dairy cows. Pages 176-180 in Modelling Nutrient Digestion and Utilization in Farm Animals. D. Sauvant, J. van Milgen, P. Faverdin, and N. Friggens, ed. Wageningen Academic Publishers, Wageningen, the Netherlands.

CVB (Centraal Veevoeder Bureau). 2007. Chemical compositions and nutritional values of feed ingredients. Centraal Veevoeder Bureau, Productschap Diervoeder, Den Haag, the Netherlands.

Diaz, M. C., M. E. Van Amburgh, J. M. Smith, J. M. Kelsey, and E. L. Hutten. 2001. Composition of growth of Holstein calves fed milk replacer from birth to 105-kilogram body weight. Journal of Dairy Science 84:830-842.

Dijkstra, J., S.M. van Zijderveld, J.A. Apajalahti, A. Bannink, W.J.J. Gerrits, J.R. Newbold, H.B. Perdok, and H. Berends. 2011. Relationships between methane production and milk fatty acid profiles in dairy cattle. Animal Feed Science and Technology 166:590-595.

Gerrits, W.J.J., J. Dijkstra, J.M.A.J. Verdonk, G. M. Beelen, and H. Boer. 1999. Effects of ammonia and starch infusion in the colon of preruminant calves. Page 55 in Book of Abstracts of VIII Int. Symp. Protein Metabolism and Nutrition, Aberdeen, UK. G.E. Lobley, A.White,. and J.C. MacRae, ed. Wageningen Pers, Wageningen, the Netherlands.

Gerrits, W.J.J., J.W. Schrama, and S. Tamminga. 1998. The marginal efficiency of utilization of all ileal digestible indispensable amino acids for protein gain is lower than 30% in preruminant calves between 80 and 240 kg live weight. The Journal of Nutrition 128: 1774-1785.

Gerrits, W. J. J., G. H. Tolman, J. W. Schrama, S. Tamminga, M. W. Bosch, and M. W. A. Verstegen. 1996. Effect of protein and protein-free energy intake on protein and fat deposition rates in preruminant calves of 80 to 240 kg live weight. Journal of Animal Science 74:2129-2139.

ISO. 1997. Animal feeding stuffs-Determination of nitrogen content and calculation of crude protein content. Kjeldahl method. ISO 5983. Int. Organ. Stand., Geneva, Switzerland.

ISO. 1998a. Animal feeding stuffs, animal products, and faeces or urine-Determination of gross calorific value. ISO 9831. Int. Organ. Stand., Geneva, Switzerland.

ISO. 1998b. Animal feeding stuffs-Determination of moisture and other volatile matter content. ISO 6496. Int. Organ. Stand., Geneva, Switzerland.

ISO. 1999. Animal feeding stuffs-Determination of fat content. ISO 6496. Int. Organ. Stand., Geneva, Switzerland.

ISO. 2002. Animal feeding stuffs-Determination of crude ash. ISO 5984. Int. Organ. Stand., Geneva, Switzerland.

Johnson, K.A., and D.E. Johnson. 1995. Methane emissions from cattle. Journal of Animal Science 73:2483-2492.

Labussière, E., S. Dubois, J. van Milgen, G. Bertrand, and J. Noblet. 2009a. Effect of solid feed on energy and protein utilization in milk-fed veal calves. Journal of Animal Science 87:1106-1119.

Labussière, E., G. Maxin, S. Dubois, J. van Milgen, G. Bertrand, and J. Noblet. 2009b. Effects of feed intake on protein and fat deposition in milk-fed veal calves. Animal 3:557-567.

Labussière, E., J. van Milgen, C.F.M. de Lange, and J. Noblet. 2011. Maintenance energy requirements of growing pigs and calves are influenced by feeding level. The Journal of Nutrition 141:1855-1861.

Marini, J. C. and M.E. Van Amburgh. 2003. Nitrogen metabolism and recycling in Holstein heifers. Journal of Animal Science 81:545-552.

Mills, J. A. N., J. Dijkstra, A. Bannink, S.B. Cammell, E. Kebreab, and J. France. 2001. A mechanistic model of whole-tract digestion and methanogenesis in the lactating dairy cow: Model development, evaluation, and application. Journal of Animal Science 79:1584-1597.

NRC. 2000. Nutrient Requirements of Beef Cattle. 7th rev. ed. Natl. Acad. Press, Washington, DC.

Ortigues, I., T. Smith, M. Gill, S.B. Cammell, and N.W. Yarrow. 1990. The effect of fishmeal supplementation of a straw-based diet on growth and calorimetric efficiency of growth in heifers. British Journal of Nutrition 64:639-651.

Reynolds, C.K., and N.B. Kristensen. 2008. Nitrogen recycling through the gut and the nitrogen economy of ruminants: An asynchronous symbiosis. Journal of Animal Science 86(Suppl. 14):E293-E305.

Rijnen, M.M.J.A., M.W.A. Verstegen, M.J.W. Heetkamp, and J.W. Schrama. 2001. Effects of dietary fermentable carbohydrates on energy metabolism in group-housed sows. Journal of Animal Science 79:148- 154.

SAS Institute. 2000. SAS User's Guide: Statistics. Release 9.20. SAS Institute, Inc., Cary, NC.

Suárez, B. J., C.G. Van Reenen, W.J.J. Gerrits, N. Stockhofe-Zurwieden, A.M. Van Vuuren, and J. Dijkstra. 2006. Effects of supplementing concentrates differing in carbohydrate composition in veal calf diets: II. Rumen development. Journal of Dairy Science 89:4376-4386.

Suárez, B. J., C.G. Van Reenen, N. Stockhofe-Zurwieden, J. Dijkstra, and W.J.J. Gerrits. 2007. Effect of roughage source and roughage to concentrate ratio on animal performance and rumen development in veal calves. Journal of Dairy Science 90:2390-2403.

Van den Borne, J.J.G.C., S.J.J. Alferink and W.J.J. Gerrits. 2004. Identifying ruminal drinking by measurement of respiratory quotient and methane production in preruminants calves. Journal of Animal Science 82(Suppl. 1):E365.

Van den Borne, J.J.G.C., J.M.A.J. Verdonk, J.W. Schrama, and W.J.J. Gerrits. 2006a. Reviewing the low efficiency of protein utilization in heavy preruminants calves - A reductionist approach. Reproductive Nutrition and Development 46:121-137.

Van den Borne, J.J.G.C., M.W.A. Verstegen, S.J.J. Alferink, R.M.M. Giebels, and W.J.J. Gerrits. 2006b. Effects of feeding frequency and feeding level on nutrient utilization in heavy preruminant calves. Journal of Dairy Science 89:3578-3586.

Van Soest, P. J., J. B. Robertson, and B.A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. Journal of Dairy Science 74:3583-3597.

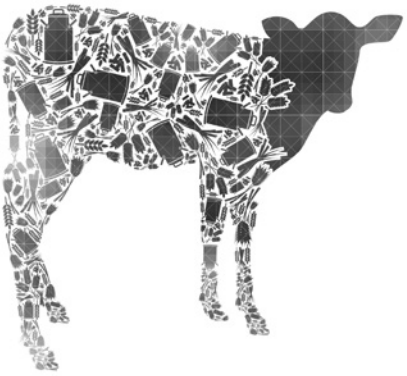
Vermorel, M., J. C. Bouvier, and Y. Geay. 1980. Utilisation de l'énergie des aliments par le veau ruminant. Influence de l'apport de lait, du niveau d'alimentation et de l'age du veau. Annales De Zootechnie 22:65-68.

Verstegen, M. W. A. and A. Hoogerbrugge. 1987. Climatic condition and health in young farm-animals. Tijdschrift voor Diergeneeskunde 112:1062-1068.

Zitnan, R. 1998. Influence of dietary concentrate to forage ratio on the development of rumen mucosa in calves. Archives of Animal Nutrition 51:279-291.

Chapter 4

Urea recycling contributes to nitrogen retention in calves fed milk replacer and low-protein solid feed



H. Berends[1], J.J.G.C. van den Borne[1], B.A. Røjen[2], J. van Baal[1], and W.J.J. Gerrits[1]

Accepted for publication in The Journal of Nutrition

[1]Animal Nutrition Group, Wageningen University, Wageningen, the Netherlands;
[2]Department of Animal Science, Aarhus University, Tjele, Denmark

ABSTRACT

Urea recycling, with urea originating from catabolism of amino acids and hepatic detoxification of ammonia, is particularly relevant for ruminant animals, in which microbial protein contributes substantially to metabolizable protein supply. However, the quantitative contribution of urea recycling to protein anabolism in calves during the transition from preruminants (milk-fed) to ruminants [solid feed (**SF**)-fed calves] is unknown. The aim was to quantify urea recycling in milk-fed calves, when provided with low-protein SF. Forty-eight calves [164 ± 1.6 kg bodyweight (**BW**)] were assigned to 1 of 4 SF levels [0, 9, 18, 27 g of dry matter (**DM**) of SF·kg $BW^{-0.75}$·d^{-1}], provided in addition to an identical amount of milk replacer. Urea recycling was quantified after a 24h intravenous infusion of [$^{15}N_2$]urea by analysis of urea isotopomers in 68h fecal and urinary collections. Real-time qPCR was used to measure gene expression levels of urea transporter-B (*bUTB*), and aquaglyceroporins-3 and -7 in rumen wall tissues. For every incremental gram of DM of SF intake (g DM·$kg^{-0.75}$), N intake increased with 0.70 g N, and N retention increased with 0.55 g N ($P < 0.01$). Of this increase in N retention, 19% could be directly explained by urea recycling. Additionally, part of the observed increase in N retention could be explained by the extra protein provided by the SF and likely by a greater efficiency of post-absorptive utilization of N for gain. *bUTB* abundance increased ($P < 0.01$) with SF provision. Aquaglyceroporin-3 expression increased ($P < 0.01$) upon SF intake, but aquaglyceroporin-7 expression did not. We conclude that, in addition to the increase in digested N, urea recycling contributes to the observed increase in N retention with increasing SF intake in milk-fed calves. Furthermore, ruminal *bUTB* and aquaglyceroporin-3 are up-regulated with SF intake, which may be associated with urea recycling.

Keywords: urea recycling, calves, urea transporter, urea nitrogen salvage

INTRODUCTION

Urea originating from catabolism of amino acids and hepatic detoxification of ammonia can enter the gastrointestinal tract in mammals, for subsequent hydrolysis by bacterial urease to ammonia. Ammonia, in turn, provides a source of nitrogen (**N**) for microbial synthesis of proteins, which can be intestinally absorbed and become available for anabolic purposes. In non-ruminants, like humans (Fouillet et al., 2008, McClelland and Jackson, 1996, Meakins and Jackson, 1996), pigs (Mosenthin et al., 1992), and cats (Russell et al., 2000), this mechanism of urea recycling is of much less importance when compared with ruminant animals, where 30 to 98% of urea produced by the liver can enter the gastrointestinal tract (Reynolds and Kristensen, 2008). In fact, urea recycling enables ruminants to survive when protein supplies are insufficient or inadequate (Lapierre and Lobley, 2001, Stewart and Smith, 2005).

In milk-fed calves, milk is usually assumed to bypass the rumen, thus providing intestinally degradable protein. When providing large quantities (48 to 60 g of DM·kg $BW^{-0.75}$ ·d^{-1}) of milk replacer (**MR**) to heavy (> 120 kg) calves, the efficiency of utilization of absorbed N is generally between 35 and 40% (Gerrits et al., 1996), thus urinary urea excretion (van den Borne et al., 2006a) and plasma urea concentrations (Quigley et al., 2006) are high. In calves exclusively fed MR, urea recycling is of little importance, as evidenced by the 80% recovery of an intravenous pulse dose of [^{13}C]urea in 48-h urine (Gerrits et al., 2001). It is likely that supplementation of a low-protein solid feed (**SF**) would provide a substrate for rumen fermentation but, at the same time, increase the demand for N in the rumen. The relatively high amount of urea in the blood circulation of milk-fed calves (van den Borne et al., 2006a) could be directed to urea recycling, resulting in an increased microbial protein supply and consequently greater protein retention. Indeed, we recently showed that provision of low-protein SF to milk-fed calves readily improved N utilization in milk-fed calves of 164 kg BW with a marginal N efficiency of 77% (i.e. for each g of N ingested from SF, 0.77 g N was retained). Based on these data, it was hypothesized that urea recycling, with urea-N originating from milk protein, allowed urea-N to be used for microbial protein synthesis and consequently for whole body protein deposition (Berends et al., 2012a).

Urea transporter proteins facilitate urea entry into the gastrointestinal tract (Stewart et al., 2005) by transporting urea across cell membranes (Smith and Rousselet, 2001). Urea transporter-B (***bUTB***) has been identified in the gastrointestinal tract of cattle (Marini and Van Amburgh, 2003, Stewart et al., 2005) and sheep (Marini et al., 2004, Ritzhaupt et al., 1997, Ritzhaupt et al., 1998). Although inhibition of bUTB has been shown to reduce trans-epithelial urea flux in isolated ruminal epithelium (Stewart et al., 2005), its exact role in the process of urea recycling, and in calves in particular, is unclear. In addition, a subclass of water channel protein family, designated aquaglyceroporins (AQPs; i.e. AQP3, AQP7, AQP9, and AQP10) are also able to transport urea (Rojek et al., 2008), and are perhaps involved in urea recycling in ruminants (Røjen et al., 2011). Nevertheless, previous studies

have produced conflicting results concerning the interrelation of diet composition, gastrointestinal entry rate of urea (**GER**), plasma urea concentrations, and the potential role of urea transporters and AQPs in ruminants (Abdoun et al., 2006, Ludden et al., 2009, Marini et al., 2004, Marini and Van Amburgh, 2003, Muscher et al., 2010, Ritzhaupt et al., 1997, Røjen et al., 2011, Simmons et al., 2009, Stewart et al., 2005). A large experimental contrast in urea recycling caused by N shortage in the rumen and coinciding with a high availability of urea in the plasma could aid in clarifying these interrelationships.
The aim was to quantify the contribution of urea recycling to protein gain in milk-fed calves, when provided with low-protein SF. In addition, the effect of low-protein SF intake on the transcript level of urea transporters and AQPs was studied. We hypothesized that supplementation of low-protein SF to milk-fed calves will increase urea recycling and N retention.

MATERIAL AND METHODS

This study was carried out at the experimental facilities of Wageningen University, the Netherlands. All procedures complied with the Dutch law for animal experiments, and the ETS123 (Council of Europe 1985 and the 86/609/EEC Directive) and were approved by Wageningen University's Committee on Animal Care and Use. The effects of low-protein SF provision on energy and N balance are published elsewhere (Berends et al., 2012a).

Calves and Treatments. Forty-eight Holstein-Frisian male calves were selected based on age, BW, uniformity, and clinical health from a commercial Dutch farm. Mean age and BW upon arrival were 53 ± 0.7 d and 55 ± 0.3 kg, respectively. Calves arrived in one batch at the research facilities, and measurement periods were staggered, with calves divided over 4 blocks. Within blocks, calves were assigned randomly to groups of 3 calves (4 groups per block).
Within blocks, groups were assigned randomly to 1 of 4 levels of SF intake: 0, 9, 18, or 27 g of DM of SF·kg $BW^{-0.75}$·d^{-1} (i.e., **SF0**, **SF9**, **SF18**, and **SF27**, respectively). Intake of MR was 41 g of DM·kg $BW^{-0.75}$·d^{-1}, irrespective of treatment. Ingredient and nutrient composition of SF components and MR are presented in Table 4.1. The SF consisted of 25% chopped wheat straw, 25% corn silage, and 50% concentrates on a DM basis. The MR and SF were provided in 2 equally sized meals at 0700 h and at 1600 h. Calves had free access to water.
Calves were exposed to their diet for at least 11 wk before the measurement period when BW averaged 164 ± 1.6 kg. Until the 5-d measurement period, calves were housed in groups of 3 (Berends et al., 2012a). During 3 d prior to the measurement period, calves were adapted to individual housing in metabolic cages, maintaining audio-visual contact between group mates. Harnesses for fecal collection bags were attached to allow quantitative collection of feces and urine. During the measurement period, groups were

housed in 1 of 2 identical, 80-m^3 indirect calorimetric chambers. Within each chamber, calves were housed individually in metabolic cages.

Table 4.1 Nutrient composition of MR and SF components[1]

		SF components		
Nutrient	**MR[2]**	**Concentrate[3]**	**Corn silage**	**Straw**
Dry matter, g/kg product	968	883	497	923
Crude protein[4], g/kg DM	212	128	77	22
Crude fat, g/kg DM	192	36	28	9
Crude ash, g/kg DM	76	66	36	66
Starch, g/kg DM	19	450	356	11
Neutral detergent fiber, g/kg DM	7	151	400	814
Gross energy, MJ/kg DM	21.7	17.7	18.7	18.0

[1] DM, dry matter; MR, milk replacer; SF, solid feed.

[2] MR ingredients: 200 g/kg whey powder concentrate, 133 g/kg delactosed whey, 357 g/kg whey powder, 20 g/kg starch, 40 g/kg wheat protein, 25 g/kg soy concentrate, 60 g/kg lard, 46 g/kg coconut oil, 60 g/kg tallow, 20 g/kg palm oil, 14 g/kg soy lecithin and 25 g/kg premix (3.5 g/kg lysine and 2 g/kg methionine)

[3] Concentrate ingredients: 361 g/kg barley, 125 g/kg carob meal, 150 g/kg lupines (CP < 335 g/kg), 300 g/kg corn, 11.5 g/kg $MgSO_4.7H_2O$, 7.7 g/kg NaCl, 21.5 g/kg $CaCO_3$, 13.5 g/kg KH_2PO_4, 10 g/kg premix (lactose carrier: 1.2 mg/kg retinol; 12.5 µg/kg cholecalciferol; 66.7 mg/kg α-tocopherol; zinc: 25 mg/kg; manganese: 20 mg/kg; iodine: 0.8 mg/kg; selenium: 0.15 mg/kg; copper: 15 mg/kg; cobalt: 0.1 mg/kg)

[4] N x 6.25

Infusion and Sample Collection. Calves were prepared with central venous catheters (Careflow, Becton Dickinson, Alphen aan den Rijn, the Netherlands) in each jugular vein for infusion and sampling, respectively. A 24 h continuous infusion of doubly ^{15}N labeled urea ([^{15}N-^{15}N]urea (99.0 atom%, T85-62005, ISOTEC, Miamisburg, OH), prepared in sterile saline (0.15 M NaCl), was conducted. The continuous infusion of 2.67 mmol/d was preceded by a priming dose of 1.07 mmol to enrich the body urea pool to approximately 0.15 mol percent excess.

Urine and fecal samples were taken before the infusion (background samples) and cumulatively for 68 h after the initiation of the infusion. Additionally, urine was collected directly after the collection period of 68 h, and it was confirmed that enrichment in urinary urea had returned to background concentrations. This check was not conducted for fecal enrichment after the collection period. Samples were stored at –20°C until analysis. Quantification of the N balance was done by subtracting N excretion with feces and urine, aerial NH_3 and water that condensed on the heat exchanger from N intake with feed. N from aerial NH_3 and water that condensed on the heat exchanger was measured per group and averaged per calf.

On day 5 of the measurement period, blood samples were taken from the right jugular vein at -30, -15, 30, 60, 90, 120, 150, 180, 240, 300, 360, 420, and 480 min relative to the

morning feeding at 0700 h. Blood was immediately transferred into lithium heparin tubes (Vacutainer, Becton Dickinson, Alphen aan de Rijn, the Netherlands), kept on ice until plasma was harvested by centrifugation at 2500 × g for 10 min. Plasma samples were stored at -20°C pending analyses.

After the end of the collection period, dietary treatments were continued until slaughter at 181±2.1 kg BW. Calves in the first block were euthanized by an intravenous injection of Na-pentobarbital, followed by exsanguination. Calves in subsequent blocks, were euthanized by stunning (captive bolt pistol), following exsanguination. Within 10 min after exsanguination, tissue samples were cut from the ventral sac of the rumen wall (1x1 cm), rinsed briefly with ice-cold saline, placed in aluminum cryotubes (Hycultec, Beutelsbach, Germany), snap frozen in liquid N, and stored at −80°C.

As MR can enter the rumen (Berends et al., 2012b, Suárez et al., 2007) we selected 12 out of the 48 calves to measure the incidence of ruminal milk. Ruminal recovery of Co from CoEDTA, provided with the last MR meal was measured at slaughter, according to procedures described previously (Berends et al., 2012b).

Analyses. Urinary urea was isolated using a cation exchange resin (AG 50WX8, 200 to 400 mesh hydrogen form, Sigma Aldrich, St. Louis, MO) and diluted to a final concentration of 1.65 mM. The eluates were analyzed according to procedures described by Sarraseca et al. (Sarraseca et al., 1998) with some modifications leading to monomolecular degradation of urea into N_2. In short, eluates and standard solutions were placed in 12-mL soda glass vials (Exetainer®; Labco Limited, Lampeter, Ceredigion, UK). To prepare these samples for Hoffman degradation, high-purity 5.0 He gas was bubbled on low pressure through the samples for 20 min and samples were frozen quickly in liquid N_2. Bubbling lines were removed and ends cut off. Once the samples were frozen, 100 µL of 10% lithium hypobromite was added and the lid was screwed on. The lithium hypobromite solution was made by adding slowly 2 mL of bromine to a 60 mL 10% (wt/wt) LiOH solution. With the Exetainer still in liquid N_2, the septum was pierced with a 19-gauge hypodermic needle connected to a helium tank on high pressure for flushing the headspace. A second 23-gauge needle allowed the helium to exit the tube. After 3 min of flushing this second needle was removed first to leave a positive pressure inside the Exetainer. The tube was removed from the liquid N and placed in a heating block at 65°C for 25 min to degrade urea into N_2 gas. This reaction is sensitive to the concentration of urea with more $^{15}N^{14}N$ gas molecules at higher concentrations due to the cross-reaction of $^{14}N^{14}N$ and $^{15}N^{15}N$ urea molecules (Sarraseca et al., 1998). The concentration of urea used in the analysis (1.65 mM) ensured suitable amounts of gas, and minimized non-monomolecular degradation of urea. The occurrence of the non-monomolecular reaction was calculated from the increase in $^{15}N^{14}N$ gas molecules when enriched urea standards were run alongside the samples as described by Sarraseca et al. (Sarraseca et al., 1998). The analysis of the samples started immediately after preparation. Enrichment of ^{15}N species in N_2 was determined according to the methods described by Marini and Attene-Ramos (Marini and Attene-

Ramos, 2006) by continuous flow-isotope ratio mass spectrometry (Finnigan Delta V Plus, Thermo Scientific, Bremen, Germany) and read as a mass-to-charge ratio of 28, 29, and 30 corresponding to the $^{14}N^{14}N$, $^{14}N^{15}N$, and $^{15}N^{15}N$ parent urea molecule, respectively.
Feces were freeze-dried and ground to pass a 1 mm sieve, and subsequently ground on a ball mill (Retsch MM 2000, Retsch GmbH & Co., Haan, Germany). Fecal ^{15}N enrichments were analyzed after combustion in continuous-flow mode using Finnigan delta V Advantage (Finnigan, San Jose, CA) and read with a Finnigan MAT C11N8 elemental analyzer (Finnigan, San Jose, CA). The multiple-entry urea kinetic model of Zuur et al. (2000) was used to calculate urea-N kinetics. Briefly, urea entry rate (UER) is calculated as the dose (e.g. priming plus infusion dose) multiplied by the enrichment of the infusate divided by the urinary enrichment minus 1. The model assumes that there are two fates for synthesized urea, namely a proportion is excreted in urine (UUE) and the remainder enters the gastrointestinal tract (**GER**: gastrointestinal entry rate). The methodology does not allow discrimination of urea entry into various sites of the gastrointestinal tract (e.g. rumen, colon, etc.). Urea entering the gastrointestinal tract is hydrolyzed to ammonia, which can: 1) return to the ornithine cycle (**ROC**), 2) be excreted in feces (**UFE**: urea-N to fecal excretion), and 3) be utilized for anabolic purposes (**UUA**: urea-N utilized for anabolism). After administration of [$^{15}N^{15}N$]urea into the body urea pool, we quantified the various fates of urea-N through analysis of urea enrichment in 68h urine and feces samples. When [$^{15}N^{15}N$]urea is not appearing in urine (**UUE**), it is assumed to have entered the gastrointestinal tract. When entering the gastrointestinal tract [$^{15}N^{15}N$]urea is hydrolyzed to labelled ammonia, part of which is returned to the ornithine cycle (ROC) with [$^{14}N^{15}N$]urea as the product. Excretion of urea-N to feces (UFE) was quantified by the enrichment of 68h feces. Urea-N utilized for anabolism was estimated by subtracting ROC and UFE from GER. Plasma urea and urinary urea were determined by an enzymatic colorimetric Berthelot method (Human, Wiesbaden, Germany). For plasma urea concentrations, the area under the curve (AUC) was calculated. Plasma urea concentrations measured at -15 and -30 min were averaged and considered to represent the pre-prandial level (i.e. t = 0 min). The N content was measured in feed, feces, urine, the acid solution containing aerial NH_3, and in water that condensed on the heat exchanger by the Kjeldahl method according to ISO 5983 (ISO, 1997). Digested N was calculated by subtracting fecal N from N intake. N digestibility was expressed as the percentage of N intake that was digested.
RNA extraction and qPCR analysis. Frozen ruminal tissues samples were ground under liquid N and total RNA was extracted using Trizol Reagent (Life Technologies, Bleiswijk, the Netherlands). The isolated RNA was subsequently subjected to an on-column DNase digestion to eliminate DNA contamination (NucleoSpin RNA II kit; Macherey-Nagel GmbH & Co. KG, Düren, Germany). Quality of RNA was assessed using a 2100 Bioanalyzer and RNA 6000 Nano LabChip kit (Agilent Technologies, Palo Alto, CA). The RNA integrity number (mean ± SD) was 8.4±0.7. Concentration of RNA was determined using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Rockland, DE). First strand cDNA

synthesis was performed using 400 ng of total RNA per 20 µL sample reaction using Superscript III reverse transcriptase (Life technologies), deoxyribonucleotide triphosphate (dNTP; Roche Diagnostics Nederland BV, Almere, the Netherlands), and random hexamer primers (Roche Diagnostics Nederland BV) for 1 h at 50°C according to the manufacturer's protocol (Life Technologies). As a control, reactions in the absence of reverse transcriptase were performed on each sample to verify the absence of genomic DNA contamination of the RNA samples and they were never positive. Subsequent qPCR was performed on a 7500 Fast Real-Time PCR System (Applied Biosystems Deutschland GmbH, Darmstadt, Germany) by using the SensiMix SYBR Low-ROX kit (Bioline UK Ltd., London, UK). Amplification conditions consisted of 95 °C for 10 min, followed by 40 cycles of 95 °C for 10 s, 60 °C for 5 s, and 72 °C for 5 s. The primers used were designed with the software program Primer Express 3.0 (Applied Biosystems) and recommended primer sets that span an intron were selected. For *bAQP3*, *bAQP7*, and *bUTB*, primers are specified in Table 4.2. For AQP10, we did not detect rumen mRNA expression by reverse transcriptase PCR (primers tested are in Table 4.3). Melting curve analysis and fractionation of the qPCR products on an ethidum bromide-stained agarose gel was carried out to determine primer specificity. Quantitative mRNA measurement was performed by establishing a linear calibration curve using 10-fold serial dilutions of cDNA template for corresponding genes. Amplification efficiency ranged between 91% and 100%. As internal standards, we analysed the expression of housekeeping genes importin 8 (*IPO8*), eukaryotic translation elongation factor 2 (*EEF2*) and β-actin (*ACTB*). Absolute expression levels of the urea transporter genes were normalized to that of *IPO8*, since both *NormFinder* (Andersen et al., 2004) and *BestKeeper* (Pfaffl et al., 2004) found this one as the most stable reference gene.

Table 4.2 Primers used in real-time qPCR in rumen tissue

Gene	Forward primer	Reverse primer
AQP3	5'-CGTGACCCTAGGCATCCTTATT-3'	5'-AGGTGTACACAGGCAGCTTGATC-3'
AQP7	5'-GGGCTTCCTGGATGAGGTGGTG-3'	5'-CAATGGCCTGCGTCCCCAACA-3'
UT-B	5'-TGGCACTCACCTGGCAAACCC-3'	5'-TGGCAATCCGACCACAGCCAT-3'
IPO8	5'-TGCCATGGTATTTCTCCTCAAA-3'	5'-ACAATCCTGCTGAAAAACCTCATAT-3'
EEF2	5'- TCACTGACACCCGGAAGGAT-3'	5'-ATGAAATTCAAGTCGTTCTCTGAAAG-3'
ACTB	5'- GCCCTGAGGCTCTCTTCCA-3'	5'-CGGATGTCGACGTCACACTT-3'

Statistical Analyses

The data set included 34 successful observations; 7 SF0 calves, 10 SF9 calves, 10 SF18 calves, and 7 SF27 calves, respectively. Calves were excluded because of failure of infusion pumps or reduced catheter patency (4 calves), clinical sickness (2 calves), incomplete urine collection (4 calves), or feed refusals exceeding 20% (4 calves).

Table 4.3 Primers used in real-time qPCR to test AQP10 (accession no. XM_003581937.2) expression in rumen tissue

Set	Forward primer	Reverse primer
1	5′-TGGTAATGTCTCAGGGGCCCACC-3′	5′-GCCCCAGCCAGCTACGTA -3′
2	5′-TGTTTGTGCTCATGCTCCTC-3′	5′-GCTCTGAGTCCTCAGGATGG-3′
3	5′-CCACCTGAATCCAGCCTTCTC-3′	5′-GATAGCATGTCGGCCAGGA-3′

Means per treatment (as presented in Table 4.4 and Figure 4.1) are mathematical means with their SEMs. Dependent variables were analyzed by mixed model analysis (PROC MIXED in SAS 9.20 by SAS Institute, Inc., Cary, NC), with calf as the experimental unit, including the fixed effect of block and SF intake (expressed as g DM·kg$^{-0.75}$·d^{-1}) as a linear regression variable. Significance of the contribution of a quadratic and cubic component in the regression analysis was evaluated, but these were omitted from the model when found to be not significant. When model residuals were not normally distributed, data were transformed (log, square root, or inverse). When data were transformed, they are presented as nontransformed means with their SEMs, and only *P*-values were derived from the transformed data. Differences were considered significant at $P < 0.05$.

RESULTS

Table 4.4 shows treatment averages, estimated intercepts, and slopes for the N balance and urea kinetics measurements.

Only estimated values for intercepts (for SF0) and slopes are reported in the text hereafter. With increasing SF intake, N intake gradually increased with 0.70 g per g DM·kg BW$^{-0.75}$ ($P < 0.001$). Fecal N excretion was 4.5 g/d for SF0 calves and increased with SF intake ($P < 0.001$). Urinary N excretion was 29 g for SF0 calves and decreased with SF intake ($P < 0.001$). Urinary excretion of non-urea N increased ($P < 0.001$) with SF intake. As a result, N retention increased with SF intake ($P < 0.001$). Apparent total tract N digestibility decreased with SF intake ($P < 0.001$). Urea entry rate (UER), assumed equal to urea synthesis, however, was not affected by SF intake. Urinary urea-N excretion (UUE) was estimated as 23.6 g urea-N per d for SF0 calves, and decreased with SF intake (-0.40±0.052 g urea-N per g DM·kg BW$^{-0.75}$: $P < 0.001$). The GER was estimated as 6.8 g urea-N per d for SF0 calves, and increased with SF intake (0.28±0.09 g urea-N per g DM·kg BW$^{-0.75}$; $P < 0.01$). Subsequently, the return of urea-N to the ornithine cycle (ROC) increased with SF intake (0.10±0.02 g urea-N per g DM·kg BW$^{-0.75}$; $P < 0.001$). Estimated fecal excretion (UFE) was 0.2 g urea-N per d for SF0 calves and increased with SF intake (0.08±0.01 g urea-N per g DM·kg BW$^{-0.75}$; $P < 0.001$). As a net result,

Table 4.4 Effects of SF intake on N balance and urea-N kinetics based on [$^{15}N^{15}N$]urea infusions in calves of 164 kg BW[1]

N balance variables	SF0 (n = 7)	SF9 (n = 10)	SF18 (n = 10)	SF27 (n = 7)
N intake, g N/d	62.0	69.8	74.6	83.2
From MR	62.1	63.6	62.4	64.1
From SF	0	6.3	12.3	19.1
N feces[5], g N/d	4.5	8.5	11.5	15.9
N urine, g N/d	29.2	26.6	24.5	22.6
Non-urea N	4.8	7.0	9.9	9.1
Urea-N	24.3	19.6	14.4	13.6
N retention, g N/d	27.6	34.0	38.2	43.7
N digestibility, %	92.8	87.9	84.6	80.9
Urea kinetic variables, g urea-N/d				
UER	33.0	26.9	28.1	28.1
UUE	24.3	19.6	14.4	13.6
GER	8.7	7.2	13.7	14.6
ROC	1.5	1.6	3.1	4.2
UFE[6]	0.4	0.8	1.6	2.6
UUA[7]	6.8	4.9	9.0	7.8

*P < 0.05; **P < 0.01; ***P < 0.001

[1] Solid feed (SF) provision was 0, 9, 18, or 27 g DM kg $BW^{-0.75}$·d^{-1} (e.g., SF0, SF9, SF18, SF27) and fed in combination with a fixed amount of milk replacer (41 g DM kg $BW^{-0.75}$·d^{-1}). Solid feed was composed of 50% concentrate, 25% straw, and 25% corn silage on a dry matter basis. BW, body weight; DM, dry matter; GER, gastrointestinal entry rate; MR, milk replacer; ROC, return to ornithine cycle; SF, solid feed; UER, urea entry rate; UFE, urea nitrogen to fecal excretion; UUA, urea nitrogen used for anabolism; UUE, urinary urea nitrogen elimination.

[2] Estimate of the response parameter in calves fed no solid feed

urea used for anabolic purposes (UUA) was estimated as 5.6 g urea-N per d for SF0 calves and increased with SF intake (0.10±0.08 g urea-N per g DM·kg $BW^{-0.75}$; $P < 0.01$). Ruminal recovery of Co, supplied as CoEDTA with the final MR meal before slaughter, averaged 5.4% and was not affected ($P > 0.05$) by SF intake.

The postprandial response of plasma urea concentrations to various levels of SF intake was analyzed. The AUC obtained from plasma urea concentrations until 8h after feeding averaged 1140 mM · 480 min for SF0 and decreased linearly ($P < 0.001$) with SF intake.

At the end of this trial, macroscopic rumen development at slaughter was scored on a 5-point scale from poor to excellent: 1 – 1.5 – 2 – 2.5 – 3 (Berends et al., 2012b). Rumen

SEM	Intercept (g urea-N/d)[2]	Regression coefficient β[3]		
		Estimate	SE	P-value[4]
1.94	62.3	0.70	0.054	<0.0001
1.65	62.5	0.04	0.044	0.32
0.38	-0.1	0.65	0.011	<0.0001
0.79	4.5	0.39	0.025	<0.0001
1.66	29.3	-0.24	0.046	<0.0001
1.06	5.4	0.17	0.037	0.0001
0.52	23.6	-0.40	0.052	<0.0001
1.99	28.0	0.55	0.065	<0.0001
1.03	92.4	-0.41	0.034	<0.0001
3.58	30.4	-0.12	0.098	0.23
0.52	23.6	-0.40	0.052	<0.0001
3.46	6.8	0.28	0.088	0.0033
0.93	1.1	0.10	0.021	<0.0001
0.27	0.2	0.08	0.008	<0.0001
2.78	5.6	0.10	0.079	0.0132

[3] Expressed in g $N \cdot d^{-1}$ per g DM·kg $BW^{-0.75} \cdot d^{-1}$ (except for N digestibility, which is expressed in % per g DM·kg $BW^{-0.75} \cdot d^{-1}$), represents the change in response parameter per increase of solid feed intake
[4] Probability for test if the regression coefficient (β) equals 0
[5] Data were transformed (sqrt) to obtain homogeneity of variance
[6] Data were transformed (inverse) to obtain homogeneity of variance
[7] Data were transformed (log) to obtain homogeneity of variance

development score averaged 1.0±0.00, 1.7±0.23, 2.1±0.17, and 2.5±0.22 for SF0, SF9, SF18, and SF27, respectively, and increased ($P < 0.05$) with SF intake.

We examined the ruminal expression of *bAQP3* and *bAQP7*, which are subtypes of the aquaglyceroporin family (39), and the expression of *bUTB*, the main member of the urea transporter family expressed in the bovine rumen, at the end of the experimental period. Real-time qPCR analysis revealed that *bUTB* expression showed a quadratic increase ($P < 0.05$) with SF intake (Figure 4.1). *bAQP3* expression increased linearly ($P < 0.05$) with SF intake, whereas the expression of *bAQP7* was low and remained unaltered.

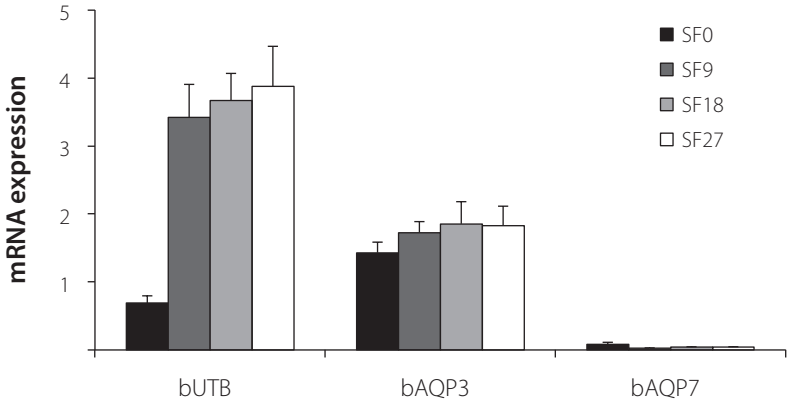


Figure 4.1 Effect of SF intake on mRNA expression of *bUTB*, *bAQP3*, and *bAQP7* in rumen tissue from calves weighing 164 kg. Absolute mRNA expression levels, presented in arbitrary units, are normalized to those of the housekeeping gene importin 8. Data are means ± SEMs of 12 calves for each treatment. Milk replacer intake was constant for all calves at 41 g DM kg $BW^{-0.75}$**·d**$^{-1}$. SF intake increased mRNA expression of *bUTB* (P , 0.05) (*P* < 0.05) and *bAQP3* (*P* < 0.05). *bAQP3*, bovine aquaglyceroporin 3; *bAQP7*, bovine aquaglyceroporin 7; *bUTB*, bovine urea transporter B; BW, body weight; DM, dry matter; SF, solid feed; SF0, 0 g DM of SF kg $BW^{-0.75}$·d^{-1}; SF9, 9 g DM of SF kg $BW^{-0.75}$·d^{-1}; SF18,18 g DM of SF kg $BW^{-0.75}$·d^{-1}; SF27, 27 g DM of SF kg $BW^{-0.75}$·d^{-1}.

DISCUSSION

Quantitative Importance of Urea Recycling

Incremental effects are hereafter consistently expressed as a net response to increasing SF intake, so as g N per g DM·kg $BW^{-0.75}$ (see also Table 4.4). N intake increased by 0.70 g N and N retention increased by 0.55 g N in response to SF intake in milk-fed calves. The marginal efficiency of N retention of 76% i.e. 0.55 g extra retained per 0.70 g extra ingested from SF, was previously reported (Berends et al., 2012a), and recycling of urea, likely originating from the deamination of amino acids originating from the MR, was suggested to be of quantitative importance. In the current study, UUA explained 0.10 g N of the 0.55 g N retained. This means that 19% of extra N retained with SF intake could be explained directly by recycling of urea. The response of UUA to SF was considered linear because of the absence of a significant quadratic component in this relationship. The log transformation needed to obtain homogeneity of variance, however, indicates that the increase in UUA was not linear, although systematic deviations from linearity were not observed (Table 4.4).

The remaining part of extra N retained (0.45 g N) is not explained by urea recycling but by other factors. In this paragraph, we speculate on the relative importance of several factors that may contribute to the observed increase in N retention with increasing SF intake. Firstly, digestible protein intake increased with increasing SF intake. The increase in digestible protein intake with SF can be estimated by subtracting undigested N, endogenous N, and microbial N from extra ingested N. Undigested N from SF in feces were estimated by subtracting extra microbial N losses (UFE, Table 4.4) and endogenous N losses from fecal N excretion (0.39 g N). Endogenous N losses from extra SF intake were estimated at 0.0048 g N based on a linear relationship of endogenous N with DM intake (NRC, 2001). By difference, digestible N intake from SF is estimated at 0.395 g N. When retained with a hypothetical efficiency of 50% (Ortigues et al., 1990), for example, this explains 44% of the extra N retention with increasing SF intake. This example shows that the increase in digestible protein intake is substantial, and likely to explain part of the observed increase in N retention.

Although an increase in N intake is commonly associated with an increase in UER in growing and lactating cattle (Reynolds and Kristensen, 2008), this response was not observed in our study. The absence of an increase in UER with N intake may indicate that direct post-absorptive utilization of N was increased. Potentially, digestible energy intake, amino acid intake, and nutrient synchronization contributed to the greater N retention with increasing SF intake. Digestible energy intake increased with increasing SF intake. In milk-fed calves in a similar weight range, extra protein-free energy intake increased N deposition (Gerrits et al., 1996). In that study, an increase of 33 kJ of protein-free DE·kg $BW^{-0.75}$ was associated with an increase of 1 g N retained. In the current study, digestible energy intake increased 11 kJ per g DM SF·kg $BW^{-0.75}$ (Gerrits et al., 1996), and could therefore be quantitatively important. In addition, amino acid intake can also increase N deposition in beef steers (Awawdeh et al., 2006). Alternatively, synchronization of nutrient supply with nutrient demand may be improved with increasing SF intake, due to attenuated nutrient absorption from rumen fermentation. In milk-fed calves, increasing the feeding frequency improved N retention (van den Borne et al., 2006b).

Ruminal Co recovery, on average 5.4%, was unaffected by SF intake and considerably lower than the range of 15 to 35% reported earlier (Berends et al., 2012b, Suárez et al., 2007). Therefore, leakage of milk into the rumen is unlikely to explain differences in N retention between treatments. Urinary ^{15}N enrichment returned to background levels after the collection period, indicating that this period was sufficiently long, but this check was not performed for fecal enrichment, possibly resulting in overestimation of UUA (Zuur et al., 2000).

Urea Recycling in Meal-fed (Pre)ruminants

Urea recycling has been measured successfully using doubly labelled ^{15}N urea in human (Jackson et al., 1993, Jackson et al., 1984, Meakins and Jackson, 1996, Wolfe, 1981), mice

(Marini et al., 2006), and cats (Russell et al., 2000). Most studies were conducted under steady-state conditions. However, such an approach may not represent the physiologically relevant situation in meal-fed animals. In the current study, we applied a 24-h tracer infusion while calves were fed twice daily. Although plasma urea concentrations were relatively constant over the 8-h postprandial period, urea production responds to meal feedings, as demonstrated previously in milk-fed calves (van den Borne et al., 2006b). This probably resulted in an isotopic nonsteady state in the current study. For example, in case urea recycling is more pronounced during periods of high urea production (i.e. lower plasma urea enrichment) then this may have underestimated GER and UUA in the current study. In addition, plasma urea concentrations indicated that the estimated priming and infusion dose were greater than needed.

In addition, UUA, but not GER, could have been overestimated by urinary excretion of ^{15}N in other forms than urea. All ^{15}N not excreted in feces or in urinary urea, is assumed to be retained. We observed that non-urea N excretion in urine increased with SF intake (0.17 g N per g DM·kg $BW^{-0.75}$; $P < 0.01$). Non-urea N in urine may include creatinine, purine derivatives, free amino acids, and free NH_3 (Bristow et al., 1992). In our study, excretion of purine derivatives potentially contributed to non-urea N excretion with increasing SF intake (Vercoe, 1976). As the increase in influx of N from intake was 2.5 times the increase in GER, and ammonia is used less efficiently for microbial protein synthesis than degradable protein (Argyle and Baldwin, 1989, Blake et al., 1983), we expect this effect to be of minor importance.

Plasma Urea Concentrations

Plasma urea concentrations averaged 1.6 to 2.4 mM, which is in the lower range of previously reported values in adult sheep and cattle (Lapierre and Lobley, 2001, Sunny et al., 2007). A possible shortage of N in the rumen may have worsened with increasing low-protein SF intake, which could limit rumen fermentation processes. Simultaneously, GER increased with SF intake, possibly due to an N deficit induced by the low-protein SF. As SF intake increased, however, factors like ruminal pH, volatile fatty acid production, and blood flow may also have affected GER. The AUC of postprandial plasma urea concentrations decreased with SF intake, and corresponded with the increase in GER. Possibly, the potential for further increases in GER may have been limited by the urea supply from blood. Studies in sheep and cattle have shown that GER increases with increasing plasma urea concentration (Kristensen et al., 2010, Sunny et al., 2007, Vercoe, 1969). Dairy cows also showed increased epithelial permeability that facilitated the transport of urea to the gastrointestinal tract in response to a low-protein diet (Røjen and Kristensen, 2012), but the absolute amount of urea entering the gastrointestinal tract did not increase due to a decreased plasma urea concentration.

Urea Transporters

The *bUTB* expression differed between calves without and calves with SF, whereas no differences were observed between SF levels. Previously, dietary N content did not affect rumen *bUTB* expression in adult dairy cows (Røjen et al., 2011) and lambs (Marini et al., 2004). In calves of 12 wk old, SF provision resulted in a twofold increase in empty rumen weight and the ratio of rumen mucosa to serosa length - a measure of absorptive surface - compared with values seen in calves fed no SF (Suárez et al., 2006). As indicated by the observed increase in macroscopic rumen development with SF intake, we expect that the rumen absorptive surface increased in response to SF intake, and likely resulted in an increased capacity to transport urea. The current study shows an increase of GER and a greater *bUTB* expression with increasing SF intake, although the latter is not linear when including calves without SF. The regulation of UT-B and its quantitative importance for urea entry into the rumen remains to be assessed in calves.

We detected rumen expression of *bAQP3* and *bAQP7*, but not of *bAQP10*, by real-time qPCR. Notably, the *bAQP3* expression increased with increasing SF intake, whereas that of *bAQP7*remained unaltered. Similarly, Røjen et al. (2011) demonstrated in lactating dairy cows that *bAQP3* expression increased with increasing dietary N level. The cows exposed to a high dietary N level also showed elevated arterial urea concentrations, which may independently have increased urea transport across the rumen wall. Walpole et al. (2013) demonstrated, using Ussing chambers, that *bAQP3* and *bUTB* expression were involved in ruminal mucosal flux of urea. Hence, AQP3 may be associated to urea transport across the gastrointestinal tract in calves. The effects of plasma urea concentrations and N shortage in the rumen on both *bAQP3* and *bUTB* expression need further study.

In conclusion, provision of a low-protein SF in amounts up to 27 g DM·kg $BW^{-0.75}$·d^{-1} to milk-fed calves resulted in increased recycling of urea into the gastrointestinal tract and a high marginal efficiency (76%) of N utilization for growth. In addition to an increase in digested N with SF intake, capture of recycled urea by the calves explained 19% of the observed increase in N retention. Furthermore, the observed increase in N retention may be explained by increased energy supply from SF, and potentially improved post-absorptive amino acid utilization for growth. Furthermore, the provision of SF, regardless of the particular amount of SF provided, elevates *bUTB* expression in the rumen wall of milk-fed calves.

ACKNOWLEDGEMENTS

We thank Michel Breuer and Leo Kruijt (both from Animal Nutrition Group, Wageningen University, the Netherlands) and Birgit Løth and Anne Krustrup (Department of Animal Science, Aarhus University, Denmark) for their skilled technical assistance.

REFERENCES

Abdoun, K., F. Stumpff, and H. Martens. 2006. Ammonia and urea transport across the rumen epithelium: a review. Animal Health Research Reviews 7(1-2):43-59.

Andersen, C. L., J. L. Jensen, and T. F. Ørntoft. 2004. Normalization of real-time quantitative reverse transcription-PCR data: a model-based variance estimation approach to identify genes suited for normalization, applied to bladder and colon cancer data sets. Cancer research 64(15):5245-5250.

Argyle, J. and R. Baldwin. 1989. Effects of amino acids and peptides on rumen microbial growth yields. Journal of Dairy Science 72(8):2017-2027.

Awawdeh, M., E. Titgemeyer, G. Schroeder, and D. Gnad. 2006. Excess amino acid supply improves methionine and leucine utilization by growing steers. Journal of Animal Science 84(7):1801-1810.

Berends, H., J. Van den Borne, S. Alferink, C. Van Reenen, E. Bokkers, and W. Gerrits. 2012a. Low-protein solid feed improves the utilization of milk replacer for protein gain in veal calves. Journal of Dairy Science95(11):6654-6664.

Berends, H., C. G. van Reenen, N. Stockhofe-Zurwieden, and W. J. J. Gerrits. 2012b. Effects of early rumen development and solid feed composition on growth performance and abomasal health in veal calves. Journal of Dairy Science 95(6):3190-3199.

Blake, J., D. Salter, and R. Smith. 1983. Incorporation of nitrogen into rumen bacterial fractions of steers given protein-and urea-containing diets. Ammonia assimilation into intracellular bacterial amino acids. British Journal of Nutrition 50(03):769-782.

Bristow, A. W., D. C. Whitehead, and J. E. Cockburn. 1992. Nitrogenous constituents in the urine of cattle, sheep and goats. Journal of the Science of Food and Agriculture 59(3):387-394.

Fouillet, H., B. Juillet, C. Bos, F. Mariotti, C. Gaudichon, R. Benamouzig, and D. Tomé. 2008. Urea-nitrogen production and salvage are modulated by protein intake in fed humans: results of an oral stable-isotope-tracer protocol and compartmental modeling. The American Journal of Clinical Nutrition 87(6):1702-1714.

Gerrits, W. J. J., Beelen, G.M., Dijkstra, J., Verdonk, J.M.A.J. 2001. Ammonia infusion and starch fermentation in preruminant calves (150-180 kg). in Internal report No V99.001. ID TNO Anmial Nutrition (in Dutch), Wageningen, the Netherlands.

Gerrits, W. J. J., G. H. Tolman, J. W. Schrama, S. Tamminga, M. W. Bosch, and M. W. A. Verstegen. 1996. Effect of protein and protein-free energy intake on protein and fat deposition rates in preruminant calves of 80 to 240 kg live weight. Journal of Animal Science 74(9):2129-2139.

ISO. 1997. Animal feeding stuffs - Determination of nitrogen content and calculation of crude protein content. ISO 5983. Int. Organ. Stand., Geneva, Switzerland.

Jackson, A. A., M. S. Danielsen, and S. Boyes. 1993. A noninvasive method for measuring urea kinetics with a single dose of [15N15N] urea in free-living humans. The Journal of Nutrition 123(12):2129.

Jackson, A. A., D. Picou, and J. Landman. 1984. The non-invasive measurement of urea kinetics in normal man by a constant infusion of 15N15N-urea. Human nutrition. Clinical Nutrition 38(5):339-354.

Kristensen, N. B., A. C. Storm, and M. Larsen. 2010. Effect of dietary nitrogen content and intravenous urea infusion on ruminal and portal-drained visceral extraction of arterial urea in lactating Holstein cows. Journal of Dairy Science 93(6):2670-2683.

Lapierre, H. and G. Lobley. 2001. Nitrogen recycling in the ruminant: A review. Journal of Dairy Science 84:E223-E236.

Ludden, P. A., R. M. Stohrer, K. J. Austin, R. L. Atkinson, E. L. Belden, and H. J. Harlow. 2009. Effect of protein supplementation on expression and distribution of urea transporter-B in lambs fed low-quality forage. Journal of Animal Science 87(4):1354-1365.

Marini, J. C. and M. S. Attene-Ramos. 2006. An improved analytical method for the determination of urea nitrogen isotopomers in biological samples utilizing continuous flow isotope ratio mass spectrometry. Rapid communications in mass spectrometry 20(24):3736-3740.

Marini, J. C., J. D. Klein, J. M. Sands, and M. E. Van Amburgh. 2004. Effect of nitrogen intake on nitrogen recycling and urea transporter abundance in lambs. Journal of Animal Science 82(4):1157-1164.

Marini, J. C., B. Lee, and P. J. Garlick. 2006. In vivo urea kinetic studies in conscious mice. The Journal of Nutrition 136(1):202-206.

Marini, J. C. and M. E. Van Amburgh. 2003. Nitrogen metabolism and recycling in Holstein heifers. Journal of Animal Science 81(2):545-552.

McClelland, I. S. and A. A. Jackson. 1996. Urea kinetics in healthy young women: minimal effect of stage of menstrual cycle, contraceptivepill and protein intake. British Journal of Nutrition 76(02):199-209.

Meakins, T. S. and A. A. Jackson. 1996. Salvage of exogenous urea nitrogen enhances nitrogen balance in normal men consuming marginally inadequate protein diets. Clinical Science 90(Pt 3):215-225.

Mosenthin, R., W. Sauer, and C. de Lange. 1992. Tracer studies of urea kinetics in growing pigs: I. The effect of intravenous infusion of urea on urea recycling and the site of urea secretion into the gastrointestinal tract. Journal of Animal Science 70(11):3458-3466.

Muscher, A., B. Schröder, G. Breves, and K. Huber. 2010. Dietary nitrogen reduction enhances urea transport across goat rumen epithelium. Journal of Animal Science 88(10):3390-3398.

NRC. 2001. Nutrient requirements of dairy cattle. 7th rev. ed. Natl. Acad. Press, Washington, DC.

Ortigues, I., T. Smith, M. Gill, S. B. Cammell, and N. W. Yarrow. 1990. The effect of fishmeal supplementation of a straw-based diet on growth and calorimetric efficiency of growth in heifers. British Journal of Nutrition 64(3):639-651.

Pfaffl, M. W., A. Tichopad, C. Prgomet, and T. P. Neuvians. 2004. Determination of stable housekeeping genes, differentially regulated target genes and sample integrity: BestKeeper–Excel-based tool using pair-wise correlations. Biotechnology letters 26(6):509-515.

Quigley, J., T. Wolfe, and T. Elsasser. 2006. Effects of additional milk replacer feeding on calf health, growth, and selected blood metabolites in calves. Journal of Dairy Science 89(1):207-216.

Reynolds, C. and N. B. Kristensen. 2008. Nitrogen recycling through the gut and the nitrogen economy of ruminants: An asynchronous symbiosis. Journal of Animal Science 86(14 suppl):E293-E305.

Ritzhaupt, A., G. Breves, B. Schröder, C. Winckler, and S. Shirazi-Beechey. 1997. Urea transport in gastrointestinal tract of ruminants: effect of dietary nitrogen. Biochemical Society Transactions 25(3):490S.

Ritzhaupt, A., I. Wood, A. Jackson, B. Moran, and S. Shirazi-Beechey. 1998. Isolation of a RT-PCR fragment from human colon and sheep rumen RNA with nucleotide sequence similarity to human and rat urea transporter isoforms. Biochemical Society Transactions 26(2):S122.

Rojek, A., J. Praetorius, J. Frøkiaer, S. Nielsen, and R. A. Fenton. 2008. A current view of the mammalian aquaglyceroporins. Annu. Rev. Physiol. 70:301-327.

Røjen, B. A. and N. B. Kristensen. 2012. Effect of time duration of ruminal urea infusions on ruminal ammonia concentrations and portal-drained visceral extraction of arterial urea-N in lactating Holstein cows. Journal of Dairy Science 95(3):1395-1409.

Røjen, B. A., S. B. Poulsen, P. K. Theil, R. A. Fenton, and N. B. Kristensen. 2011. Effects of dietary nitrogen concentration on messenger RNA expression and protein abundance of urea transporter-B and aquaporins in ruminal papillae from lactating Holstein cows. Journal of Dairy Science 94(5):2587-2591.

Russell, K., G. Lobley, J. Rawlings, J. Millward, and E. Harper. 2000. Urea kinetics of a carnivore, Felis silvestris catus. British Journal of Nutrition 84(05):597-604.

Sarraseca, A., E. Milne, M. Metcalf, and G. Lobley. 1998. Urea recycling in sheep: effects of intake. British Journal of Nutrition 79(1):79-88.

Simmons, N., A. Chaudhry, C. Graham, E. Scriven, A. Thistlethwaite, C. Smith, and G. Stewart. 2009. Dietary regulation of ruminal bovine UT-B urea transporter expression and localization. Journal of Animal Science 87(10):3288-3299.

Smith, C. P. and G. Rousselet. 2001. Facilitative urea transporters. Journal of Membrane Biology 183(1):1-14.

Stewart, G. S., C. Graham, S. Cattell, T. P. L. Smith, N. L. Simmons, and C. P. Smith. 2005. UT-B is expressed in bovine rumen: potential role in ruminal urea transport. American Journal of Physiology-Regulatory Integrative and Comparative Physiology 289(2):R605-R612.

Stewart, G. S. and C. P. Smith. 2005. Urea nitrogen salvage mechanisms and their relevance to ruminants, non-ruminants and man. Nutr Res Rev 18(1):49-62.

Suárez, B., C. Van Reenen, W. Gerrits, N. Stockhofe-Zurwieden, A. Van Vuuren, and J. Dijkstra. 2006. Effects of supplementing concentrates differing in carbohydrate composition in veal calf diets: II. Rumen development. Journal of Dairy Science 89(11):4376-4386.

Suárez, B. J., C. G. Van Reenen, N. Stockhofe-Zurwieden, J. Dijkstra, and W. J. J. Gerrits. 2007. Effect of roughage source and roughage to concentrate ratio on animal performance and rumen development in veal calves. Journal of Dairy Science 90(5):2390-2403.

Sunny, N., S. Owens, R. Baldwin, S. El-Kadi, R. Kohn, and B. Bequette. 2007. Salvage of blood urea nitrogen in sheep is highly dependent on plasma urea concentration and the efficiency of capture within the digestive tract. Journal of Animal Science 85(4):1006-1013.

Van den Borne, J., J. Verdonk, J. W. Schrama, and W. J. J. Gerrits. 2006a. Reviewing the low efficiency of protein utilization in heavy preruminant calves - a reductionist approach. Reproduction Nutrition Development 46(2):121-137.

Van den Borne, J., M. W. A. Verstegen, S. J. J. Alferink, R. M. M. Giebels, and W. J. J. Gerrits. 2006b. Effects of feeding frequency and feeding level on nutrient utilization in heavy preruminant calves. Journal of Dairy Science 89(9):3578-3586.

Vercoe, J. 1969. The transfer of nitrogen from the blood to the rumen in cattle. Crop and Pasture Science 20(1):191-197.

Vercoe, J. 1976. Urinary allantoin excretion and digestible dry-matter intake in cattle and buffalo. Journal of Agricultural Sciences Cambridge 86:613-615.

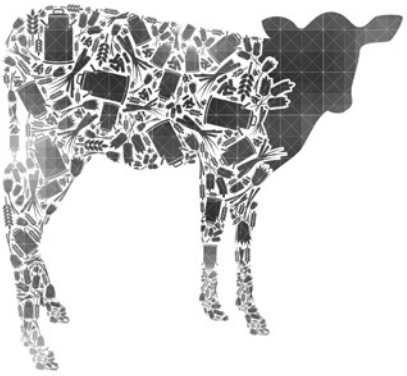
Walpole, M., B. Schurmann, P. Górka, G. Penner, M. Loewen, and T. Mutsvangwa. 2013. Functional roles of aquaporins and urea transporters in urea flux across the ruminal epithelium. Pages 269-270 in Energy and protein metabolism and nutrition in sustainable animal production. Springer.

Wolfe, R. R. 1981. Measurement of urea kinetics in vivo by means of a constant tracer infusion of di-15N-urea. American Journal of Physiology-Endocrinology And Metabolism 240(4):E428-E434.

Zuur, G., K. Russell, and G. Lobley. 2000. Multiple-entry urea kinetic model: effects of incomplete data collection. In: McNamara, J.P., J. France, and D. Beever, editors. Modelling nutrient utilization in farm animals. Walingford (United Kingdom): CABI Publishing. p. 145–61.

Chapter 5

Dietary preferences, health, and growth performance in Holstein-Friesian calves provided free diet selection



H. Berends[1], W.J.J. Gerrits[1], L.E. Webb[2], E.A.M. Bokkers[2], M. S. Gilbert[1], B. Engel[3], and C.G. van Reenen[4]

To be submitted

[1]Animal Nutrition Group, Wageningen University, Wageningen, the Netherlands; [2]Animal Production Systems Group, Wageningen University, Wageningen, the Netherlands; [3]Biometris, Wageningen University, Wageningen, the Netherlands; [4]Livestock Research, Animal Sciences Group, Wageningen University and Research Centre, Lelystad, the Netherlands

ABSTRACT

This study aimed to gain insight in the dietary preferences and intake of calves developing towards ruminants, and in the associations of dietary preferences to health and growth performance. Twenty-three male Holstein-Friesian calves (17 ± 0.5 days of age, 46 ± 0.4 kg bodyweight) could select their own diet through a free choice feeding system with *ad libitum* access to milk replacer (**MR**), water, hay, barley straw, concentrate, and corn silage. Individual feed intake of each diet component was recorded at 3 and 6 months of age. The number of days of medical treatments for respiratory and gastrointestinal disorders was recorded for each calf. *Post mortem* observations performed at 27 weeks of age included visual examination of rumen and abomasal wall, as well as rumen weight with and without contents, and warm carcass weight. Principal component analysis (**PCA**) was used to summarize dietary preferences recorded at 3 and 6 months of age, and PCA regression analysis was used to examine relationships of these with health and growth performance parameters. At 3 months, gross energy intake averaged 37.4 MJ per d (or 1.09 MJ/kg$^{0.75}$ per d) including 51% MR, 29% concentrates, 15% hay, 3% straw, and 2% corn silage. At 6 months, gross energy intake increased to 86.5 MJ per d (or 1.45 MJ/kg$^{0.75}$ per d) including 30% MR, 48% concentrates, 12% hay, 1% straw, and 9% corn silage. Water intake was 2.5 L and 8.3 L at 3 and 6 months, respectively. Feeding frequency of MR was 6.5 times and 8.2 times per d at 3 and 6 months, respectively. Individual differences in measures of dietary preference were highly variable and dietary preferences were not consistent between 3 and 6 months. Intake of MR at 3 months was positively related to warm carcass weight and empty rumen weight at 27 weeks. Water intake at 3 months was positively associated to respiratory disorders, while intake of fibrous feed at 3 months was negatively associated to medical treatments for respiratory disorders. Medical treatments for gastro-intestinal disorders were negatively associated to intake of energy-rich feed types at 6 months and positively to fibrous feed intake at 3 months. In conclusion, free diet selection resulted in large individual variation in dietary preferences. Dietary preferences changed with age, and results indicate that feed intake pattern is associated to health factors.

Keywords: Calf, Feed preference, Health, Voluntary feed intake

INTRODUCTION

Diet formulations for calves, during their development from preruminant to ruminant, aim at efficient growth performance and gastrointestinal development. These aims can be in conflict with each other. Maximizing growth rate can be achieved by feeding large amounts of milk replacer (**MR**) (Bartlett et al., 2006, Brown et al., 2005) and, in dairy calves, maximizing growth rate during the first weeks of life has been shown to increase milk production in later life (Soberon et al., 2012). On the other hand, calves drinking large amounts of MR have a low intake of starter (Jasper and Weary, 2002, Terré et al., 2007), which is required for production of volatile fatty acids to stimulate rumen development (Sander et al., 1959). Additionally, roughage intake promotes muscular development of the rumen (Tamate et al., 1962) and stimulates rumination (Hodgson, 1971). Furthermore, early rumen development is beneficial for growth performance (Berends et al., 2012b). The combination of high amounts of MR and solid feeds (**SF**) in a calf ration is associated to gastrointestinal problems such as abomasal lesions, plaque formation of the rumen wall (Berends et al., 2012b, Prevedello et al., 2012).

So far, studies on calf nutrition have primarily used experimental contrasts to study separate effects of roughage sources, MR formulation, weaning strategies, feeding frequency, or particular combinations of MR and SF. Alternatively, studying the intake of dietary components in a free-choice setting can provide important information about between-animal variation, and inherent changes in the priorities of calves with age. In addition, identification of factors driving the motivation for intake of these components can provide valuable information for designing future feeding strategies. This has been demonstrated in free-choice experiments in sheep (Villalba et al., 2011, Villalba and Provenza, 1999) and in weaned calves (Atwood et al., 2001). Probably for practical reasons, MR has never been a ration component in such a free choice setting, despite its obvious potential contribution to energy intake. In addition, providing young calves a free-choice setting for 6 months, enables us to study development and consistency of dietary preferences over time. Therefore, the aim of the current study was to gain insight in the dietary preference and intake of calves developing towards ruminants, when given free and *ad libitum* choice between MR and different types of SF until 6 months of age. In particular, consistency of dietary preference and associations between individual dietary preferences and health and growth performance parameters were examined.

MATERIAL AND METHODS

Measurements on behavior are described in another paper (Webb et al., unpublished).

Calves, Experimental design, and Housing

Twenty-four Holstein-Friesian calves were included in the experiment. Because of commercial availability, only male calves were included. Each calf originates from another commercial dairy farm. Calves were purchased via a contracted dealer in two batches from to obtain a homogenous group of calves of similar bodyweight (46 ± 0.4 kg) and age (17 ± 0.5 days). All calves could select their own diet through a free choice feeding system with *ad libitum* access to different ration components: MR, water, hay, barley straw, concentrate, and corn silage (Table 5.1). Individual feed intake was recorded during two 4-d measurement periods, at 3 and 6 months of age. Calves were checked daily by animal caretakers and treated with antibiotics as and when required according to a veterinary protocol. Calves with diarrhoea were not treated with electrolytes, because interfere with dietary choice was minimized

Calves were housed in one of two groups of 12 calves in pens measuring 23.0 m^2, with wooden slatted floors without bedding material, with an automated milk dispenser (TAP5-VH1-50-F2, Förster Technik®, Egen, Germany), four feed troughs, and a water bowl. The barn was mechanically ventilated and lit by lamps from 0600 h to 2200 h. Average minimum and maximum temperature was 17 ± 0.1 °C and 21 ± 0.1 °C. Average minimum and maximum humidity was 58 ± 0.5% and 73 ± 0.5%.

Table 5.1 Analysed nutrient composition of milk replacer (MR), concentrate, and roughages

Nutrient[1]	MR[2]	Concentrate[3]	Barley straw	Hay	Corn silage
Dry matter, g/kg product	965	881	922	908	303
Crude Protein	210	179	31	92	84
Crude fat	173	40	8	15	36
Crude ash	80	54	61	104	37
Neutral detergent fibre	-	209	787	590	399
Sugars	-	43	51	132	2
Starch	31	380	1	2	352

[1] Presented as g/kg dry matter unless specified otherwise.

[2] Ingredient composition of MR: 22.2% whey protein concentrate, 38.1% whey powder, 14.7% palm oil, 8.8% low lactose whey powder, 4.6% soy concentrate, 3.7% coconut oil, 3.0% wheat protein, 2.0% starch, 2.9% premix

[3] Ingredient composition of concentrate: 26% corn, 25% barley, 25% lupines (crude fat < 70, crude protein < 335), 20% wheat middlings, 4% premix (lactose carrier, provided per kg concentrate: vitamin A: 4000 IU; vitamin D: 500 IU; vitamin E: 100 IU; zinc: 25 mg; manganese: 20 mg; iodine: 0.8 mg; selenium: 0.15 mg; copper: 15 mg; cobalt: 0.1 mg), 1.2% $MgSO_4.7H_2O$, 0.5% NaCl, 1.6% $CaCO_3$, 2% KH_2PO_4.

Diets and Feeding

MR and concentrate compositions (Table 5.1) were designed to meet recommendations for beef cattle concerning minerals and vitamins (NRC, 2000) so that calves did not depend

on MR for their mineral and vitamin supply. Solid feed components were offered *ad libitum* in separate troughs. Left-overs were weighed once daily. Solid feed components were administered into different troughs daily in the home pens to avoid confounding effects of positional preference. The MR was provided at 120 g DM/L, at approximately 42 °C, and at a maximum drinking speed of 0.3 L/min. After arrival, the MR allowance was kept constant at 5 L/d for 7 days, then gradually increased to *ad libitum* provision in the following 14 days.

Measurements

Feed intake was recorded during measurement periods at 3 and 6 months of age. Calves were moved in groups of four calves to a test-pen (12 m^2) for a period of 7 days: 3 days of habituation followed by 4 days of measurements. The test-pen provided identical ration components, and allowed individual registration of feed intake. Feed left-overs were weighed daily to validate computer-recorded intake data at group level in the test-pen. From week 8 to 26, calves were weekly clinically examined by one researcher and scored for the respiration pattern, urine drinking victim - being suggested by others as determined by the appearance of prepuce - , and showing clinical signs of bloat (Table 5.2). These aspects are further specified in Table 5.2. For bloat, urine drinking victim, and respiration pattern, the maximum value of wk 10, 11, 12 and 13 was determined for 3 months of age, and for 6 months of age the maximum value of wk 23, 24, 25 and 26 was determined (Table 5.2).
At 27 weeks of age, calves were slaughtered. The rumen was dissected along the dorsal line, emptied, and rinsed with cold water. Rumen weight was recorded with and without contents. The mucosal surface of the rumen wall was examined visually by one veterinarian and the presence, density, and development of rumen papillae were scored on a 5-point scale from poor to excellent: 1 – 1.5 – 2 – 2.5 – 3 according to Suárez et al., 2007. The presence of plaque formation on the rumen wall was assessed visually (Table 5.2) according to Suárez et al., 2007. The abomasal wall was inspected macroscopically for lesions (erosions, ulcers and scars) in the fundic or pyloric region, in the following size categories: small = lesion ≤ 0.5 cm^2; medium = 0.5 cm^2 < lesion ≤ 1.0 cm^2; large = lesion > 1.0 cm^2. Area of pyloric damage was estimated by multiplication of counts and size category, where average size per category was assumed 0.25, 0.75 and 1.25 cm^2 for small, medium and large lesions, respectively. Live weight and warm carcass weights (Berends *et al.*, 2012a) were recorded. Feed samples (MR, concentrates, straw, hay, corn silage) were collected every 8 weeks, pooled and analysed for dry matter, crude ash, crude protein, crude fat, neutral detergent fibre, starch, and reducing sugars according to Berends *et al.*, 2012b.

Statistical Analysis

Observations on twenty-three calves were included in the analysis, because one calf died before the first measurement period at 3 months due to volvulus of the small intestine. All calculations were performed with GenStat (Genstat Committee, 2000). Multiple regression

Table 5.2 Health and growth performance parameters of 23 calves (3 and 6 months of age), provided free and ad libitum access to 6 ration components: milk replacer, water, hay, straw, corn silage, concentrate

Item	Description
Clinical health	
Treatments for respiratory disorders	Number of days treated for clinical signs of respiratory disorders
Treatments for gastrointestinal disorders	Number of days treated for clinical signs of gastrointestinal disorders
Total days of medical treatment	Number of days that an animal was treated based on clinical signs of sickness
Bloat	Excessive abdomen fill due to accumulation of gas in the rumen.1: no bloat, 2: mild bloat, 3: severe bloat. Max. score of 4 weeks
Respiration pattern	0: normal respiration, 1: increased or abnormal rate of respiration. Max. score of 4 weeks. Expressed as prevalence (%) of calves
Urine drinking victim	0: normal, 1: sugged prepuce of the calf. Max. score of 4 weeks. Expressed as prevalence (%) of calves
Rumen development	
Full rumen weight	Rumen weight plus fresh rumen content, in kg
Empty rumen weight	Rumen weight after dissection, in kg (e.g. 'empty rumen weight')
Rumen development score	Rumen development scored from 1 (poor) to 4 (fully developed)
Rumen plaque	Plaque: rumen mucosa containing focal or multifocal patches with coalescing and adhering papillae covered by a sticky mass of feed, hair and cell debris. 0: no plaque, 1: plaque. Expressed as prevalence in % of calves
Abomasal damage	
Lesions in fundic region	Number of lesions present in the fundic region of the abomasum
Small	Lesion area ≤ 0.5 cm^2
Medium	0.5 cm^2 < Lesion area < 1 cm^2
Large	Lesion area ≥ 1.0 cm^2
Lesions in pyloric region	Number of lesions present in the pyloric region of the abomasum
Small	Lesion area ≤ 0.5 cm^2
Medium	Lesion area: 0.5 cm^2 - 1 cm^2
Large	Lesion area ≥ 1.0 cm^2
Area of pyloric damage	Area estimated by multiplication of counts and size category. Average size per category is assumed to be 0.25 cm^2 for small lesions, 0.75 cm^2 for medium lesions and 1.25 cm^2 for large lesions
Area of fundic damage	Area estimated by multiplication of counts and size category. Average size per category is assumed to be 0.25 cm^2 for small lesions, 0.75 cm^2 for medium lesions and 1.25 cm^2 for large lesions
Growth performance	
Live weight	Live weight at slaughter, kg
Warm carcass weight	kg

	Age at scoring, months	Mean or %	SEM	Min	Max
	0-6	4.3	0.62	1	11
	0-6	3.9	1.74	0	17
	0-6	8.5	1.50	2	32
	3	2.1		2	3
	6	2.0		2	3
	3	9		0	1
	6	9		0	1
	3	39	10.4	0	1
	6	0		0	0
	6	19.1	1.03	12.8	37.8
	6	4.3	0.16	2.8	5.8
	6	2.9	0.15	1.5	4.0
	6	10	6.6	0	1
	6				
	6	0.17	0.174	0	4
	6	0	0	0	0
	6	0	0	0	0
	6				
	6	1.83	0.502	0	8
	6	1.22	0.350	0	7
	6	1.39	0.401	0	7
	6	3.11	0.731	0	14
	6	0.04	0.043	0	1
	6	253	7.2	164	296
	6	140	4.1	89	164

analysis was used for the analysis of the relationship between feed intake related variables and health parameters. Feed intake related variables recorded in the present experiment were significantly interrelated (results not shown). Therefore, Principal Component Analysis (PCA) (Joliffe, 1986) was used to condense nine correlated feed intake related measures (intake of MR, water, concentrate, hay, corn silage, straw, roughage proportion of SF, NDF intake and MR feeding frequency) into mutually independent (orthogonal) principal components (**PC**). PCs are linear combinations of the original measurements and the coefficients are referred to as loadings. Prior to PCA, feed intake related measures were corrected for possible test-pen effects by subtracting the mean of the associated test-pen group from each individual measurement. This is equivalent to the use of residuals after analysis of variance of feed intake related measures with an experimental factor for test-pens in the model. The PCs were assumed to reflect independent characteristics (or dimensions) underlying the residual correlation matrix. The residual correlation matrix reflects correlation between feed intake related measures within test-pens. The outcomes of the PCs, scores, were calculated from the original measurements, and not from the residuals, using the same loadings. PCA was performed separately for the data recorded at 3 and at 6 months. After extraction, PCs were scaled by their standard deviations (square roots of associated eigenvalues) and subjected to varimax rotation, to enhance interpretation of components. The first five PCs obtained at each age, collectively explaining at least 90% of the total variance (see Results section), were used as regressors in a stepwise multiple regression approach (principal component regression or PCR). Potentially relevant PCs ($P < 0.15$) were selected after regression analyses, with each PC successively introduced as a single regressor. Selected PCs were included in subsequent multiple regression analyses, with health parameters as dependent variables, and principal components as independent variables from feed intake related variables. Forward and backward selection approaches were applied, aiming for highest adjusted R^2. Components selected by either of the two approaches were submitted to best subset selection, again aiming for highest adjusted R^2. Only components contributing significantly ($P < 0.05$, t-test) to the final regression model were retained. Days of treatment were analysed as count data employing log linear regression, specifying a logarithmic link function, and a variance proportional to the mean. Initially, all regression models, including the log linear regression models, comprised random test-pen effects and a fixed effect for batch. Because the impact of random test-pen effects was negligible, i.e. because of a small or negative associated component of variance (results not shown), random test-pen effects were omitted from the final regression models. Analyses including random test-pen effects were either performed by restricted maximum likelihood (REML) (McCulloch et al., 2008) or penalized quasi likelihood (PQL) (Breslow and Clayton, 1993). The final analyses without random test-pen effects were either performed by least squares or maximum quasi-likelihood. In order to assess the extent to which individual patterns of dietary preference were consistent over time, the intake of each ration component was first

expressed as % of total DM at each age. Next, Spearman rank correlations were calculated for pairs of the same intake variables between ages. To correct for possible test-pen effects, in all instances rank numbers were assigned within each test-pen separately.

RESULTS AND DISCUSSION

Average SF intake reached 987 ± 100.0 g DM/d at 3 months and 3205 ± 174.6 g DM/d at 6 months, and both intake and preferences showed large individual variation (Table 5.3), as previously shown in lambs and calves offered free dietary choice (Atwood et al., 2001, Provenza et al., 1996). The average voluntary MR intake and growth observed in the current study is in line with studies with (dairy) calves provided whole milk *ad libitum* (Jasper and Weary, 2002, Khan et al., 2007). At 3 months, gross energy (**GE**) intake averaged 37 MJ per d (or 1.09 MJ/kg$^{0.75}$ per d) and was composed of 51% MR, 29% concentrates, 15% hay, 3% straw, and 2% corn silage. At 6 months, GE intake was increased to 86 MJ per d (or 1.45 MJ/kg$^{0.75}$ per d) and was composed of 48% concentrates, 30% MR, 12% hay, 9% corn silage and 1% straw. Digestible energy (**DE**) intake of calves in the current study was estimated approximately 72 MJ of DE/d at 6 months, of which 65% originates from SF and 35% from MR (Berends et al., 2012a, CVB, 2007). The DE intake of calves at 6 months and growth observed in the current study are high when compared to commercial feeding systems like veal where calves are fed ca. 58-62 MJ digestible energy per d at 6 months (Berends et al., 2012b).

Table 5.3 Descriptive data of bodyweight and feed intake related parameters recorded at 3 and 6 months of age for 23 calves, provided free and ad libitum access to 6 ration components: milk replacer (MR), water, hay, straw, corn silage, concentrate

	3 months of age				6 months of age			
Item	**mean**	**SEM**	**min**	**max**	**mean**	**SEM**	**min**	**max**
Bodyweight, kg	111	1.3	80	131	232	2.9	154	278
MR intake, g DM/d[1]	905	83.6	509	1579	1250	80.0	488	1864
Feeding frequency MR, #/d	6.5	0.49	3.0	9.5	8.2	0.48	5.0	12.3
Water intake[2], L/d	2.5	0.50	0.4	10.4	8.3	0.96	2.5	20.6
Hay intake, g DM/d[1]	313	32.1	24	671	571	59.4	61	1298
Straw intake, g DM/d[1]	72	11.8	6	194	41	9.3	0	120
Corn silage intake, g DM/d[1]	34	7.8	1	165	432	85.4	18	1722
Concentrate intake, g DM/d[1]	568	90.8	68	1392	2165	145.8	678	3777
Solid feed intake, g DM/d[1]	987	100.0	126	1850	3205	174.6	1405	4950

[1] DM: dry matter.
[2] Water used from a drinking bowl. Excludes water intake with MR.

Ruminants regulate feed intake based on factors including nutritional needs (Atwood et al., 2001, Provenza et al., 1996, Villalba and Provenza, 1999), flavour (Villalba et al., 2011), and post-ingestive feedback (Provenza and Balph, 1987). In the current study, we speculated that the opportunity to freely select ration components may have increased total SF intake. In addition, calves chose a roughage proportion of the SF of 42% at 3 months and 33% at 6 months, which is substantially more than commonly supplied to dairy, fattening, and veal calves. Previously, Castells et al. (2012) provided combined diets containing different roughage sources in addition to concentrate to young (8-71 d) calves, and observed an increase in total feed intake and growth performance. In comparison, when calves were provided with predetermined percentages of hay and started in a mixture, total feed intake declined with increasing percentage of hay inclusion (Hill et al., 2008). Potentially, free and/or separate provision of ration components may stimulate feed intake and consequently growth performance.

PCA produced at 3 and 6 months at least three PCs with an eigenvalue larger than 1 (Table 5.4) and the first three PCs explained over 65% of total variation. Cross loading of variables was minimal, except for concentrate intake at 6 months, which had high loadings at both PC2 and PC3, and corn silage intake at PC1 and PC5 at 6 months. At 3 months, the first PC had a high positive loading for concentrate intake, combined with a high negative loading for roughage proportion. Therefore, PC1 at 3 months is referred to as 'Concentrate for roughage', representing the replacement of roughage for concentrate. Similarly, for all PCs, names were chosen to summarize the loadings of each PC.

Consistency of Dietary Preference

Only for corn silage intake, a significant Spearman rank correlation was found between 3 and 6 months (correlation = 0.72; $P < 0.01$). The absence of any other significant Spearman rank correlations indicates that individual differences in the intake of different dietary sources were in general not consistent over time between 3 and 6 months. This is in line with the study of Atwood *et al.* (2001), where beef calves (365 kg) given free access to 3 SF components changed preferences constantly throughout a 63-d period. In addition, the PCA (Table 5.4) demonstrates that loading patterns of feed intake related variables at 3 months were different from those at 6 months. This means that feed intake related variables were differentially interrelated at each age. Likely, patterns of dietary preference of individual calves had not stabilized between 3 and 6 months. Whether dietary preferences are not consistent over time in ruminants, or whether dietary preferences further mature as the calf develops remains unclear from this study. We speculate that incompletion of the voluntary weaning process, which did not occur within the timeframe of 6 months in the present experiment, could play a role in stabilizing nutrient requirements and dietary preference. Furthermore, as calves originated from different farms, health factors may have been more important for motivating dietary choices at 3 than at 6 months.

Consequences for Health

Although abomasal ulcers and erosions are of concern to all calf-raising systems, prevalence rates highly differ for the different systems: 0.2 to 5.7% of beef calves, 32 to 76% in veal calves, 1.0 to 2.6% in dairy cows (Marshall, 2009). In the current study, 83% of calves had one or more lesions in the pyloric area (results not shown), although the average number of lesions was low and the size small when compared to calves in a veal system (Berends et al., 2012b). The provision of roughages to milk-fed calves increased the prevalence of abomasal lesions (Brscic et al., 2011, Mattiello et al., 2002). The aetiology of abomasal lesions is uncertain and multifactorial, but suggested to be primarily associated to prolonged inappetence and sustained low abomasal pH (Marshall, 2009). The prevalence of abomasal lesions in the present study was low when compared with prevalences observed in veal calves (Brscic et al., 2011, Gottardo et al., 2002, Mattiello et al., 2002), commonly fed MR twice daily. Increasing feeding frequency of MR, as also observed in the current study, results in increased luminal pH (Ahmed et al., 2002), and may decrease the risk for abomasal lesions. Additionally, rumen development and stimulation of fermentative degradation of potentially sharp particles may reduce their mechanically abrasive effect on the abomasal mucosa. Berends et al. (2012b) showed that early compared with late rumen development reduced the number of large scars in the abomasum in veal calves. It was suggested that rumen development may provide a form of protection to abomasal lesions. An association between the area of pyloric damage and water intake at 3 and 6 months was found: PC's with high loadings for water intake, i.e. 'Water' at 3 months and 'Hay and water for corn' at 6 months, had positive regression coefficients (Table 5.5). Previously, Gottardo *et al.* (2002) found no relationship between administration of drinking water and the number of animals with abomasal lesions, which suggests that the increase in water consumption observed in the present experiment is a consequence of abomasal damage, rather than a cause. This hypothesis is further supported by the finding that abomasal damage in sheep leads to increased water consumption (Rowe et al., 1988). Notably, the relationship between the area of pyloric damage and PC's associated with high fibre intake, i.e. 'Fibre' at 3 months and 'Straw' at 6 months, suggested that the area of pyloric damage increased with increasing voluntary intake of fibrous feeds. A reduced intake of fibrous feeds could be a cause or a consequence of pyloric damage. Data from previous studies in milk-fed calves suggest that the provision of fibrous feeds increased the prevalence of abomasal lesions (Brscic et al., 2011, Mattiello et al., 2002).

At 27 weeks, plaque formation was observed in 10% of the calves. The latter is low when compared with data from Suárez et al. (2007), who observed plaque formation in 63% of calves fed MR plus SF composed of 40% concentrate and 60% corn silage. The macroscopic rumen development score observed in the current study was high when compared with other studies using a similar scoring system in veal calves (Berends et al., 2012b, Suárez et

Table 5.4 Loadings of principal components (PC) extracted by principal component analyses of nine feed intake related variables recorded in 23 calves† at 3 and 6 months of age, and the eigenvalues and proportions of total variation explained by each PC†

	3 months of age				
	PC1	PC2	PC3	PC4	PC5
Item	Concentrate for roughage	Fibre	Milk	Corn	Water
Milk replacer (MR) intake, g DM/d[1]	-0.20	-0.39	**0.81**	0.03	-0.28
Feeding frequency of MR, #/d	0.41	0.32	**0.59**	-0.03	0.44
Water intake, L/d	0.19	0.11	-0.09	0.09	**0.95**
Concentrate intake, g DM/d[1]	**0.87**	0.36	0.02	0.11	0.20
Hay intake, g DM/d[1]	-0.13	**0.93**	-0.15	-0.11	0.05
Corn silage intake, g DM/d[1]	0.09	-0.01	-0.01	**0.94**	0.06
Straw intake, g DM/d[1]	-0.40	0.43	0.44	0.48	0.08
Roughage proportion of solid feed, %	**-0.95**	0.17	0.10	0.01	-0.14
Neutral detergent fibre intake, g/d	0.30	**0.91**	0.04	0.20	0.17
Eigenvalues	3.20	1.95	1.38	0.93	0.73
Variance explained, %	36	22	15	10	8

†23 Holstein-Friesian male calves from 0 to 6 months of age were provided free and *ad libitum* access to 6 ration components: milk replacer (MR), water, hay, straw, corn silage, concentrate.
[1] DM: dry matter.

al., 2007). Although calves chose on average a solid diet with a roughage:concentrate ratio of 42:58 at 3 months and 33:67 at 6 months (Table 5.3), with a high between-animal variation, clinical signs of severe bloat remained low. When comparing these data to other studies, it is important to consider the *ad libitum* and continuous availability of all ration components in the current experiment. An increased feeding frequency as a result of *ad libitum* access in the current study may provide a more constant supply of nutrients to the rumen, which could result in more constant fermentation. This may stimulate rumen development, and lower the risks of bloat, and plaque. In a commercial setting, restricted feeding may affect rumen pH and fermentation kinetics.

Regression analysis with PCs as independent variables and health parameters as dependent variables, provided insight into interrelations while avoiding multicollinearity between feed intake related variables. Warm carcass weight was positively related to 'Milk' at 3 months (Table 5.4). 'Milk' at 3 months was also positively related to empty rumen weight. Potentially, calves that consume more MR – the highest contributor to DE intake – early in life, have a greater growth potential, and therefore also a higher SF intake at 6

	6 months of age				
	PC1	PC2	PC3	PC4	PC5
Item	Hay and water for corn	Concentrate and fibre	Roughage for concentrates	Straw	Milk and hay for corn
Milk replacer (MR) intake, g DM/d[1]	-0.10	-0.09	0.19	-0.04	**0.92**
Feeding frequency of MR, #/d	**-0.77**	-0.13	-0.33	-0.31	-0.07
Water intake, L/d	**0.75**	0.31	-0.22	-0.42	-0.11
Concentrate intake, g DM/d[1]	-0.01	**0.64**	**-0.71**	0.09	-0.21
Hay intake, g DM/d[1]	**0.51**	0.49	0.29	0.00	**0.61**
Corn silage intake, g DM/d[1]	**-0.53**	0.42	0.30	0.01	**-0.63**
Straw intake, g DM/d[1]	-0.03	-0.02	0.19	**-0.94**	0.05
Roughage proportion of solid feed, %	0.08	0.18	**0.94**	-0.16	0.10
Neutral detergent fibre intake, g/d	0.03	**0.99**	0.07	-0.04	-0.05
Eigenvalues	2.53	2.10	1.64	1.09	0.99
Variance explained, %	28	23	18	12	11

months, as suggested by the positive relation between empty rumen weight and both 'Concentrate and fibre' and 'Roughage for concentrates' at 6 months. Increasing SF intake likely improves muscular (fibre) and absorptive (concentrates) capacity of the rumen.

The total number of treatments is in the upper range of therapeutic treatments when compared to reported data for veal calves (Timmerman et al., 2005). In the current study, we distinguished respiratory and gastrointestinal disorders, the two major contributors to the total number of treatments for both dairy calves (Svensson et al., 2003) and veal calves (Pardon et al., 2012). The number of days calves were treated for gastrointestinal disorders was positively related to 'Fibre' at 3 months, while negatively to 'Concentrate and fibre' and 'Milk and hay for corn' at 6 months. The number of days calves were treated for gastrointestinal disorders was positively related to intake of fibre-rich sources at 3 months and negatively related to intake of energy-rich sources at 6 months (MR and concentrate intake). Because the majority of these treatments were applied before 3 months of age (results not shown), these data suggest that calves responded to gastrointestinal disorders by adapting their feed intake pattern. Increased MR feeding was previously shown to

Table 5.5 Multiple regression models with health parameters as dependent variables, and principal components† as independent variables from feed intake related variables

Dependent variable	Independent variable			β	SE (β)	Adjusted R^2	P
	PC	Age, months	Interpretation				
Warm carcass weight, kg	Intercept			151.9	4.03	52.8	
	PC3_3mo	3	Milk	8.0	2.58		***
Empty rumen weight, g	Intercept			4558	141.0	70.1	
	PC2_3mo	3	Fibre	- 86	38.8		**
	PC3_3mo	3	Milk	293	80.5		***
	PC2_6mo	6	Concentrate and fibre	194	54.4		***
	PC3_6mo	6	Roughage for concentrates	121	51.0		**
Days of treatment for gastrointestinal disorders	Intercept			1.00	0.437	28.1	
	PC2_3mo	3	Fibre	0.47	0.273		*
	PC2_6mo	6	Concentrate and fibre	- 0.78	0.362		**
	PC5_6mo	6	Milk and hay for corn	- 0.79	0.314		**
Days of treatment for respiratory disorders	Intercept			1.37	0.183	39.1	
	PC2_3mo	3	Fibre	- 0.28	0.077		***
	PC5_3mo	3	Water	0.42	0.099		***
Area of pyloric damage, cm^2	Intercept			0.33	0.480	22.9	
	PC2_3mo	3	Fibre	0.43	0.186		**
	PC5_3mo	3	Water	0.75	0.322		**
	PC1_6mo	6	Hay and water for corn	0.47	0.228		*
	PC4_6mo	6	Straw	-0.73	0.333		**
	PC5_6mo	6	Milk and hay for corn	-0.43	0.217		*

*$P < 0.10$; **$P < 0.05$; ***$P < 0.01$.

†Principal components (PC) were extracted by principal component analysis (PCA), after varimax rotation, of 9 feed intake related variables. See Table 5.4 for loadings of feed intake related variables on PCs.

increase number of days calves were treated for clinical gastrointestinal disorders in dairy calves during the first 3 months of age (Quigley et al., 2006), although other studies observed no effect of MR intake when considering occurrence of diarrhoea (Jasper and Weary, 2002, Khan et al., 2007). Previously, Coverdale et al. (2004) found no effect of dietary inclusion of hay on diarrhoea scores and general antibiotic use in calves upon weaning, which could indicate that the increased hay and fibre intake at 3 months is a consequence rather than a cause of gastrointestinal disorders.

Respiratory disorders are a relevant health problem in young calves housed indoor (Virtala et al., 1996), especially in calves gathered from different farms at an early stage of life, as in our study. Respiratory disorders are the primary cause of mortality in veal calves (Pardon et al., 2012). The aetiology of respiratory disorders is considered multifactorial (Brscic et al., 2012); important factors include housing system, humidity, ammonia concentration, and innate and adaptive immunity. In the current study, the number of days calves were treated for respiratory disorders was negatively related to 'Fibre' intake at 3 months and positively to 'Water' intake at 3 months. This may indicate that only feed intake related components at 3 months were associated with respiratory disorders, which is in line with the observation that the prevalence of hampered respiration tends to decrease as calves age (Brscic et al., 2012). Furthermore, the associations found suggest that calves with an increased intake of (fibrous) SF show less respiratory abnormalities. Likely, the depressed feed intake was a consequence of the occurrence of health disorders rather than a cause. In line with this idea, it has been shown, for example, that calves reduced the intake of hay, which was provided *ad libitum* on top of a concentrate diet, after an infection with intestinal worms (Verstegen et al., 1988). Thus, the associations found between dietary preferences and health parameters in the current study suggest that individual calves seem to be able to adjust their dietary preferences and intake according to their health status. Alternatively, a particular pattern of dietary preference may have been an underlying cause or risk factor for health disorders among calves. Interestingly, Prevedello *et al.* (2012) found a tendency for a decreased prevalence of respiratory disorders among milk-fed calves fed 20 to 28% of roughage proportion in the SF compared with a full-concentrate diet on top of an identical MR diet, which supports this latter suggestion. Regardless of the underlying mechanism behind the association between measures of feed intake and days of treatment for respiratory or digestive disorders within the calves, the data suggest that calves adapt their dietary preference to health factors.

In conclusion, the current study provided unique insight in the dietary preferences of young calves, given free access to five sources of feeds, including MR, and the associations with health and growth performance parameters. We observed large between-animal variation in dietary preferences. The absence of consistency of dietary preferences between 3 and 6 months of age suggests that dietary preferences of calves alter with age. The associations found between dietary preferences and health parameters in the current

study suggest that individual calves adjust their dietary preferences and intake according to health factors.

ACKNOWLEDGEMENTS

The authors thank the Division of Earth and Life Sciences (ALW) of the Netherlands Organization for Scientific Research (NWO, The Hague, the Netherlands) and the Product Board Animal Feed (The Hague, the Netherlands) for financial support. We thank Bert Beukers (Wageningen University, Wageningen, the Netherlands), Norbert Stockhofe-Zurwieden, Ralph Kok, Pieter Roskam (Central Veterinary Institute, Lelystad, the Netherlands) and Anne Raats for their skilled assistance.

REFERENCES

Ahmed, A. F., P. D. Constable, and N. A. Misk. 2002. Effect of feeding frequency and route of administration on abomasal luminal pH in dairy calves fed milk replacer. Journal of Dairy Science 85(6):1502-1508.

Atwood, S. B., F. D. Provenza, R. D. Wiedmeier, and R. E. Banner. 2001. Influence of free-choice vs mixed-ration diets on food intake and performance of fattening calves. Journal of Animal Science 79(12):3034-3040.

Bartlett, K., F. McKeith, M. VandeHaar, G. Dahl, and J. Drackley. 2006. Growth and body composition of dairy calves fed milk replacers containing different amounts of protein at two feeding rates. Journal of Animal Science 84(6):1454-1467.

Berends, H., J. J. G. C. van den Borne, S. J. J. Alferink, C. G. van Reenen, E. A. M. Bokkers, and W. J. J. Gerrits. 2012a. Low-protein solid feed improves the utilization of milk replacer for protein gain in veal calves. Journal of Dairy Science 95(11):6654-6664.

Berends, H., C. G. van Reenen, N. Stockhofe-Zurwieden, and W. J. J. Gerrits. 2012b. Effects of early rumen development and solid feed composition on growth performance and abomasal health in veal calves. Journal of Dairy Science 95(6):3190-3199.

Breslow, N. E. and D. G. Clayton. 1993. Approximate inference in generalized linear mixed models. Journal of the American Statistical Association 88(421):9-25.

Brown, E., M. VandeHaar, K. Daniels, J. Liesman, L. Chapin, D. Keisler, and M. Nielsen. 2005. Effect of increasing energy and protein intake on body growth and carcass composition of heifer calves. Journal of Dairy Science 88(2):585-594.

Brscic, M., L. F. M. Heutinck, M. Wolthuis-Fillerup, N. Stockhofe-Zurwieden, B. Engel, E. K. Visser, F. Gottardo, E. A. M. Bokkers, B. J. Lensink, G. Cozzi, and C. G. Van Reenen. 2011. Prevalence of gastrointestinal disorders recorded at postmortem inspection in white veal calves and associated risk factors. Journal of Dairy Science 94(2):853-863.

Brscic, M., H. Leruste, L. F. M. Heutinck, E. A. M. Bokkers, M. Wolthuis-Fillerup, N. Stockhofe-Zurwieden, F. Gottardo, B. J. Lensink, G. Cozzi, and C. G. Van Reenen. 2012. Prevalence of respiratory disorders in veal calves and potential risk factors. Journal of Dairy Science 95(5):2753-2764.

Castells, L., A. Bach, G. Araujo, C. Montoro, and M. Terre. 2012. Effect of different forage sources on performance and feeding behavior of Holstein calves. Journal of Dairy Science 95(1):286-293.

Coverdale, J. A., H. D. Tyler, J. D. Quigley Iii, and J. A. Brumm. 2004. Effect of various levels of forage and form of diet on rumen development and growth in calves. Journal of Dairy Science 87(8):2554-2562.

CVB. 2007. Chemical compositions and nutritional values of feed ingredients. Centraal Veevoeder Bureau, Productschap Diervoeder, Den Haag, the Netherlands.

Gottardo, F., S. Mattiello, G. Cozzi, E. Canali, E. Scanziani, L. Ravarotto, V. Ferrante, M. Verga, and I. Andrighetto. 2002. The provision of drinking water to veal calves for welfare purposes. Journal of Animal Science 80(9):2362-2372.

Genstat Committee. 2000. Reference Manual. Procedure Library PL12. R. G. Payne and G. M. Arnold, ed. VSN International, Oxford, UK.

Hill, T. M., H. G. Bateman Ii, J. M. Aldrich, and R. L. Schlotterbeck. 2008. Effects of the amount of chopped hay or cottonseed hulls in a textured calf starter on young calf performance. Journal of Dairy Science 91(7):2684-2693.

Hodgson, J. 1971. The development of solid food intake in calves 4. The effect of the addition of material to the rumen, or its removal from the rumen, on voluntary food intake. Animal Production 13(04):581-592.

Jasper, J. and D. Weary. 2002. Effects of ad libitum milk intake on dairy calves. Journal of Dairy Science 85(11):3054-3058.

Joliffe, I. T. 1986. Principal component analyses. Springer-Verlag, New York.

Khan, M., H. Lee, W. Lee, H. Kim, S. Kim, K. Ki, J. Ha, H. Lee, and Y. Choi. 2007. Pre-and postweaning performance of Holstein female calves fed milk through step-down and conventional methods. Journal of Dairy Science 90(2):876-885.

Marshall, T. S. 2009. Abomasal ulceration and tympany of calves. Veterinary Clinics of North America: Food Animal Practice 25(1):209-220.

Mattiello, S., E. Canali, V. Ferrante, M. Caniatti, F. Gottardo, G. Cozzi, I. Andrighetto, and M. Verga. 2002. The provision of solid feeds to veal calves: II. Behavior, physiology, and abomasal damage. Journal of Animal Science 80(2):367-375.

McCulloch, C. E., S. R. Searle, and J. M. Neuhaus. 2008. Generalized, Linear, and Mixed Models - 2nd Edition. John Wiley & Sons, Inc., NJ, USA.

NRC. 2000. Nutrient Requirements of Beef Cattle. 7th rev. ed. Natl. Acad. Press, Washington, DC.

Pardon, B., K. De Bleecker, M. Hostens, J. Callens, J. Dewulf, and P. Deprez. 2012. Longitudinal study on morbidity and mortality in white veal calves in Belgium. BMC veterinary research 8(1):26.

Prevedello, P., M. Brscic, E. Schiavon, G. Cozzi, and F. Gottardo. 2012. Effects of the provision of large amounts of solid feeds to veal calves on growth and slaughter performance and intravitam and postmortem welfare indicators. Journal of Animal Science 90(10):3538-3546.

Provenza, F. and D. Balph. 1987. Diet learning by domestic ruminants: theory, evidence and practical implications. Applied Animal Behavioral Sciences 18(3):211-232.

Provenza, F. D., C. B. Scott, T. S. Phy, and J. J. Lynch. 1996. Preference of sheep for foods varying in flavors and nutrients. Journal of Animal Science 74(10):2355-2361.

Quigley, J., T. Wolfe, and T. Elsasser. 2006. Effects of additional milk replacer feeding on calf health, growth, and selected blood metabolites in calves. Journal of Dairy Science 89(1):207-216.

Rowe, J., J. Nolan, G. De Chaneet, E. Teleni, and P. Holmes. 1988. The effect of haemonchosis and blood loss into the abomasum on digestion in sheep. British Journal of Nutrition 59(01):125-139.

Sander, E. G., R. G. Warner, H. N. Harrison, and J. K. Loosli. 1959. The stimulatory effect of sodium butyrate and sodium propionate on the development of rumen mucosa in the young calf. Journal of Dairy Science 42(9):1600-1605.

Soberon, F., E. Raffrenato, R. Everett, and M. Van Amburgh. 2012. Preweaning milk replacer intake and effects on long-term productivity of dairy calves. Journal of Dairy Science 95(2):783-793.

Suárez, B. J., C. G. Van Reenen, N. Stockhofe-Zurwieden, J. Dijkstra, and W. J. J. Gerrits. 2007. Effect of roughage source and roughage to concentrate ratio on animal performance and rumen development in veal calves. Journal of Dairy Science 90(5):2390-2403.

Svensson, C., K. Lundborg, U. Emanuelson, and S.-O. Olsson. 2003. Morbidity in Swedish dairy calves from birth to 90 days of age and individual calf-level risk factors for infectious diseases. Preventive Veterinary Medicine 58(3):179-197.

Tamate, H., A. D. McGilliard, N. L. Jacobson, and R. Getty. 1962. Effect of various dietaries on the anatomical development of the stomach in the calf. Journal of Dairy Science 45(3):408-420.

Terré, M., M. Devant, and A. Bach. 2007. Effect of level of milk replacer fed to Holstein calves on performance during the preweaning period and starter digestibility at weaning. Livestock Science 110(1):82-88.

Timmerman, H., L. Mulder, H. Everts, D. Van Espen, E. Van der Wal, G. Klaassen, S. Rouwers, R. Hartemink, F. Rombouts, and A. Beynen. 2005. Health and growth of veal calves fed milk replacers with or without probiotics. Journal of Dairy Science 88(6):2154-2165.

Verstegen, M., W. Hel, G. Albers, and A. Kloosterman. 1988. Effect of trichostrongylid infection on feed intake, metabolic rate and protein gain of calves. Journal of Animal Physiology and Animal Nutrition 60(1-5):209-218.

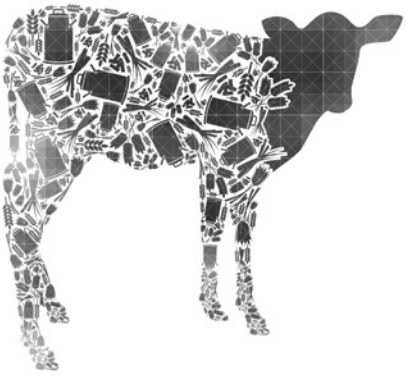
Villalba, J., A. Bach, and I. Ipharraguerre. 2011. Feeding behavior and performance of lambs are influenced by flavor diversity. Journal of Animal Science 89(8):2571-2581.

Villalba, J. J. and F. D. Provenza. 1999. Nutrient-specific preferences by lambs conditioned with intraruminal infusions of starch, casein, and water. Journal of Animal Science 77(2):378-387.

Virtala, A. M. K., G. Mechor, Y. Gröhn, and H. Erb. 1996. The effect of calfhood diseases on growth of female dairy calves during the first 3 months of life in New York State. Journal of Dairy Science 79(6):1040-1049.

Chapter 6

Estimation of milk leakage into the rumen of milk-fed calves through an indirect and repeatable method



E. Labussière[1], H. Berends[2], M.S. Gilbert[2], J.J.G.C. van den Borne[2], and W.J.J. Gerrits[2]

Accepted for publication by Animal

[1]Institut national de la recherche agronomique (INRA), Saint-Gilles, France; [2]Animal Nutrition Group, Wageningen University, Wageningen, the Netherlands

ABSTRACT

In milk-fed calves, quantification of the milk that enters the rumen (**RMV**) due to malfunction of the esophageal groove reflex may explain part of the variability observed between animals in their growth performance. The RMV can directly be quantified by adding an indigestible marker to the diet and measuring its recovery in the rumen at slaughter, but this technique cannot be repeated in time in the same animal. The objective of the study was to evaluate three indirect methods for estimating RMV. The first method was based on the assumption that ruminal drinking delays and limits acetaminophen appearance in blood after ingestion of milk supplemented with acetaminophen. The second method was based on a negative linear relationship between RMV and urinary recovery of non-metabolizable monosaccharides (3-o-methylglucose, L-rhamnose, and D-xylose) added to the milk, due to rumen fermentation. In the third method, RMV was calculated as the difference between total milk intake and the increase in abomasal milk volume (**AMV**) at feeding, measured through ultrasonography shortly after feeding, or estimated from the mathematical extrapolation of AMV to feeding time, based on consecutive measurements. These methods were tested in three experiments where calves (n=22, n=10 and n=13) were bucket-fed, or partly tube-fed (i.e. by inserting milk replacer into the rumen via a tube to mimic ruminal drinking). Additionally, CoEDTA and CrEDTA were used as an indigestible marker in one experiment to trace bucket-fed or tube-fed milk replacer, respectively to measure RMV. The relationship between AMV measured by ultrasonography and AMV measured at slaughter improved when kinetics of AMV were extrapolated to the time of slaughter by mathematical modeling (error between predicted and measured AMV equaled 0.49 L). With this technique, RMV during feeding averaged 17 and 24% of intake in experiments 2 and 3, respectively. Plasma acetaminophen kinetics and recovery of non-metabolizable monosaccharides in urine were partly associated with ruminal drinking but these techniques are not considered quantitatively accurate without further information of rumen degradation and absorption. The recovery of indigestible marker measured at slaughter gave a quantitative estimate of RMV (2% in experiment 3), but improper measurement of emptying rate of fluid from the rumen may lead to underestimation. In conclusion, measuring changes in AMV by ultrasonography in response to milk feeding was the most promising indirect method to quantify RMV in veal calves.

Keywords: calf, milk replacer, ruminal drinking, ultrasonography, abomasum size.

INTRODUCTION

In calves, milk replacer (**MR**) by-passes the rumen and enters the abomasum directly due to closure of the esophageal groove (Guilhermet et al., 1975). It is therefore commonly accepted that digestive processes in milk-fed calves resemble those in true monogastric animals. Nonetheless, even in calves that are not clinically identified as ruminal drinkers, considerable amounts of MR may enter the rumen (called "ruminal milk"; up to 25% of milk intake, Suárez et al., 2007), which may induce ruminal and metabolic acidosis in a clinical case (Gentile et al., 2004, Herrli-Gygi et al., 2008). Due to fermentation of nutrients from MR in the rumen, ruminal drinking will decrease nutrient availability and the efficiency of nutrient utilization for protein and fat retention (Armstrong, 1969, Herrli-Gygi et al., 2006), hence reducing growth performance in calves. Therefore, identifying ruminal drinkers and quantifying the volume of MR that leaks into the rumen (**RMV**) in non-clinical ruminal drinkers is of importance in nutritional practice and in nutritional studies, and is required to identify age related developments, within and between animal variation and risk factors in the occurrence of RMV.

Quantification of RMV requires measuring the volume of MR which is recovered in the rumen after feeding. This can be achieved by providing a soluble indigestible marker with the last feeding before slaughter, and then measuring marker recovery in the calf's rumen at slaughter (Suárez et al., 2007, Berends et al., 2012). This direct measurement requires quantitative collection of rumen contents, and does not allow repeated measurements on the same animal. Furthermore; it may be biased by post-mortem equilibration of hydrostatic pressure. Repeatable and less-invasive methods to identify ruminal drinkers have also been proposed, such as the acetaminophen absorption test or imaging techniques. The acetaminophen absorption test has been successfully used to identify, but not to quantify, ruminal drinking in lambs and calves (Schaer et al., 2005, Herrli-Gygi et al., 2008, Sharifi et al., 2009). This test involves provision of acetaminophen with MR and assumes that the appearance of acetaminophen in blood is delayed by ruminal drinking (Herrli-Gygi et al., 2008). In addition, it is assumed that acetaminophen cannot be absorbed by the rumen and abomasum, but is absorbed quickly from the proximal intestinal lumen. Although the mechanisms and sites of acetaminophen absorption are poorly documented, the delay in absorption (time to reach maximal concentration, T_{max}) or the area under the plasma concentration curve (area under the curve; **AUC**) may relate to the RMV (Schaer et al., 2005, Herrli-Gygi et al., 2008). Imaging techniques of the digestive tract can also be used to quantify transit of digesta in the gastrointestinal tract. For instance, ultrasonography has been proposed as a method to identify ruminal drinkers (Braun and Gautschi, 2013) and it offers the opportunity to measure abomasal volume (**AMV**) in calves (Wittek et al., 2005). This may allow quantification of RMV by subtracting the increase in AMV after feeding from total milk volume intake. Until now, studies that estimated AMV from ultrasonography have been conducted in calves younger than 50 days, whereas the age of

veal calves in fattening stage may reach 240 days. It is currently not known if ultrasonography can also be used for measuring AMV accurately in heavy calves. In addition to these methods, the urinary recovery of a pulsed dose of a non-metabolizable monosaccharide could be used to quantify RMV. To account for differences in clearance time, 3-O-methyl-glucose – **3-O-MG**, L-rhamnose – **L-R**, and D-xylose – **D-X** in MR can be used as potential candidates. These monosaccharides are added to the MR, absorbed by different pathways (Wijtten et al., 2011) but not metabolized by the calf and thus excreted in the urine. Recovery of these monosaccharides in urine should therefore be close to 100% when MR bypasses the rumen. Ruminal drinking, however, will result in microbial degradation of these monosaccharides in the rumen, thereby reducing urinary recovery. The objective of the current study was to evaluate these three indirect, repeatable methods to quantify RMV in veal calves, and to compare these methods to the direct method, i.e. recovery of indigestible markers in the rumen at slaughter.

MATERIAL AND METHODS

All procedures were in agreement with the Dutch Law on Experimental Animals, which complies with ETS123 (Council of Europe 1985 and the 86/609/EEC Directive) and were approved by the Animal Care and Use Committee of Wageningen University.

Experimental Design

Three experiments were designed to evaluate indirect methods for measuring RMV in veal calves, and to compare the most promising indirect method with the direct method in which an indigestible marker added to the MR is recovered in the rumen at slaughter. In Experiment 1, effects of induced ruminal milk on kinetic parameters of blood acetaminophen concentration were studied. Milk replacer was introduced in the rumen of calves (hereafter referred to as tube milk) or fed via a bucket (hereafter referred to as bucket milk), and kinetics of blood acetaminophen were assessed after tracing either the tube milk or the bucket milk, or both (Table 6.1). In Experiment 2, effects of an induced contrast in ruminal milk on changes in AMV (ultrasonography), abomasal emptying (acetaminophen absorption test), and urinary recovery of non-metabolizable monosaccharides in urine were studied (Table 6.2). Based on the results of Experiments 1 and 2, the most promising indirect method was selected and compared with the direct method of indigestible marker recovery in the calf's rumen at slaughter (Suárez et al., 2007), which is considered to be the 'gold standard', in Experiment 3.

Experiment 1. The objective was to determine the effects of induced ruminal milk on kinetics of plasma acetaminophen concentration when tracing either bucket milk, tube milk or both by acetaminophen (Table 6.1). Measurements were conducted in 24 calves (mean BW: 160.8 ± 6.7 kg) housed individually and allocated to one of four dietary

treatments. In treatment 1A, a whey-based MR was provided in a bucket (without nipple), assuming that the vast majority of MR from the bucket directly enters the abomasum. In treatments 1B, 1C and 1D, 50% of the MR was provided via a bucket, and 50% was provided directly into the rumen of the calves via a tube that was inserted in the esophagus unto the rumen (length of the tube : 150 cm), after the calf finished drinking MR from the bucket. Acetaminophen (Sigma Aldrich, 50 mg/kg BW) was dissolved in boiling water and mixed with the MR before feeding. It was offered with the bucket milk for treatments 1A and 1D, with the tube milk for treatment 1C, or divided equally over the bucket and the tube milk for treatment 1B (Table 6.1). The total amount of liquid MR equaled 6.8 kg/calf, which was prepared by dissolving a standard commercial MR in hot water at 65°C (147 g of powder/kg of liquid). It was offered to the calves at a temperature of about 42°C. Energy allowance was 850 kJ of digestible energy (**DE**) per kg $BW^{0.75}$ per d. Calves were not offered solid feed during the experiment. Blood samples were taken by venipuncture from the jugular vein at 30 min before and at 30, 60, 90, 120, 150, 180, 300 and 420 min after feeding. Plasma was harvested and stored at -20°C. Two calves on treatment 1A were excluded because they showed clinical signs of ruminal drinking (clay-like feces and abdominal distension; Breukink et al., 1988), hence data from 22 calves were considered in the analyses.

Experiment 2. The objective was to determine the effects of induced ruminal milk on AMV estimated from ultrasonography, recovery of non-metabolizable monosaccharides (3-O-MG, L-R, and D-X) in urine and kinetics of plasma acetaminophen. In addition, the predictive quality of these variables for RMV was evaluated through correlation analysis. Measurements were conducted in 10 calves (mean BW: 203 ± 11 kg) that were housed individually in metabolic cages and allocated to one of two treatments. Calves received 100% of the MR via a bucket without nipple (treatment 2A; 5 calves; Table 6.2) or 50% of the MR via a bucket and 50% via a tube (treatment 2B; 5 calves). Acetaminophen (50 mg/kg BW) and monosaccharides (46 mg/kg $BW^{0.75}$ of 3-O-MG, 103 mg/kg $BW^{0.75}$ of D-X and 218 mg/kg $BW^{0.75}$ of L-R) were dissolved in boiling water, mixed with the MR before feeding, and offered to the calves via bucket and tube according to treatments. A catheter was inserted in the jugular vein to allow frequent blood sampling and the abdominal site (from the xiphoid process to the navel) was shaved to allow ultrasonography. The calves originated from another experiment, where they were fed 1064 kJ DE/kg $BW^{0.75}$ per d, using two whey-based MR differing in fat and lactose content. Calves were divided over treatments so that experimental diets were equally divided over treatments. The powder was dissolved in hot water at 65°C (174 g of powder/kg of liquid) and offered to the calves at a temperature of 43°C. Total liquid MR allowance averaged 8.1 kg/calf. The calves had no access to solid feed during the experiment.

Blood samples were taken at 30 min before and at 30, 60, 90, 120, 150, 180, 300, 420 and 600 min after feeding via the jugular vein catheter. Plasma was harvested and stored at -20°C pending analysis. Width, height and length of the abomasum were measured by

ultrasonography (3.5 MHz ultrasound linear transducer, MyLab 30, Pie Medical) after local application of a gel to the skin of the abdominal site (from the xiphoid process to the navel; Wittek et al., 2005). Measurements were conducted three times during the first hour after feeding and once per hour during three subsequent hours. All dimensions were measured in duplicate for each timepoint. Urine was collected quantitatively for 24 h after feeding, weighed, sampled, and stored at -20°C.

Experiment 3. The objective was to compare the most promising indirect method from Experiments 1 and 2 with the direct method, i.e. indigestible marker recovery in the rumen at slaughter (Suárez et al., 2007). From Experiments 1 and 2, ultrasonography was considered the best indirect method for estimating RMV (see Results), and was therefore included in Experiment 3. The experiment included 16 calves that were housed individually in metabolic cages and allocated to one of four treatments (n=4 per treatment) for the meal preceding slaughter. In treatments 3A, 3C and 3D, calves received 100% of their MR in a bucket without nipple (Table 6.4). In treatment 3B, calves received 75% of their MR in a bucket without nipple and the remaining 25% was directly introduced into the rumen via a tube. In all treatments, calves received 36 g of CoEDTA with the bucket milk. In addition, calves at treatment 3B received on average 821 g of CrEDTA solution (5 mg of Cr/g) with the tube milk. Calves on treatments 3A and 3B were slaughtered at 121 and 124 min after feeding, respectively and calves on treatments 3C and 3D were slaughtered at 248 and 365 min after feeding, respectively. The calves were fed the same MR that were used in Experiment 2. Milk replacer was offered to the calves at a concentration of 167 g of powder/kg of liquid at a temperature of 43°C so that the daily energy intake was 1064 kJ DE/kg $BW^{0.75}$ (calves received 9.8 kg of liquid MR on average). Calves had no access to solid feed during the experiment.

From feeding to slaughter, kinetics of abomasal sizes were determined by ultrasonography, with three measurements during the first hour after feeding and then once per hour until slaughter. After transportation (approximately 5 min), calves were euthanized by an intravenous injection of Na-pentobarbital and lifted by the forelegs (Suárez et al., 2007). The digestive tract was divided into three compartments (forestomachs, abomasum, and small intestine plus colon) that were closed using collars before removing the digestive tract from the body. The content of each compartment was collected quantitatively, weighed, homogenized, sampled, and stored for laboratory analyses. Because three calves (two from treatment 3B and one from treatment 3D) refused to drink their MR, measurements were conducted on 13 calves (mean BW: 237.7 ± 24.1 kg; 2 to 4 calves per treatment; Table 6.4).

Chemical Analyses

The density of each liquid MR was measured and, for calculation of AMV, assumed to be similar in the abomasum. Plasma acetaminophen concentration was measured by colorimetry (kit K8002, Cambridge Life Sciences, Ely, Cambs, UK). The concentration of

3-O-MG, D-X, and L-R in urine was determined by gas chromatography employing flame ionization for detection and D-glucoheptose as an internal standard (Jansen et al., 1986). The dry matter (**DM**) content in the digestive contents was determined according to standard methods (AOAC, 1990). The Cr and Co concentrations in the contents from each gastrointestinal compartment were measured by atomic spectroscopy after acid hydrolysis (Williams et al., 1962).

Calculations

The AMV in the shape of an ellipsoid was calculated from the height, width, and length of the abomasum as: height × width × length × $\pi/6$ (Wittek et al., 2005). Two calculations were used for estimating RMV. First, RMV was determined (**RMV1**) by the difference between the volume of MR provided through the bucket and the first measurement of AMV after feeding (on average 16 and 12 min after feeding for Experiments 2 and 3, respectively). For tube-fed calves, the volume of MR provided via the tube was added to RMV1. Second, the kinetics of AMV were fitted by a compartmental model to determine which proportion of the MR provided via the bucket enters the rumen because of leakage during drinking. The model consists of two compartments, representing rumen and abomasum. The model assumes that the rumen compartment fills at feeding with all the MR provided via the tube and a proportion (x) of the MR provided via the bucket due to leakage during drinking. The rumen compartment then empties into the abomasum at a constant fractional rate. The abomasum compartment fills with a proportion (1-x) of MR provided via the bucket directly through the esophageal groove plus the liquid originating from rumen emptying. The liquid in the abomasum then empties at a constant fractional rate. Ordinary differential equations were solved using the package deSolve in the R software (Soetaert et al., 2010) and parameters of the model (x: proportion of the MR provided via the bucket that entered in the rumen due to leakage during drinking and constant fractional emptying rates from the rumen and the abomasum) were estimated for each calf to minimize the sum of squared differences between actual and predicted AMV (Nelder and Mead, 1965). Volumes of the contents of the rumen and abomasum before feeding were based on volumes measured at slaughter in Experiment 3 and equaled 9300 and 100 mL, respectively for Experiment 2 and 10000 and 100 mL, respectively for Experiment 3. The RMV was then calculated (**RMV2**) as the sum of the MR provided via the tube and the proportion of MR provided via the bucket which leaked into the rumen (x). In Experiment 3, the AMV at slaughter (**AMVs**) was calculated as the last measurement of volume by ultrasonography before slaughter (**AMV1**) or by extrapolating the kinetics of AMV to the time of slaughter (**AMV2**). Because of an insufficient number of successful measurements, parameters of the model could not be estimated on 2 and 4 calves in Experiments 2 and 3, respectively. In Experiment 3, abomasal volume at slaughter (**AMV3**) was also calculated from Co recovery in the abomasum, assuming that the concentration of Co in the MR remained constant after feeding.

In Experiments 1 and 2, the increase in plasma acetaminophen concentration after feeding was calculated assuming that the baseline equaled the plasma acetaminophen concentration measured at 30 min before feeding. The urinary recovery of 3-o-MG, D-X, and L-R was calculated as the proportion of monosaccharide intake which was excreted in urine during the 24-h collection period after feeding.

Statistics

Experiment 1. For each calf, kinetics of plasma acetaminophen concentration in time after feeding were described by a Michaelis-Menten model (Lopez et al., 2000) using PROC NLIN of SAS (2004) with a scale parameter (**a**). The model estimated the maximal acetaminophen concentration ($\mathbf{C_{max}}$), the time at which C_{max} was obtained ($\mathbf{T_{max}}$), the ratio between C_{max} and T_{max}, and the area under the concentration curve until 420 min after feeding ($\mathbf{AUC_{420}}$):

$$Acetaminophen\ concentration = \frac{C_{max} \times T_{max}}{a-1} \times \frac{a \times time^{a-1}}{\frac{T_{max}{}^{a}}{a-1} + time^{a}}$$

The mean squared predictive error equaled 21% of the mean acetaminophen concentration. Parameters of the blood acetaminophen concentration response curve were analyzed for a treatment effect using PROC GLM of SAS (2004).

Experiment 2. Kinetics of plasma acetaminophen concentrations in time were analyzed using the model described for Experiment 1. The mean squared predictive error equaled 24% of the mean acetaminophen concentration. The AUC was calculated for 100, 420, and 600 min after feeding ($\mathbf{AUC_{100}}$, $\mathbf{AUC_{420}}$, and $\mathbf{AUC_{600}}$, respectively).

Data from kinetics of plasma acetaminophen concentration, RMV1, RMV2, and monosaccharide recoveries were analyzed for a treatment effect using PROC GLM of SAS (2004). Additionally, a principal component analysis was performed using the package FactoMineR (Husson et al., 2008), and including the data on kinetics of plasma acetaminophen concentration and monosaccharide recoveries, considering RMV1 and RMV2 as supplementary quantitative variables. A linear relationship between RMV1 or RMV2 and kinetic parameters of blood acetaminophen concentration and monosaccharide recoveries was tested using PROC GLM of SAS (2004).

Experiment 3. Data from marker recovery were analyzed for the effect of treatment using PROC GLM of SAS (2004). Predicted AMV1 and AMV2 were compared with the actual AMVs, considering a linear relationship (PROC GLM; SAS, 2004).

RESULTS

Experiment 1

The increase in plasma acetaminophen concentration after acetaminophen intake is shown in Figure 6.1. The C_{max} tended to be the lowest when acetaminophen was partly or totally fed with tube milk (treatments 1B and 1C; 15.3 mg/L on average) and the highest when acetaminophen was fed with bucket milk (21.5 mg/L; $P = 0.08$; Table 6.1). Time to reach C_{max} was affected by treatment ($P < 0.01$) and was lowest when all acetaminophen was fed with half of the milk via the bucket (116 min; treatment 1D) and highest when all acetaminophen was fed with half of the milk via the tube (280 min; treatment 1C). The ratio between C_{max} and T_{max} is indicative for the slope of the ascending phase of the kinetics and gives an indication of the rate of acetaminophen appearance.

Table 6.1 Effect of route of administration (bucket vs. intraruminal tube) of acetaminophen and milk replacer on kinetic parameters of plasma acetaminophen concentration in milk-fed calves (Experiment 1)

	Treatment					
	1A	1B	1C	1D	Rsd[1]	P
Number of calves	4	6	6	6		
Intake of milk replacer (kg/calf)						
Bucket	6.8	3.4	3.4	3.4		
Tube	-	3.4	3.4	3.4		
Acetaminophen (mg/kg BW)						
Bucket	50	25	-	50		
Tube	-	25	50	-		
C_{max}[1] (mg/L)	16.8	16.0	14.5	21.5	4.5	0.08
T_{max}[1] (min)	167[x]	131[x]	280[w]	116[x]	59	<0.01
Ratio C_{max}/T_{max} (mg/L per min)	0.10[x]	0.12[x]	0.08[x]	0.23[w]	0.08	0.03
AUC_{420} (g/L during 420 min)	5.75[wx]	5.02[x]	4.67[x]	6.67[w]	1.08	0.02

[1] Rsd: residual standard deviation; C_{max}: maximum plasma acetaminophen concentration; T_{max}: time to reach maximum plasma acetaminophen concentration; AUC_{420}: area under the concentration curve between 0 and 420 min after feeding.

[wxy] Within each row, means with different subscripts differ ($P < 0.05$).

This ratio was higher ($P = 0.03$) for treatment 1D than for the other treatments (0.23 vs 0.10 mg/L per min on average for treatments 1A, 1B and 1C). The AUC_{420} increased ($P = 0.02$) from 4.85 g/L on average for treatments 1B and 1C (all or half of the acetaminophen with half of the milk fed via the tube) to 6.67 g/L for treatment 1D (all the acetaminophen with half of the milk fed via the bucket).

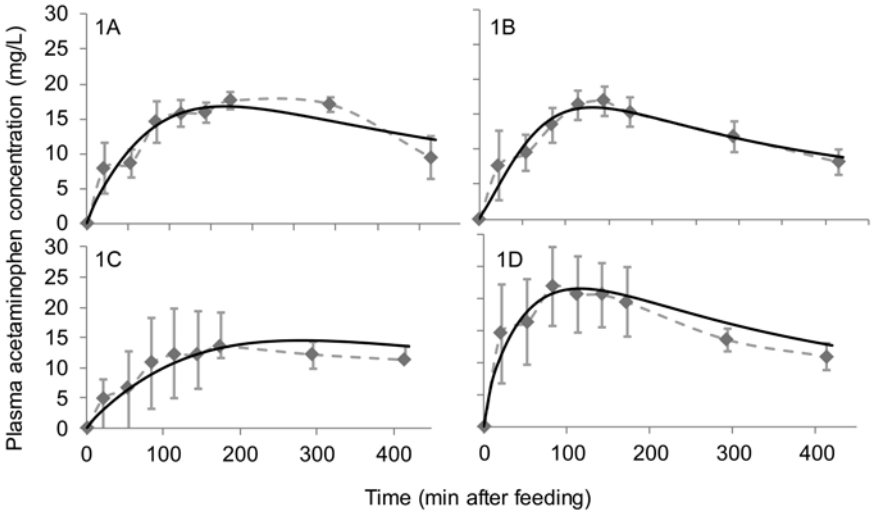


Figure 6.1 Effect of route of administration (bucket vs. intraruminal tube) of acetaminophen and milk replacer on kinetics of plasma acetaminophen concentration (Experiment 1)[a]

[a] 1A: milk replacer and acetaminophen were provided via bucket; 1B: milk replacer and acetaminophen were equally provided via bucket and via tube; 1C: milk replacer was equally provided via bucket and via tube, acetaminophen was provided via tube; 1D: milk replacer was equally provided via bucket and via tube, acetaminophen was provided via bucket. Dotted line: mean measured plasma acetaminophen concentration per treatment (n = 4 for treatment 1A and n = 6 for treatments 1B, 1C and 1D). Solid line: predicted plasma acetaminophen concentration by the model of Lopez et al. (2000) using mean parameters per treatments (see Table 6.1 for details).

Experiment 2

Estimated RMV1 (from the first ultrasonography measurement of abomasal volume) increased from 1.87 to 5.31 L ($P < 0.01$; treatments 2A and 2B, respectively; Table 6.2), whereas estimated RMV2 (from mathematical modeling of the kinetics of abomasal volume after feeding) increased from 1.37 to 6.62 L ($P < 0.01$; treatments 2A and 2B, respectively) when ruminal drinking was simulated by tube-feeding. The modified Michaelis-Menten equation used to model the increase in plasma acetaminophen concentration after feeding indicated a decrease in C_{max} when RMV increased ($P = 0.05$), but the T_{max} and the ratio C_{max}/T_{max} did not differ between treatments. The AUC did not differ between treatments from 0 to 100 min after feeding but it was 18% lower in treatment 2B than in treatment 2A ($P < 0.01$) when the integral duration equaled 420 or 600 min. The average 24-h recoveries of non-metabolizable monosaccharides (3-O-MG,

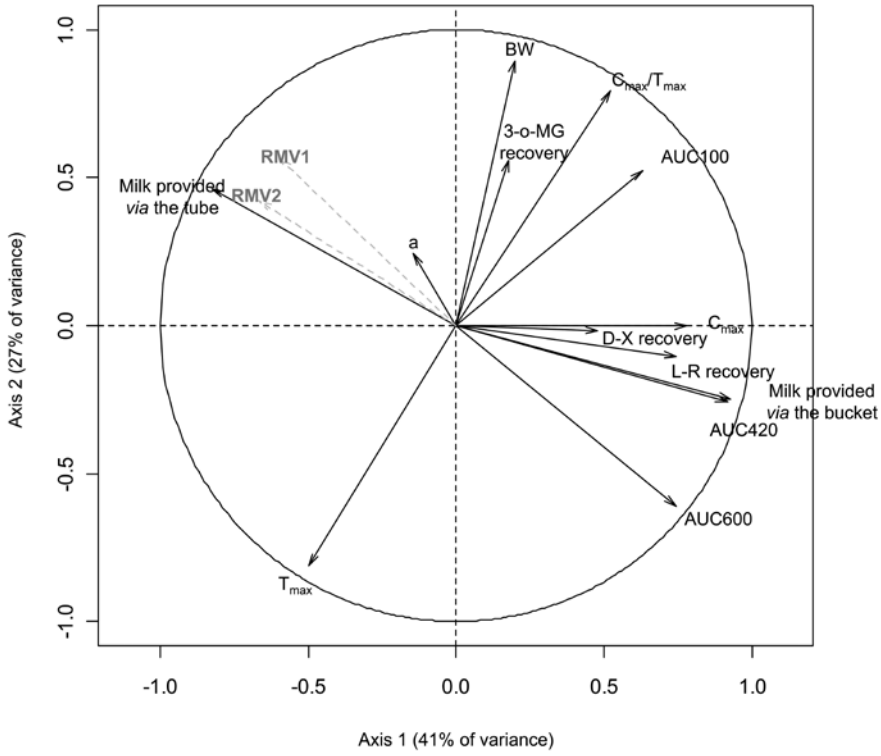


Figure 6.2 Principal component analysis between body weight (BW), volume of milk replacer provided via the bucket or the tube, estimated ruminal milk volume (RMV1 and RMV2; see text for details), 3-*O*-methylglucose (3-o-MG), D-xylose (D-X), and L-rhamnose (L-R) recoveries in urine and kinetics of blood acetaminophen concentration in milk-fed calves (a: scale parameter; C_{max}: maximal plasma acetaminophen concentration; T_{max}: time to reach C_{max}; $AUC_{100\text{-}600}$: area under the curve of plasma acetaminophen concentration between 0 and 100-600 min after feeding; Experiment 2).

D-X and L-R) did not exceed 25% of intake. The 24-h recoveries of 3-O-MG and D-X were not affected by treatment, whereas the 24-h recovery of L-R decreased when ruminal drinking was simulated by tube-feeding (7.9 vs 13.8%, respectively; $P = 0.01$).

Data from individual calves in Experiment 2 were also included in a principal component analysis (Figure 6.2), in which the first and the second principal components of the analysis explained 41 and 27% of total variation, respectively. In accordance with the experimental design, the first axis of the analysis was positively correlated with the volume of milk

Table 6.2 Effects of route of administration (bucket vs. intraruminal tube) of milk replacer on ruminal milk volume, kinetic parameters of plasma acetaminophen concentration and recoveries of 3-O-methylglucose, D-xylose, and L-rhamnose in 24h urine of milk-fed calves (Experiment 2)

	Treatment			
Variable	**2A**	**2B**	**Rsd[1]**	**P**
Number of calves	5	5		
Intake of milk replacer (kg/calf)				
Bucket	8.1	3.9		
Tube	-	4.2		
Acetaminophen (mg/kg BW)				
Bucket	51	25		
Tube	-	25		
Non-metabolizable monosaccharides (mg/kg $BW^{0.75}$)				
Bucket	372[2]	182[3]		
Tube	-	182[3]		
RMV1[1] (L)	1.87	5.31	1.50	<0.01
RMV2[1,4] (L)	1.37	6.62	1.54	<0.01
C_{max}[1] (mg/L)	16.6	14.5	1.4	0.05
T_{max}[1] (min)	174	187	63	0.75
Ratio C_{max}/T_{max} (mg/L per min)	0.10	0.09	0.03	0.77
AUC_{100}[1] (g/L)	0.81	0.69	0.20	0.38
AUC_{420}[1] (g/L)	5.52	4.53	0.31	<0.01
AUC_{600}[1] (g/L)	7.34	6.05	0.42	<0.01
24-h recovery (% of intake)				
3-*O*-methylglucose[4]	17.7	20.4	13.5	0.77
D-xylose	24.5	15.9	9.7	0.20
L-rhamnose	13.8	7.9	3.0	0.01

[1] Rsd: residual standard deviation; RMV1: ruminal milk volume estimated from the first measurement of abomasum volume by ultrasonography; RMV2: ruminal milk volume estimated from the kinetics of abomasum volume; Cmax: maximum plasma acetaminophen concentration; Tmax: time to reach maximum plasma acetaminophen concentration, $AUC_{100\text{-}600}$: area under the curve of plasma acetaminophen concentration between 0 and 100, 420 or 600 min after feeding.

[2] Composition: L-rhamnose, 59.4%; D-xylose, 28.2%; and 3-*O*-methylglucose, 12.4%.

[3] Composition: L-rhamnose, 59.3%; D-xylose, 28.0%; and 3-*O*-methylglucose, 12.6%.

[4] Data from one calf per treatment were missing for RMV2; data from one calf on treatment 2B were missing for 3-*O*-methylglucose recovery; see text for details.

provided via the bucket (R = 0.93) and negatively correlated with the volume of milk provided via the tube (R = -0.83). Additionally, the first axis was correlated ($P < 0.05$) with AUC_{420} (R = 0.92), C_{max} (R = 0.78), L-R recovery (R = 0.75), AUC_{600} (R = 0.75), and RMV2 (R = -0.67). Furthermore, the first axis tended to be correlated with RMV1 (R = -0.60; P = 0.07). The second axis was correlated ($P < 0.05$) with BW (R = 0.89), the ratio C_{max}/T_{max} (R = 0.79) and T_{max} (R = -0.81). The results of the principal component analysis were used to select possible candidates for predicting RMV1 and RMV2. The slopes of the relationships between RMV1 or RMV2 and AUC_{420}, AUC_{600}, or L-R recovery were all negative (Table 6.3) whereas the slopes of the relationship between RMV1 or RMV2 and C_{max} did not significantly differ from 0 (data not shown). Based on the residual standard deviation, the correlation of RMV1 was better than the correlation of RMV2, irrespective of the predictor. The best predictor for RMV1 or RMV2 was AUC_{600}, which resulted in the lowest residual standard deviation (1.83 and 1.88 L for prediction of RMV1 and RMV2, respectively).

Table 6.3 Linear relationships between parameters of the plasma acetaminophen response curve and L-rhamnose recovery in urine with ruminal milk volume in (Experiment 2, 8 calves)

	Prediction of RMV1[1,2]			Prediction of RMV2[1,2]		
Predictor	**Intercept**	**Slope**	**Rsd[1]**	**Intercept**	**Slope**	**Rsd[1]**
AUC_{420}[1]	16.42*	-2.58†	2.12	24.05*	-3.97*	2.17
AUC_{600}[1]	20.10**	-2.46*	1.83	27.58**	-3.47*	1.88
L-rhamnose recovery (% of intake)	7.75**	-414*	1.84	9.35**	-507*	2.34

[1] Rsd: residual standard deviation; RMV1: ruminal milk volume estimated from the first measurement of abomasum volume by ultrasonography (L); RMV2: ruminal milk volume estimated from the kinetics of abomasum volume (L); $AUC_{420\text{-}600}$: area under the curve of plasma acetaminophen concentration between 0 and 420 or 600 min after feeding (mg/L).

[2] The intercept and slope of each equation were tested for their difference with 0: †$P < 0.10$; *$P < 0.05$; **$P < 0.01$.

Experiment 3

The volume of milk that entered the rumen did not differ between treatments and averaged 2762 mL when calculated from the first ultrasonography measurement (RMV1) and 2337 mL when calculated from extrapolation of mathematical modeling of the kinetics of AMV (RMV2; Table 6.4). When CoEDTA was added to the milk offered to the calves via the bucket during the last meal before slaughter, Co recovery in the abomasum was highest at 121 min after feeding (treatment 3A; 64.7% of intake; Table 6.4) and lowest at 365 min after feeding (treatment 3D; 6.0% of intake; $P < 0.01$). In the rumen, Co recovery did not differ between treatments and showed large variations. In the small intestine and colon, the Co recovery increased from 34.1% (treatment 3A) to 90.6% (treatment 3D). In two calves, Cr recovery was measured after administrating CrEDTA into the rumen

Table 6.4 Effects of route of administration (bucket vs intraruminal tube) of milk replacer and indigestible markers (CoEDTA and CrEDTA) and interval between feeding and slaughter on ruminal milk volume and marker recoveries in segments of the digestive tract of milk-fed calves (Experiment 3)

	Treatment					
	3A	**3B**	**3C**	**3D**	**Rsd[1]**	**P**
Number of calves	4	2	4	3		
Interval feeding to slaughter (min)	121	124	248	365		
Intake of milk replacer (kg/calf)						
Bucket	9.1	8.0	9.9	9.9		
Tube	-	2.7	-	-		
Markers with feeding before slaughter (g/calf)						
CoEDTA in bucket	36	36	36	36		
CrEDTA in tube[2]	-	821	-	-		
RMV1[1] (L)	2.59	4.89	2.36	2.11	1.79	0.38
RMV2[1,3](L)	0.47	3.84	2.75	2.63	0.97	0.10
Co recovery (% of intake)						
Rumen	1.6	2.4	0.8	2.4	2.2	0.77
Abomasum	64.7^{w}	31.9^{x}	21.8^{xy}	6.0^{y}	11.8	<0.01
Small intestine + colon	34.1^{y}	58.8^{x}	70.8^{x}	90.6^{w}	11.4	<0.01
Cr recovery (% of intake)[4]						
Rumen		87.7±11.5				
Abomasum		0.4±0.1				
Small intestine + colon		6.2±8.2				

[1] Rsd: residual standard deviation, RMV1: ruminal milk volume estimated from the first measurement of abomasal volume by ultrasonography; RMV2: ruminal milk volume estimated from the kinetics of abomasal volume.
[2] Solution of CrEDTA at 5 mg Cr/g.
[3] Data from two calves on treatment 3A and one calf on treatment 3B and 3D were missing; see text for details.
[4] Values are means ± standard deviation for two calves.
^{wxy} Within each row, means with different subscripts differ ($P < 0.05$).

(treatment 3B). At slaughter (124 min after feeding), 87.7% of Cr intake was recovered in the rumen, and 0.4 and 6.2% of Cr intake was recovered in the abomasum and the small intestine plus colon, respectively (Table 6.4). The AMV estimated from ultrasonography measurements (AMV1 and AMV2) were regressed against abomasal volume measured at slaughter (AMVs, Figure 6.3). The intercept and the slope of the linear relationships between AMV1 or AMV2 and AMVs did not significantly differ from zero and unity, respectively. However, the intercept of the linear relationship between AMV3 and AMVs

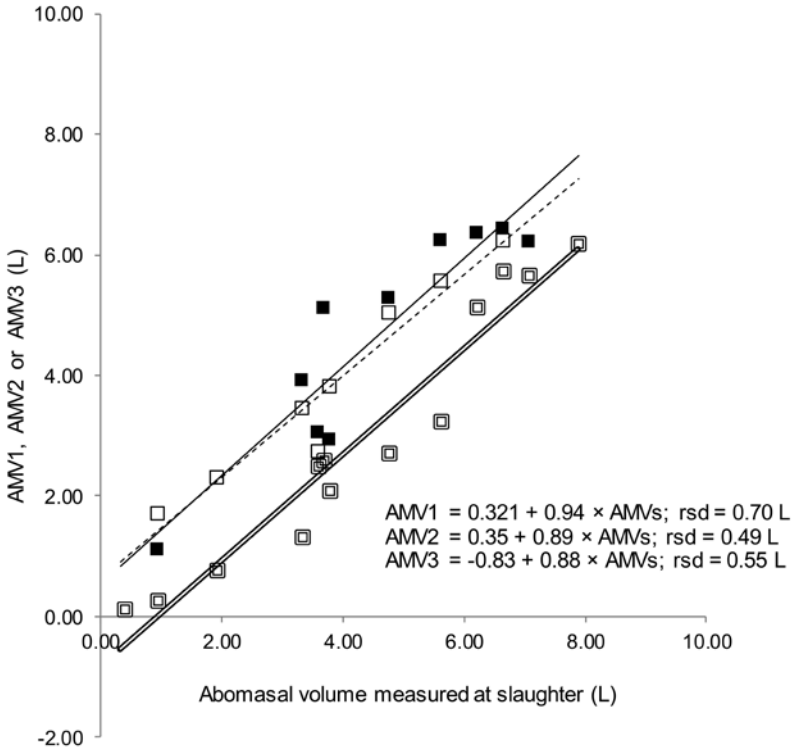


Figure 6.3 Relationship between abomasal volume estimated from ultrasonography (AMV1: ■ and AMV2: □ – 8 calves) or from indigestible marker recovery (AMV3: ⧈ – 13 calves; see text for details) and abomasal volume measured at slaughter (AMVs; Experiment 3) in milk-fed calves. Solid line: linear relationship between AMV1 and AMVs. Dotted line: linear relationship between AMV2 and AMVs. Double line: linear relationship between AMV3 and AMVs: the intercept differed from zero (P = 0.03) and slope tended to differ from unity (P = 0.10).

was significantly lower than zero (P = 0.03) and the slope of the relationship tended to differ from unity (P = 0.10). The residual standard deviation for the relationships between AMVs and AMV1, AMV2, or AMV3 equaled 608, 442 or 551 mL, respectively.

DISCUSSION

The aim of this study was to propose a method to quantify RMV in calves, which is defined as the volume of milk that enters the rumen due to leakage of milk during drinking or due to backflow of milk from the abomasum. In veal calves that received large amounts of MR, individual values may account for more than 25% of intake in subclinical cases (Suárez et al., 2007, Berends et al., 2012), which induces reduced growth performances and ruminal and metabolic acidosis in clinical cases (Gentile et al., 2004, Herrli-Gygi et al., 2006). Identification of clinical ruminal drinkers can be achieved through the occurrence of reduced appetite and clay-like feces (Breukink et al., 1988) but the latter indicators do not give any information regarding the amount of milk that enters the rumen. Nevertheless, both in clinical and subclinical cases nutrients from milk are subject to ruminal fermentation and are metabolized in and absorbed by the rumen instead of being absorbed from the intestinal lumen, which decreases utilization of nutrients for growth (Herrli-Gygi et al., 2006). Accordingly, the effects of ruminal drinking on the metabolism of calves can vary depending on the proportion of ruminal milk, which was highly variable in our study (Table 6.2 and Table 6.4). A repeatable and non-invasive quantification of RMV is then required to explain variation between animals in metabolic studies, or to validate methods to reduce ruminal drinking.

Ultrasonography as a Tool to quantify RMV

In this paper, we propose to calculate RMV as the difference between milk intake and volume of milk that is recovered in the abomasum after drinking. This method allows calculating the leakage of milk during drinking but does not account for the backflow of milk from the abomasum after feeding, at least with RMV1. Nevertheless, no evidence of backflow has been reported in calves (Guilhermet et al., 1975) and the occurrence of milk in the rumen can be largely attributed to malfunction of the esophageal groove. Ultrasonography offers the opportunity to measure AMV, assuming that the filled abomasum takes the shape of an ellipsoid (Wittek et al., 2005). A good estimation of milk leakage during drinking then requires the measurement of AMV shortly after feeding (less than 3 min postprandial; Wittek et al., 2005) because 30% of ingested liquid may have already be emptied from the abomasum during the first 30 min after feeding (measured as duodenal appearance of liquid 30 min after feeding; Toullec et al., 1971). This appears difficult to realize because ultrasonography needs physical restriction of the calf and local application of an ultrasonography gel, which cannot be performed before or during drinking because this may affect the behavior of the calf, cause stress, and increase the amount of ruminal milk (Herrli-Gygi et al., 2008). Therefore, we propose to measure the kinetics of AMV after feeding and to predict changes in AMV during and just after drinking by mathematical modeling. Mathematical models were previously proposed to describe AMV kinetics in calves (Wittek et al., 2005), but they assumed that AMV does not increase after feeding

whereas ruminal drinking can lead to an increase in AMV after feeding, when the ruminal milk empties into the abomasum. We used a two-compartment mathematical model for describing the kinetic patterns of AMV and for extrapolating this to AMV at feeding or at slaughter. The comparison of extrapolated abomasal volume (AMV2) to abomasal volume measured at slaughter (AMVs) indicates that the use of the mathematical model improves the prediction of AMVs by reducing the prediction error from 0.70 to 0.49 L (Figure 6.3). Moreover, AMVs was calculated by weighing the abomasum contents and assuming that its density equals the density of ingested milk. Nevertheless, abomasal volume calculated from indigestible marker recovery (AMV3) was always lower than AMVs (Figure 6.3), which may be explained by dilution of milk in the abomasum with abomasal secretions (Toullec et al., 1971). To account for this dilution, pre-prandial AMV in the mathematical model was fixed at 100 mL because this value was in line with AMV measured in the third experiment at 6 h after feeding and in accordance with previously reported data (from 20 to 137 mL; Wittek et al., 2005), but which might be underestimated when calves receive large amounts of solid feed, that increase the flow of digesta from the rumen to the abomasum. Nevertheless, the relationship between measured AMVs and predicted AMV2 indicates that the use of ultrasonography and mathematical modelling of kinetics of AMV gives a reliable estimate of AMV to calculate RMV. Using this technique, RMV averaged 16 (from 0 to 33%) and 21% (from 0 to 39%) of intake for bucket-fed calves in experiments 2 and 3, respectively, which was in a similar range of values previously reported (14 to 35%; Suárez et al., 2007, Berends et al., 2012).

Acetaminophen and Non-Metabolizable Monosaccharides to quantify RMV

In this study, we also considered the use of metabolic tracers (acetaminophen and non-metabolizable monosaccharides) as potential candidates for quantifying RMV. In these methods it is assumed that the transient retention of milk in the rumen increases the delay between intake and intestinal absorption of nutrients. In addition it is assumed that metabolism of monosaccharides by microbes of the rumen decreases their recovery in urine. From the principal component analysis, AUC_{420}, AUC_{600}, and L-R recovery were identified as the best predictors for RMV. The negative slope of the predictive linear relationships (Table 6.3) confirms that an increase in AUC_{420}, AUC_{600}, or L-R recovery is associated with a decrease in RMV, which agrees with our hypothesis. Nevertheless, the residual standard deviation of the predictive mathematical relationships, which was higher than 1.8 L (Table 6.3), was similar to the average RMV in case of natural ruminal drinking (i.e. values measured in calves from treatment 2A; Table 6.2). Additionally, T_{max} in calves that received acetaminophen via the intraruminal tube (treatment 1C; 280 min after feeding) was higher than T_{max} in calves that were partly or totally bucket-fed, but the values were in accordance with bibliographic data (Schaer et al., 2005, Herrli-Gygi et al., 2008). However, T_{max} of bucket-fed calves did not differ from T_{max} of calves where milk and acetaminophen were equally spread between bucket-feeding and tube-feeding. This

may indicate limitations in the sensitivity of the acetaminophen test, possibly caused by absorption of acetaminophen from gastric compartments or by natural occurrence of ruminal drinking in bucket-fed calves (20% of milk intake, from calves on treatment 2A in experiment 2). Moreover, plasma acetaminophen concentrations at the end of the measurements (420 and 600 min after feeding in experiments 1 and 2, respectively) had not returned to pre-prandial levels (Figure 6.1), suggesting that predictive relationships may be further improved by increasing the time span of blood sampling. Nevertheless, our study confirms the potential of using blood parameters related to acetaminophen absorption (T_{max}, ratio C_{max}/T_{max} and AUC) to identify ruminal drinking in calves (Schaer et al., 2005, Herrli-Gygi et al., 2008).

Non-metabolizable monosaccharides were used in experiment 2 to quantify RMV, assuming that they are quantitatively and totally recovered in 24-h urine when not subjected to fermentation in the rumen. Nevertheless, we found low recoveries of monosaccharides in urine (<25% of intake; Table 6.2). These values were lower than recovery of D-X in urine from veal calves during the first 6 post-prandial hours only (26% of intake; Lallès et al., 1995). These recoveries question the completeness of digestive absorption and urinary excretion of monosaccharides (Bjarnason et al., 1995). Additionally, dehydration associated with ruminal drinking (Herrli-Gygi et al., 2008) may decrease the volume of urine, inducing irregular emptying of the bladder. An increase in time of urine collection or the use of a urinary catheter in the bladder may increase the accuracy of predictive relationships between urinary recoveries of non-metabolizable monosaccharides and RMV. In our study, urinary recovery of actively absorbed monosaccharides (3-O-MG and D-X) was not affected by the intraruminal infusion of tracers, whereas urinary recovery of passively absorbed L-R was decreased when it was introduced into the rumen. These results suggest that absorption of 3-O-MG and D-X through the ruminal wall may occur (Aschenbach et al., 2000) whereas L-R could be more sensitive to ruminal degradation.

Estimates of RMV in Heavy Calves

In the third experiment, estimates of Co recovery in the rumen contents when CoEDTA was added as an indigestible marker to the milk fed through the bucket, varied between 0 to 6.1% of intake (mean: 1.8% of intake; Table 6.4). These values were lower than those previously reported for veal calves that received large amounts of solid feed (from 13 to 25 % of intake; Suárez et al., 2007, Berends et al., 2012). In our study, calves receiving Co were slaughtered more than two h after feeding allowing liquid to leave the rumen. This indicates that milk leakage while drinking might have been higher. Indeed, results from the two calves that were tube-fed with Cr as an indigestible marker indicate that only 88% of the milk introduced into the rumen via the tube remained in the rumen two h after administration, which corresponds to a rumen emptying rate of liquids of 6.2% per h. Using this passage rate of milk from the rumen and assuming that it is constant after feeding, RMV at the time of feeding would vary between 0 and 9% of intake (2% on

average). Nevertheless, it should be noted that this value for emptying rate of milk from the rumen was obtained with only two calves in particular feeding conditions because of tube-feeding. This value is lower than emptying rates reported for dairy cows (from 6.4 to 20.6%/h; Seo et al., 2007), whereas no indication for rumen emptying and rumen motility has been previously reported for veal calves. Consequently, the utilization of Co recovery in the rumen at slaughter would underestimate RMV. Calculated from ultrasonography measurements, RMV in experiments 2 and 3 were higher (20% of intake on average).

CONCLUSIONS

Plasma acetaminophen kinetics and recovery of non-metabolizable monosaccharides in urine were weakly associated with ruminal drinking, but these techniques cannot be used to quantify RMV. Ultrasonography allowed accurate measurements of changes in AMV in response to feeding in veal calves. Leakage of milk into the rumen can then be estimated by subtracting the change in AMV from total milk intake. The accuracy of the AMV measurement, and thus the estimated RMV, was improved when kinetics of abomasal volume after feeding were extrapolated to the time of slaughter by mathematical modeling. With this technique, the volume of milk that leaked into the rumen during feeding averaged 20% in heavy veal calves. The recovery of an indigestible marker in milk measured in the rumen at slaughter gave a quantitative estimate of RMV (2% in experiment 3), but improper measurement of emptying rate of fluid from the rumen may lead to underestimation. In conclusion, measuring changes in AMV by ultrasonography in response to feeding was the most promising indirect method to quantify RMV in veal calves.

ACKNOWLEDGEMENTS

The authors thank Laurie Poussier, Laure Hygonnet, Sven Alferink and the staff members of the experimental facilities at Wageningen University (Wageningen, the Netherlands) and VanDrie Group (Scherpenzeel, the Netherlands) for their assistance. The authors acknowledge Walter Grünberg (Department of Farm Animal Health, Utrecht University, the Netherlands) and Norbert Stockhofe-Zurwieden (Central Veterinary Institute, Lelystad, the Netherlands) for their help in designing the experiments. The experiments were done during the stay of E. Labussière at Wageningen University and Research Center, which was founded by a scholarship from the Wageningen Institute of Animal Sciences (WIAS).

REFERENCES

AOAC, 1990. Official methods of analysis, 15th edition. AOAC. Arlington, VA, USA.

Armstrong, D.G. 1969. Cell bioenergetics and energy metabolism. In Handbuch der Tierernährung (eds W Lenkeit, K Breirem and E Crasemann), pp. 385-414. Verlag P. Parey, Hamburg, Germany.

Aschenbach, J.R., S.K. Bhatia, H. Pfannkuche and G. Gäbel. 2000. Glucose is absorbed in a sodium-dependent manner form forestomach contents of sheep. The Journal of Nutrition 130:2797-2801.

Berends, H., C.G. Van Reenen, N. Stockhofe-Zurwieden and W.J.J. Gerrits. 2012. Effects of early rumen development and solid feed composition on growth performance and abomasal health in veal calves. Journal of Dairy Science 95:3190-3199.

Bjarnason, I., A. Macpherson and D. Hollander. 1995. Intestinal permeability: An overview. Gastroenterology 108, 1566-1581.

Braun, U. and A. Gautschi. 2013. Ultrasonographic examination of the forestomachs and the abomasum in ruminal drinker calves. Acta Veterinaria Scandinavica 55.

Breukink, H.J., T. Wensing, A. van Weeren-Keverlink, E.G. van Bruinessen-Kapsenberg and N.A.P.C. de Visser. 1988. Consequences of failure of the reticular groove reflex in veal calves fed milk replacer. Veterinary Quarterly 10:126-135.

Gentile, A., S. Sconza, I.O.G. Lorenz, G. Rademacher, P. Famigli-Bergamini and W. Klee. 2004. D-lactic acidosis in calves as a consequence of experimentally induced ruminal acidosis. Journal of Veterinary Medicine A 51:64-70.

Guilhermet, R., C.M. Mathieu and R. Toullec. 1975. Transit des aliments liquides au niveau de la gouttière oesophagienne chez le veau préruminant et ruminant. Annales De Zootechnie 24:69-79.

Herrli-Gygi, M., H.M. Hammon, Y. Zbinden, A. Steiner and J.W. Blum. 2006. Ruminal drinkers: endocrine and metabolic status and effects of suckling from a nipple instead of drinking from a bucket. Journal of Veterinary Medecine A 53:215-224.

Herrli-Gygi, M., A. Steiner, M.G. Doherr, J.W. Blum, M. Kirchhofer and P. Zanolari. 2008. Digestive processes in ruminal drinkers characterized by means of the acetaminophen absorption test. The Veterinary Journal 176:369-377.

Husson, F., J. Josse and S. Lê. 2008. FactoMineR: An R package for multivariate analysis. Journal of Statistical Software 25:1-18.

Jansen, G., F.A. Muskiet, H. Schierbeek, R. Berger and W. van der Slik. 1986. Capillary gas chromatographic profiling of urinary, plasma and erythrocyte sugars and polyols as their trimethylsilyl derivatives, preceded by a simple and rapid prepurification method. Clinica Chimica Acta 157:277-293.

Lallès, J.P., R. Toullec, P.B. Pardal and J.W. Sissons. 1995. Hydrolyzed soy protein isolate sustains high nutritional performance in veal calves. Journal of Dairy Science 78:194-204.

Lopez, S., J. France, W.J.J. Gerrits, M.S. Dhanoa, D.J. Humphries and J. Dijkstra. 2000. A generalized Michaelis-Menten equation for the analysis of growth. Journal of Animal Science 78:1816-1828.

Nelder, J.A. and R. Mead. 1965. A simplex method for function minimization. The Computer Journal 7:308-313.

SAS 2004. SAS/STAT® 9.1 User's Guide. SAS Institute Inc. Cary, NC, USA.

Schaer, S., M. Herrli-Gygi, N. Kosmeas, H. Boschung and A. Steiner. 2005. Characteristics of acetaminophen absorption in healthy unweaned calves as an indirect measurement of the oroduaodenal transit rate of liquid meals. Journal of Veterinary Medicine A 52:325-332.

Seo, S., C. Lanzas, L.O. Tedeschi and D.G. Fox. 2007. Development of a mechanistic model to represent the dynamics of liquid flow out of the rumen and to predict the rate of passage of liquid in dairy cattle. Journal of Dairy Science 90:840-855.

Sharifi, K., W. Gründberg, S. Soroori, M. Mohri and M.S. Ahrari-Khafi. 2009. Assessment of the acetaminophen absorption test as a diagnostic tool for the evaluation of the reticular groove reflex in lambs. American Journal of Veterinary Research 70:820-825.

Soetaert, K., T. Petzoldt and R.W. Setzer. 2010. Solving differential equations in R: package deSolve. Journal of Statistical Software 33:1-25.

Suárez, B.J., C.G. Van Reenen, N. Stockhofe-Zurwieden, J. Dijkstra and W.J.J. Gerrits. 2007. Effect of roughage source and roughage to concentrate ratio on animal performance and rumen development in veal calves. Journal of Dairy Science 90:2390-2403.

Toullec, R., P. Thivend and C.M. Mathieu. 1971. Utilisation des protéines du lactosérum par le veau préruminant à l'engrais. 1. Vidange stomacale comparée du lait entier et de deux laits de remplacement ne contenant que des protéines de lactosérum comme source de matières azotées. Annales De Biologie Animale Biochimie Biophysique 11:435-453.

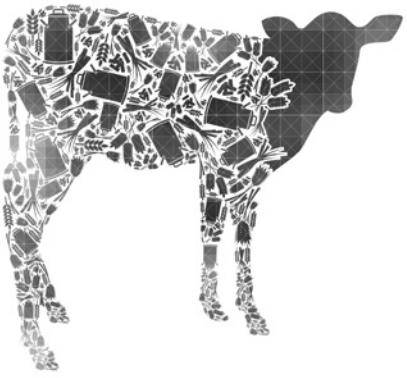
Wijtten, P.J.A., J.J. Verstijnen, T.A.T.G. van Kempen, H.B. Perdok, G. Gort and M.W.A. Verstegen. 2011. Lactulose as a marker of intestinal barrier function in pigs after weaning. Journal of Animal Science 89:1347-1357.

Williams, C.H., D.J. David and O. Lismaa. 1962. The determination of chromic oxide in faeces samples by atomic absorption spectrophotometry. Journal of Dairy Science 59:381-385.

Wittek, T., P.D. Constable, T.S. Marshall and S.S. Crochik. 2005. Ultrasonographic measurement of abomasal volume, location and emptying rate in calves. American Journal of Veterinary Research 66:537-544.

Chapter 7

The utilization of roughages and concentrates relative to that of milk replacer increases strongly with age in veal calves



H. Berends[1], J.J.G.C. van den Borne[1], H. Mollenhorst[2,3], C.G. van Reenen[4], E.A.M. Bokkers,[2] and W.J.J. Gerrits[1]

Accepted for publication by Journal of Dairy Science

[1]Animal Nutrition Group, Wageningen University, Wageningen, the Netherlands;
[2]Animal Production Systems Group, Wageningen University, Wageningen, the Netherlands;
[3]Business Economics Group, Wageningen University, the Netherlands; [4]Livestock Research, Animal Sciences Group, Wageningen University and Research Centre, Lelystad, the Netherlands

ABSTRACT

We aimed to investigate the feeding values of milk replacer (**MR**), roughage, and concentrates for veal calves in a paired-gain setting, thus avoiding any prior assumptions in feeding values and major differences in nutrient intakes. One hundred sixty male Holstein-Friesian calves of 2 wk of age and 45 ± 0.2 kg bodyweight (**BW**) were included in the experiment. Calves were allocated to pens (5 calves per pen) and pens were randomly assigned to 1 of 4 solid feed (**SF**) levels: SF1, SF2, SF3, or SF4, respectively, and to 1 of 2 roughage-to-concentrate (**R:C**) ratios: 20:80 or 50:50. An adaptation period from wk 1 to 10 preceded the experimental period from wk 11 to 27. Total DMI from SF was targeted to reach 20 kg DM for SF1, 100 kg DM for SF2, 180 kg DM for SF3, and 260 kg DM for SF4 during the 16-wk experimental period, and increased with pre-planned, equal weekly increments. Roughage was composed of 50% corn silage and 50% chopped wheat straw based on DM. The quantity of MR provided was adjusted every 2 wk based on BW to achieve similar targeted rates of carcass gain across treatments.

The reduction in MR provided (in kg DM) to realize equal rates of carcass gain with inclusion of SF (in kg DM) differed between the R:C ratio of 50:50 (0.41 kg MR/kg SF) and the R:C ratio of 20:80 (0.52 kg MR/kg SF). As carcass gain unintentionally increased with SF intake, the paired-gain objective was not fully achieved. When adjusted for realized rates of carcass gain, calves fed an R:C ratio of 20:80 still required 10% less MR than calves fed an R:C ratio of 50:50 for equal rates of carcass gain, indicating that the utilization of SF for gain increased with concentrate inclusion. Averaged of the 16-wk experimental period, the feeding value of MR relative to that of concentrates and roughages was close to that predicted based on their respective digestible energy contents. Nevertheless, the feeding value of SF relative to that of MR increased substantially with age. Therefore, additivity in feeding values of these ration components cannot be assumed. The results of the current study can contribute to the development of new concepts for formulation of veal calf diets with substantial amounts of SF.

Keywords: veal calves, growth performance, nutrient utilization, forage

INTRODUCTION

Provision of a minimum daily amount (50 to 250 g) of fibrous feed for veal calves is compulsory according to guidelines of the EU. Solid feed (**SF**) provision reduces nonnutritive oral behaviors (Kooijman et al., 1991, Veissier et al., 1998, Webb et al., 2012), thereby contributing to improved calf welfare. In addition, increasing prices of milk replacer (**MR**) ingredients provide an economic incentive to replace MR by SF.

When combining SF and MR, interactions occurring at the level of digestion or post-absorption may influence nutrient utilization and growth performance in veal calves. Milk-fed calves provided with concentrate feed as the only SF source had signs of parakeratosis and so-called 'plaque' formation (i.e. patches of focal mucosa inflammation with coalescing and adhering papillae covered by SF particles, hair and cell debris), which may inhibit rumen development and nutrient uptake (Suárez et al., 2006, Suárez et al., 2007). Early rumen development was suggested to increase utilization of SF in veal calves, especially towards the end of the fattening period (Berends et al., 2012b). However, the provision of SF to veal calves may increase the prevalence of abomasal lesions (Brscic et al., 2011, Mattiello et al., 2002, Welchman and Baust, 1987) and concentrate provision has been associated with ruminal drinking, leakage of MR into the rumen (Berends et al., 2012b). Recently, it has been shown that urea recycling contributes to nitrogen retention in milk-fed calves provided with a low-protein SF (Berends et al., 2012a) but this contribution decreases with increasing protein content in the SF (Berends et al, unpublished data).

Such interactions between MR and SF complicate an accurate prediction of the feeding value of a ration consisting of MR and SF in veal calves when based on well documented separate effects of SF only (e.g. Ortigues et al., 1990) and MR only (e.g. Gerrits et al., 1996). Studying the impact of substantial exchanges of MR for SF raises methodological problems. The incremental feeding value of a particular mixture of SF is typically evaluated using a dose-response approach. When MR intake is fixed and an incremental dose of SF is provided (see e.g. Berends et al., 2012a), the assumption that differences in BW gain do not influence the feeding value of the SF provided, may be violated. Furthermore, SF intake will likely affect the carcass weight:live weight ratio which needs to be considered when comparing feeding strategies. When MR is exchanged for SF on digestible energy (**DE**) basis (see e.g. Labussière et al., 2009a), assumptions have to be made with regard to the DE content of all dietary ingredients prior to the start of the experiment. With increasing quantities of MR being replaced by SF, these design problems are exacerbated. To circumvent these methodological issues, the current study assesses the feeding values of MR, concentrates, and roughages for veal calves in a paired-gain set-up. Therefore, groups of calves were subjected to pre-set levels of concentrate and roughage intake, whereas the level of MR was adjusted to target equal rates of carcass gain across treatments. In this way, a substantial interchange of MR, concentrates, and roughages can be addressed while avoiding undesirable changes in the rate of BW gain. Differences in

feeding values between these main ration components are addressed within the bounds of a particular choice of roughage and composition of concentrates or MR.

MATERIAL AND METHODS

This study was conducted at the research facilities of VanDrie Group (Scherpenzeel, the Netherlands). Procedures complied with the Dutch Law on Experimental Animals and the ETS123 (Council of Europe 1985 and the 86/609/EEC Directive) and were approved by the Animal Care and Use Committee of Wageningen University.

Animals, Experimental Design, and Housing

One hundred sixty male Holstein-Friesian calves were purchased from commercial dairy farms at 2 wk of age, selected based on BW, uniformity, and clinical health. Mean BW upon arrival was 45 ± 0.2 kg. Calves were randomly allocated to pens (5 calves per pen). Pens were randomly assigned to 1 of 4 SF levels: SF1, SF2, SF3, or SF4, respectively, and to a roughage-to-concentrate (**R:C**) ratio of 20:80 or 50:50 on DM basis (Table 7.1). The experiment consisted of 2 successive periods; an adaptation period from wk 1 to 10 and an experimental period from wk 11 to 27. During the adaptation period all pens were exposed to their assigned SF level but an R:C ratio of 50:50 was used for all pens to allow for optimal rumen development as assessed in earlier studies (Berends et al., 2012b, Suárez et al., 2007). At the onset of the experimental period, i.e. at wk 11, we employed a 2 x 4 factorial arrangement of treatments with 4 pens per treatment combination and pen as the experimental unit. Animal health was checked daily. Hemoglobin concentration in blood was monitored throughout the trial, i.e. at wk 3, 7, 11, 15, 19, 23 and at slaughter, and corrected to comply with the minimum EU level of 4.5 mmol/L at the end of the fattening period.

Table 7.1 Experimental design and number of pens per treatment

Solid feed level	Target DMI[1]	No. of pens	
		R:C 20:80[2]	R:C 50:50[2]
SF1	20	4	4
SF2	100	4	4
SF3	180	4	4
SF4	260	4	4

[1] Cumulative DMI from solid feed (kg DM) during the 17-wk experimental period.
[2] R:C: roughage-to-concentrate ratio on a DM basis. Roughage consisted of 50% corn silage and 50% chopped wheat straw on a DM basis.

Pens measured 3 x 3 m and were equipped with open fences and wooden-slatted floors, without bedding material. During the first 4 wk, calves were kept individually in 0.9-m^2 temporary pens placed inside the group pen to facilitate monitoring of individual feeding behavior and health. Calves were exposed to daylight and artificial light from 0500 h to 2300 h and to darkness during the remainder of the day.

Diets and Feeding

Roughage consisted of 50% corn silage and 50% chopped wheat straw on a DM basis (Table 7.2). During the first 4 wk of the adaptation period, corn silage was replaced on a DM basis by alfalfa hay to stimulate SF intake. The SF offered to calves increased with pre-planned, equal weekly increments. Cumulative DMI from SF during the experimental period was targeted to reach 20 kg DM for SF1, 100 kg DM for SF2, 180 kg DM for SF3, and 260 kg DM for SF4. The quantity of MR was calculated to achieve similar targeted rates of carcass gain across treatments, and was adjusted biweekly based on the realized BW gain in the preceding 2-wk period. Rates of carcass gain were estimated from the measured rates of BW gain. We assumed rumen tissue mass and contents to represent the most relevant difference between live weight gain and carcass weight gain (Berends et al., 2012b), and assumed these proportional to SF intake. Therefore data from previous trials were used to predict rumen mass and content for each SF level (Suárez et al., 2007; Berends et al., 2012a; Berends et al., unpublished data).

Table 7.2 Analyzed nutrient composition (g/kg DM) of concentrate, corn silage, and wheat straw, fed during the 17-wk experimental period

Nutrient	Concentrate[1]	Corn silage	Wheat straw
Dry matter, g/kg product	898	297	931
Crude protein[2]	137	69	31
Crude fat	67	29	9
Starch	429	312	11
Neutral detergent fibre	127	421	794

[1] Concentrate composition: 36.2% corn, 20.6% lupins, 20.3% barley, 12.5% carob meal, 4.4% corn gluten meal, 6% premix (lactose carrier, provided per kg concentrate: vitamin A: 4,000 IU; vitamin D: 500 IU; vitamin E: 100 IU; zinc ($ZnSO_4$): 25 mg; manganese (MgO): 20 mg; iodine (KI): 0.8 mg; iron ($FeSO_4$): 63 mg, selenium (Na_2SeO_3): 0.15 mg; copper ($CuSO_4$): 15 mg; cobalt: 0.1 mg, 1.13 g magnesium (magnesium sulphate), 3.0 g sodium (NaCl), 8.6 g calcium ($CaCO_3$), 3.9 g potassium (KH_2PO_4).
[2] N × 6.25.

Concentrate composition (Table 7.2) was designed to meet mineral and vitamin requirements for beef cattle (NRC, 2000). MR was reconstituted with water and concentration increased gradually from 125 to 188 g/L for all treatments simultaneously

during the experiment. MR was supplied in buckets at 40 to 41°C, and was provided twice daily in equally sized meals at 0600 h and 1600 h. Calves were allowed 15 min to consume the milk; MR refusals were collected and weighed. During the first 8 wk of the adaptation period, calves received a commercial starter MR based on 32% whey powder and 30% skimmed milk powder with 223 g/kg DM crude protein and 180 g/kg DM crude fat. The experimental MR (Table 7.3) was provided during the last 2 wk of the adaptation period and during the experimental period. The experimental MR was introduced over 3 feedings. The SF was provided as a mixture in a long feed trough in front of the pen, directly after the morning meal. Feed refusals were weighed once daily. During the first 4 wk of the adaptation period, calves were allowed ad libitum access for 20 min to water supplied in buckets around noon. From wk 5 onwards calves had free access to water via drinking nipples.

Table 7.3 Ingredient composition and analyzed nutrient composition of the experimental milk replacer

Ingredient, g/kg		Nutrient[4], g/kg DM	
Fat-filled whey powder[1]	394.0	Dry matter (g/kg product)	969.5
Whey protein concentrate	193.9	Crude protein[5]	210.0
Whey	143.3	Crude fat	211.6
Delactosed whey	135.0	Starch	22.3
Soy protein isolate	37.7		
Soy protein concentrate	25.0		
Acidified whey[2]	20.0		
Pregelatinized wheat starch	20.0		
Vitamin and mineral premix[3]	10.0		
Calcium formate	9.8		
L-lysine.HCl	5.2		
DL-methionine	2.7		
Citric acid	2.0		
Mono ammonium phosphate	1.4		
L-threonine	0.2		

[1] Contained 50% fat from palm oil and coconut oil (80/20, w/w).

[2] Acidified with lactate, provided 5.9 g lactate per kg of the experimental diet.

[3] Provided per kg of the experimental diet: 16.6 g K, 15.5 g Cl, 8.3 g Fe, 6.1 g P, 5.9 g Cu ($CuSO_4$), 5.7 g Na, 1.3 g Mg (MgO), 25,000 IU vitamin A, 4,000 IU vitamin D3, 100 IU vitamin E.

[4] Expressed in g/kg DM unless specified otherwise.

[5] Calculated as N × 6.25.

Measurements

Feed Intake and Performance. Intake of MR and SF was recorded daily. Calves were weighed every 2 wk.

Slaughter Procedure. At 27 wk, calves were transported for approximately 40 min (45 km) to a slaughter facility and euthanized by stunning (captive bolt pistol) and subsequent exsanguination. Measurement of carcass weight and classification of meat color and fat score were performed by a certified employee of the Central Office for Slaughter Livestock Services (BV CBS, 2007). Carcass gain was calculated by subtracting the estimated carcass weight at the start of the experimental period from the carcass weight. To estimate carcass weight at the start of the experimental period, dressing percentages obtained at the end of the experiment for the associated SF levels with R:C ratios of 50:50 were used. Meat color was scored on a 10-point scale from 1: pale to 10: red on the Musculus Rectus Abdominis of the carcass using a handheld spectrophotometer Konica-Minolta CR400, as previously described by (Hulsegge et al., 2001). Fat score of the carcasses was assessed by hypodermic fat coverage at the outside of the carcass and inside of the chest cavity on a 5-point scale from 1: no or very low amounts of fat, to 5: the carcass as well as the chest cavity is covered with fat. The empty rumen weight was recorded. Rumen development was assessed by visual examination of the rumen mucosal surface and the presence and density of rumen papillae, and scored on a 5-point scale from 1 (poor; few papillae or short papillae) to 5 (excellent; numerous, long and well developed papillae), by an experienced pathologist-anatomist. Individual scores (i.e. fat, color, rumen development) were averaged per pen and subsequently treated as continuous variables. Abomasal damage was assessed by counting and scoring the size of lesions in the pyloric area of the abomasal wall, and was expressed as a product of counts and size. Lesions (erosions, ulcers, and scars) were categorized as small (≥ 0.5 cm^2), medium (0.5 - 1.0 cm^2), or large (≥ 1.0 cm^2) and were assumed to be 0.25, 0.75 and 1.25 cm^2, respectively.

Chemical Analyses. Samples of MR, concentrate, corn silage, and straw were collected weekly. Corn silage samples were pooled by month; other samples were pooled for the experimental period. All samples were analyzed for DM, crude protein, starch, NDF (except for MR), and crude fat content. For determination of DM content, corn silage and concentrate samples were freeze-dried. Concentrate, straw, and corn silage samples were ground to pass a 1-mm screen. DM content was determined by drying to a constant weight according to ISO Standard 6496 (ISO, 1998). Kjeldahl N content was determined according to ISO 5983 (ISO, 1997). Crude fat content was determined after acid hydrolysis according to ISO 6496 (ISO, 1999). The NDF content was analyzed according to Van Soest et al. (1991). Starch content was determined enzymatically as described by Rijnen et al. (2001). Prior to starch analysis, reducing sugars were extracted from the samples according to the Luff-Schoorl method (NEN, 1974), using 40% ethanol and subsequent hydrolysis in a weak acid environment.

Statistical Analysis

In total, 15 calves were excluded from the experiment: 6 calves during the adaptation period, 9 calves during the experimental period due to clinical sickness (n = 7; e.g. lung infection, diarrhea) or death (n = 2). Pen was considered the experimental unit, and dependent variables were averaged per pen prior to statistical evaluation. Dependent variables were analyzed using analysis of covariance (SAS 9.20 by SAS Institute, Inc, Cary, NC). The model comprised SF intake as a covariate and the interaction between SF intake and R:C ratio, to estimate regression coefficients for both R:C ratios. The main effect of R:C ratio was excluded to obtain a single intercept, which represents the value of the dependent variable when SF intake is zero (i.e. R:C ratio of SF is meaningless). The number of calves in a pen (3, 4, or 5) was included in a weight statement to account for differences in reliability of pen means. Residual carcass gain was included as a covariate in the model to account for within-treatment variation of carcass gain when the dependent variable was DMI from MR, fat score, color score, or live weight gain. Residual carcass gain was calculated for each pen by difference between the pen mean and the treatment mean. When model residuals were not normally distributed, data were transformed to obtain homogeneity of variance. Data are presented as raw means with their SEM and *P*-values were obtained from analysis of the normally distributed data (after transformation if required). Regression coefficients are presented with their common intercept.

To evaluate time-related changes in MR intake to achieve similar rates of carcass gain, bi-weekly data were analyzed. Bi-weekly rates of MR intake, expressed as MR intake per kg of carcass gain (kg DM MR/kg carcass gain), was considered the dependent variable. Fixed effects of SF intake (kg DM/d) as co-variable, and the interaction between SF intake and R:C ratio on the dependent variable were analyzed for every 2-wk period. The significance of the decline in regression coefficients was determined by analysis of covariance (SAS 9.20 by SAS Institute, Inc, Cary, NC) with time (i.e. 2-wk period) as a covariate, including the interaction between R:C ratio and time. Differences were considered significant at $P < 0.05$.

RESULTS

Realized cumulative DMI from SF were very close to targeted DMI (Table 7.4). Figure 7.1 shows the effect of R:C ratio on the relationship between total DMI from MR and total DMI from SF over the 17-wk experimental period. The regression coefficients in Figure 7.1 represent the change in MR intake (in kg DM) to achieve similar rates of carcass gain per kg increase in DMI from SF for each R:C ratio. The regression coefficient for the R:C ratio of 50:50 was -0.41 kg MR/kg SF and differed ($P < 0.001$) from the regression coefficient for the R:C ratio of 20:80, which was -0.53 kg MR/kg SF. Carcass gain during the experimental period, however, increased slightly with SF intake ($P < 0.01$; Table 5), independent of the R:C ratio.

Table 7.4 Dry matter intake (DMI) from solid feed (SF), milk replacer (MR), and performance of veal calves in a 17-wk experiment aimed to achieve similar rates of carcass gain across levels of SF intake at each of 2 roughage-to-concentrate (R:C) ratios[1].

SF level	SF1				SF2				SF3				SF4			
Target DMI SF, kg	20				100				180				260			
R:C[2]	50:50		20:80		50:50		20:80		50:50		20:80		50:50		20:80	
No. of pens	4		4		4		4		4		4		4		4	
	mean	SE	mean	SE	mean	SE	mean	SE	mean	SE	mean	SE	mean	SE	mean	SE
DMI from SF, kg	20	0.1	20	0.0	93	2.9	94	0.3	167	1.4	172	0.8	239	0.9	248	1.6
DMI from MR, kg	274	6.6	271	0.9	251	6.2	232	1.4	215	3.8	194	1.9	184	1.7	150	0.9
Live weight gain, kg	133	6.9	127	3.2	144	9.2	145	2.9	158	5.1	154	2.9	158	3.2	159	3.8
Carcass gain, kg	74	3.4	74	2.6	80	5.6	80	2.1	88	3.6	84	1.9	87	1.4	85	2.3
Color score[3]	4.2	0.06	3.9	0.44	4.8	0.48	4.0	0.56	4.8	0.57	4.5	0.47	5.3	0.14	5.5	0.43
Fat score[3]	2.0	0.09	2.2	0.06	2.4	0.31	1.8	0.23	2.2	0.08	2.1	0.06	1.9	0.08	2.1	0.08
Blood Hb, mM	4.3	0.15	4.3	0.06	4.7	0.19	5.0	0.14	5.5	0.11	5.6	0.04	5.8	0.18	5.9	0.02
Carcass weight, kg	131	2.6	130	3.3	131	2.1	137	2.2	139	1.5	144	4.4	139	1.7	142	1.8
Live weight, kg	230	3.3	232	6.8	246	3.2	243	8.6	253	2.0	260	6.1	259	3.8	259	4.7

[1] Statistical analysis of these data is included in Table 7.5.

[2] Roughage consisted of 50% corn silage and 50% chopped wheat straw on a DM basis.

[3] Meat color was scored on a 10-point scale from 1: pale to 10: red on the Musculus Rectus Abdominis of the carcass using a handheld spectrophotometer. Fat score of the carcasses was assessed by hypodermic fat coverage at the outside of the carcass and inside of the chest cavity on a 5-point scale from 1: no or very low amounts of fat, to 5: the carcass as well as the chest cavity is covered with fat.

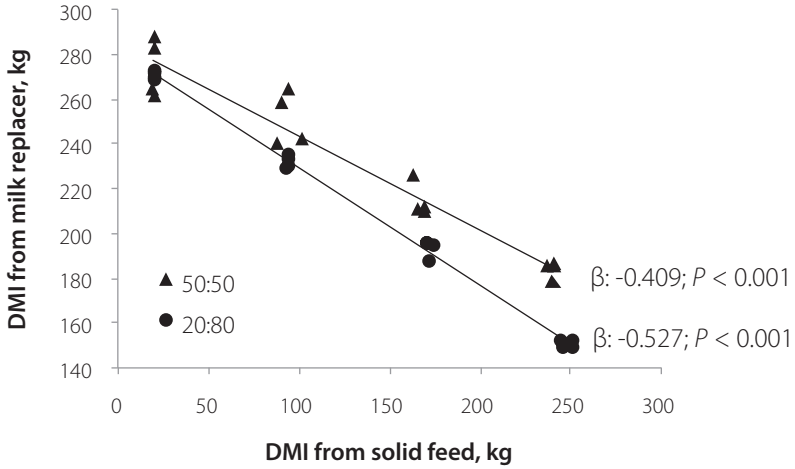


Figure 7.1 Relationship between total DMI from solid feed and DMI from milk replacer at 2 roughage-to-concentrate ratios (▲: 50:50, ●: 20:80) in veal calves in a paired-gain design from wk 11 to 27 of the experiment. Each observation includes a pen of 3-5 animals. Roughage consisted of 50% corn silage and 50% chopped wheat straw based on DM. The regression coefficient (β) represents the change in milk replacer intake required to maintain growth rate when increasing solid feed intake (kg DM/kg DM). Regression coefficients differed ($P < 0.01$) between roughage-to-concentrate ratios (see also Table 7.5).

Due to unexpected high rates of BW gain of calves at the high SF intakes, the paired-gain objective was not completely achieved. The MR allowance required to achieve the same BW gain for calves at the low SF intakes was beyond the intake capacity of these calves. In order to correct for differences in carcass gain, the effect of R:C ratio on MR intake per kg carcass gain was analyzed. The regression coefficients were -0.0077 and -0.0086 kg MR/kg carcass gain per kg SF for R:C ratios of 50:50 and 20:80, respectively (Table 7.5), which differed ($P < 0.05$) from each other.

The effect of time on the relationship between MR intake, expressed per kg of carcass gain, and SF intake is shown in Figure 7.2. These bi-weekly regression coefficients were obtained from 2-wk averages of SF intake (Figure 7.3; supplementary figures), MR intake (Figure 7.4; supplementary figures), and carcass gain (Figure 7.5; supplementary figures). The regression coefficients, representing the reduction in MR intake per kg SF per kg carcass gain for a 2-wk period, decreased with time ($P < 0.05$). The decrease with time was greater for the R:C ratio of 20:80 than for 50:50 ($P < 0.01$).

Carcass color score tended to increase with SF intake ($P < 0.10$; Table 7.5). Fat score of the carcass was unaffected by SF intake and R:C ratio. Live weight gain increased with SF

Table 7.5 Effects of incremental DMI from solid feed (SF) on selected response parameters in veal calves at 2 roughage-to-concentrate (R:C) ratios (50:50 and 20:80). The 17-wk experiment aimed to achieve similar rates of carcass gain across levels of SF intake

Response variable	R:C ratio[1]	Intercept[2]	Beta[3]			P-value[5]
			Estimate	SE	*P*-value[4]	SF*R:C ratio
DMI from MR, kg	50:50	283	-0.409	0.0147	***	***
	20:80		-0.527	0.0146	***	
Carcass gain[6], kg	50:50	73.2	0.071	0.0137	***	NS
	20:80		0.055	0.0136	**	
DMI from MR per carcass gain, kg/kg	50:50	3.83	-0.0077	0.00048	***	*
	20:80		-0.0086	0.00048	***	
Color score[6]	50:50	3.84	0.0061	0.00191	**	NS
	20:80		0.0049	0.00190	*	
Fat score[6]	50:50	2.09	0.0000	0.00060	NS	NS
	20:80		-0.0003	0.00060	NS	
Live weight gain, kg	50:50	129	0.144	0.0113	***	NS
	20:80		0.131	0.01133	***	

[1] Roughage consisted of 50% corn silage and 50% chopped wheat straw on a DM basis.
[2] Estimate of the response variable for calves without SF.
[3] Regression coefficient β ($y = a + \beta \cdot x$) represents the change in response variable per increase in DMI from SF.
[4] Probability for test whether the regression coefficient (β) equals 0.
[5] A significant interaction between the DMI from SF and the R:C ratio indicates that the regression coefficient differs between the R:C ratios. The effect of residual carcass gain (observed carcass gain – average carcass gain per treatment) was included for DMI from MR, color score, fat score and live weight gain, and found significant for DMI from MR, fat score, and for live weight gain.
[6] Meat color was scored on a 10-point scale from 1: pale to 10: red on the Musculus Rectus Abdominis of the carcass using a handheld spectrophotometer. Fat score of the carcasses was assessed by hypodermic fat coverage at the outside of the carcass and inside of the chest cavity on a 5-point scale from 1: no or very low amounts of fat, to 5: the carcass as well as the chest cavity is covered with fat. Data were transformed (arsin, sqrt) to obtain homogeneity of variance.
NS: not significant; †$P < 0.10$; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

intake ($P < 0.001$) and this increase did not differ between R:C ratios. Rumen development score increased with SF intake ($P < 0.001$), and this increase tended to be greater for the R:C ratio of 20:80 (Table 7.6). Empty rumen weight increased ($P < 0.001$) with SF intake and ranged between 2.24 kg and 5.18 kg. The surface of abomasal damage ranged from 0.1 to 4.3 cm^2, and increased with SF intake for R:C ratio 20:80 ($P < 0.05$), but not for 50:50, although the interaction was not significant ($P = 0.11$).

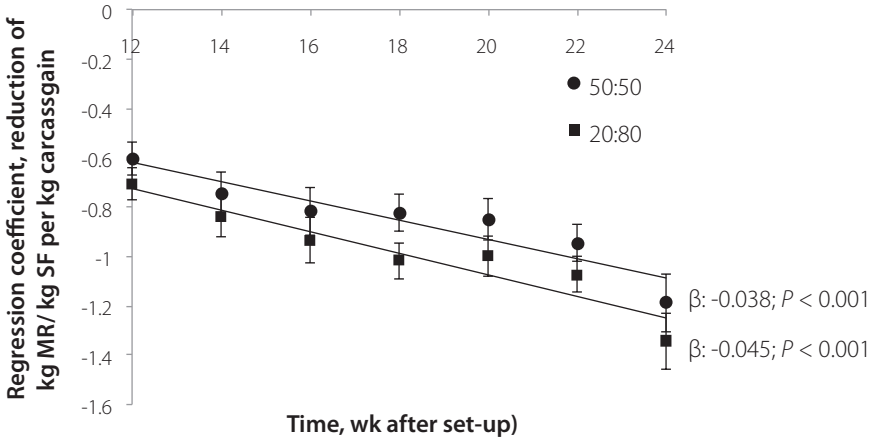


Figure 7.2 Linear regression between the regression coefficients describing the relationship between milk replacer (MR, kg DM/d) intake, expressed per kg carcass gain, and solid feed (SF, kg DM/d) intake at 2 roughage-to-concentrate ratios (▲: 50:50, ●: 20:80) from wk 11 to 27 after set-up in veal calves in a paired-gain design. Each observation represents the regression coefficient at a given time point, based on a 2-wk period, after set-up at 2 wk of age. For both R:C ratios, regression coefficients decreased with time ($P < 0.001$) and this decline was smaller ($P < 0.01$) for an R:C ratio of 50:50 than for 20:80. Roughage consisted of 50% corn silage and 50% chopped wheat straw based on DM.

Table 7.6 Effects of incremental DMI from solid feed (SF) on post-mortem variables at 2 roughage-to-concentrate (R:C) ratios (50:50 and 20:80) in veal calves in a paired-gain set-up

Response variable	R:C ratio[1]	Intercept[2]	Beta[3]			P-value[5] SF*R:C ratio
			Estimate	SE	*P*-value[4]	
Rumen development score	50:50	1.38	0.0035	0.00062	***	†
	20:80		0.0046	0.00062	***	
Empty rumen weight, kg	50:50	2.73	0.0066	0.00115	***	NS
	20:80		0.0059	0.00115	***	
Abomasal damage, cm^2	50:50	0.80	0.0038	0.00250	NS	NS
	20:80		0.0052	0.00249	*	

[1] Roughage consisted of 50% corn silage and 50% chopped wheat straw on a DM basis.
[2] Estimate of the response parameter for calves without solid feed.
[3] Regression coefficient β ($y = a + \beta \cdot x$) represents the change in response variable per increase in DMI from solid feed.
[4] Probability for test if the regression coefficient (β) equals 0.
[5] A significant interaction between the DMI from SF and the R:C ratio indicates that the regression coefficient differs between the R:C ratios. The effect of residual carcass gain was not significant for any response parameter.
NS: not significant; †$P < 0.10$; *$P < 0.05$; ***$P < 0.001$.

DISCUSSION

Utilization of SF for Growth and Effect of R:C ratio

There is a lack of data on the feeding values of MR, roughages, and concentrates fed to veal as well as dairy calves. We aimed to investigate the feeding values of MR, roughage, and concentrates for veal calves in a paired-gain setting, thus avoiding any prior assumptions in feeding values and large differences in nutrient intakes. The feeding value of SF is expressed as the reduction in MR intake (kg DM) to realize equal rates of carcass gain with increasing SF intake (kg DM), and can be derived by regression of MR intake and SF intake (Figure 7.1). For an R:C ratio of 50:50, MR intake decreased by 409 g DM for each extra kg DM SF, and for an R:C ratio of 20:80 this was 529 g DM MR. Thus, the amount of MR required to realize equal rates of carcass gain was almost 25% lower for the R:C ratio of 20:80 than for 50:50, indicating that utilization of SF for growth is increased with concentrate proportion in the SF. After correction for the observed increase in carcass gain with SF intake (6.3 kg per 100 kg DM SF; $P < 0.001$; Table 7.5), the average reduction of MR intake to realize equal rates of carcass gain was still lower for the R:C ratio of 50:50 (0.63 kg MR/kg SF) than for the R:C ratio of 20:80 (0.70 kg MR/kg SF).

Differences in digestibility of energy from concentrates, roughages, and MR contribute to a large extent to variation in their feeding value. When assuming additivity of MR and SF, it would not make a difference whether DE originates from MR or SF. Based on this assumption, we calculated the decrease in DE from MR with increasing DE from SF at each of the 2 R:C ratios. The estimated DE content of MR was 18.5 MJ/kg DM, as calculated from the analyzed GE value and a digestibility of GE of 96% (Labussi**è**re et al., 2009b, van den Borne et al., 2006b). The estimated DE content of SF was 10.8 MJ/kg DM for an R:C ratio of 50:50 (Berends et al., 2012a) and 13.1 MJ/kg DM for an R:C ratio of 20:80 (van den Borne et al., unpublished data). These digestibility studies in veal calves (Berends et al., 2012a; van den Borne et al., unpublished data) used identical R:C ratios and roughage and concentrate sources as in the current study. Based on these estimates the reduction of MR intake with increasing SF intake would then be 0.58 and 0.71 kg MR/kg SF for 50:50 and 20:80, respectively. These calculated values are only slightly different from the average values obtained in the current study (0.62 and 0.69 kg MR/kg SF for 50:50 and 20:80, respectively). Differences between SF and MR in post-absorptive utilization of DE could be affected by energy used by visceral tissues (Reynolds et al., 1991a, b), and methane losses with SF intake in veal calves (Berends et al., 2012a). The reduction in MR intake to realize equal rates of carcass gain with increasing SF intake is close to those calculated based on DE, assuming additivity of feeding values of MR, roughages, and concentrates.

If digestible crude protein (**DCP**) would be the main driving force for carcass gain, it would not matter whether the DCP originates from MR or SF. Therefore, we calculated the decrease in DCP from MR required for equal carcass gain with increasing DCP for SF at each of the 2 R:C ratios. When assuming DCP contents of MR, SF 50:50, and SF 20:80 at 197,

42 and 66 g/kg based on calf studies (Labussière et al., 2009a, Moody et al., 2007, Ortigues et al., 1990), the reduction in MR intake with increasing SF intake would be 0.21 kg MR/kg SF for an R:C ratio of 50:50 and 0.33 kg MR/kg SF for an R:C ratio of 20:80. Hence, the theoretical reduction in MR intake with increasing SF intake is only 34 to 48% of the reduction observed in the current study, suggesting that digestible energy from SF was more limiting than digestible crude protein when exchanging MR for SF. In dairy calves, it was shown that at weaning, increasing metabolizable energy intake resulted in increased weight gain whereas increasing protein supply had no effect (Journet, 1984). Potential effects of urea recycling were excluded from this calculation. N from MR may be reutilized for protein gain due to urea recycling when providing SF (Berends et al., unpublished data), but the quantitative contribution of urea recycling to protein gain depends on the N level of SF (Berends et al., unpublished data).

Thus, the average reduction in MR intake with increasing SF intake realized throughout the experimental period is in close accordance with the theoretical predictions by exchanging SF for MR based on DE supply. These data indicate that feeding value of SF for calf diets can be estimated from DE content.

However, this average reduction realized throughout the experimental period does not account for the large variation observed in time. The regression coefficients, representing the reduction in MR intake with increasing SF intake (kg MR/kg SF per kg carcass gain per day), decreased during the experiment for both R:C ratios (Figure 7.2), indicating that the utilization of SF for growth relative to MR increased with age. The decline was greater for the R:C ratio of 20:80 than for 50:50 ($P < 0.01$), which may be explained by the inclusion of concentrate or a reduced efficiency of MR utilization or both. Utilization of protein from MR for growth is known to decrease with increasing age in milk-fed calves (Gerrits et al., 1996, van den Borne et al., 2006a), whereas the utilization of SF increased with age in veal calves between 108 to 164 kg BW (Berends et al., 2012a). Like in dairy calves (Hodgson, 1971), SF intake may increase rumen volume resulting in greater rumen retention time and degradation. Furthermore, rumen functioning and/or fermentation were shown to improve with age in calves fed exclusively SF (Devant et al., 2000, Rotger et al., 2005). The latter corresponds with previous observations in veal calves, where early initiation of rumen development was suggested to result in increased nutrient utilization, especially during the second half of the fattening period (Berends et al., 2012c). Additionally, leakage of MR into the rumen, referred to as ruminal drinking, may increase with age (Guilhermet et al., 1975). Increased ruminal drinking could reduce utilization of MR for gain and thus a relatively higher utilization of SF when exchanging SF for MR to obtain equal growth rates. In addition, ruminal drinking may interfere with microbial fermentation processes in the rumen and could thus affect utilization of SF. Finally, urea recycling in milk-fed calves provided with SF increased with increasing age (Berends et al., 2012a), which has been associated with increased utilization of N from MR (Berends et al., unpublished data).

Rumen Development and Abomasal Damage

An increased supply of end-products from rumen fermentation, either due to increased SF intake or concentrate inclusion, can be expected to increase papillae development (Tamate et al., 1962). Rumen development score and empty rumen weight both increased with increasing SF intake, which is agreement with previous studies in veal calves (Berends et al., 2012b, Morisse et al., 2000). Empty rumen weights were in the upper range or above previously reported values in veal calves of similar age, which may be explained by the lower level of SF intake (< 750 g DM/d) than in the current study (< 3000 g DM/d) (Berends et al., 2012b, Morisse et al., 2000). Inclusion of 30 to 60% roughage (from various sources) at the expense of concentrates decreased empty rumen weight in veal calves at 10 wk of age (Suárez et al., 2007). In the current study, the increase in rumen development score tended to be ($P < 0.10$) greater for an R:C ratio of 20:80 than for 50:50, which may be explained by greater concentrations of VFA in the rumen with increasing concentrate supply (Berends et al., 2012b). The surface of abomasal damage increased with SF intake for an R:C ratio of 20:80 ($P < 0.05$), but not for 50:50 (interaction SF intake*R:C ratio: $P = 0.11$). Other studies showed that provision of SF to milk-fed calves increased the prevalence of abomasal damage, including pyloric ulcers, when compared with calves fed MR only (Breukink et al., 1991, Brscic et al., 2011, Mattiello et al., 2002). SF particles may exert mechanically abrasive effects on the abomasal mucosa, which may be sensitized due to provision of large volumes of MR. Promoting early rumen development was previously hypothesized to stimulate fermentative degradation of particles and thereby reducing abomasal damage in veal calves (Berends et al., 2012b). In that study, early rumen development reduced the prevalence of large scars in the abomasum compared with late rumen development (6% vs. 20% of calves) (Berends et al., 2012c). The current study used a larger contrast in SF intake, but did not include roughage proportions over 50%.

CONCLUSIONS

The aim of the current study was to investigate the feeding values of MR, roughage, and concentrates for the formulation of veal calf diets with substantial amounts of SF. In a paired-gain setting, thus avoiding any prior assumptions in feeding values and large differences in nutrient intakes, calves were exposed to 4 levels of SF intake (20, 100, 180, or 260 kg DM during the 17-wk experimental period) and 2 roughage-to-concentrate ratios (20:80 vs. 50:50), with roughage consisting of wheat straw and corn silage. The quantity of MR fed to realize equal rates of carcass gain was 10% lower for the 20:80 than for the 50:50 R:C ratio, indicating that the utilization of SF for gain increased with concentrate inclusion. Averaged of the 16 wk experimental period, the feeding value of MR relative to that of concentrates and roughages was close to that predicted based on their respective DE contents. Nevertheless, the feeding value of SF relative to that of MR increased substantially

with age. Therefore, additivity in feeding values of these ration components cannot be assumed.
The surface of abomasal damage increased with SF intake for an R:C ratio of 20:80, but not for 50:50. Rumen development score increased with SF intake.

ACKNOWLEDGEMENTS

The authors thank the Division for Earth and Life Sciences (ALW) of the Netherlands Organisation for Scientific Research (NWO, The Hague, the Netherlands), and the Product Board Animal Feed (The Hague, the Netherlands) for financially supporting this research. We thank Klaas Boeder, Bart Evers, Ewart van Voorst (VanDrie Group, Scherpenzeel, the Netherlands), Bert Beukers, André Jansen, Joswita van der Velde (Wageningen University, Wageningen, the Netherlands), Norbert Stockhofe-Zurwieden, Ralph Kok, Pieter Roskam (Central Veterinary Institute, Lelystad, the Netherlands), and Bas Engel (Biometris, Wageningen, the Netherlands) for their contributions.

REFERENCES

Berends, H., J. J. G. C. van den Borne, S. J. J. Alferink, C. G. van Reenen, E. A. M. Bokkers, and W. J. J. Gerrits. 2012a. Low-protein solid feed improves the utilization of milk replacer for protein gain in veal calves. Journal of Dairy Science 95(11):6654-6664.

Berends, H., C. G. van Reenen, N. Stockhofe-Zurwieden, and W. J. J. Gerrits. 2012b. Effects of early rumen development and solid feed composition on growth performance and abomasal health in veal calves. Journal of Dairy Science 95(6):3190-3199.

Breukink, H. J., T. Wensing, and J. M. Mouwen. 1991. Abomasal ulcers in veal calves: pathogenesis and prevention. Pages 118-122 in Proc. International Symposium on Veal Calf Production. Pudoc Wageningen, Wageningen, the Netherlands.

Brscic, M., L. F. M. Heutinck, M. Wolthuis-Fillerup, N. Stockhofe-Zurwieden, B. Engel, E. K. Visser, F. Gottardo, E. A. M. Bokkers, B. J. Lensink, G. Cozzi, and C. G. Van Reenen. 2011. Prevalence of gastrointestinal disorders recorded at postmortem inspection in white veal calves and associated risk factors. Journal of Dairy Science 94(2):853-863.

Devant, M., A. Ferret, J. Gasa, S. Calsamiglia, and R. Casals. 2000. Effects of protein concentration and degradability on performance, ruminal fermentation, and nitrogen metabolism in rapidly growing heifers fed high-concentrate diets from 100 to 230 kg body weight. Journal of Animal Science 78(6):1667-1676.

Gerrits, W. J. J., G. H. Tolman, J. W. Schrama, S. Tamminga, M. W. Bosch, and M. W. A. Verstegen. 1996. Effect of Protein and Protein-Free Energy Intake on Protein and Fat Deposition Rates in Preruminant Calves of 80 to 240 kg Live Weight. Journal of Animal Science 74(9):2129-2139.

Guilhermet, R., C. Mathieu, and R. Toullec. 1975. Transit des aliments liquides au niveau de la gouttiere oesophagienne chez le veau preruminant et ruminant. Annales De Zootechnie 24:69-79.

Hodgson, J. 1971. The development of solid food intake in calves 3. The relation between solid food intake and the development of the alimentary tract. Animal Science 13(03):449-460.

Hulsegge, B., B. Engel, W. Buist, G. Merkus, and R. Klont. 2001. Instrumental colour classification of veal carcasses. Meat Science 57(2):191-195.

ISO. 1997. Animal feeding stuffs - Determination of nitrogen content and calculation of crude protein content. ISO 5983. Int. Organ. Stand., Geneva, Switzerland.

ISO. 1998. Animal feeding stuffs - Determination of moisture and other volatile matter content. ISO 6496. Int. Organ. Stand., Geneva, Switzerland.

ISO. 1999. Animal feeding stuffs - Determination of fat content. ISO 6492. Int. Organ. Stand., Geneva, Switzerland.

Journet, M. 1984. Nutrition des veaux au cours du sevrage. I.-Evolution de la consommation d'aliments et des concentrations sanguines de divers métabolites énergétiques. Annales de Zootechnie 33:427-444.

Kooijman, J., H. K. Wierenga, and P. R. Wiepkema. 1991. Development of abnormal oral behavior in group-housed veal calves: Effects of roughage supply. New Trends in Veal Calf Production:54-58.

Labussière, E., S. Dubois, J. van Milgen, G. Bertrand, and J. Noblet. 2009a. Effect of solid feed on energy and protein utilization in milk-fed veal calves. Journal of Animal Science 87(3):1106-1119.

Labussière, E., G. Maxin, S. Dubois, J. van Milgen, G. Bertrand, and J. Noblet. 2009b. Effect of feed intake on heat production and protein and fat deposition in milk-fed veal calves. Animal 3:557-567.

Mattiello, S., E. Canali, V. Ferrante, M. Caniatti, F. Gottardo, G. Cozzi, I. Andrighetto, and M. Verga. 2002. The provision of solid feeds to veal calves: II. Behavior, physiology, and abomasal damage. Journal of Animal Science 80(2):367-375.

Moody, M., G. Zanton, J. Daubert, and A. Heinrichs. 2007. Nutrient utilization of differing forage-to-concentrate ratios by growing Holstein heifers. Journal of Dairy Science 90(12):5580-5586.

Morisse, J. P., D. Huonnic, J. P. Cotte, and A. Martrenchar. 2000. The effect of four fibrous feed supplementations on different welfare traits in veal calves. Animal Feed Science and Technology 84(1-2):129-136.

NEN. 1974. Onderzoekingsmethoden voor veevoeders - Bepaling van het gehalte aan reducerende suiker, ruwe totaal suiker, saccharose en ruwe lactose. NEN (Nederlandse Norm), Delft, the Netherlands.

NRC. 2000. Nutrient Requirements of Beef Cattle. 7th rev. ed. Natl. Acad. Press, Washington, DC.

Ortigues, I., T. Smith, M. Gill, S. B. Cammell, and N. W. Yarrow. 1990. The effect of fishmeal supplementation of a straw-based diet on growth and calorimetric efficiency of growth in heifers. British Journal of Nutrition 64(3):639-651.

Reynolds, C. K., H. F. Tyrrell, and P. J. Reynolds. 1991a. Effects of diet forage-to-concentrate ratio and intake on energy metabolism in growing beef heifers: net nutrient metabolism by visceral tissues. The Journal of Nutrition 121(7):1004.

Reynolds, C. K., H. F. Tyrrell, and P. J. Reynolds. 1991b. Effects of diet forage-to-concentrate ratio and intake on energy metabolism in growing beef heifers: whole body energy and nitrogen balance and visceral heat production. The Journal of Nutrition 121(7):994-1003.

Rijnen, M., M. Verstegen, M. Heetkamp, J. Haaksma, and J. Schrama. 2001. Effects of dietary fermentable carbohydrates on energy metabolism in group-housed sows. Journal of Animal Science 79(1):148-154.

Rotger, A., A. Ferret, S. Calsamiglia, and X. Manteca. 2005. Changes in ruminal fermentation and protein degradation in growing Holstein heifers from 80 to 250 kg fed high-concentrate diets with different forage-to-concentrate ratios. Journal of Animal Science 83(7):1616-1624.

Suárez, B., C. Van Reenen, W. Gerrits, N. Stockhofe-Zurwieden, A. Van Vuuren, and J. Dijkstra. 2006. Effects of supplementing concentrates differing in carbohydrate composition in veal calf diets: II. Rumen development. Journal of Dairy Science 89(11):4376-4386.

Suárez, B. J., C. G. Van Reenen, N. Stockhofe-Zurwieden, J. Dijkstra, and W. J. J. Gerrits. 2007. Effect of roughage source and roughage to concentrate ratio on animal performance and rumen development in veal calves. Journal of Dairy Science 90(5):2390-2403.

Tamate, H., A. D. McGilliard, N. L. Jacobson, and R. Getty. 1962. Effect of various dietaries on the anatomical development of the stomach in the calf. Journal of Dairy Science 45(3):408-420.

Van den Borne, J., J. Verdonk, J. W. Schrama, and W. J. J. Gerrits. 2006a. Reviewing the low efficiency of protein utilization in heavy preruminant calves - a reductionist approach. Reproduction Nutrition Development 46(2):121-137.

Van den Borne, J., M. W. A. Verstegen, S. J. J. Alferink, R. M. M. Giebels, and W. J. J. Gerrits. 2006b. Effects of feeding frequency and feeding level on nutrient utilization in heavy preruminant calves. Journal of Dairy Science 89(9):3578-3586.

Van Soest, P. J., J. Robertson, and B. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. Journal of Dairy Science 74(10):3583-3597.

Veissier, I., A. R. Ramirez De La Fe, and P. Pradel. 1998. Nonnutritive oral activities and stress responses of veal calves in relation to feeding and housing conditions. Applied Animal Behavior Science 57(1-2):35-49.

Webb, L. E., E. A. Bokkers, B. Engel, W. J. Gerrits, H. Berends, and C. G. van Reenen. 2012. Behavior and welfare of veal calves fed different amounts of solid feed supplemented to a milk replacer ration adjusted for similar growth. Applied Animal Behavior Science 136(2):108-116.

Welchman, D. D. and G. N. Baust. 1987. A survey of abomasal ulceration in veal calves. Veterinary Record 121(25-26):586-590.

SUPPLEMENTARY FIGURES

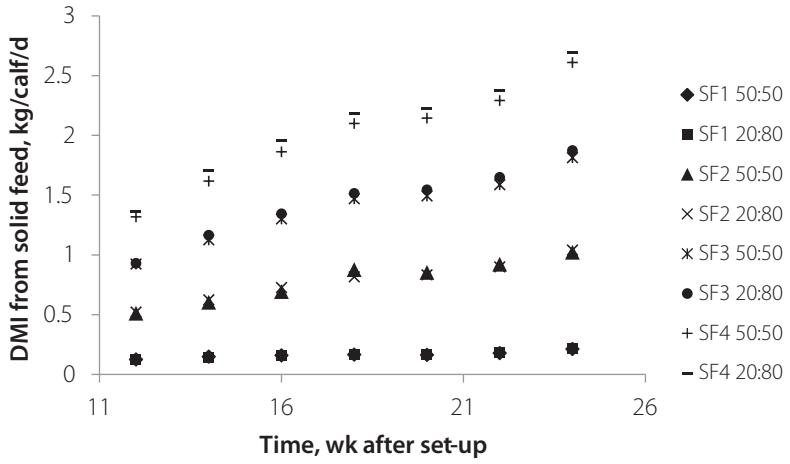


Figure 7.3 Realized DMI from solid feed in veal calves during experimental period. Treatments included 4 solid feed intake levels (SF1, SF2, SF3, SF4) and 2 roughage-to-concentrate ratios (50:50, 20:80).

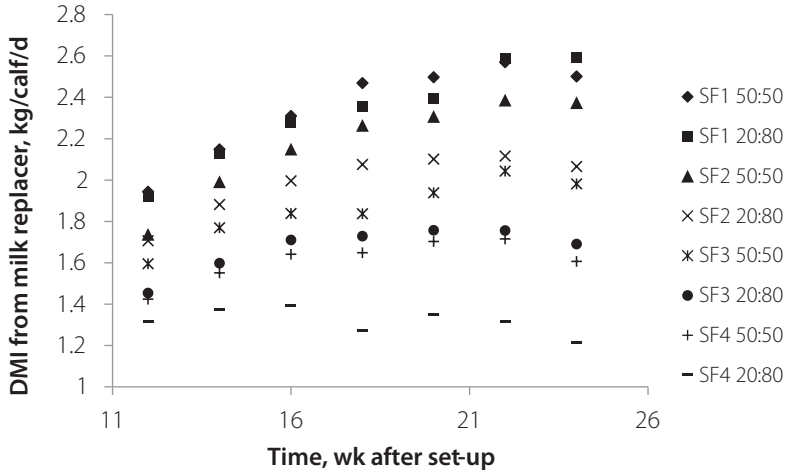


Figure 7.4 Realized DMI from milk replacer in veal calves during experimental period. Treatments included 4 solid feed intake levels (SF1, SF2, SF3, SF4) and 2 roughage-to-concentrate ratios (50:50, 20:80). The experimental set-up was paired-gain, while treatment were set, and DMI from milk replacer was adjusted bi-weekly to correct for differences in gain.

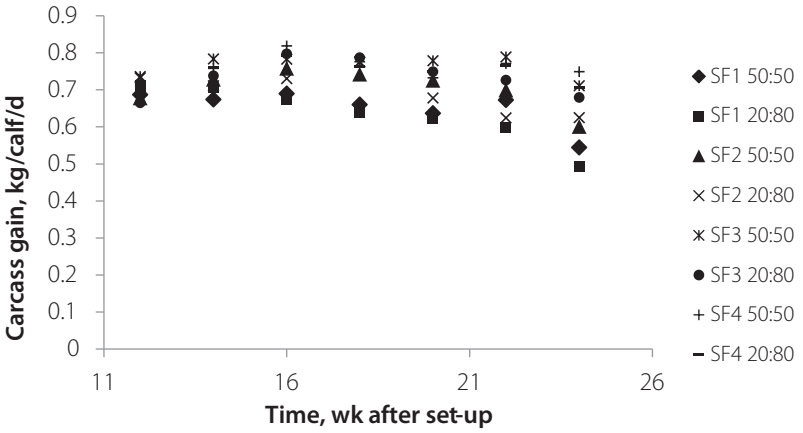
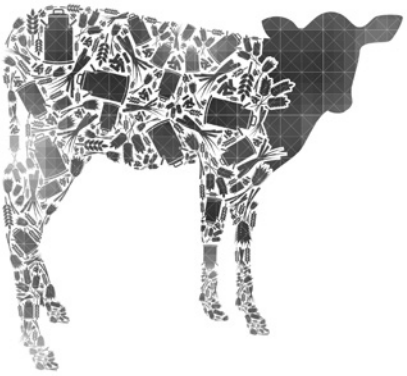


Figure 7.5 Realized carcass gain in veal calves during experimental period. Rates of carcass gain were estimated from the measured rates of BW gain, assuming that carcass: liveweight ratio was constant throughout the experimental period. Treatments included 4 solid feed intake levels (SF1, SF2, SF3, SF4) and 2 roughage-to-concentrate ratios (50:50, 20:80).

Chapter 8

Effects of solid feed level and roughage-to-concentrate ratio on ruminal drinking and passage kinetics of concentrate, straw, and milk replacer in veal calves



H. Berends[1], J.J.G.C. van den Borne[1], N. Stockhofe-Zurwieden[2], M.S. Gilbert[1], T. Zandstra[1], W.F. Pellikaan[1], C.G. van Reenen[3], E.A.M. Bokkers[4], and W.J.J. Gerrits[1]

To be Submitted

[1]Animal Nutrition Group, Wageningen University, Wageningen, the Netherlands;
[2]Central Veterinary Institute, Wageningen University and Research Centre, the Netherlands;
[3]Livestock Research, Animal Sciences Group, Wageningen University and Research Centre, Lelystad, the Netherlands;
[4]Animal Production Systems Group, Wageningen University, Wageningen, the Netherlands

ABSTRACT

This study aimed to investigate effects of solid feed (**SF**) level and roughage-to-concentrate (**R:C**) ratio on ruminal drinking and passage kinetics of milk replacer, concentrates, and straw in veal calves. In total, 80 male Holstein-Friesian calves (2 wk of age, 45 ± 0.2 kg bodyweight) were divided over 16 pens (5 calves per pen). Pens were randomly assigned to either a low (**LSF**) or a high (**HSF**) SF level, and to one of two R:C ratios; 20:80 or 50:50 on DM basis. Roughage was composed of 50% corn silage and 50% chopped wheat straw on DM basis. At 27 wk of age, measurements were conducted in 32 calves (2 calves per pen). During the measurement period, SF intake was 1170 g DM/d for LSF and 3000 g DM/d for HSF. Indigestible markers were supplied with the feed as a single dose to estimate passage kinetics of milk replacer (CoEDTA, supplied 4 h before slaughter), concentrates (hexatriacontane, C_{36}, supplied 24 h before slaughter), and straw (Cr-NDF, supplied 48 h before slaughter). At slaughter, marker recovery was measured in gastrointestinal compartments, and rumen development and abomasal damage were assessed.

Results showed that ruminal recovery of Co averaged 20% of the last milk replacer meal. Passage kinetics of milk replacer were largely unaffected by SF level and R:C ratio. Rumen fractional passage rate of concentrates was estimated from rumen C_{36} recovery and increased ($P < 0.001$; SEM: 0.445) from 3.30 %/h for LSF to 4.93 %/h for HSF. Rumen fractional passage rate of straw was estimated from rumen Cr recovery and increased from 1.30%/h for LSF to 1.67%/h for HSF. An increase in SF intake was accompanied by an increase in fresh and dry rumen contents. R:C ratio did not affect passage of concentrates or straw. Rumen development score, but not empty rumen weight increased with SF intake. Abomasal damage increased with SF intake, but was unaffected by R:C ratio. In HSF, but not in LSF calves, pH decreased with increasing concentrate proportion, whereas VFA concentrations increased, indicating increased fermentation. The similar ratio between Cr and C_{36} in the small and large intestine indicates that the digesta flow of concentrate and straw is mainly determined by rumen and abomasum emptying.

In conclusion, intake of SF introduces large variation in passage kinetics of digesta fractions in veal calves, predominantly in the rumen compartment. The level of SF, and not its R:C ratio, increases ruminal passage rates of concentrates and roughages in veal calves. These data provide insight in passage kinetics of milk replacer, roughages, and concentrates, and may contribute to the accuracy of diet formulation in calves fed milk replacer and SF.

Keywords: calves, passage rates, nutrient utilization, forage-to-concentrate ratio

INTRODUCTION

Provision of a minimum daily amount (50 to 250 g) of fibrous feed for veal calves is compulsory according to guidelines of the EU. Solid feed (**SF**) provision reduces abnormal oral behaviors (Kooijman et al., 1991, Veissier et al., 1998, Webb et al., 2012), thereby contributing to improved calf welfare. In addition, increasing prices of milk replacer (**MR**) ingredients provide an economic incentive to replace MR by SF in veal calf diets above these EU requirements. With increasing amounts of SF provision, veal calves become more dependent upon the products from rumen fermentation for maintenance requirements and growth than upon long chain fatty acids and glucose originating from MR. Recently, we showed that the contribution of SF and MR to body weight gain of veal calves largely follows its contribution to digestible energy (**DE**) supply, but strong effects of age exist (Berends et al., unpublished). Underlying mechanisms are not clear, but it is likely that variation in rumen passage kinetics of diet components largely contributes to variation in DE supply. To the best of our knowledge, there is no information on passage kinetics of digesta in veal calves fed a combination of MR and SF. In dairy calves, it was shown that reducing MR intake (up to 60% compared with a control) had only minor effects on rumen passage kinetics (Broesder et al., 1990). In addition, roughage-to-concentrate (**R:C**) ratio (Colucci et al., 1982, Poore et al., 1990, Rotger et al., 2005) as well as SF level (Colucci et al., 1990) may affect passage kinetics, as observed in ruminants fed only SF. In calves, MR is generally assumed to bypass the reticulorumen by means of the esophageal groove reflex, but ruminal drinking (i.e. leakage of MR to the rumen) can be substantial in dairy calves (0-25%) (Abe et al., 1979, Guilhermet et al., 1975) and veal calves (14 to 35%) (Berends et al., 2012, Suárez et al., 2007). The objective was to study the effect of SF level and R:C ratio on ruminal drinking and digesta kinetics in veal calves fed MR, roughages and concentrates. Indigestible markers were used to estimate kinetics of MR (CoEDTA), concentrates (hexatriacontane; C_{36}), and straw (Cr-NDF) within the digestive tract.

MATERIAL AND METHODS

This study was conducted at the research facilities of VanDrie group (Scherpenzeel, the Netherlands). Procedures complied with the Dutch Law on Experimental Animals and the ETS123 (Council of Europe 1985 and the 86/609/EEC Directive) and were approved by the Animal Care and Use Committee of Wageningen University.

Animals, Experimental Design, and Housing

Eighty male Holstein-Friesian calves (2 wk of age; 45 ± 0.2 kg bodyweight) were purchased from commercial dairy farms, and selected based on uniformity and clinical health. Calves were allocated to pens (5 calves per pen; 16 pens) based on bodyweight (**BW**). Pens were

randomly assigned to one of two SF levels: LSF or HSF, respectively, and to one of two R:C ratios: 20:80 or 50:50 (Table 8.1). Upon arrival, calves were adapted to the designated SF intake levels at a fixed R:C of 50:50 until wk 10. During the experimental period (wk 11 to 27), calves were exposed to their assigned SF level and R:C ratio. During the last 2 d of the experimental period, passage kinetics were measured in 32 calves.

Table 8.1 Daily DM intake from milk replacer (g DM/calf/d) in calves (n = 8 per treatment combination) fed at one of two levels of solid feed (SF) intake, at one of two roughage-to-concentrate ratios (R:C)[1] at 27 wk of age

	R:C ratio	
SF intake level	**20:80**	**50:50**
Low[2]	2224	2370
High[2]	1058	1458

[1] Roughage consisted of 50% corn silage and 50% chopped wheat straw on a DM basis.
[2] SF intake in the measurement period was 1170 and 3000 g DM/d for the low and high SF level, respectively.

During the experimental period, calves were housed in groups of 5 calves in pens (3 x 3 m) equipped with wooden-slatted floors and open fences, and without bedding material. During the last 2 d of the experimental period, 2 calves per pen were randomly selected and housed in individual pens placed inside the group pen to facilitate individual feeding and monitoring. Calves were exposed to daylight and artificial light from 0500 h to 2300 h and to darkness during the remainder of the day. Animal health was checked daily. Hemoglobin concentration in blood was monitored across the trial at 11, 15, 19, and at 23 wk and corrected to comply with the minimum EU level of 4.5 mmol/L at the end of the fattening period.

Diets and Feeding

Roughage was composed of 50% corn silage and 50% chopped wheat straw on a DM basis. Provision of SF increased bi-weekly and linearly. The quantity of MR was calculated to achieve similar rates of carcass gain across treatments, and adjusted biweekly based on the realized BW gain in the preceding 2 wk period (Berends et al., unpublished). During the experimental period, SF intake averaged 780 g DM/d for LSF and 2000 g DM/d for HSF. Ingredient and nutrient composition of SF components and MR are presented in Table 8.2. The concentration of MR increased gradually from 125 g/L to 188 g/L. The MR was supplied in buckets at 40 to 41°C, provided twice daily in equally sized meals at 0600 h and at 1600 h, respectively. During the first 8 wk after arrival, calves were provided a commercial starter MR based on 32.2% whey powder and 30.0% skimmed milk powder with 223 g/kg DM crude protein and 180 g/kg DM crude fat. Subsequently, the MR as presented in Table 8.3

was provided. The SF was provided as a mixture in a long feed trough in front of the pen directly after the morning meal; refusals were removed and weighed once daily. During the first 4 wk of the adaptation period, calves were allowed ad libitum access to water supplied in buckets around noon. Thereafter, calves had free access to water provided via drinking nipples. During the 2-d measurement period (wk 27), calves were provided free access to water at 1200 h and at 2200 h.

Table 8.2 Analysed nutrient composition of concentrate, corn silage, and straw

Nutrient[1]	Concentrate[2]	Corn silage	Straw
Dry matter, g/kg product	898	297	931
Crude protein[3]	137	69	31
Crude fat	67	29	9
Starch	429	312	11
Neutral detergent fibre	127	421	794

[1] Expressed in g/kg DM unless specified otherwise.
[2] Concentrate composition: 36.2% corn, 20.6% lupins, 20.3% barley, 12.5% carob meal, 4.4% corn gluten meal, 6% premix (lactose carrier, provided per kg concentrate: vitamin A: 4,000 IU; vitamin D: 500 IU; vitamin E: 100 IU; zinc: 25 mg; manganese: 20 mg; iodine: 0.8 mg; selenium: 0.15 mg; copper: 15 mg; cobalt: 0.1 mg, 11.5 g $MgSO_4.7H_2O$, 7.7 g NaCl, 21.5 g $CaCO_3$, 13.5 g KH_2PO_4).
[3] N × 6.25.

Measurements

Feed intake and performance. MR intake and SF intake was measured daily. Calves were weighed every 2 wk.

Markers. Passage kinetics were assessed by measuring the recovery of external markers in digesta in four compartments of the gastrointestinal tract; reticulorumen, abomasum, small intestine, and large intestine at slaughter. To assess passage kinetics of straw, chromium mordanted fiber straw (Cr-NDF, 50 g) was provided with the SF at 48 h before slaughter. The Cr-NDF was prepared as described by Udén et al. (1980) and had the same particle size as the chopped straw (Table 8.2). To assess ruminal drinking (leakage of MR into the rumen) and passage kinetics of MR, 36 g Co(II)EDTA (Udén et al., 1980), was dissolved into the final MR meal, and provided 4 h before slaughter. To assess passage kinetics of concentrates was enriched with 6% hexatriacontane (C_{36}; > 95.0% w/w, MINAKEM, Beuvry-la-Forêt, France) and provided with the SF 24 h before slaughter.

Slaughter Procedure. At the end of the measurement period, calves were culled at the experimental facilities at one of two consecutive slaughter days. Euthanasia by pentobarbital was followed by exsanguination. To avoid reflux of MR from the abomasum into the forestomachs at slaughter, calves were lifted by the forelegs immediately following exsanguination. Subsequently, ligations were made at the level of the thoracic entrance,

Table 8.3 Ingredient composition and analyzed nutrient composition of milk replacer

Ingredient, g/kg		Nutrient[4], g/kg DM	
Fat-enriched whey[1]	394.0	Dry matter, g/kg product	969.5
Whey protein concentrate	193.9	Crude protein[5]	210.0
Whey	143.3	Crude fat	211.6
Delactosed whey	135.0	Starch	22.3
Soy protein isolate	37.7		
Soy protein concentrate	25.0		
Acidified whey[2]	20.0		
Pregelatinized wheat starch	20.0		
Vitamin and mineral premix[3]	10.0		
Calcium formate	9.8		
L-lysine.HCl	5.2		
DL-methionine	2.7		
Citric acid	2.0		
Mono ammonium phosphate	1.4		
L-threonine	0.2		

[1] Fat-enriched whey contained 50% fat from palm oil and coconut oil (80/20, %w/w).
[2] Acidified with lactate, provided 5.9 g lactate per kg of the experimental diet.
[3] Provided per kg of the experimental diet: 16.6 g K, 15.5 g Cl, 8.3 g Fe, 6.1 g P, 5.9 g Cu, 5.7 g Na, 1.3 g Mg, 25,000 IU vitamin A, 4,000 IU vitamin D3, 100 IU vitamin E.
[4] Expressed in g/kg DM unless specified otherwise.
[5] N × 6.25.

the omasal-cardia of the abomasum and the reticulo-omasal orifice. The gastrointestinal tract was divided into four segments: reticulorumen, abomasum, small intestine, and large intestine. The weight of each segment was recorded with and without contents. Rumen contents were quantitatively collected and solid and liquid phases were separated using a metal sieve (1.5 mm). A reconstituted sample of rumen contents was prepared by proportional sampling of the liquid and solid phases, which was oven-dried and stored for analysis. After mixing, samples of abomasal contents, small intestinal contents, and large intestinal contents were taken and stored at -20 °C pending analysis. The length of small intestine was measured. In addition, a sample of the rumen liquid was used for pH measurement, then acidified with H_3PO_4 (5%) and stored at -20 °C pending analysis of volatile fatty acids (**VFA**).

The rumen mucosal surface was examined visually and the presence and density of rumen papillae were scored on a 5-point scale from poor to excellent: 1 – 1.5 – 2 – 2.5 – 3 according to Suárez et al. (2007). The abomasal wall was visually inspected macroscopically for lesions (erosions, ulcers, and scars) in the following size categories: small = modification

≤ 0.5 cm^2; medium = 0.5 cm^2 < modification < 1.0 cm^2; large = modification ≥ 1.0 cm^2. Surface area of lesions in the pyloric region was then estimated by multiplication of counts and size category, where average size per category was assumed to be 0.25 cm^2 for small-sized lesions, 0.75 cm^2 for medium-sized lesions and 1.25 cm^2 for large-sized lesions.

Chemical Analyses. Samples of MR, concentrate, corn silage, and straw were collected weekly. Corn silage samples were pooled by month; other samples were pooled for the experimental period. Feed components (MR, corn silage, concentrate, straw) were analysed for DM, ash, N, starch, NDF (except for MR), and crude fat content. For determination of DM content, corn silage and concentrate samples were freeze-dried. Concentrate, straw, and corn silage samples were ground to pass a 1-mm screen. Dry matter content was determined by drying to a constant weight according to ISO Standard 6496 (ISO, 1998). Crude ash content was determined by incineration in a muffle furnace by combustion at 550°C according to ISO 5984 (ISO, 2002). Kjeldahl N content was determined according to ISO 5983 (ISO, 1997). Starch content was determined enzymatically as described by Rijnen et al. (2001). The NDF content was analysed according to Van Soest et al. (1991). Crude fat content was determined after acid hydrolysis according to ISO 6496 (ISO, 1999). Concentrations of VFA were quantified by gas chromatography (GC; Fisons HRGC Mega 2, CE Instruments, Milan, Italy) according to Pellikaan et al., 2011 with a split/splitless injector operated in split mode (split ratio 1:10) and fitted to a flame ionisation detector (FID), using a capillary column (EC-1000, Alltech; 30 m, i.d. 0.53 mm, film thickness 1.00 µm) with He as the carrier gas (50 kPa pressure). The starting temperature of the column was set at 110 °C for 2 min followed by an 18 °C /min increase to 200 °C at which point the temperature was maintained for 1 min. Isocaproic acid was included as the internal standard.

The Cr and Co concentrations were determined in the contents from each gastrointestinal compartment by atomic absorption spectrometry according to Pellikaan et al., 2013. The external marker was oxidized by wet-destruction at 350 °C for 1 h in an HNO_3 (65%, Fluka Chemie GmbH, Buchs, Switzerland) and $HClO_4$ (70% to 72%, Merck KgaA) solution and absorbance of Cr^{6+} was measured at 357.8 nm in a nitrous oxide acetylene flow using an atomic absorption spectrophotometer (AA240FS, Varian, Palo Alto, CA, USA).

Extraction and analysis of *n*-alkanes was carried out according to Bezabih et al. (2011). In short, ground samples were pulverized using a bullet mill (MM 2000; 4 min at 80 Hz; Retsch Technology GmbH, Haan, Germany) before extraction and analysis as described by Mayes et al. (1986) with modifications of Salt et al. (1992) using tetratriacontane as an internal standard. The extracted samples were analyzed for C_{36} using a gas chromatograph. The concentration of C_{36} was calculated from peak areas using an internal standard (Bezabih et al., 2011).

Calculations. Rumen fractional passage rates were estimated for concentrates and straw from rumen recoveries of C_{36} and Cr according to the formula:

$$C_t = C_0 \cdot e^{-kp \cdot t},$$

where C_t is the marker concentration at time t, C_0 is the marker concentration at time 0, kp is the fractional passage rate and t is time in h.

Statistical Analysis. The dataset included 32 observations. For Co recovery, 8 calves were excluded because of MR refusals exceeding 10% (1 LSF 50:50; 5 LSF 20:80; 2 HSF 50:50). Dependent variables were analyzed by mixed model analysis in PROC GLIMMIX for proportions and in PROC MIXED (SAS 9.20 by SAS Institute, Inc, Cary, NC) for continuous data, with calf as experimental unit, including pen as random effect, SF level, R:C ratio, and day of slaughter as fixed effects, and the interaction between SF level and R:C ratio. Model residuals were checked for normal distribution. Results are presented as least squared means and their SEM. Differences were considered significant at $P < 0.05$.

RESULTS

During the experimental period, LSF calves consumed 93 (R:C ratio 50:50) and 94 kg DM of SF (R:C ratio 20:80) and 251 and 232 kg DM of MR, respectively. In the same period, HSF calves consumed 239 (R:C ratio 50:50) and 248 kg DM of SF (R:C ratio 20:80) and 184 and 150 kg DM of MR, respectively (results not shown). Live weight was greater ($P < 0.05$) for calves fed HSF (258 kg) than for calves fed LSF (239 kg), but growth performance was not affected by R:C ratio (results not shown).Rumen Co recovery showed large variation and was unaffected by SF level and R:C ratio (Table 8.4). Abomasal Co recovery was greater ($P < 0.05$) in calves fed LSF than in calves fed HSF. Rumen recovery of C_{36} was unaffected by R:C ratio, but decreased by 15% points ($P < 0.01$) with increasing SF intake. This difference was reflected in the cumulative C_{36} recovery. Rumen recovery of Cr was unaffected by R:C ratio, but was decreased by 8% points ($P < 0.01$) with increasing SF intake. This difference was reflected in the cumulative Cr recovery.

Table 8.4 Effects of level of solid feed (SF; low vs. high) and roughage-to-concentrate ratio (R:C; 20:80 vs. 50:50) on the recovery of pulse dosed digesta markers Co, Cr and C_{36}, in gastrointestinal compartments of veal calves of 249 ± 4.0 kg BW[1]

	Low SF		High SF			P-value		
Item	**20:80**	**50:50**	**20:80**	**50:50**	**SEM**	**SF**	**R:C**	**SF × R:C**
Co recovery, %								
Rumen	18.3	11.4	16.3	34.4	9.59	NS	NS	NS
Abomasum	11.2	14.8	9.2	3.3	3.11	*	NS	†
Small intestine	27.6	44.7	39.4	31.0	8.05	NS	NS	†
Large intestine	23.1	16.7	20.4	17.9	4.89	NS	NS	NS
Total	78.4	86.2	84.0	85.6	3.17	NS	NS	NS
C_{36} recovery, %								
Rumen	48.4	44.2	30.0	32.4	4.10	***	NS	NS
Abomasum	1.2	2.0	2.0	1.6	0.47	NS	NS	†
Small intestine	3.3	1.7	2.6	4.5	0.91	NS	NS	**
Large intestine	13.5	14.3	14.5	13.5	2.43	NS	NS	NS
Total	66.4	62.2	49.1	52.1	4.74	**	NS	NS
Cr recovery, %								
Rumen	55.6	53.6	48.4	42.2	3.78	**	NS	NS
Abomasum	1.6	2.2	1.6	0.6	0.79	NS	NS	**
Small intestine	1.6	0.9	0.9	2.2	0.51	NS	NS	**
Large intestine	5.2	6.9	5.0	5.3	1.23	NS	NS	NS
Total	63.9	63.5	56.9	51.1	3.78	**	NS	NS

[1] Dietary treatments as explained in Table 8.1. Co was pulse dosed as CoEDTA with milk replacer 4 h before slaughter; C_{36} was pulse dosed with concentrates in SF 24 h before slaughter; Cr was pulse dosed as Cr-NDF with straw in SF 48 h before slaughter.

NS: not significant; †$P < 0.10$; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

At high SF intake, a low R:C ratio resulted in a 1 point lower pH ($P < 0.05$) and a higher total VFA concentration (+37 mmol/L; SF×RC, $P < 0.01$; Table 8.5). The level of SF level did not affect empty weights of the gastrointestinal compartments, but rumen contents (fresh as well as dry) increased ($P < 0.001$) in response to an increase in SF level. DM content decreased with increasing concentrate inclusion, in the rumen, abomasum, and numerically in the large intestine. The empty weight of the abomasum increased ($P < 0.05$) with increasing R:C ratio. Small intestinal weight, contents, or length were unaffected by SF level or R:C ratio.

Table 8.5 Effects of level of solid feed (SF; low vs. high) and roughage-to-concentrate ratio (R:C; 20:80 vs. 50:50) on weights of gastrointestinal compartments, contents, and DM content in veal calves of 249 ± 4.0 kg BW[1]

	Low SF		High SF			*P*-value		
Item	20:80	50:50	20:80	50:50	SEM	SF	R:C	SF × R:C
Rumen								
Ruminal pH	6.4	6.5	6.0	6.9	0.19	NS	**	*
Total VFA, mmol/L	125	127	161	124	9.3	†	†	*
Empty weight, kg	5.1	4.6	5.4	6.0	1.00	NS	NS	NS
Contents, kg	16.9	15.7	23.4	22.9	2.04	***	NS	NS
DM content, g/kg	137	152	163	174	12.12	***	*	NS
Abomasum								
Empty weight, kg	1.8	2.4	2.0	2.1	0.16	NS	*	*
Contents, kg	1.5	2.6	2.5	1.2	0.47	NS	NS	**
DM content, g/kg	44	69	81	83	11.0	***	*	*
Small intestine								
Empty weight, kg	5.0	5.4	5.0	5.0	0.35	NS	NS	NS
Contents, kg	3.7	4.0	4.7	3.8	0.52	NS	NS	NS
DM content, g/kg	66	65	61	74	7.69	NS	NS	NS
Length, m	28.2	29.9	29.5	28.4	0.65	NS	NS	NS
Large intestine								
Empty weight, kg	2.4	2.5	2.6	2.2	0.19	NS	NS	*
Contents, kg	2.0	1.7	2.1	2.1	0.18	†	NS	NS
DM content, g/kg	113	130	143	137	11.0	*	NS	NS

[1] Dietary treatments as explained in Table 8.1.
NS: not significant; †$P < 0.10$; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

From rumen recoveries of Cr and C_{36}, fractional rates of passage for these markers were estimated (Table 8.6). Ruminal passage rate of C_{36}, representing concentrates, increased ($P < 0.001$) with SF level, from 3.30%/h for LSF to 4.93%/h for HSF calves. Ruminal passage rate of Cr, representing straw, increased ($P < 0.001$) with SF level, from 1.30%/h for LSF to 1.67%/h for HSF calves. Ruminal passage rates of concentrates and straw were unaffected by R:C ratio.

Table 8.6 Effects of level of solid feed (SF; low vs. high) and roughage-to-concentrate ratio (R:C; 20:80 vs. 50:50) on estimated fractional ruminal passage rates for concentrates and straw in veal calves of 249 ± 4.0 kg BW[1]

	Low SF		High SF			*P*-value		
	20:80	50:50	20:80	50:50	SEM	SF	R:C	SF × R:C
Kp concentrates, %/h	3.17	3.44	5.07	4.78	0.445	***	NS	NS
Kp straw, %/h	1.27	1.32	1.53	1.80	0.160	**	NS	NS

[1] Dietary treatments as explained in Table 8.1.
NS: not significant; ** $P < 0.01$; *** $P < 0.001$.
Characteristics describing abomasal damage are presented in Table 8.7. The estimated area of abomasal damage in the pyloric area was greater ($P < 0.05$) in calves fed HSF than in calves fed LSF. Rumen development score increased ($P < 0.05$) with SF intake but was unaffected by R:C ratio.

Table 8.7 Effects of level of solid feed (SF; low vs. high) and roughage-to-concentrate ratio (R:C; 20:80 vs. 50:50) on abomasal damage in veal calves of 249 ± 4.0 kg BW[1]

	Low SF		High SF			*P*-value		
	20:80	50:50	20:80	50:50	SEM	SF	R:C	SF× R:C
Pyloric area estimate, cm^2	0.63	1.47	2.89	3.07	1.74	*	NS	NS
Rumen development score	1.75	1.75	2.69	1.81	0.353	*	†	†

[1] Dietary treatments as explained in Table 8.1.
NS: not significant; † $P < 0.10$; * $P < 0.05$; ** $P < 0.01$.

DISCUSSION

Ruminal Drinking and Passage of MR

Although the majority of MR is assumed to bypass the rumen by means of the esophageal groove reflex, ruminal drinking, i.e. the leakage of MR in the rumen, is frequently observed in veal calves. In the current study, ruminal recovery of Co, supplied with the MR, averaged 20% (Table 8.4), which is in line with previous studies (Berends et al., 2012, Suárez et al., 2007), but exceeding estimates from others (< 5%; Berends et al., unpublished; Labussière et al. (unpublished). Although SF level and R:C ratio did not affect ruminal drinking, the large variation (20 ± 9.6) complicates interpretation of the effect of SF level and R:C ratio on passage of MR through the remaining part of the digestive tract. In order to gain more insight in the gastrointestinal transit of MR, Co recovery in the abomasum, small intestine, and large intestine was expressed as a percentage of the oral dose corrected for rumen recovery (Figure 8.1), thereby neglecting passage of the marker from the rumen compartment within the 4-h period between administration and slaughter.

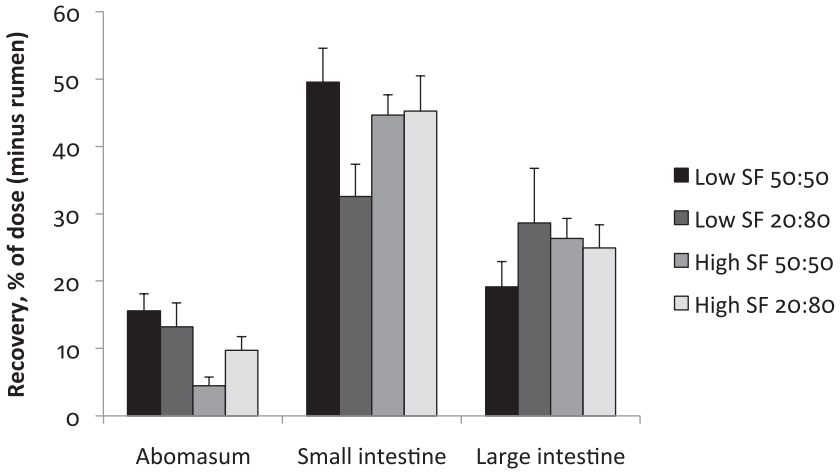


Figure 8.1 Co recovery as a percentage of the dose administered, corrected for rumen recovery (see Material and Methods section). Abomasal recovery was affected by level of SF ($P < 0.01$) but not by R:C ratio or the interaction. Small and large intestinal recoveries were unaffected by SF level, R:C ratio or the interaction.

Results show that passage kinetics of MR were hardly affected by SF intake or composition. In the current study, there was a large contrast in MR intake between treatments (Table 8.1), with LSF 50:50 calves receiving the largest amounts of MR and HSF 20:80 receiving the lowest amounts of MR. In ruminants, abomasal volume and the rate of digesta flow from the abomasum and along the small intestine are linearly related to the level of intake (Gregory et al., 1985). For MR, abomasal emptying rate is negatively related to meal volumes (Bell and Razig, 1973). The significant effect of SF intake on abomasal recovery (Table 8.4; Figure 8.1) may therefore be partly explained by the associated reduction in MR intake.

Total Co recovery averaged 84% (Table 8.4), indicating, part of the MR was already excreted with the feces within 4 h after feeding. Alternatively, the assumption that CoEDTA is not absorbed may be debated. Although this marker has been used to estimate the total emptying rate (Gregory et al., 1985) and ruminal milk (Berends et al., 2012, Suárez et al., 2007), an underestimation on CoEDTA recovery may be possible due to Co excretion via urine, which was found to be 2-3% in ruminants (Udén et al., 1980).

In summary, strong effects of SF intake and R:C ratio on passage kinetics of MR were not found in this study. Results confirm earlier indications that ruminal drinking is quantitatively important, but also highly variable, in calves fed SF and MR.

Passage Rates of Concentrates and Straw

The observed increase in ruminal fractional passage rate with increasing SF intake and decreasing MR intake was previously observed in dairy calves (Broesder et al., 1990) and with increasing SF intake in adult ruminants (Colucci et al., 1990, Owens and Goetsch, 1986, Robinson et al., 1987). However, increasing SF intake, confounded with age, in young ruminants did not affect passage of particles (Hodgson, 1971a, Lallès and Poncet, 1990). Fractional passage rates observed in the current study are in line with passage rates reported previously for dairy calves around weaning (Hart and Polan, 1984, Lallès and Poncet, 1990, Vazquez-Anon et al., 1993) and adult ruminants cattle fed only SF (Colucci et al., 1990, Owens and Goetsch, 1986; Warner et al., 2013), when considering differences in BW, rumen volume, and SF intake. SF intake in the current study was rather low because a substantial part of the DE was still supplied by MR. A relatively low SF intake results in a lower passage rate. Potentially, rumen motility is also different in these calves when compared to calves fed only SF. Furthermore, increasing SF intake increased rumen contents (Table 8.5), which is in accordance with the positive relationship observed between rumen fluid volume and SF intake in young calves during weaning (Hodgson, 1971b) and in dairy cows (Owens and Goetsch, 1986). With an identical rate of passage, a greater rumen volume enables a longer retention time and thus greater feed degradation. Gaining insight into these aspects could substantially contribute to the accuracy of feed evaluation.

Ruminal passage rate of concentrates is typically greater than ruminal passage rate of roughages like straw (Colucci et al., 1990) which can be explained by increased rumination and communition to decrease particle size of roughages. The absence of an effect of R:C ratio on passage rates of concentrates (Poore et al., 1990, Warner et al., 2013) and roughages (Poore et al., 1990, Rotger et al., 2005) is supported by literature in steers, heifers, and dairy cows, although the effect of R:C ratio may depend on the range of concentrate inclusion and on the level of total SF intake (Colucci et al., 1990, Offer and Dixon, 2000). A negative effect of concentrate proportion on rumen volume, as observed in ruminants (Owens and Goetsch, 1986), was not found in the current study.

The relationship between C_{36} and Cr recovery in the different post-rumen compartments was significant ($P < 0.001$) and strong ($R^2 \geq 0.80$) for the tubular parts of the gut (Figure 8.2), but this was less clear for the abomasum ($R^2 = 0.49$; Figure 8.2) and the rumen ($R^2 = 0.40$; results not shown). These results suggest that rumen and abomasal emptying differ between concentrates and straw, whereas passage through the remaining part of the gut is similar for concentrates and straw. This observation is in line with previous studies on passage behavior of solid and liquid markers through the post-rumen sections of the gastrointestinal tract in dairy cows (Pellikaan, 2004, Wylie et al., 2000).

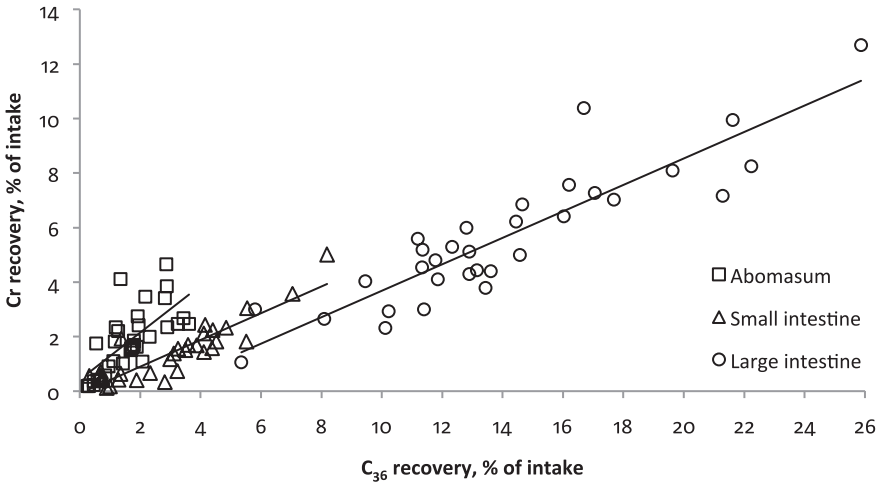


Figure 8.2 Relationship between hexatriacontane (C_{36}) recovery and chromium (Cr) recovery in abomasum (□, y = 0.86 x + 0.42; R^2 = 0.49), small intestine (Δ, y = 0.49 x – 0.08; R^2 = 0.80), and large intestine (□, y = 0.49 x – 1.17; R^2 = 0.80). Co was pulse dosed as CoEDTA with milk replacer 4 h before slaughter; C_{36} was pulse dosed with concentrates 24 h before slaughter; Cr was pulse dosed as Cr-NDF with straw 48 h before slaughter. Recoveries were determined in gastrointestinal compartments of veal calves of 249 ± 4.0 kg BW. For each segment, the estimated regression coefficient was significant ($P < 0.001$).

In conclusion, the low passage rates found for concentrates and straw in the current study can be explained by the low SF intake (or high MR intake) and by an increase in rumen volume. The strong relationship between Cr and C_{36} recovery in the small and large intestine indicates that the digesta flow of concentrate and straw is determined by rumen and abomasum emptying and rather similar in the tubular parts of the gut.

Gastrointestinal Development

Ruminal VFA concentrations observed in the current study (Table 8.5) were in the upper range of VFA concentrations observed in growing calves and heifers (Hart and Polan, 1984, Moody et al., 2007, Rotger et al., 2005, Vazquez-Anon et al., 1993). This could be explained by ruminal drinking in these calves (Table 8.4). The levels of MR fed to the calves (Table 8.1) and the associated ruminal drinking may have masked effects of SF level and R:C ratio on rumen fermentation parameters. Furthermore, variation in rumen volume (Table 8.5) may have contributed to variation in VFA concentrations. The greater ruminal VFA concentration observed in calves fed a high level of SF with a 20:80 R:C ratio indicates a greater rumen fermentation which can be explained by the large amount of readily degradable fiber

originating from concentrates in that diet. The increase in total VFA concentration is accompanied by a reduction in rumen pH in these calves. Rumen development tended to be improved with a greater concentrate inclusion (from 50 to 80%). In previous work, we showed that concentrate inclusion from 0 to 50% increased the rumen development score (Berends et al., 2012). Also, the increase in rumen development score with increasing SF intake was reported previously (Berends et al., unpublished), but this effect was not accompanied by an increase in empty rumen weight. Therefore, we speculate that papillae development rather than muscular development is affected by SF intake, as the latter is more likely to directly contribute to weight gain. Abomasal weight decreased with concentrate inclusion in LSF calves (Table 8.5). Possibly, the abomasum adapts its capacity and weight to R:C ratio.

The area of abomasal damage (including ulcers, erosions, and scars) was estimated from counts and size categories and increased with increasing SF intake, regardless the R:C ratio (Table 8.7). We previously hypothesized that early rumen development may protect the abomasum against diet-induced damage, as indicated by a decrease in large scars at 27 wk of age in calves with a well-developed rumen at 14 wk of age (Berends et al., 2012). In the current study, however, the increase in rumen development was accompanied by an increase in abomasal damage. These data indicate that rumen development cannot prevent the increase in abomasal damage with increasing SF intake.

CONCLUSIONS

Ruminal recovery of Co, indicating ruminal drinking, averaged 20% of the last MR meal and was not affected by SF level and R:C ratio. Rumen fractional passage rate of concentrates increased from 3.30 %/h for LSF to 4.93 %/h for HSF. Rumen fractional passage rate of straw increased from 1.30%/h for LSF to 1.67%/h for HSF. In addition to an increase in rumen passage rate, increasing SF intake also increased fresh and dry rumen contents. Rumen development score, but also abomasal damage, increased with increasing SF intake, and were unaffected by R:C ratio. The strong relationship between Cr and C_{36} recovery in small and large intestine indicates that the digesta flow of concentrate and straw is determined by rumen and abomasum emptying and that transit of concentrates and straw is similar in the tubular parts of the gut. These data provide insight in passage kinetics of MR, roughages, and concentrates, and may contribute to the accuracy of diet formulation in calves fed MR and SF.

ACKNOWLEDGEMENTS

We thank Klaas Boeder, Bart Evers, Ewart van Voorst (VanDrie Group, Scherpenzeel), Bert Beukers, André Jansen, Joswita van der Velde (Wageningen University, the Netherlands), Pieter Roskam, and Ralph Kok (Central Veterinary Institute, the Netherlands) for their skilled

technical assistance. The Division for Earth and Life Sciences (ALW) of the Netherlands Organisation for Scientific Research (NWO, The Hague, the Netherlands), and the Product Board Animal Feed (The Hague, the Netherlands) are gratefully acknowledged for financially supporting this research.

REFERENCES

Abe, M., T. Iriki, K. Kondoh, and H. Shibui. 1979. Effects of nipple or bucket feeding of milk-substitute on rumen by-pass and on rate of passage in calves. British Journal of Nutrition 41(01):175-181.

Bell, F. and S. Razig. 1973. Gastric emptying and secretion in the milk-fed calf. The Journal of physiology 228(2):499-512.

Berends, H., C. G. van Reenen, N. Stockhofe-Zurwieden, and W. J. J. Gerrits. 2012. Effects of early rumen development and solid feed composition on growth performance and abomasal health in veal calves. Journal of Dairy Science 95(6):3190-3199.

Bezabih, M., W. Pellikaan, A. Tolera, and W. Hendriks. 2011. Evaluation of n-alkanes and their carbon isotope enrichments (δ13C) as diet composition markers. Animal 5(01):57-66.

Broesder, J. T., M. B. Judkins, L. J. Krysl, S. A. Gunter, and R. K. Barton. 1990. Thirty or sixty percent milk replacer reduction for calves: effects on alfalfa hay intake and digestibility, digestive kinetics and ruminal fermentation.Journal of Animal Science 68(9):2974-2985.

Colucci, P., L. Chase, and P. Van Soest. 1982. Feed intake, apparent diet digestibility, and rate of particulate passage in dairy cattle. Journal of Dairy Science 65(8):1445-1456.

Colucci, P., G. Macleod, W. Grovum, I. McMillan, and D. Barney. 1990. Digesta kinetics in sheep and cattle fed diets with different forage to concentrate ratios at high and low intakes. Journal of Dairy Science 73(8):2143-2156.

Gregory, P., S. Miller, and A. Brewer. 1985. The relation between food intake and abomasal emptying and small intestinal transit time in sheep. British Journal of Nutrition 53(02):373-380.

Guilhermet, R., C. Mathieu, and R. Toullec. 1975. Transit des aliments liquides au niveau de la gouttiere oesophagienne chez le veau preruminant et ruminant. Annales De Zootechnie 24:69-79.

Hart, S. P. and C. E. Polan. 1984. Effect of sodium bicarbonate and disodium phosphate on animal performance, ruminal metabolism, digestion, and rate of passage in ruminating calves. Journal of Dairy Science 67(10):2356-2368.

Hodgson, J. 1971a. The development of solid food intake in calves 3. The relation between solid food intake and the development of the alimentary tract. Animal Science 13(03):449-460.

Hodgson, J. 1971b. The development of solid food intake in calves. 2. Studies on the volume of rumen fluid, determined by an indirect method. Animal Production 13(01):25-36.

ISO. 1997. Animal feeding stuffs - Determination of nitrogen content and calculation of crude protein content. ISO 5983. Int. Organ. Stand., Geneva, Switzerland.

ISO. 1998. Animal feeding stuffs - Determination of moisture and other volatile matter content. ISO 6496. Int. Organ. Stand., Geneva, Switzerland.

ISO. 1999. Animal feeding stuffs - Determination of fat content. ISO 6492. Int. Organ. Stand., Geneva, Switzerland.

ISO. 2002. Animal feeding stuffs - Determination of crude ash. ISO 5984. Int. Organ. Stand., Geneva, Switzerland.

Kooijman, J., H. K. Wierenga, and P. R. Wiepkema. 1991. Development of abnormal oral behavior in group-housed veal calves: Effects of roughage supply. New Trends in Veal Calf Production:54-58.

Lallès, J. and C. Poncet. 1990. Rate of passage of digesta during and after weaning in calves fed concentrate diets containing pea or soya-bean meal. Livestock Production Science 24(4):333-345.

Mayes, R.W., C.S. Lamb, and P.M. Colgrove. 1986. The use of dosed and herbage n-alkanes as markers for the determination of herbage intake. Journal of Agricultural Science, Cambridge 107:161-170.

Moody, M., G. Zanton, J. Daubert, and A. Heinrichs. 2007. Nutrient utilization of differing forage-to-concentrate ratios by growing Holstein heifers. Journal of Dairy Science 90(12):5580-5586.

Offer, N. and J. Dixon. 2000. Factors affecting outflow rate from the reticulo-rumen. Pages 833-844 in Proc. Nutrition Abstracts and Reviews. Series B, Livestock Feeds and Feeding. CAB International.

Owens, F. and A. Goetsch. 1986. Digesta passage and microbial protein synthesis. Pages 196-223 in Control of Digestion and Metabolism in Ruminants. L. Milligan, W. Grovum, and A. Dobson, ed. Prentice-Hall, Englewood Cliffs, New Yersey, USA.

Pellikaan, W. F. 2004. Passage of ^{13}C-labelled feed components through the digestive tract of dairy cows. Pages 119-144 in Wageningen Institute of Animal Sciences, Animal Nutrition Group. PhD thesis. Wageningen University, Wageningen, the Netherlands.

Pellikaan, W.F., Verstegen, M. W. A., Tamminga, S, Dijkstra J. and W. H. Hendriks. 2013. δ^{13}C as a marker to study digesta passage kinetics in ruminants: a combined in vivo and in vitro study. Animal 7(5):754–767.

Pellikaan, W.F., Stringano, E., Leenaars, J., Bongers, D.J.G.M., van Laar-van Schuppen, S., Plant, J., and I. Mueller-Harvey. 2011. Evaluating effects of tannins on extent and rate of in vitro gas and CH_4 production using an automated pressure evaluation system (APES). Animal Feed Science and Technology 166– 167:377– 390

Poore, M., J. Moore, and R. Swingle. 1990. Differential passage rates and digestion of neutral detergent fiber from grain and forages in 30, 60 and 90% concentrate diets fed to steers. Journal of Animal Science 68(9):2965-2973.

Rijnen, M., M. Verstegen, M. Heetkamp, J. Haaksma, and J. Schrama. 2001. Effects of dietary fermentable carbohydrates on energy metabolism in group-housed sows Journal of Animal Science 79(1):148-154.

Robinson, P., S. Tamminga, and A. Van Vuuren. 1987. Influence of declining level of feed intake and varying the proportion of starch in the concentrate on rumen ingesta quantity, composition and kinetics of ingesta turnover in dairy cows. Livestock Production Science 17:37-62.

Rotger, A., A. Ferret, S. Calsamiglia, and X. Manteca. 2005. Changes in ruminal fermentation and protein degradation in growing Holstein heifers from 80 to 250 kg fed high-concentrate diets with different forage-to-concentrate ratios. Journal of Animal Science 83(7):1616-1624.

Salt, C.A., R.W. Mayes, and D.A. Elston. 1992. Effects of season, grazing intensity and diet composition on the radiocaesium intake by sheep on re-seeded hill pasture. Journal of Applied Ecology 29:378-387.

Suárez, B. J., C. G. Van Reenen, N. Stockhofe, J. Dijkstra, and W. J. J. Gerrits. 2007. Effect of roughage source and roughage to concentrate ratio on animal performance and rumen development in veal calves. Journal of Dairy Science 90(5):2390-2403.

Udén, P., P. E. Colucci, and P. J. Van Soest. 1980. Investigation of chromium, cerium and cobalt as markers in digesta. Rate of passage studies. Journal of the Science of Food and Agriculture 31(7):625-632.

Van Soest, P. J., J. Robertson, and B. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. Journal of Dairy Science 74(10):3583-3597.

Vazquez-Anon, M., A. Heinrichs, J. Aldrich, and G. Varga. 1993. Postweaning age effects on rumen fermentation end-products and digesta kinetics in calves weaned at 5 weeks of age. Journal of Dairy Science 76(9):2742-2748.

Veissier, I., A. R. Ramirez De La Fe, and P. Pradel. 1998. Nonnutritive oral activities and stress responses of veal calves in relation to feeding and housing conditions. Applied Animal Behavior Science 57(1-2):35-49.

Warner, D., J. Dijkstra, S. Tamminga, and W. Pellikaan. 2013. Passage kinetics of concentrates in dairy cows measured with carbon stable isotopes. Animal 7(12):1935-1943.

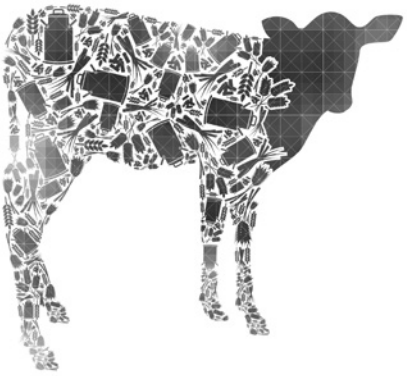
Webb, L. E., E. A. Bokkers, B. Engel, W. J. Gerrits, H. Berends, and C. G. van Reenen. 2012. Behavior and welfare of veal calves fed different amounts of solid feed supplemented to a milk replacer ration adjusted for similar growth. Applied Animal Behavior Science 136(2):108-116.

Williams, C., D. David, and O. Iismaa. 1962. The determination of chromic oxide in faeces samples by atomic absorption spectrophotometry. The Journal of Agricultural Science 59(03):381-385.

Wylie, M., W. Ellis, J. Matis, E. Bailey, W. James, and D. Beever. 2000. The flow of forage particles and solutes through segments of the digestive tracts of cattle. British Journal of Nutrition 83(03):295-306.

Chapter 9

Effect of route of administration of protein on N metabolism in veal calves fed milk replacer and solid feeds



H. Berends[1], J.J.G.C. van den Borne[1], B.A. Røjen[2], W.H. Hendriks[1], and W.J.J. Gerrits[1]

Submitted to Journal of Dairy Science

[1]Animal Nutrition Group, Wageningen University, Wageningen, the Netherlands;
[2]Department of Animal Science, Aarhus University, Tjele, Denmark

ABSTRACT

The current study evaluated the effects of the route of protein administration to calves fed a combination of solid feed (**SF**) and milk replacer (**MR**) at equal total protein intake on urea recycling and N retention. Thirty Dutch Holstein-Friesian male calves (44 ± 0.5 kg BW, 2 wk of age) were randomly assigned to one of 3 treatments: a low level of SF with a low protein content (SF providing 12% of total N intake), a high level of SF with a low protein content (SF providing 22% of total N intake), or a high level of SF with a high protein content (SF providing 36% of total N intake). Total N intake was equalized to 1.8 g of N·kg $BW^{-0.75}$·d^{-1} by adjusting N intake via MR. The SF mixture consisted of 50% concentrates, 25% corn silage, and 25% straw on a DM basis. At 180 ± 3.7 kg BW, N balance was measured over a period of 5 d, and urea recycling was assessed from [$^{15}N_2$]urea kinetics.

Increasing low-protein SF intake at equal total protein intake resulted in a shift from urinary to fecal N excretion but did not affect N retention (0.71 g N·kg $BW^{-0.75}$·d^{-1}). Increasing low-protein SF intake increased urea recycling but urea re-used for anabolism remained unaffected. Total tract NDF digestibility decreased (-9%) with increasing low-protein SF intake, indicating reduced rumen fermentation. Increasing the N content of SF at equal total protein intake decreased urea production, excretion, and return to ornithine cycle, and increased N retention by 17%. This increase was likely related to an effect of energy availability on N retention, in part related to an increase in total tract NDF digestion (> 10%) and to increased energy supply via the MR.

In conclusion, increasing low-protein SF intake at the expense of protein intake from MR, did not affect N retention efficiency in calves. Increasing the N content of SF at equal total protein intake decreased urea production, increased N retention, and coincided with improved fibre degradation. Therefore, results suggest that low N availability in the rumen limits microbial growth in calves fed low-protein SF (93 g CP/kg DM), and this effect cannot be compensated for by recycling of urea originating from MR.

Keywords: urea kinetics, digestibility, concentrates, milk replacer

INTRODUCTION

Urea recycling is a mechanism essential to the N economy of ruminants, where 30 to 98% of urea-N produced by the liver enters the gastrointestinal tract, is subsequently hydrolyzed by bacterial urease to ammonia-N, and potentially used for microbial protein synthesis (Reynolds and Kristensen, 2008). In milk-fed calves, milk replacer (**MR**) allowance ranges between 40 to 60 g DM·kg $BW^{-0.75}$·d^{-1}, thus providing large amounts of intestinally degradable protein. In these calves, urea recycling is of little importance (Hayashi et al., 2006), as shown by the 80% recovery of an intravenous pulse dose of [^{13}C]urea in 48-h urine (Gerrits et al., 2001, van den Borne et al., 2006a). When milk-fed calves were supplemented with increasing amounts of low-protein solid feed (**SF**), the marginal efficiency of N retention was high (76%) (Berends et al., 2012), especially when compared with marginal efficiencies of 30 to 40% reported for calves fed only MR (van den Borne et al., 2006a). The contribution of a low-protein SF to the N economy of milk fed calves was explained by recycling of urea, likely from MR origin, and by an effect of an increase in absorbed energy on post-absorptive N efficiency (Berends et al., unpublished).

At equal protein intake, however, it is currently not known how interactions between SF and MR affect protein deposition in calves. Also, the route of protein supply (SF vs. MR) may impact whole body protein metabolism. For example, ruminal microbes may prefer amino acids-N from SF over ammonia-N from urea recycling, because ammonia is used less efficiently for microbial protein synthesis than degradable true protein (Argyle and Baldwin, 1989, Blake et al., 1983). In addition, due to diurnal fluctuations in rumen fermentation, urea recycling may not fully complement the actual N deficit in the rumen at specific times of the day. Improved insight in the utilization of N from MR and SF is required to mitigate N emissions from calves into the environment and to adapt feeding strategies to large fluctuations in MR ingredient prices. We hypothesize that utilization of N from SF for growth differs from that of N from MR. Apparent N digestibility of SF can be rather low: 45% in calves fed exclusively SF (Ortigues et al., 1990), whereas apparent N digestibility of MR ranges between 92 and 95% (Labussière et al., 2009a, van den Borne et al., 2006b). In addition, SF intake will contribute to energy supply of calves. Increased energy supply from lactose and fat (Gerrits et al., 1996) and timing of energy relative to protein availability (van den Borne et al., 2006b; 2007; 2012) have been shown to contribute to changes in efficiency of N utilization for growth in calves.

Therefore, the aim of the current study was to evaluate the effects of the route of protein administration (SF *vs.* MR) at equal total protein intake on urea recycling, N retention, and apparent total tract digestibility in calves. We hypothesized that at equal protein intake, an increased proportion of protein intake via a high-protein SF increases microbial protein production, but at the same time reduces urea recycling.

MATERIAL AND METHODS

This study was conducted at the research facilities of the VanDrie Group (Scherpenzeel, the Netherlands). Procedures complied with the Dutch Law on Experimental Animals, and the ETS123 (Council of Europe 1985 and the 86/609/EEC Directive) and were approved by the Animal Care and Use Committee of Wageningen University.

Animals, Experimental Design, and Housing

Thirty Dutch Holstein-Friesian male calves were included in the experiment. Calves were gathered from commercial dairy farms at 2 wk of age, and selected based on bodyweight (**BW**), uniformity, and clinical health. Mean BW upon arrival was 44 ± 0.5 kg. Calves were allocated to pens (5 calves per pen) and treatments based on BW. Treatments included: a low level of SF with a low protein content providing 12% of total N intake (**LPLS**), a high level of SF with a low protein content providing 22% of total N intake (**LPHS**), or a high level of SF with a high protein content providing 36% of total N intake (**HPHS**). Total N intake of all treatments was equalized to 1.8 g of N·kg $BW^{-0.75}$·d^{-1} by adjusting N intake via MR (Table 9.1). From wk 10 after arrival onwards, calves were exposed to their treatments. Measurements were conducted at an average BW of 180 ± 3.7 kg. Measurement periods were staggered from 20 and 25 wk of age, with 6 calves - 2 of each treatment - per measurement period due to limited availability of metabolic cages and infusion devices. The 5-d measurement period was preceded by a 9-d adaptation period. Animal health was checked daily. Hemoglobin concentration in blood was monitored across the trial at 3, 7, 11, 15, 19, 23 wk and corrected to comply with a minimum level of 4.5 mmol/L at the end of the fattening period.

Table 9.1 Nitrogen provision from milk replacer and solid feed[1] to veal calves

Treatment[2]	n	Milk replacer		Solid feed		Total N
		g DM·kg $BW^{-0.75}$·d^{-1}	g N/kg DM	g DM·kg $BW^{-0.75}$·d^{-1}	g N/kg DM	g N·kg $BW^{-0.75}$·d^{-1}
LPLS	10	46.8	33.9	15.6	13.7	1.8
LPHS	10	41.5	33.9	28.9	13.7	1.8
HPHS	10	46.8	24.5	28.9	22.6	1.8

[1] Solid feed consisted of 50% concentrates, 25% corn silage, and 25% wheat straw on a DM basis.
[2] LPLS: low level of solid feed with a low level of protein, LPHS: high level of solid feed with a low level of protein, HPHS: high level of solid feed with a high level of protein.

Before the adaption and measurement periods, calves were housed in groups of 5 calves in pens measuring 3 x 3 m equipped with wooden-slatted floors and open fences, and without bedding material. Calves were exposed to daylight and to artificial light from

0500 h to 2300 h and to darkness during the remainder of the day. During the 9-d adaptation period, calves were housed for 7 d in individual pens placed inside the group pen to facilitate individual feeding and monitoring, and for 2 d on metabolic cages (0.79 x 1.85 m), equipped with wooden-slatted floors and open fences. When entering the metabolic cages, calves were harnessed to allow quantitative, separate collection of feces and urine. Calves remained on the metabolic cages during the subsequent 5-d measurement period. Cages enabled audio-visual contact between calves.

Diets and Feeding

Dietary treatments were realized by adjusting N level of MR and MR intake as shown in Table 9.1. N content was 13.7 g N/kg DM for the low-protein SF and 22.6 g N/kg DM for the high-protein SF (Table 9.1), which was achieved by exchanging starch-rich sources (corn and barley) for corn gluten meal in the concentrate, aiming for similar rumen degradation rates of crude protein (**CP**), starch, neutral detergent fibre (**NDF**), and non-starch polysaccharides (CVB, 2007). The concentrate compositions are presented in Table 9.2. The SF was composed of 50% concentrates, 25% corn silage, and 25% chopped wheat straw on a DM basis. Provision of SF started at 10 d after arrival. DMI from SF increased with equal weekly increments. Total DMI from SF during wk 10 to 27 was targeted to reach 100 kg for LPLS and 260 kg for LPHS and HPHS (Berends et al., unpublished). The quantity of MR was slightly adjusted to achieve similar BW across treatments at the onset of the measurement period. The MR was reconstituted with water and the concentration increased gradually from 125 g/L to 188 g/L. The MR (Table 9.3) was supplied in buckets at ca. 40 °C and provided twice daily in equally sized meals at 0600 h and at 1600 h. Until the adaptation period, SF

Table 9.2 Analyzed nutrient composition of low- and high-N concentrate, corn silage, and wheat straw

Nutrient[1]	Concentrate[2]		Corn silage	Wheat straw
	Low-protein	High-protein		
Dry matter, g/kg product	899	908	284	927
Crude protein, N x 6.25	138	246	67	29
Crude fat	66	69	29	9
Starch	424	332	322	9
Neutral detergent fibre	116	116	427	813

[1] Expressed in g/kg DM unless specified otherwise.

[2] Concentrate composition for low-protein concentrate: 42.0% corn, 21.0% lupins, 23.6% barley, 5.0% carob meal, 2.5% coconut oil, 6% premix (lactose carrier, provided per kg concentrate: vitamin A: 4,000 IU; vitamin D: 500 IU; vitamin E: 100 IU; zinc: 25 mg; manganese: 20 mg; iodine: 0.8 mg; selenium: 0.15 mg; copper: 15 mg; cobalt: 0.1 mg, 1.13 g magnesium (magnesium sulphate), 3.0 g sodium (NaCl), 8.6 g calcium ($CaCO_3$), 3.9 g K (KH_2PO_4). Concentrate composition for high-protein concentrate: 31.8% corn, 21.0% lupins, 17.9% barley, 15.8% corn gluten meal, 5.0% carob meal, 2.5% coconut oil, 6% premix (identical to premix in low-protein concentrate).

was provided as a mixture in a long feed trough in front of the pen directly after the morning meal; refusals were collected once daily, before the morning MR meal, and weighed. During the adaptation and measurement periods, SF was supplied individually twice daily in a feed trough directly after the MR feeding; refusals were collected before each MR meal and weighed. Calves had ad libitum access to water during the experiment via drinking nipples.

Table 9.3 Ingredient and analyzed nutrient composition of the milk replacers (MR)

	Low-protein MR	High-protein MR
Ingredient composition, g/kg		
Fat-enriched whey[1]	410.9	394.0
Whey protein concentrate	0.0	193.9
Whey	354.1	143.3
Delactosed whey	118.2	135.0
Soy protein isolate	25.8	37.7
Soy protein concentrate	15.0	25.0
Acidified whey[2]	20.0	20.0
Pregelatinized wheat starch	24.0	20.0
Vitamin and mineral premix[3]	10.0	10.0
Calcium formate	10.4	9.8
L-lysine.HCl	4.8	5.2
DL-methionine	2.0	2.7
Citric acid	2.0	2.0
Mono ammonium phosphate	2.0	1.4
L-threonine	1.0	0.2
Nutrient composition[4], g/kg DM		
Dry matter, g/kg product	971.1	969.7
Crude protein, N x 6.25	158.6	203.0
Crude fat	211.7	206.4
Starch	26.1	21.0

[1] Fat-enriched whey contained 50% fat from palm oil and coconut oil (80/20, %w/w).
[2] Acidified with lactate, provided 5.9 g lactate per kg of the experimental diet.
[3] Provided per kg of the experimental diet: 16.6 g K, 15.5 g Cl, 8.3 g Fe, 6.1 g P, 5.9 g Cu, 5.7 g Na, 1.3 g Mg, 25,000 IU vitamin A, 4,000 IU vitamin D3, 100 IU vitamin E.
[4] Expressed in g/kg DM unless specified otherwise.

Isotope Infusion. On the first day of the measurement period, calves were prepared with central venous catheters (Careflow, Becton Dickinson, Alphen aan den Rijn, the Netherlands) for infusion. A 24-h continuous infusion of doubly ^{15}N labeled urea ([$^{15}N^{15}N$]urea, >99.0

atom%, ISOTEC, Miamisburg, OH) prepared in sterile saline (0.15 M NaCl) was conducted. The continuous infusion of 0.039 mL/min (2.09 mmol/d) was preceded by a priming dose of 1.05 mmol to enrich the body urea pool to approximately 0.15 mol percent excess.

Measurements. Urine samples were taken before the infusion (background samples) and cumulatively for 72 h after the initiation of the infusion. Feces were quantitatively collected in plastic bags that were attached to calves before infusion and cumulatively for 72 h after the initiation of the infusion. N balance was calculated from quantitative measurements of MR and SF intake, and feces and urine excretion for 120 h. Urine was collected in buckets and acidified to pH < 2 with sulfuric acid to prevent microbial activity and ammonia volatilization. During each measurement period, MR, and SF components were sampled. Each calf was weighed before and after the measurement period.

Analyses. Urinary urea was isolated using a cation exchange resin (AG 50WX8, 200 to 400 mesh hydrogen form, Sigma Aldrich, St. Louis, MO) and diluted to a final concentration of 1.65 mmol/L. The eluates were analyzed according to procedures described by Sarraseca et al. (1998) with some modifications leading to monomolecular degradation of urea into N_2. In short, eluates and standard solutions were placed in 12-mL soda glass vials (Exetainer®; Labco Limited, Lampeter, Ceredigion, UK). To prepare these samples for Hoffman degradation, high-purity 5.0 He gas was bubbled on low pressure through the samples for 20 min and samples were frozen quickly in liquid N_2. Bubbling lines were removed and ends cut off. Once the samples were frozen, 100 µL of 10% LiOBr was added and the lid was screwed on. The LiOBr solution was made by adding slowly 2 mL of bromine to a 60 mL 10% (wt/wt) LiOH solution. With the Exetainer still in liquid N_2, the septum was pierced with a 19-gauge hypodermic needle connected to a helium tank on high pressure for flushing the headspace. A second 23-gauge needle allowed the helium to exit the tube. After 3 min of flushing this second needle was removed first to leave a positive pressure inside the Exetainer. The tube was removed from the liquid N and placed in a heating block at 65 ^{0}C for 25 min to degrade urea into N_2 gas. This reaction is sensitive to the concentration of urea with more $^{15}N^{14}N$ gas molecules at higher concentrations due to the cross-reaction of $^{14}N^{14}N$ and $^{15}N^{15}N$ urea molecules (Sarraseca et al., 1998). The concentration of urea used in the analysis (1.65 mmol/L) ensured suitable amounts of gas, and minimized non-monomolecular degradation of urea. The occurrence of the non-monomolecular reaction was calculated from the increase in $^{15}N^{14}N$ gas molecules when enriched urea standards were run alongside the samples, as described by Sarraseca et al. (1998). The analysis of the samples started immediately after preparation. Enrichment of ^{15}N species in N_2 was determined according to the methods described previously (Marini and Attene-Ramos, 2006) for continuous flow-isotope ratio mass spectrometry (Finnigan Delta V Plus, Thermo Scientific, Bremen, Germany) and read as a mass-to-charge ratio of 28, 29, and 30 corresponding to the $^{14}N^{14}N$, $^{14}N^{15}N$, and $^{15}N^{15}N$ parent urea molecule, respectively. Feces were freeze-dried and ground to pass a 1 mm sieve, and subsequently ground on a ball mill (Retsch MM 2000, Retsch GmbH & Co., Haan, Germany). Fecal ^{15}N

enrichments were analyzed after combustion in continuous-flow mode using Finnigan Delta V Advantage (Finnigan, San Jose, CA) and read with a Finnigan MAT C11N8 elemental analyzer (Finnigan, San Jose, CA).

Urinary urea was determined by an enzymatic colorimetric Berthelot method (Human, Wiesbaden, Germany). Samples of MR, concentrates, corn silage, and straw were collected weekly. All feed components, except for corn silage, were pooled for the five consecutive measurement periods. Feed components (MR, corn silage, concentrates, straw) were analyzed for DM, N, starch, NDF (except for MR), and crude fat content.

For determination of DM content, corn silage and concentrate samples were freeze-dried. Concentrates, straw, and corn silage samples were ground to pass a 1-mm screen. Dry matter content was determined by drying to a constant weight according to ISO Standard 6496 (ISO, 1998). Kjeldahl N content was determined according to ISO 5983 (ISO, 1997). Crude fat content was determined after acid hydrolysis according to ISO 6496 (ISO, 1999). The NDF content was analyzed according to Van Soest et al. (1991). Starch content was determined enzymatically as described by Rijnen et al. (2001).

Calculations. The multiple-entry urea kinetic model of Zuur et al. (2000) was used to calculate urea-N kinetics. Briefly, urea entry rate (**UER**) is calculated as the dose (e.g. priming plus infusion dose) multiplied by the enrichment of the infusate divided by the urinary enrichment minus 1. The model assumes that there are two fates for synthesized urea, namely a proportion is excreted in urine (**UUE**) and the remainder enters the gastrointestinal tract (**GER**: gastrointestinal entry rate). The methodology does not allow discrimination of urea entry into various sites of the gastrointestinal tract (e.g. rumen, colon, etc.). Urea entering the gastrointestinal tract is hydrolyzed to ammonia, which can: 1) return to the ornithine cycle (**ROC**), 2) be excreted in feces (**UFE**: urea-N to fecal excretion), and 3) be utilized for anabolic purposes (**UUA**: urea-N utilized for anabolism). After administration of [$^{15}N^{15}N$]urea into the body urea pool, we quantified the various fates of urea-N through analysis of urea enrichment in urine and feces samples. When [$^{15}N^{15}N$]urea is not appearing in urine (UUE), it is assumed to have entered the gastrointestinal tract. When entering the gastrointestinal tract [$^{15}N^{15}N$]urea is hydrolyzed to labelled ammonia, part of which is returned to the ornithine cycle (**ROC**) with [$^{14}N^{15}N$]urea as the product. Excretion of urea-N to feces (UFE) was quantified by the enrichment of feces. Urea-N utilized for anabolism was estimated by subtracting ROC and UFE from GER.

Statistical Analysis

For the N balance, the dataset included 26 successful observations; 10 LPLS calves, 8 LPHS calves, and 8 HPHS calves, respectively. Calves were excluded because of clinical sickness (1 calf), or feed refusals exceeding 10% of targeted total N intake (3 calves; 2 LPHS calves and 1 LPLS calf). For urea kinetics, 1 extra observation (1 calf LPHS) was excluded from the dataset because of a missing sample. Dependent variables were analyzed by mixed model analysis (PROC MIXED in SAS 9.20 by SAS Institute, Inc, Cary, NC), with calf as experimental

unit, including pen before the adaption period as random effect, treatment and measurement period as fixed effects, and the interaction between the latter two factors. The random effect of pen and the interaction between measurement period and treatment were not significant and therefore excluded from the model. Pairwise differences, with Tukey adjustments for multiple comparisons, were determined when the effect of treatment appeared significant. Model residuals were checked for normal distribution. Results are presented as least squared means and their SEM. Differences were considered significant at $P < 0.05$.

RESULTS

The effect of low-protein SF level, the effect of N content of SF, and the interaction between these two are described below in separate subsections. Average BW at the start of the measurement period was 180 ± 3.7 kg. Due to SF and MR refusals and slight differences between estimated and analyzed N contents of SF and MR, the targeted total N intake of 1.80 g N·kg $BW^{-0.75}$·d^{-1} was not fully achieved for LPLS and LPHS (Table 9.4). Therefore, the realized proportion of total N intake originating from SF was 12% for LPLS, 23% for LPHS, and 34% for HPHS.

Effect of low-protein SF level. Fecal N excretion was greater for LPHS than for LPLS (0.38 *vs.* 0.29 g N·kg $BW^{-0.75}$·d^{-1}; $P < 0.001$). As a result, digestible N intake was 0.11 N·kg $BW^{-0.75}$·d^{-1} greater ($P < 0.001$) for LPLS than for LPHS. Despite this difference, N retention was identical for LPLS and LPHS (0.71 g N·kg $BW^{-0.75}$·d^{-1}). Urea production was similar for LPLS and LPHS, and averaged 0.95 g urea-N·kg $BW^{-0.75}$·d^{-1} (Table 9.5). Re-use of urea-N for anabolism did not differ between LPLS and LPHS, although urea return to the ornithine cycle and loss to feces were greater ($P < 0.05$) for LPHS. Apparent total tract nutrient digestibility was generally greater ($P < 0.05$) for LPLS than for LPHS, as evidenced by a greater apparent total tract digestibility of starch (+1%), crude protein (+5%), and NDF (+9%) (Table 9.6).

Effect of N content of SF. The effect of protein content of the SF was assessed by comparing LPHS with HPHS. N intake from LPHS was slightly lower than from HPHS, due to SF and MR refusals. Digestible N intake was 5% greater ($P < 0.05$) for HPHS than for LPHS (Table 9.4). In addition, N retention was 17% greater ($P < 0.05$) for HPHS than for LPHS. Despite a greater digestible N intake in HPHS calves (Table 9.4), urea production was 25% lower for HPHS than for LPHS ($P < 0.05$, Table 9.5). Urinary urea excretion (+29%), return to ornithine cycle (+78%), and loss to feces (+50%) were greater ($P < 0.05$) for LPHS than for HPHS. Finally, urea re-used for anabolism did not differ between LPHS and HPHS (Table 9.5). Apparent total tract digestibility of crude fat (+2%) tended to be greater ($P < 0.10$) for LPHS than for HPHS, whereas apparent total tract digestibility of starch (+1%; $P < 0.01$) and NDF (+11%; $P < 0.05$) were greater for HPHS than for LPHS (Table 9.6).

Table 9.4 Effects of level of solid feed (SF) intake and level of protein in SF and milk replacer (MR) on N balance in veal calves

	Treatments[1]						*P*-value		
	LPLS		LPHS		HPHS		LPLS vs. LPHS	LPHS vs. HPHS	LPLS vs. HPHS
	mean	SE	mean	SE	mean	SE			
No. of calves	10		8		8		-	-	-
Bodyweight, kg	178	5.1	181	5.8	181	5.8	-	-	-
N balance, g N·kg $BW^{-0.75}$·d^{-1}									
N intake	1.73	0.012	1.71	0.013	1.80	0.013	-	-	-
from MR	1.52	0.010	1.31	0.012	1.19	0.012	-	-	-
from SF	0.21	0.009	0.40	0.011	0.62	0.011	-	-	-
Fecal N excretion	0.29	0.013	0.38	0.014	0.41	0.014	***	NS	***
Digestible N intake	1.44	0.016	1.33	0.018	1.40	0.018	***	*	†
Urinary N excretion	0.73	0.022	0.62	0.025	0.56	0.025	**	NS	***
N retention	0.71	0.028	0.71	0.032	0.83	0.032	NS	*	**
N retention, as % of N intake	41	1.5	42	1.8	46	1.8	NS	†	*
N retention, as % of digestible N intake	49	1.7	53	1.9	60	1.9	NS	*	***

NS: not significant; †$P < 0.10$; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

[1] Treatments are described in Table 9.1. LPLS (n = 10): low level of solid feed with a low level of protein, LPHS (n = 8): high level of solid feed with a low level of protein, HPHS (n = 8): high level of solid feed with a high level of protein.

Table 9.5 Effects of N level of solid feed and milk replacer, and solid feed intake on urea-N kinetics based on [$^{15}N^{15}N$]urea infusions in veal calves[1]

	Treatments[2]						*P*-value		
	LPLS		LPHS		HPHS		LPLS vs. LPHS	LPHS vs. HPHS	LPLS vs. HPHS
	mean	SE	mean	SE	mean	SE			
Urea kinetic variables, in g urea-N·kg $BW^{0.75}$·d^{-1}									
Production (UER)	0.94	0.043	0.95	0.049	0.76	0.053	NS	*	*
Urinary excretion (UUE)	0.52	0.025	0.45	0.029	0.35	0.031	†	*	**
Entry to GIT (GER)	0.42	0.045	0.50	0.051	0.41	0.055	NS	NS	NS
Return to ornithine cycle (ROC)	0.08	0.018	0.16	0.021	0.09	0.022	*	*	NS
Loss to faeces (UFE)	0.01	0.003	0.03	0.003	0.02	0.004	***	*	*
Re-use for anabolism (UUA)	0.32	0.048	0.30	0.055	0.29	0.059	NS	NS	NS

[1] UER, urea-N entry rate; GER, gastrointestinal entry rate; ROC, return to ornithine cycle; UFE, loss to feces; UUA, urea-N utilized for anabolism; GIT, gastrointestinal tract.
[2] Treatments are described in Table 9.1. LPLS (n = 10): low level of solid feed with a low level of protein, LPHS (n = 7): high level of solid feed with a low level of protein, HPHS (n = 8): high level of solid feed with a high level of protein.
NS: not significant; †$P < 0.10$; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

Table 9.6 Effects of N level of solid feed and milk replacer, and solid feed intake on apparent total tract digestibility of crude fat, starch, crude protein, and NDF in veal calves

Digestibility, % of intake	Treatments[1]						*P*-value		
	LPLS		LPHS		HPHS		LPLS vs. LPHS	LPHS vs. HPHS	LPLS vs. HPHS
	mean	SE	mean	SE	mean	SE			
Crude fat	89.4	0.89	89.1	1.02	86.6	1.02	NS	†	†
Starch	98.0	0.14	97.1	0.16	97.8	0.16	***	**	NS
Crude protein	83.3	0.71	77.7	0.81	77.4	0.81	***	NS	***
NDF	58.4	2.85	49.4	3.24	60.2	3.47	*	*	NS

NS: not significant; †$P < 0.10$; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.
[1] Treatments are described in Table 9.1. LPLS (n = 10): low level of solid feed with a low level of protein, LPHS (n = 8): high level of solid feed with a low level of protein, HPHS (n = 8): high level of solid feed with a high level of protein.

Effect of SF level and N content of SF. LPLS and HPHS calves differed in SF level and the protein content of the SF supplied. Fecal N excretion was 41% greater ($P < 0.001$) for HPHS than for LPLS. As a result, digestible N intake tended to be greater ($P < 0.10$) for LPLS. As urinary N excretion was 30% greater ($P < 0.001$) in LPLS calves, N retention was 17% greater ($P < 0.01$) in HPHS calves. Also as a percentage of intake, N retention was greater ($P < 0.05$) for HPHS calves than for LPLS calves (46 *vs.* 41%; Table 9.4). Despite a greater digestible N intake in LPLS calves, urea production was 0.94 g urea-N·kg $BW^{-0.75}$·d^{-1} for LPLS calves, and greater ($P < 0.05$) than 0.76 g N·kg $BW^{-0.75}$·d^{-1} observed for HPHS calves. Digestibility of crude fat tended to be greater ($P < 0.10$) and digestibility of crude protein was greater ($P < 0.001$) in LPLS than in HPHS calves.

DISCUSSION

When fed exclusively on MR, the marginal efficiency of protein utilization for growth typically is 30 to 40% in heavy calves (> 100 kg BW), and decreases with age (van den Borne et al., 2006a). When combining MR and low-protein SF, urea originating from deamination of amino acids from MR can, through urea recycling, contribute to protein gain in calves. As a consequence, incremental amounts of N from low-protein SF were retained with an efficiency of 76% (Berends et al., 2012). The aim of the current study was to evaluate the effects of the route of administration of protein, through SF or MR, on urea recycling, whole body N retention, and apparent total tract digestibility in calves at equal total protein intake.

In general, values for N retention observed in the current study were similar to values previously reported values in milk-fed calves (Labussière et al., 2009b, van den Borne et al., 2006b, Zanton and Heinrichs, 2008), SF-fed calves (Ortigues et al., 1990), or calves fed both MR and SF (Berends et al., 2012, Labussière et al., 2009b). N retention was identical for LPLS and LPHS (0.71 g N·kg $BW^{-0.75}$·d^{-1}) and 17% greater for HPHS calves.

Increasing Low-protein SF provision

At equal total protein intake, the effects of increasing low-protein SF intake were evaluated by comparing LPLS and LPHS. The results showed that increasing the proportion of N intake via low-protein SF, while maintaining total N intake by adjusting MR provision, increased N excretion in feces and decreased N excretion in urine. This shift from urinary to fecal N excretion was reported previously in milk-fed calves provided with incremental amounts of low-protein SF (Berends et al., 2012; unpublished), and is commonly found when comparing milk-fed and SF-fed calves (Zanton and Heinrichs, 2008). This is likely due to a lower digestibility of N from SF than from MR, greater endogenous N losses associated with DM intake from SF (NRC, 2001), and greater losses of urea-N that entered the gut and got lost in feces (UFE; Table 9.5). Potentially, differences in nutrient synchrony affect N

utilization from amino acids from SF compared with MR. Results show that N retention was identical for LPLS and LPHS calves, whereas digestible N intake was greater (+0.11 g N·kg $BW^{-0.75}$·d^{-1}; $P < 0.001$) for LPLS than for LPHS. Potentially, an increase in low-protein SF intake was associated with a greater demand for N in the rumen. Indeed, gut entry rate increased numerically (+0.08 g N·kg $BW^{-0.75}$·d^{-1}) and return to ornithine cycle (+0.08 g N·kg $BW^{-0.75}$·d^{-1}; $P < 0.05$) and loss to feces (+0.02 g N·kg $BW^{-0.75}$·d^{-1}; $P < 0.001$) increased in LPHS compared with LPLS. These results suggest that fecal N excretion and urea recycling increased in LPHS compared with LPLS, whereas urea re-used for anabolism was the same for both treatments. Increasing low-protein SF intake at equal protein intake resulted in identical N retention.

In general, the apparent digestibility of a SF decreases with increasing SF intake (NRC, 2001). Therefore, the greater starch and NDF digestibility in LPLS calves could be related to a lower SF intake or alternatively, to an increased urea recycling (from deamination of amino acids originating predominantly from MR) to complement a potential N deficit for microbial fermentation in the rumen. In dairy cows, rumen degradation of carbohydrates is impaired at a protein content of SF below 16% (Oldham, 1984). In the current study, the increased level of low-protein SF and the reduced N intake from MR may have limited rumen fermentation in LPHS calves.

From this isonitrogenous comparison, it can be concluded that increasing low-protein SF intake led to a shift from urinary to fecal excretion but did not affect whole body N balance in veal calves. Results showed that, within the range applied in the current study, 1 g of N from SF was utilized as efficiently as 1 g of N from MR for protein deposition in calves at equal total protein intake.

Low-protein vs. High-protein SF

When comparing LPHS and HPHS, the main difference is the route of N intake (23 vs. 34% of N intake via SF) at equal total N and SF intake. Increasing N supply through SF increased N retention by 17%. Due to feed refusals, N intake was slightly greater in HPHS calves. When N retention was expressed as a percentage of N intake, however, N retention was still 13% greater for HPHS calves than for LPHS calves. The efficiency of N retention is known to be quadratically related to N intake in milk-fed and SF-fed calves, reaching a maximum at approximately 1.8 g·kg $BW^{-0.75}$·d^{-1} (Zanton and Heinrichs, 2008), which is close to the N retention found in the current study. Although digestible N intake was 5% greater in HPHS calves (Table 9.4), urea production (UER) was 25% greater for LPHS calves than for HPHS calves (Table 9.5). Post-absorptive utilization of N for growth therefore increased substantially when N content of the SF increased at equal total protein intake, which may be explained by various mechanisms.

Firstly, the (numerical) increase in urea recycling may have been insufficient to complement the N deficit in the rumen for calves fed low-protein SF. Fermentable organic matter content was slightly greater in low-protein SF than in high-protein SF (467 *vs.* 451 g/kg

DM), and 19% more rumen degradable protein was available per g fermentable organic matter in the high-protein SF. Therefore, it may be expected that there was a greater need for N in rumen with low-protein SF. The numerical increase in GER, and significant increases in ROC and UFE suggest that urea recycling was increased but may not have been able to fully complement the N deficit in the rumen, as indicated by the substantial (> 10%) increase in NDF digestibility of the HPHS compared with the LPHS. It has been shown previously in dairy cattle that increasing dietary CP content increased ruminal digestion (Belanche et al., 2012, Doreau et al., 1990). In addition, rumen microbes may prefer N from amino acids over N from urea (Argyle and Baldwin, 1989, Blake et al., 1983) and the supply of dietary amino acids to the rumen was greater for HPHS calves.

Secondly, digestible energy (DE) intake differed between treatments, which is known to affect protein deposition in calves, independently of protein intake (Gerrits et al., 1996, Ortigues et al., 1990, Schroeder et al., 2006). DE intake could be estimated from GE values (Berends et al., 2012; CVB, 2007) and assumed digestibilities of GE from MR (Labussière et al., 2009a, van den Borne et al., 2006b) and SF (Berends et al., 2012): 1027, 1117, and 1228 kJ·kg $BW^{-0.75}$·d^{-1} for LPLS, LPHS, and HPHS, respectively. If the effect of protein-free energy intake on protein deposition is assumed to be 33 kJ of DE·kg $BW^{-0.75}$·g N^{-1} (Gerrits et al, 1996), approximately two-third of the observed increase in N retention for HPHS relative to LPHS may be explained by an increase in DE intake. In addition, the increase in apparent NDF digestibility in calves fed HPHS compared with calves fed LPHS may have resulted in a further increase of DE intake.

In summary, at equal protein and SF intake, an increase in protein content of SF resulted in an increase in N retention of 17% in calves. The utilization of apparently digestible N for N retention was greater at a higher protein content of the SF, which is partly explained by increased energy supply. Furthermore, NDF digestibility increased with more than 10% which may be explained by the preferential use of protein-N over urea-N for microbial protein synthesis in the rumen.

CONCLUSIONS

1) Increasing low-protein SF intake at the expense of N intake from MR leads to a shift from urinary to fecal N excretion, but does not alter whole body N retention in calves. Urea recycling increases with increasing low-protein SF intake, but re-use of recycled N for anabolism is not affected.
2) Increasing the protein content of SF at equal total protein and equal SF intake increased N retention by 17% in calves. Results indicate that rumen fermentation, and thus fibre degradation of a low-protein SF (93 g CP/kg DM) is hampered by the low protein availability. Recycling of urea cannot compensate for this effect. Furthermore, energy availability contributed substantially to the increase in N retention.

ACKNOWLEDGEMENTS

The authors thank the Division for Earth and Life Sciences (ALW) of the Netherlands Organisation for Scientific Research (NWO, The Hague, the Netherlands), and the Product Board Animal Feed (The Hague, the Netherlands) for financially supporting this research. Klaas Boeder, Bart Evers, Ewart van Voorst (VanDrie Group, Scherpenzeel, the Netherlands), Joswita van de Velde, Teun Keizer, Sven Alferink, Tamme Zandstra, Michel Breuer (Animal Nutrition Group, Wageningen University, the Netherlands), André Jansen, Bert Beukers (Department of Animal Sciences, Wageningen University, the Netherlands), Birgit Løth and Anne Krustrup (Department of Animal Health and Bioscience, Aarhus University, Denmark) are gratefully acknowledged for their skilled technical assistance.

REFERENCES

Argyle, J. and R. Baldwin. 1989. Effects of amino acids and peptides on rumen microbial growth yields. Journal of Dairy Science 72(8):2017-2027.

Belanche, A., M. Doreau, J. E. Edwards, J. M. Moorby, E. Pinloche, and C. J. Newbold. 2012. Shifts in the rumen microbiota due to the type of carbohydrate and level of protein ingested by dairy cattle are associated with changes in rumen fermentation. The Journal of Nutrition 142(9):1684-1692.

Berends, H., J. J. G. C. van den Borne, S. J. J. Alferink, C. G. van Reenen, E. A. M. Bokkers, and W. J. J. Gerrits. 2012. Low-protein solid feed improves the utilization of milk replacer for protein gain in veal calves. Journal of Dairy Science 95(11):6654-6664.

Blake, J., D. Salter, and R. Smith. 1983. Incorporation of nitrogen into rumen bacterial fractions of steers given protein-and urea-containing diets. Ammonia assimilation into intracellular bacterial amino acids. British Journal of Nutrition 50(03):769-782.

Cotta, M. A. and J. B. Russell. 1982. Effect of peptides and amino acids on efficiency of rumen bacterial protein synthesis in continuous culture. Journal of Dairy Science 65(2):226-234.

CVB, 2007. Chemical compositions and nutritional values of feed ingredients. Centraal Veevoeder Bureau, Productschap Diervoeder, Den Haag, the Netherlands.

Doreau, M., A. Delacroix, J. Jouany, C. Durier, and B. Rémond. 1990. The influence of physiological state and dietary nitrogen supply on digestion in the dairy cow. Journal of Animal Science 68(11):3853-3860.

Gerrits, W. J. J., Beelen, G.M., Dijkstra, J., and J.M.A.J. Verdonk. 2001. Ammonia infusion and starch fermentation in preruminant calves (150-180 kg). In: Internal report No. V99.001. ID TNO Anmial Nutrition (in Dutch), Wageningen, the Netherlands.

Gerrits, W. J. J., G. H. Tolman, J. W. Schrama, S. Tamminga, M. W. Bosch, and M. W. A. Verstegen. 1996. Effect of protein and protein-free energy intake on protein and fat deposition rates in preruminant calves of 80 to 240 kg live weight. Journal of Animal Science 74(9):2129-2139.

Hayashi, H., M. Kawai, I. Nonaka, F. Terada, K. Katoh, and Y. Obara. 2006. Developmental changes in the kinetics of glucose and urea in holstein calves. Journal of Dairy Science 89(5):1654-1661.

ISO. 1997. Animal feeding stuffs - Determination of nitrogen content and calculation of crude protein content. ISO 5983. Int. Organ. Stand., Geneva, Switzerland.

ISO. 1998. Animal feeding stuffs - Determination of moisture and other volatile matter content. ISO 6496. Int. Organ. Stand., Geneva, Switzerland.

ISO. 1999. Animal feeding stuffs - Determination of fat content. ISO 6492. Int. Organ. Stand., Geneva, Switzerland.

Labussière, E., S. Dubois, J. van Milgen, G. Bertrand, and J. Noblet. 2009a. Effect of solid feed on energy and protein utilization in milk-fed veal calves. Journal of Animal Science 87(3):1106-1119.

Labussière, E., G. Maxin, S. Dubois, J. van Milgen, G. Bertrand, and J. Noblet. 2009b. Effect of feed intake on heat production and protein and fat deposition in milk-fed veal calves. Animal 3(4):557-567.

Marini, J. C. and M. S. Attene-Ramos. 2006. An improved analytical method for the determination of urea nitrogen isotopomers in biological samples utilizing continuous flow isotope ratio mass spectrometry. Rapid Communications in Mass Spectrometry 20(24):3736-3740.

NRC. 2001. Nutrient requirements of dairy cattle. 7th rev. ed. Natl. Acad. Press, Washington, DC.

Oldham, J. 1984. Protein-energy interrelationships in dairy cows. Journal of Dairy Science 67(5):1090-1114.

Ortigues, I., T. Smith, M. Gill, S. B. Cammell, and N. W. Yarrow. 1990. The effect of fishmeal supplementation of a straw-based diet on growth and calorimetric efficiency of growth in heifers. British Journal of Nutrition 64(3):639-651.

Reynolds, C. and N. B. Kristensen. 2008. Nitrogen recycling through the gut and the nitrogen economy of ruminants: An asynchronous symbiosis. Journal of Animal Science 86(14):E293-E305.

Rijnen, M., M. Verstegen, M. Heetkamp, J. Haaksma, and J. Schrama. 2001. Effects of dietary fermentable carbohydrates on energy metabolism in group-housed sows. Journal of Animal Science 79(1):148-154.

Sarraseca, A., E. Milne, M. Metcalf, and G. Lobley. 1998. Urea recycling in sheep: effects of intake. British Journal of Nutrition 79(1):79-88.

Schroeder, G., E. Titgemeyer, M. Awawdeh, J. Smith, and D. Gnad. 2006. Effects of energy source on methionine utilization by growing steers. Journal of Animal Science 84(6):1505-1511.

Soto, R. C., S. A. Muhammed, C. Newbold, C. Stewart, and R. Wallace. 1994. Influence of peptides, amino acids and urea on microbial activity in the rumen of sheep receiving grass hay and on the growth of rumen bacteria in vitro. Animal Feed Science and Technology 49(1):151-161.

Van den Borne, J., J. Verdonk, J. W. Schrama, and W. J. J. Gerrits. 2006a. Reviewing the low efficiency of protein utilization in heavy preruminant calves - a reductionist approach. Reproductive Nutrition and Development 46(2):121-137.

Van den Borne, J., M. W. A. Verstegen, S. J. J. Alferink, R. M. M. Giebels, and W. J. J. Gerrits. 2006b. Effects of feeding frequency and feeding level on nutrient utilization in heavy preruminant calves. Journal of Dairy Science 89(9):3578-3586.

Van den Borne, J. J., S. J. Alferink, M. J. Heetkamp, A. A. Jacobs, M. W. Verstegen, and W. J. Gerrits. 2012. Asynchronous supply of indispensable amino acids reduces protein deposition in milk-fed calves. The Journal of Nutrition 142(12):2075-2082.

Van den Borne, J. J. G. C., J. F. Hocquette, M. W. A. Verstegen, and W. W. J. Gerrits. 2007. Whole body and muscle energy metabolism in preruminant calves: Effects of nutrient synchrony and physical activity. British Journal of Nutrition 97(4):667-675.

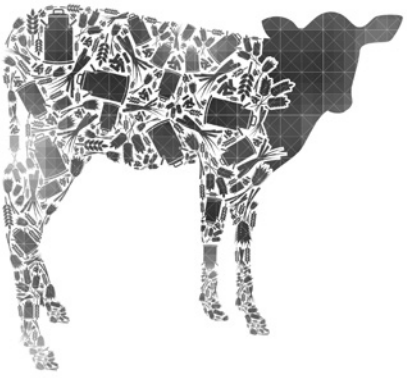
Van Soest, P. J., J. Robertson, and B. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. Journal of Dairy Science 74(10):3583-3597.

Zanton, G. and A. Heinrichs. 2008. Analysis of nitrogen utilization and excretion in growing dairy cattle. Journal of Dairy Science 91(4):1519-1533.

Zuur, G., K. Russell, and G. Lobley. 2000. Multiple-entry urea kinetic model: effects of incomplete data collection. In: McNamara, JP, France, J, Beever, D, editors. Modelling nutrient utilization in farm animals. Walingford (UK): CABI Publishing;2000:145-161.

Chapter 10

General Discussion



INTRODUCTION

For both welfare and economic reasons, SF increasingly represents an important portion of the diet for veal calves. However, interactions between MR and SF complicate the prediction of their nutritional value, and adverse effects on health may occur. These effects include poor rumen development (Brscic et al., 2011, Suárez et al., 2006a), ruminal drinking (Suárez et al., 2007), and abomasal lesions (Brscic et al., 2011, Mattiello et al., 2002). Interactions between nutrients occur either at the level of digestion or post-absorption, and the age-dependency of these interactions complicate an accurate prediction of growth performance throughout the fattening period.

These partly interrelated issues currently limit further improvements in welfare and economic performance that could originate from increasing SF intake in veal calf diets. The aim of this thesis was to provide a scientific basis for the development of novel feeding strategies combining MR and SF, optimizing the use of feed resources, and alleviating health problems related to interactions between SF and MR.

In the following paragraphs, the work described in this thesis, as well as conclusions and implications for veal production are discussed. Firstly, the methodologies applied to assess nutrient utilization of SF and MR are evaluated. Furthermore, findings on nutrient utilization and interactions are discussed. Secondly, the quantitative measurement of ruminal drinking is evaluated and results from a meta-analysis of factors influencing ruminal drinking are discussed. Thirdly, the effects of free diet selection (Chapter 5) are compared with a reference diet for milk-fed calves. In addition, a comparison is made between free and separate provision of SF components. Finally, recommendations are provided for future research and for practice for the development of novel feeding strategies combining MR and SF. This chapter concludes with an overview of the main conclusions of the research. Throughout this chapter, new information from studies in other chapters is presented in textboxes.

NUTRIENT UTILIZATION AND INTERACTIONS

In this thesis, the consequences of increasing SF intake for protein and energy metabolism of veal calves were investigated. Feeding values of SF cannot be predicted from protein and energy utilization for growth in exclusively SF-fed calves, because (age-dependent) interactions occur between MR and SF at the level of digestion or post-absorption.

Methodological Considerations

In a series of large scale animal experiments (Chapters 2, 3, 4, 7, and 9), various combinations of MR, concentrates, and roughages were fed to veal calves in order to study the nutritional consequences of combining these ration components into calf diets and their effects on

nutrient utilization. Each of these experimental designs (Figure 10.1) has advantages and disadvantages that need consideration to enable proper understanding of its outcome. In Chapter 2, the effects of partially replacing MR by SF during the first half of the fattening period and partially replacing corn silage and barley straw by concentrate during the second half of the fattening period were tested. The calves that consumed more SF and less MR during the first period, reached identical carcass weight gains at 25 wk. Although reaching identical carcass weight was not specifically aimed for, utilization of SF and MR could be compared and appeared to be similar. A disadvantage of this approach was that rates of (carcass) gain differed between treatments throughout the conduct of the experiment, due to differences in onset of SF provision between treatments. Differences in bodyweights and maintenance requirements throughout the experiment may have affected nutrient utilization. Furthermore, composition of gain is known to be affected by age (Gerrits et al., 1996) and may therefore be confounded with treatment. In Chapter 3 and 4, the objective was to quantify the marginal response of SF intake on nutrient utilization when provided in addition to MR. To study the marginal response of SF intake, incremental amounts of SF where provided while maintaining identical intake of MR. The advantage of this approach is that responses found can be directly attributed to SF intake. A disadvantage is that treatments differed in total nutrient intake and thus growth rate. An increased growth rate is accompanied by an increased ratio in fat-to-protein retention. Therefore, large differences in growth rates between treatments should be avoided.

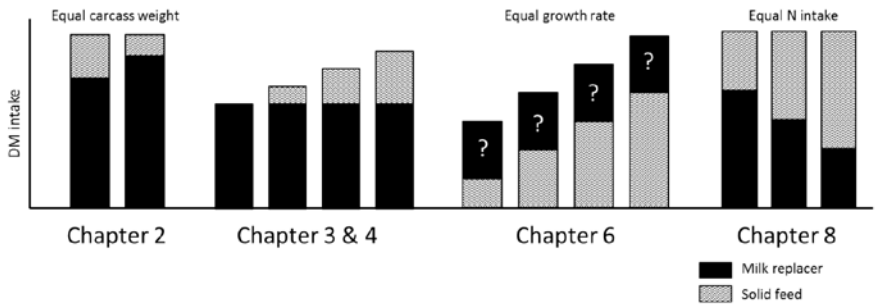


Figure 10.1 Schematic representations of experimental designs to study nutrient utilization in this thesis.

Throughout the studies in this thesis it became clear that exchanging a considerable part of MR for SF is complex in terms of experimental design. Either SF is dosed in addition to a constant quantity of MR (Chapter 3, 4), or SF is exchanged for MR (Chapter 2). Interactions between MR and SF complicate the calculation of feeding values to be used for SF and

MR, and isocaloric feeding is impossible due a lack of insight in digestibility. Therefore, the experiment in Chapter 7 was designed using a paired-gain set-up. Calves were subjected to pre-set levels of concentrate and roughage intake, whereas the level of MR was adjusted to target equal rates of carcass gain across treatments. In this way, a substantial interchange of MR, concentrates, and roughages was addressed while avoiding undesirable changes in the rate of BW gain. The amounts of MR needed to realize identical rates of gain was the relevant outcome of this study, and would provide insight in the feeding values of concentrates and roughages in comparison to MR. All interactions occurring between SF and MR during digestion and post-absorption, including the time-dependent interactions, that affect growth rate are accounted for. A major drawback, however, is that no insight in the quantitative importance of each of these separate aspects could be gained. Secondly, carcass gain needs to be predicted from bodyweight gain. The carcass:bodyweight proportion will differ throughout the fattening period due to factors like rumen development and gastrointestinal contents. Furthermore, differences in feeding values between these main ration components were addressed within the bounds of a particular choice of roughage and composition of concentrates or MR, and may not be representative for other diet compositions.

Finally, in Chapter 9, the effects of route of protein administration (via low-protein SF, high-protein SF or MR) on urea recycling and N retention were examined. Total N provision was equalized across treatments, because data on digestibility of the various N sources and post-absorptive interactions like urea recycling were lacking. A disadvantage of this experiment is that variation in digestibility and efficiency of N utilization results in different growth rates. In addition, total energy supply could not be equalized across treatments due to the lack of data to accurately predict digestible energy (**DE**) supply. Estimated DE intake differed across treatments (Chapter 9) and likely contributed to differences in N retention (Gerrits et al., 1996).

In conclusion, several experimental designs were applied throughout the studies in this thesis to assess nutrient utilization in calves fed a diet with MR and SF. In order to correctly interpret the outcomes of these studies, it is crucial to consider their differences, advantages, and disadvantages. Aspects of major importance include intake levels (energy, protein, DM), growth rates, carcass:bodyweight proportion and time-dependent interactions.

Interactions between Nutrients when Combining SF and MR in Calf Rations

The provision of low-protein SF results in a high marginal efficiency of N utilization (76% at 164 kg BW; Chapter 3), which can be explained by an increased supply of digestible N, urea recycling, and increased post-absorptive N utilization for growth (Chapter 4). In Chapter 9, it was shown that rumen fermentation benefits from increasing N content of the SF. In Chapter 4 and 9, a large part of the observed increase in N retention with SF intake was explained by the increase in DE supply, based on relationships described between

protein-free energy intake and protein retention in milk-fed veal calves (Gerrits et al., 1996). The increase in N retention with increasing DE supply was assumed to be independent of the composition of DE. However, the origin of DE could affect its metabolic fate. There are important metabolic differences between milk-fed and SF-fed calves in the substrates available for energy metabolism. In milk-fed calves, protein, carbohydrates, and fat provide approximately 20, 45, and 35% of ME intake, respectively (Palmquist et al., 1992). In SF-fed calves, these nutrients typically provide 15, 75, and less than 10% of ME intake. The carbohydrate portion of the diet results in absorption of primarily lactose in milk-fed calves, and primarily volatile fatty acids in SF-fed calves. These energy sources may affect post-absorptive utilization of amino acids differently. In (heavy) veal calves, postprandial responses are indicative of the development of insulin resistance (Hugi et al., 1997), as further indicated by glucosuria (Hostettler-Allen et al., 1994, Hugi et al., 1998). Glucosuria is associated with postprandial hyperglycaemia, hyperinsulinaemia, and likely caused by a low insulin sensitivity of liver, muscle, and adipose tissue (Hostettler-Allen et al., 1994, Hugi et al., 1997). Volatile fatty acids (**VFA**) represent up to two-thirds of DE intake in SF-fed cattle (Huntington and Reynolds, 1987). Acetate, propionate, and butyrate are used as energy sources. Acetate is the principal substrate for lipogenesis, whereas propionate is predominantly used for gluconeogenesis, because net absorption of glucose from the intestinal tract is small in SF-fed cattle. The extent of gluconeogenesis from propionate when calves are provided with both MR and SF is unknown, but data indicate that in ruminants, gluconeogenesis still has a high metabolic priority even if glucose is exogenously supplied (Aschenbach et al., 2010). In addition, no differences were observed in the capacity to metabolize glucose from propionate when comparing hepatocytes from preruminant with hepatocytes from ruminating calves (Donkin and Armentano, 1995). Furthermore, fat deposition may also be affected by a different supply in energy sources. In SF-fed calves, body fat is mainly synthesized from acetate (Hanson and Ballard, 1967), whereas in milk-fed calves, body fat is synthesized from dietary lipids. In comparison to carbohydrate, dietary energy from fat increases fat deposition in milk-fed calves (Tikofsky et al., 2001). In addition, fat deposition in milk-fed calves increases in response to protein intake (Gerrits et al., 1996).

To summarize, further insight in the effects of protein-free energy sources (fat, lactose, VFA), energy intake, and protein-energy interactions from SF and MR is needed to optimize nutrient utilization. Furthermore, the effects of VFA from SF and long chain fatty acids from MR on insulin sensitivity need to be assessed.

Amino Acid Composition. A substantial part of N from MR can be successfully substituted by N from SF (Chapter 9). When the quantity of N originating from SF increases, it is important to accurately estimate the supply and metabolism of amino acids from SF and potential interactions with MR. In milk-fed calves, the marginal efficiency of utilization of dietary ileal digestible amino acids for deposition in the body did not exceed 25% for all

(semi)indispensable amino acids except for cysteine and arginine (Gerrits et al., 1998). In SF-fed ruminants, amino acids can be utilized for the production of precursors for gluconeogenesis. As discussed in the previous paragraph, it is unknown to what extent this may happen for calves fed both SF and MR, although some data suggest that gluconeogenesis from amino acids could be independent from dietary glucose supply. The effect of route of protein administration on utilization and gluconeogenesis in calves fed rations with both MR and SF needs further study. Furthermore, the amino acid composition and production of (microbial) protein in the rumen needs to be assessed. The quality of amino acid supply in SF-fed cattle can be improved by increasing net microbial protein synthesis, supplementing rumen degradable protein sources, or provide ruminally protected amino acids (Merchen and Titgemeyer, 1992). Amino acids from MR are in fact ruminally protected amino acid when bypassing the rumen through the reticular groove reflex, and provide an opportunity to complement amino acid supply from SF or microbial protein. In future research, the adjustment of amino acids profiles of MR and microbial protein to meet amino acid requirements for growth could be considered in calf nutrition. Accurate estimation of the amino acids available from microbial protein synthesized in the rumen requires reliable estimation of ruminal microbial yield, amino acid composition of microbial protein, and the digestibility of microbial amino acids.

Nutrient Synchrony. Veal calves are commonly fed MR twice daily. MR is digested and absorbed rapidly after a meal and, therefore, patterns of nutrient availability show large diurnal variation. With increasing SF intake, calves will spend more time consuming their feed, which results in a more sustained pattern of rumen fermentation. It is, therefore, hypothesized that synchronization of nutrient supply with nutrient demand may be improved with increasing SF intake, due to delayed nutrient absorption from rumen fermentation following passage rate. The postprandial kinetics in methane production reported in Chapter 3 provide some insight in effects of SF intake on the extent and time of fermentation processes, although the time needed to consume the SF was not recorded. In addition, SF intake in the other experiments (Chapter 5, 7, 8, 9) exceeded the SF levels used in Chapter 3. With increasing SF intake, the post-absorptive supply of nutrients is likely to be more constant during the day and may have comparable positive effects on nutrient utilization in calves similar to increasing feeding frequency of MR (van den Borne et al., 2006). In addition, increasing feeding frequency of SF is of interest as well, but not studied in this thesis. Feeding frequency of SF may enhance rumen digestion (Aikman et al., 2008), amongst others through increased saliva production (Beauchemin et al., 2008), which may improve fibre digestion. This may benefit post-absorptive utilization of nutrients from SF compared with MR.

Rumen Fermentation. Rumen development is crucial for degradation of SF, and has been studied extensively in dairy calves (Coverdale et al., 2004, Harrison et al., 1960, Khan et al.,

2007, Sander et al., 1959, Stobo et al., 1966a, b, Tamate et al., 1962, Zitnan, 1998, 1999, Zitnan et al., 1998, Zitnan et al., 1999), and in veal calves (Suárez et al., 2006a, Suárez et al., 2006b, Suárez et al., 2007). In order to optimize nutrient utilization for growth in calves provided with substantial amounts of SF, future research should focus on understanding and optimizing rumen fermentation processes in veal calves, as the rumen is the major site for degradation of SF sources. Therefore, measurements of rumen development in veal calves should shift from macroscopic measurements of development towards functional measurements (e.g. VFA production and absorption, microbial protein supply to the duodenum, fractional passage rate). Further optimizing rumen fermentation requires a thorough understanding of dietary manipulation of microbial protein synthesis and volatile fatty acid production, and, more specifically, the development towards maturity of rumen fermentation processes. Energy and N availability and synchronization in the rumen are considered to be the most important limiting factors for optimal microbial growth (Firkins, 1996). Both the nature of carbohydrates (Fernando et al., 2010) and the protein level of the diet modify the rumen microbial population in dairy cows (Belanche et al., 2012). Furthermore, the absorption of VFA in relation to hyperkeratosis and plaque formation, as well as rumen pH could be further investigated. Finally, the effect of ruminal fermentation of MR, as a result of ruminal drinking, on rumen fermentation kinetics needs to be assessed. These aspects may be the first to consider while optimizing rumen fermentation in veal calves.

Passage Rate. The accuracy of predicting diet degradation depends on estimation of passage rates, since passage and degradation are competing rumen processes. The fractional passage rate is affected by dietary composition and feed intake in cattle (Seo et al., 2006a, Seo et al., 2006b). Likely, meal size, feed intake pattern, age, and ruminant species play a role as well. In dairy cows, diet digestibility is negatively related to feed intake, because a reduced rumen retention time reduces the extent of microbial digestion of feeds (NRC, 2001). In calves, rumen size may be more adaptable to the diet than in dairy cows, and therefore, passage rate may be less sensitive to SF intake. Broesder et al. (1990) found that level of MR and forage in calves had a minor influence on passage rates of particles and fluid. Potentially, rumen capacity increased in response to DMI. Indeed, in the studies reported here, increasing SF intake was shown to increase rumen contents (Chapter 8). Based on the results of Chapter 8, it is unclear whether the passage rate of MR is affected by the provision of SF. The comparison made in Textbox I, however, suggests that the passage rate of MR is increased by feeding SF.

Growth Model. The objective of the thesis was to study growth performance from rations consisting of a combination of MR and SF. In Chapter 7, it was concluded that it is not possible to provide common feeding values for MR and SF, due to interactions between SF and MR that are affected by age. However, the data in this thesis do provide valuable

Textbox I. Influence of SF provision on passage rate of MR

For MR, a pilot study was performed to quantify recoveries of two meals in four gastrointestinal compartments. Twelve male Holstein-Friesian calves (251 ± 5.5 kg bodyweight) were housed individually on metabolic cages and allocated to one of three time periods between their last MR meal and slaughter: 2h, 4h, or 6h. In the last meal before slaughter, calves received Cobalt-EDTA with the MR. In the meal preceding the last meal, calves received Chromium-EDTA with the MR. Volume of liquid MR was ca. 10 L. Calves had no access to SF during the experiment.

Cumulative Cobalt (Co) recovery was close to 100% for all treatments (Table 10.1). There was a clear shift in Co recovery from abomasum (2h), to small intestine (4h), to large intestine (6h) with time. The cumulative Chromium (Cr) recovery indicated that three-quarters of the milk meal was excreted in feces by within 12h. The remainder was recovered in the rumen (i.e. as a result of ruminal drinking) or in the large intestine. The recovery of Co in the rumen at 4h after feeding was substantially lower than the average of 20% observed in Chapter 8. Furthermore, recoveries of Co in the abomasum (+12%), small intestine (+14%), and large intestine (+7%) were greater than in Chapter 8, and these differences cannot be exclusively attributed to the difference in ruminal drinking (i.e. recovery of Co in the rumen). Potentially, the large amounts of SF consumed by the calves in Chapter 8, or the different volumes of MR consumed could be involved. These results indicate that abomasal passage rate of MR is increased by SF intake.

Table 10.1 Recovery of Co en Cr, supplemented with milk replacer (MR), in gastrointestinal compartments in veal calves fed exclusively on MR (251 ± 5.5 kg)[1,2].

	Treatments						
Time feeding to slaughter	2h		4h		6h		Treatment effect[1]
No. of successful observations	2		3		3		
	mean	SEM	mean	SEM	mean	SEM	
Co recovery (provided with MR meal at day 0), % of intake							
Rumen	3	2.5	1	0.6	2	1.9	NS
Abomasum	52[a]	0.1	21[b]	6.2	6[b]	4.2	**
Small intestine	41	4.0	49	13.7	35	6.6	NS
Large intestine[3]	2[a]	1.3	26[a,b]	12.1	57[b]	5.9	*
Total	98	2.9	96	5.5	100	6.9	NS
Cr recovery (provided with MR meal at day -1), % of intake							
Rumen	5	3.8	3	1.4	4	3.4	NS
Abomasum	0	0.2	0	0.0	0	0.0	NS
Small intestine	0	0.1	0	0.0	0	0.1	NS
Large intestine[3]	15	1.1	25	9.7	22	8.2	NS
Total	20	5.2	28	11.0	26	11.7	NS

[1] The effect of treatment was tested for each segment and for their total using a general linear model (PROC GLM in SAS 9.20, Inc., Cary, NC) with calf as experimental unit, including treatment (time feeding to slaughter) as fixed effect.

[2] Within a row, means with different superscripts (a, b and c) differ ($P < 0.05$).

[3] Including cecum.

*$P < 0.05$; **$P < 0.01$

insight in these interactions and allow evaluation of their effects on growth performance. Designing a growth simulation model to predict protein and energy utilization in veal calves fed a combination of SF and MR is, therefore, considered to be a useful next step towards feed evaluation for veal calves. Such a model could be based on models currently available for nutrient utilization of MR, e.g. Gerrits et al. (1997a,b) extended with a rumen model (e.g. Bannink et al., 2008) to include SF utilization and interactions of SF with MR. The model should include effects of MR and SF on rumen fermentation processes, passage rates, ruminal drinking, and post-absorptive interactions as described in this thesis.

RUMINAL DRINKING

Ruminal drinking (**RD**) or leakage of MR into the rumen, was shown to be substantial in veal calves. Suárez et al. (2007) found that, on average, 21 to 35% of an orally supplied dose of CoEDTA, supplied with the MR, was recovered in the rumen at slaughter. In Chapter 2, RD averaged 14 to 18%, and in Chapter 8, RD averaged 20% of the final MR meal. Assuming that nutrients from MR are subject to rumen fermentation in case of RD, subclinical RD may increase the incidence of bloat, reduce post-absorptive availability of nutrients, and increase variation in growth rate. In this thesis, increasing SF intake did not affect RD. In Chapter 2, concentrate inclusion from 0 to 50% of SF increased (+5%) RD. In all experiments described in this thesis, RD may have contributed to variation in response parameters, such as urea recycling, rumen pH, nutrient utilization, rumen development score, and potentially plaque formation.

Method Evaluation

Because the measurement of Co recovery at slaughter to quantify RD is invasive and does not allow repetitive measurements within one animal, alternative methods were evaluated in Chapter 6. The acetaminophen absorption test identified ruminal drinkers, but could not quantify the amount of MR in the rumen. Measuring the abomasal volume using echography was more successful and results have shown that volume estimates and volumes measured at slaughter were in close agreement. In addition to these methods, a small scale trial was conducted with two calves using radiography and barium sulphate which was previously used in lambs to visualize the kinetics of milk passage (Sharifi et al., 2009). Although not quantitative, the pictures obtained with this technique would visualize the size of the problem. Unfortunately, the equipment used was not sufficiently powerful to produce clear output. Finally, a dual tracer technique was developed and tested during the studies described in Chapter 3 and 4 (Textbox II). However, this method needs further refinement and validation.

Textbox II. Quantifying ruminal drinking using a dual tracer technique

Ruminal drinking (RD) was measured by combining oral administration of [^{13}C]urea and intravenous administration of [^{15}N^{15}N]urea (Figure 10.2). Incomplete recovery of an oral dose of [^{13}C]urea, provided with MR, in 48-h urine can be explained by RD or by re-entry of absorbed [^{13}C]urea into the gastrointestinal tract, both resulting in fermentation of urea, and the consequent loss of the labelled carbon atom as $^{13}CO_2$. To correct for the gut entry rate of previously absorbed [^{13}C]urea, gut entry rate was quantified using [^{15}N^{15}N]urea which was infused for 24 h and the recovery of urea isotopomers was analysed in 68h urine and faeces, as described in Chapter 4. Infusion of [^{15}N^{15}N]urea was conducted two days after oral administration of [^{13}C]urea.

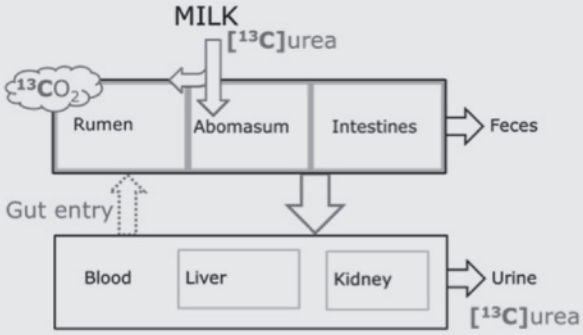


Figure 10.2 Schematic representation of quantification of ruminal drinking by a single dose of [^{13}C]urea, provided with the milk meal. In case of ruminal drinking, the carbon atom of [^{13}C]urea is lost as $^{13}CO_2$ due to microbial fermentation in the rumen. The recovery of [^{13}C]urea in urine is an estimate of ruminal drinking. In order to correct for potential re-entry of absorbed [^{13}C]urea into the gastrointestinal tract (indicated by dotted arrow), a 24-h intravenous administration of [^{15}N^{15}N]urea (Chapter 4) was used.

Subclinical RD was estimated at 0.5±0.6 L, being 7% of the MR provided, which is markedly lower than previously reported values (Suárez et al., 2007; Chapter 2). In total, 45% of the calves had subclinical RD of 10% of ingested milk or more but this varied substantially between calves (SEM: 9%). However, negative estimates for RD were obtained for 15 out of 38 calves: 9 calves ranging between -1 and -12%, and 6 calves ranging between -29 and -59%). It is hypothesized that these errors occurred for two reasons. Firstly, [^{13}C]urea was administered as a pulse dose at meal time, while [^{15}N^{15}N]urea was dosed continuously for 24h. Therefore, the estimate of gut entry rate is an average of fluctuations within one day. To accurately correct the recovery of [^{13}C]urea in urine for gut entry rate, the measurement of gut entry rate should represent the actual gut entry rate at meal time. Secondly, measurement of [^{13}C]urea recovery occurred 2 days after the measurement of gut entry rate, and potential effects of day-to-day variation cannot be excluded.
It can be concluded that quantification of subclinical ruminal drinking by the dual tracer method (i.e. [^{13}C] and [^{15}N^{15}N]urea) needs refining, and requires comparison to a golden standard, like the Cobalt recovery. The method could be improved when the fate of orally dosed [^{13}C]urea and the gut entry rate are studied simultaneously, to avoid effects of variation between and within days.

Factors Affecting Ruminal Drinking: a Meta-Analysis

Increasing SF intake from 1170 to 3000 g DM/d did not affect RD (Chapter 8). In the study reported in Chapter 2, additional treatments were included but not reported, with calves fed MR only (n = 10), or MR plus 500 (n = 20) or 1000 g DM SF per d (n = 20). RD at 27 wk of age averaged 18, 19, and 13%, respectively, and was not affected by SF intake. Increasing concentrate proportion from 50 to 80% of SF in Chapter 8 did not affect RD, but increasing concentrate proportion from 0 to 50% of SF in Chapter 2 increased RD. However, MR intake and volume was different in these Chapters.

The aim of the current paragraph was to gain insight in associations between SF and MR intake related variables and RD in veal calves. Therefore, a meta-analysis using data of 328 calves (Textbox III) was conducted to determine associations between RD (% of marker recovery), SF intake, the concentrate proportion of the SF, average daily gain, milk volume, and the time between feeding and slaughter.

RD averaged 17% and is a quantitatively relevant problem exhibiting large variation. The meta-analysis showed that the large variation observed in RD is not associated to the selected SF and MR intake related variables, or average daily gain (Textbox III). It is therefore recommended to further assess the within-animal variation in RD, as well as other variables (a.o. age, stress, feeding frequency, MR composition) that may influence RD.

CONSEQUENCES AND REGULATION OF VOLUNTARY FEED INTAKE

Comparing Free Choice of Ration Components with a Reference for Milk-Fed Calves

Free diet selection results in large individual variation in dietary preferences (Chapter 5). Dietary preferences change with age, and are associated with health aspects. Results indicate that DE intake and ADG of calves at 6 months of age were high (72 MJ DE per d; 1.18 kg/d ADG) when compared to commercial feeding systems where calves are fed ca. 58-62 MJ DE per day at a similar age (e.g. Chapter 2). Potentially, ad libitum DE intake would be stimulated by the choice fed situation or by the ad libitum availability of feeds. In Textbox IV, the outcome of the free choice study from Chapter 5 is compared with a reference situation for milk-fed calves. From this comparison, it was concluded that free diet selection increased total DM intake by 83% at 6 months of age and consequently warm carcass weight by 15% when compared with a reference system for milk-fed calves. Health parameters were numerically improved, except for abomasal damage in the pyloric region, when compared with a reference diet for milk-fed calves.

Table 10.2 Overview of experimental data included in the meta-analysis on ruminal drinking (RD), quantified by the rumen recovery (%) of a soluble marker supplemented with the final milk replacer (MR) meal before slaughter

Experiment	Described in	No. of calves	No. of successful observations	No. of treatments	Solid feed provision, g DM/d	% concentrate in solid feed	MR volume, L/meal	BW, kg	RD, %	Average daily gain, g/d
1	Chapter 2 (partly)	28	26	3	0 vs. 750	0 vs. 50	6.8-8.5	109 ± 1.3	15 ± 2.4	1331 ± 57.1
2	Chapter 2 (partly)	140	135	7	0 vs. 500 vs. 1000	0 vs. 50	8.2-9.0	250 ± 2.0	16 ± 1.0	1802 ± 55.7
3	Chapter 3, 4	12	12	4	0 vs. 400 vs. 800 vs. 1200	50	5.7-7.6	172 ± 2.5	5 ± 1.3	1028 ± 73.3
4	General Discussion	12	8	2	400	50	9.0–11.1	251 ± 5.5	2 ± 0.8	1490 ± 170.1
5	Chapter 8	32	24	4	1200 vs. 3000	50 vs. 80	2.9-6.5	249 ± 4.0	20 ± 4.0	1070 ± 54.9
6	Gilbert et al., unpublished	40	38	5	0		8.2–10.9	236 ± 2.4	10 ± 2.1	1033 ± 32.1
7	Suárez et al., 2007	64	64	6	750 vs. ad libitum	40 vs. 70 vs. 100	7.0	103 ± 0.8	27 ± 2.5	739 ± 10.4

Textbox III. Meta-analysis on ruminal drinking

A meta-analysis was conducted using all available data on RD in veal calves in order to study relationships between RD (% of marker recovery), and SF intake, concentrate proportion of the SF, average daily gain, milk volume, and time between feeding and slaughter. Data of 328 calves originating from 7 experiments, of which 4 reported in this thesis, were included in the analysis and are described in Table 10.2. In all calves, RD was measured using identical slaughter procedures and averaged 17%. Because of multicollinearity between these variables, straightforward multiple regression analysis was not a viable option. Instead, Principal Component Analysis (PCA) (Joliffe, 1986) was used to examine patterns of intercorrelations between multiple variables. PCA produces so-called principal components (PC), which are linear combinations of the original variables, and represent independent characteristics underlying the correlation matrix. The importance of each variable for a PC is indicated by its loading – which is similar to a coefficient of a linear equation. Variables with high loadings of the same sign on the same PC are positively correlated, and variables with high loadings of the opposite sign on the same PC are negatively correlated. Thus, if RD is mediated by one or more of the other variables included in this analysis, one would expect PCA to result in at least one PC with high loadings of multiple variables including RD. Variables were scaled prior to PCA, i.e. PCA was performed on the Pearson correlation matrix.

Table 10.3 Loadings of principal components (PC) extracted by principal component analyses of six variables recorded in calves†, and the eigenvalues and proportions of total variation explained by each PC

	PC1	PC2	PC3
Item	**SF vs. MR and ADG**	**RD vs. time meal-slaughter**	**Average daily gain**
Ruminal drinking, % of intake	0.39	**-0.64**	0.40
Concentrate proportion in solid feed, %	**0.76**	0.03	-0.36
Solid feed provision, g DM/d	**0.78**	0.37	0.44
Milk volume, L/feeding	**-0.90**	-0.22	-0.15
Time between last meal and slaughter, min	-0.13	**0.81**	-0.06
Average daily gain, g/d	**-0.67**	0.22	**0.55**
Eigenvalues	2.62	1.31	0.82
Variance explained, %	44	22	14

†Ruminal drinking was quantified in 328 Holstein-Friesian male calves from 2 to 6 months of age by assessment of the rumen recovery of a soluble marker administered with the final milk replacer meal before slaughter.

The PCA was successively performed on RD related data, with the first two PC with an eigenvalue larger than 1 (Table 10.3). The first three PC explained 80% of total variation. Cross loading of variables was minimal, except for average daily gain, which had high loadings on both PC1 and PC3. The first PC explained 44% of variation observed and had high positive loadings for concentrate proportion in the SF, SF provision, and high negative loadings for milk volume and average daily gain. The second PC explained 22% of variation observed and has a high negative loading for ruminal drinking and a high positive loading for time between the last meal and slaughter. Finally, the third PC explained 14% of variation and had a high positive loading for average daily gain. The interdependence of MR and SF in PC1 is explained by the experimental design (Table 10.3). These results further indicate that RD (PC2) is not related to average daily gain (PC3) or MR- and SF-related parameters (PC1). The association between RD and time between last meal and slaughter can be explained by the passage of MR (and the marker) recovered in the rumen with time (Table 10.1).

Does Free Choice Increase Feed Intake?

Based on the observation that preference calves consumed 83% more DM and gained 15% more carcass than reference calves (Textbox IV), an experiment was designed to study the effect of free selection in comparison to mixed provision of SF components on performance and intake (Textbox V). From this study, it was concluded that providing SF sources separately gave a slight increase in SF intake compared with providing SF sources in a mixture, but performance parameters remained unaffected. On a group-level, calves made a very consistent choice in SF components throughout the experimental period. Possibly, the high energy intake observed in Chapter 5 is stimulated by ad libitum access to MR as a ration component and/or a greater diversity of SF sources.

Nutritional Regulation of Voluntary Intake of Ration Components

Satiety is the process that leads to inhibition of further eating, decline in hunger, and increase in fullness after a meal (Blundell et al., 2010). In ruminants, distension of the reticulorumen is considered the major regulator of satiety (Allen, 1996). In addition, the absorption of metabolic fuels, like VFA, has been shown to increase satiety in dairy cows (Mbanya et al., 1993). There is lack of information on diet-related factors involved in feed intake regulation in veal calves. Compared to adult ruminants, effects of SF intake on distension of the reticulorumen may be different in veal calves. In addition, calves may regulate feed intake based on specific nutrient intake. In weaned calves, large individual differences in feed preferences were observed (Atwood et al., 2001).

Intake data of the Preference calves were further explored to study the large individual differences in the choice and intake of ration components. In Chapter 5, calves could select a diet from 6 sources, including MR for 6 months. In Figure 10.5, variation in intake of MR, SF, SF components and DM between individual calves is visualized by the coefficient of variation (CV) at 3 and 6 months of age. Variation in intake of individual diet components

Textbox IV. Free diet selection compared with a reference diet

Although not published, a reference group was included in the experiment described in Chapter 5. In this textbox, calves allowed to exhibit dietary preference (now called "Preference") are compared with calves fed a reference diet for milk-fed calves (now called "Reference"). The reference group consisted of 16 male Holstein Friesian calves that had restricted access to MR and restricted access to SF, composed of 65% corn silage, 30% concentrate, and 5% chopped barley straw on a DM basis, and ad libitum access to water. The MR was provided via an automated milk dispenser and increased gradually from 411 g DM/d at 2 wk of age to 3014 g DM/d at 27 wk of age. This MR scheme is slightly lower than veal calves raised in a commercial situation. SF was provided twice daily and increased gradually from 50 to 500 g DM/d at 22 wk of age, and subsequently decreased gradually to 395 g DM/d at 27 wk of age. Measurements were performed as described in Chapter 5. The data presented here do not allow a formal statistical comparison between treatments, because of the limited number of home pens (2) and batches (2).

At 3 months of age, Reference calves consumed on average 1325 g DM MR per d divided over 9.5 feedings, whereas Preference calves consumed only 68% of that, divided over 6.5 feedings (Table 10.4). In addition, Reference calves consumed 247 g DM SF per d, which was only 25% of the SF intake of Preference calves. At 6 months of age, differences in intake became larger. SF intake of Preference calves reached 3205 g DM/d, whereas Reference calves only consumed 11% of that, and MR intake for Preference calves was only 60% of the MR intake of Reference calves. Water consumption was greater in Preference than in Reference calves at both ages. The observed increase in water intake coincided with the increased intake of SF, and it does not reflect a difference in liquid intake from MR.

Table 10.4 Descriptive data for feed intake for Preference* and Reference* calves recorded at 3 and 6 months of age

	3 months of age				6 months of age			
	Reference		Preference		Reference		Preference	
Item	mean	SEM	mean	SEM	mean	SEM	mean	SEM
Milk replacer intake, g DM/d	1325	84.8	905	83.6	2079	120.3	1250	80.0
Feeding frequency milk replacer, #/d	9.5	1.67	6.5	0.49	13.0	2.27	8.2	0.48
Water intake†, L/d	1.8	0.28	2.5	0.50	4.5	0.78	8.3	0.96
Solid feed intake, g DM/d	247	61.5	987	100.0	351	50.1	3205	174.6

*Holstein-Friesian male calves were studied from 0 to 6 months of age in two treatments; Preference (n=23) and Reference (n=16). Within the Preference treatment, calves had free and ad libitum access to 6 ration components: hay, straw, corn silage, concentrate, milk replacer, and water. Within the Reference treatment, a reference feeding strategy for milk-fed calves was chosen, with restricted access to MR and SF, composed of 65% corn silage, 30% concentrate, and 5% chopped barley straw on a DM basis.

†Measured using a water bowl. Water intake excludes water intake with milk replacer.

Bodyweight, rumen weight with and without contents, and rumen development score were higher in Preference calves than in Reference calves (Table 10.5). The macroscopic rumen development score in Preference calves was higher than in other studies using a similar scoring system (Suárez et al., 2007; Chapter 2), which may be due to the continuous availability of all ration components and greater SF intake in Preference calves. At 27 wk, plaque formation in the rumen was observed in 10% of the Preference and 46% of the Reference calves (Table 10.5). The latter is low when compared with Suárez et al. (2007), who observed plaque formation in 63% of calves fed a diet composed of 40% concentrate and 60% corn silage. The difference could be explained by the inclusion of straw in the Reference diet, which was shown previously to be effective in reducing plaque formation (Brscic et al., 2011, Suárez et al., 2007).
Hyperkeratosis was not observed in Preference calves and occurred in 8% of the Reference calves (Table 10.5). The latter was comparable to the prevalence of 6% reported for veal calves in commercial practice (Brscic et al., 2011).

Table 10.5 Descriptive data of rumen development, parameters of clinical health, abomasal damage, and performance for Preference* and Reference* calves

	Reference		Preference	
Variable	**Mean**	**SEM**	**Mean**	**SEM**
Rumen development				
Rumen weight with contents, kg	10.6	1.23	19.1	1.03
Rumen weight without contents, kg	2.55	0.117	4.29	0.158
Rumen development score	1.3	0.11	2.9	0.15
Rumen plaque, % of calves	46	14.4	10	6.6
Hyperkeratosis, % of calves	8.3	8.3	0	0
Clinical health				
Days of treatment for respiratory disorders, d	8.8	1.20	4.3	0.62
Days of treatment for gastrointestinal disorders, d	2.0	0.58	3.9	1.74
Total days of medical treatment, d	16.4	2.78	8.5	1.50
Abomasal damage				
Lesions in torus pylorus, % of calves	62	14.0	27	9.7
Pyloric area estimate, cm^2	1.91	0.525	3.11	0.731
Fundic area estimate, cm^2	1.04	0.567	0.04	0.043
Performance				
Live weight at 6 months, kg	217	6.6	253	7.2
Warm carcass weight, kg	122	4.0	140	4.1

*Holstein-Friesian male calves were studied from 0 to 6 months of age in two treatments; Preference (n=23) and Reference (n=16). Within the Preference treatment, calves had free and ad libitum access to 6 ration components: hay, straw, corn silage, concentrate, milk replacer, and water. Within the Reference treatment, a reference feeding strategy for milk-fed calves was chosen, with restricted access to milk replacer and solid feed, composed of 65% corn silage, 30% concentrate, and 5% chopped barley straw on a DM basis.

In comparison with the Reference treatment, the number of days that calves were treated for respiratory disorders was lower, and the number of days calves were treated for gastrointestinal disorders was higher in the Preference treatment. Overall, Reference calves had more days of medical treatment than Preference calves (Table 10.5). Abomasal damage in the fundic region was for highest Reference calves, but in the pyloric region, abomasal damage was highest for Preference calves. The greater SF intake in Preference calves may have increased the prevalence of abomasal lesions, as was shown previously (Brscic et al., 2011, Mattiello et al., 2002, Welchman and Baust, 1987). Promoting rumen development and stimulating the fermentative degradation of potentially sharp particles was hypothesized to reduce the incidence of abomasal lesions. Indeed, early compared with late rumen development reduced the number of large scars in the abomasum at 25 wk of age (Chapter 2). Although the contrast in rumen development was large in the current study, this hypothesis is not further supported by the findings in Reference and Preference calves.

Textbox V. Mixed vs. separate provision of SF sources

In addition to the experiment reported in Chapter 7, 50 Holstein-Friesian male calves (2 wk of age, 45 ± 0.3 kg BW) were divided over 10 pens of 5 calves each. Pens were randomly assigned to one of 2 treatments: ad libitum provision of pelleted concentrates, chopped corn silage, and chopped wheat straw ('Free-choice'), or ad libitum provision of a mixture of concentrates, chopped corn silage, and chopped wheat straw ('Mixed-ration'). Treatments were provided from wk 12 to 27. The proportion of SF sources provided to calves in the Mixed-ration treatment was identical to the choice of the calves in the Free-choice treatment from the previous wk. All calves were provided with MR according to the scheme of the SF3 treatment in Chapter 7. For the Mixed-ration treatment, SF was provided as a mixture in a long feed trough in front of the pen directly after the morning meal; refusals were weighed once daily. For the Free-choice treatment, the feed trough was divided in three sections; one section for each of the three SF sources. SF sources changed position daily to avoid confounding effects of positional preference. Calves had free access to water provided via drinking nipples.

Milk intake did not differ between Free-choice and Mixed-ration calves in any of the wk. SF intake tended to be greater ($P < 0.10$) for Free-choice calves than for Mixed-ration calves for several wk of the experimental period after wk 16 of the experiment (Figure 10.3). Throughout the experimental period, total DM intake from SF was 298 kg for Free-choice calves, which tended to be greater ($P = 0.09$) than for Mixed-ration calves (275 kg). Total DM intake from MR was similar for both treatments (211 and 216 kg, respectively). Within the Free-choice treatment, calves (pen averages) were very consistent in their preference over time (Figure 10.4). Concentrates, corn silage, and wheat straw contributed for respectively 70, 20, and 10% to total DM intake from SF. Despite a slight increase in voluntary SF intake in Free-choice calves, no differences were observed in bodyweight (292 vs. 291 kg for Free-choice vs. Mixed-ration), carcass weight (158 vs. 160 kg for Free-choice vs. Mixed-ration), blood haemoglobin, carcass color or fat score.

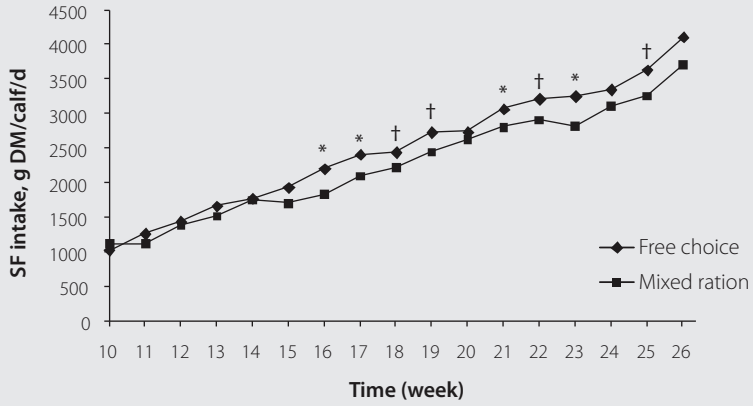


Figure 10.3 Average SF intake per wk for calves with ad libitum access to SF sources, provided separately ('Free choice') or as a mix ('Mixed ration'). SF intake data were analysed by analysis of variance (PROC GLM in SAS 9.20 by SAS Institute, Inc., Cary, NC), with pen as experimental unit, incl. treatment and slaughter day as fixed effects, for each wk. †: $P < 0.10$; *: $P < 0.05$.

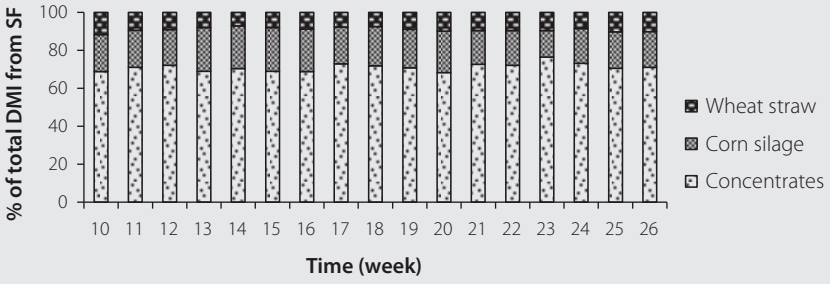


Figure 10.4 Contribution of concentrates, corn silage, and wheat straw to total DMI from SF in veal calves provided ad libitum access to three SF sources ('Free choice')

is very large, as evidenced by the coefficient of variation ranging from 25% for MR (6 months of age) up to 110% for corn silage (3 months of age). However, for total DM intake, the CV was reduced to 18% at 3 months of age and to 16% at 6 months of age. When expressing intake as total DMI per metabolic bodyweight (MBW), as DE intake per MBW, or as digestible crude protein intake per MBW, the CV is further reduced to 13-14%, 12-13%, or 13%, respectively. Expressing dietary choice as a ratio between digestible crude protein intake to DE intake (estimated from Chapter 2; van den Borne et al., 2006; CVB, 2007), this ratio averaged 9.5 at 3 months of age and 8.8 at 6 months of age, with a CV of 4.6 and 4.9%

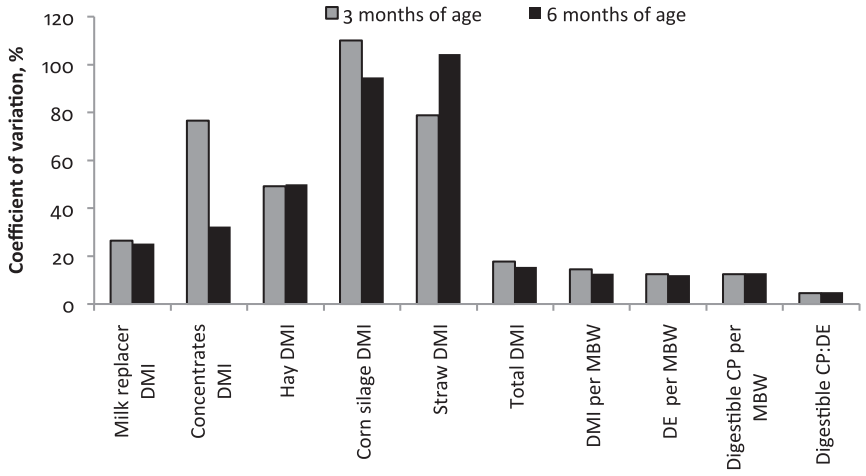


Figure 10.5 Coefficient of variation in feed intake related parameters observed in 23 Holstein-Friesian male calves with free and ad libitum access to 6 ration components (hay, straw, corn silage, concentrate, milk replacer, and water) from 0 to 6 months of age. Individual intakes of each diet component were recorded at 3 and 6 months of age. DMI: dry matter intake (g/d). MBW: metabolic bodyweight ($kg^{0.75}$). DE: digestible energy intake (MJ/d). CP: crude protein (g CP/d).

at 3 and 6 months of age, respectively. The constant ratio in DCP:DE ratio is remarkable when considering the large differences in DCP and DE contents and their ratio for the diet components. DE contents were estimated at 7.7 (straw), 11.1 (hay), 13.7 (corn silage), 15.2 (concentrates), and 19.1 (MR) MJ/kg DM. DCP contents were estimated at 7 (straw), 46 (corn silage), 48 (hay), 136 (concentrates) and 202 (MR) g digestible CP per kg DM. These results indicate that calves choose ration components to achieve a remarkably constant ratio of DCP:DE while selecting a diet.

Although calves selected a diet with a rather constant digestible CP:DE ratio, the variation observed in total DMI was highly different. In an attempt to find cues that drive voluntary feed intake in calves, the importance of bulkiness in the control of feed intake was evaluated, as distension and hypertonicity in the reticulo-rumen have been proposed as major satiating factors of ruminants (Allen, 2000). The ratio between gross energy and digestible energy (GE:DE) was chosen as an indicator for the bulkiness of ration components, and was estimated based on literature data (Chapter 2, van den Borne et al., 2006, INRA, 2007, CVB 2007). DE intake was included as a dependent variable by mixed model analysis (PROC MIXED in SAS 9.20 by SAS Institute, Inc., Cary, NC), including fixed effects of age, and independent variables (i.e. bulkiness, DE from MR, DE from concentrates)

as a co-variable with a random age term to account for repeated observations on the same calf. The interaction between the independent variable and age was included in all models. Figure 10.6 shows that there is no effect of bulkiness of the diet on estimated DE intake at 3 and 6 month of age. The same was true for DE from MR and DE from concentrates as independent variables. These data indicate that the variation in DM intake cannot be explained by diet bulkiness or DE from MR or concentrates.

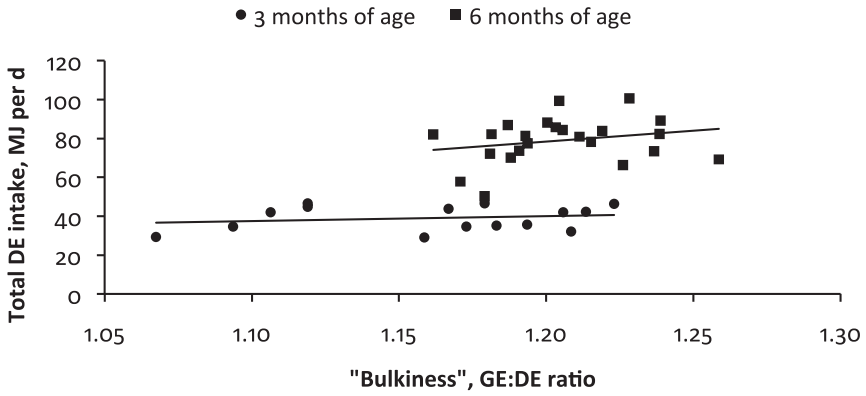


Figure 10.6 Effect of 'bulkiness' (ratio of gross energy:digestible energy) of the diet on estimated total DE intake (MJ/day), as observed in 23 Holstein-Friesian male calves with free and ad libitum access to 6 ration components (hay, straw, corn silage, concentrate, milk replacer, and water) from 0 to 6 months of age. Individual intakes of each diet component were recorded at 3 and 6 months of age. The effect of bulkiness was not significant (NS; $P > 0.05$).

CONTRIBUTION TO SUSTAINABLE VEAL PRODUCTION

Effects of SF Provision on Veal Meat Color and Quality

Although crucial for commercial acceptance of SF-based feeding strategies, few data are available regarding the effect of SF on meat color and quality attributes. Prevedello et al. (2010) evaluated the effects of the provision of two sources of SF on blood haemoglobin and iron metabolism. Blood haemoglobin concentration has been shown to highly and positively correlate to muscle iron and muscle heme pigment concentrations (Miltenburg et al., 1992). Prevedello et al. (2010) found that at an average SF intake of 830 g DM/d with respective iron contents of 33 (for a 100% corn grain diet) or 100 ppm (SF consisting of 82% corn grain, 10% straw and 8% soy), haemoglobin concentration was increased with greater iron content of the SF. However, for both treatments, haemoglobin concentration remained within ranges that allowed acceptable meat color. These results are in accordance with the

study of Cozzi et al. (2002), who have shown that provision of straw, low in iron, did not affect meat color, whereas the provision of beet pulp, rich in iron, did. Although the pale color of meat is a major criterion by which consumers judge veal quality, the effect of SF provision on sensory and instrumental meat quality attributes is of importance as well. Blokhuis et al. (2000) evaluated veal meat quality of milk-fed calves supplemented with either iron, ad libitum hay, straw, corn silage, and corn cob silage or nothing (control). Meat color, pH, shear force, cooking losses, and the sensorial evaluation of tenderness, juiciness, and flavour were evaluated in a double-blind setting in Italy by a trained taste panel. Except for differences in meat lightness, other physical as well as sensorial meat quality parameters were not affected by the provision of any source of SF (Blokhuis et al., 2000). In this thesis, SF intake largely exceeded the levels of previous studies (except for the ad libitum hay treatment). Although haemoglobin levels increased and meat color changed with increasing SF intake (e.g. Chapter 7), average color scores did not exceed 5.5 and were therefore still within the acceptable range for commercial practice. These findings suggest that SF provision can be safely increased while maintaining veal meat color and quality characteristics, provided that attention is paid to iron levels of ingredients.

Economic, Environmental, and Welfare Evaluation of Veal Calf Rations

This research was conducted within a project that also investigated consequences of feeding strategies combining SF and MR in terms of welfare (Webb et al., in preparation), economics, and environmental impact (Mollenhorst et al., in preparation). Welfare constitutes both health and behavioral aspects. It has been well established that increasing SF, particularly roughage intake, effectively reduces abnormal oral behaviors (Webb et al., 2012), and considering behavioral implications should be an integral part of the development of any feeding strategy in veal calves. The effects of increasing SF intake and SF composition on abomasal lesions are not clear from this thesis, despite the large contrasts in SF composition and intake. Early rumen development may provide some protection against abomasal damage (Chapter 2), but increasing SF intake also increased abomasal damage (Chapter 8). It is hypothesized that the aetiology of abomasal ulcers is multifactorial and that SF and MR intake are amongst others factors to take into account. In order to find mitigation strategies for abomasal ulceration and erosions, it is crucial to gain a more detailed understanding of the mucosal defence and possible impairments caused by each separate environmental factor. The associations found between health measurements and dietary preferences in Chapter 5 provide further indications for future research leads.

To evaluate the consequences of SF-based diets for farm economics, all changes in feed, labor, and equipment costs need to be carefully evaluated. Increasing SF intake is expected to decrease total feed costs, but costs for labor and equipment to provide SF may increase and need investigation. Then, manure composition and volume will change which may have an impact as well.

Finally, the effects of increasing SF intake on environmental impact need to be assessed. The results from this thesis provide a solid background for the start of a life cycle assessment (LCA) approach, which can lead to calculation of trade-offs between positive and negative aspects of the feeding of SF. For example, increasing SF intake may reduce N excretion in urine and total N output (Chapter 3, 4, 8), but SF intake increases CH_4 emissions (Chapter 3). In addition, the dietary change with increasing SF intake and decreasing MR intake may affect the carbon footprint, because MR ingredients may have a greater footprint than concentrate and roughages sources. The development of a growth model, as suggested in this chapter, may contribute to the further optimization of the use of resources, and could be extended with an economical and environmental assessment.
In conclusion, the studies reported in this thesis deal extensively with the nutritional and health consequences of combining SF and MR in feeding strategies for veal calves. They highlight how combinations of ration components differentially affect energy and nitrogen metabolism, methane emissions, ruminal passage rates, ruminal drinking, rumen development, and (abomasal) health. For the development of novel feeding strategies for veal calves, the impact on animal welfare, farm economy, and environmental impact should be considered.

CONCLUSIONS

In a series of large-scale animal experiments we fed various combinations of MR, concentrates and roughages to veal calves. To the best of our knowledge, the studies reported in this thesis are among the first ones extensively dealing with the nutritional consequences of combining these ration components into calf diets and their effect on nutrient utilization and health. They provide a solid basis for the development of growth simulation models or for further development of feed evaluation systems for calves. Briefly, the studies have led to the following conclusions:

- Early rumen development is important for maximizing nutrient yield from SF. In addition, there are leads indicating it may be beneficial for abomasal health.
- When calves are fed low protein SF in addition to MR, N efficiency is increased. Detailed studies revealed that about 20% of this effect is related to the recycling of urea-N (mainly originating from protein in MR) to the rumen, thus providing a second opportunity to contribute to protein deposition in the calf's body.
- Increasing the intake of low-protein SF at the expense of protein intake from MR does not affect N efficiency or N retention. Increasing protein intake via SF at the expense of protein intake via MR increases N retention and N efficiency and coincides with improved fibre degradation.
- When offered a choice between MR, concentrates, maize silage, hay, and straw, calves show large individual variation in dietary preferences. These preferences change with

age, and calves seem to adjust their dietary preferences and intake according to health cues. Despite the large variation in intake, calves select a diet with a constant digestible crude protein:digestible energy ratio. Providing SF sources separately or as a mixture does not affect free intake or performance.

- On average, 17% of the last MR meal is recovered in the rumen. Therefore, ruminal drinking is a problem of quantitative importance. An evaluation of methods to perform repeated measures of RD revealed ultrasonography and a dual tracer method to be promising, but not satisfactory for quantitative measurement of RD. A meta-analysis on slaughter data indicated that RD is not associated with SF or MR intake related variables or with average daily gain.
- The utilization of SF for growth increases with age, irrespective of forage-to-concentrate ratio. This effect is partly related to improved fermentation of fibre from the SF.
- Finally, SF intake but not R:C ratio affects passage rates of concentrates and straw through the calf's digestive tract. A greater SF intake increases passage rates of feedstuffs but also rumen contents, development and abomasal damage.

REFERENCES

Aikman, P., C. Reynolds, and D. Beever. 2008. Diet digestibility, rate of passage, and eating and rumination behavior of Jersey and Holstein cows. Journal of Dairy Science 91(3):1103-1114.

Allen, M. S. 1996. Physical constraints on voluntary intake of forages by ruminants. Journal of Animal Science 74(12):3063-3075.

Allen, M. S. 2000. Effects of diet on short-term regulation of feed intake by lactating dairy cattle. Journal of Dairy Science 83(7):1598-1624.

Aschenbach, J. R., N. B. Kristensen, S. S. Donkin, H. M. Hammon, and G. B. Penner. 2010. Gluconeogenesis in dairy cows: the secret of making sweet milk from sour dough. IUBMB life 62(12):869-877.

Atwood, S. B., F. D. Provenza, R. D. Wiedmeier, and R. E. Banner. 2001. Influence of free-choice vs mixed-ration diets on food intake and performance of fattening calves. Journal of Animal Science 79(12):3034-3040.

Bannink, A., J. France, S. Lopez, W.J.J. Gerrits, E. Kebreab, S. Tamminga, and J. Dijkstra. 2008. Modelling the implications of feeding strategy on rumen fermentation and functioning of the rumen wall. Animal Feed Science and Technology 143:3-26.

Beauchemin, K., L. Eriksen, P. Nørgaard, and L. Rode. 2008. Salivary Secretion During Meals in Lactating Dairy Cattle. Journal of Dairy Science 91(5):2077-2081.

Belanche, A., M. Doreau, J. E. Edwards, J. M. Moorby, E. Pinloche, and C. J. Newbold. 2012. Shifts in the rumen microbiota due to the type of carbohydrate and level of protein ingested by dairy cattle are associated with changes in rumen fermentation. The Journal of Nutrition 142(9):1684-1692.

Blokhuis, H. J., C. G. Van Reenen, J. J. Heeres, L. F. M. Heutinck, A. M. Van Vuuren, N. Stockhofe-Zurwieden, A. E. van de Braak, J. J. de Groot, I. Veissier, B. J. Lensink, L. Florand, M. S. Cockram, G. Cozzi, F. Gottardo, M. Verga, S. Mattiello, E. Canali, S. Raussi, M. Pyykkönen, H. Saloniemi, H. Hepola, and L. Hänninen. 2000. Chain Management of Veal Calf Welfare. Final report. EU project, Contract Number FAIR 3 PL96–2049, 174 pp.

Blundell, J., C. De Graaf, T. Hulshof, S. Jebb, B. Livingstone, A. Lluch, D. Mela, S. Salah, E. Schuring, and H. Van Der Knaap. 2010. Appetite control: methodological aspects of the evaluation of foods. Obesity reviews 11(3):251-270.

Broesder, J. T., M. B. Judkins, L. J. Krysl, S. A. Gunter, and R. K. Barton. 1990. Thirty or sixty percent milk replacer reduction for calves: effects on alfalfa hay intake and digestibility, digestive kinetics and ruminal fermentation. Journal of Animal Science 68(9):2974-2985.

Brscic, M., L. F. M. Heutinck, M. Wolthuis-Fillerup, N. Stockhofe-Zurwieden, B. Engel, E. K. Visser, F. Gottardo, E. A. M. Bokkers, B. J. Lensink, G. Cozzi, and C. G. Van Reenen. 2011. Prevalence of gastrointestinal disorders recorded at postmortem inspection in white veal calves and associated risk factors. Journal of Dairy Science 94(2):853-863.

Coverdale, J. A., H. D. Tyler, J. D. Quigley Iii, and J. A. Brumm. 2004. Effect of various levels of forage and form of diet on rumen development and growth in calves. Journal of Dairy Science 87(8):2554-2562.

Cozzi, G., F. Gottardo, S. Mattiello, E. Canali, E. Scanziani, M. Verga, and I. Andrighetto. 2002. The provision of solid feeds to veal calves: I. Growth performance, forestomach development, and carcass and meat quality. Journal of Animal Science 80(2):357-366.

CVB, 2007. Chemical compositions and nutritional values of feed ingredients. Centraal Veevoeder Bureau, Productschap Diervoeder, Den Haag, the Netherlands.

Donkin, S. and L. Armentano. 1995. Insulin and glucagon regulation of gluconeogenesis in preruminating and ruminating bovine. Journal of Animal Science 73(2):546-551.

Fernando, S. C., H. Purvis, F. Najar, L. Sukharnikov, C. Krehbiel, T. Nagaraja, B. Roe, and U. DeSilva. 2010. Rumen microbial population dynamics during adaptation to a high-grain diet. Applied and Environmental Microbiology 76(22):7482-7490.

Firkins, J. L. 1996. Maximizing microbial protein synthesis in the rumen. The Journal of Nutrition 126(4 Suppl):1347S-1354S.

Gerrits, W. J. J., J. W. Schrama, and S. Tamminga. 1998. The marginal efficiency of utilization of all ileal digestible indispensable amino acids for protein gain is lower than 30% in preruminant calves between 80 and 240 kg live weight. The Journal of Nutrition 128(10):1774-1785.

Gerrits, W. J. J., G. H. Tolman, J. W. Schrama, S. Tamminga, M. W. Bosch, and M. W. A. Verstegen. 1996. Effect of Protein and Protein-Free Energy Intake on Protein and Fat Deposition Rates in Preruminant Calves of 80 to 240 kg Live Weight. Journal of Animal Science 74(9):2129-2139.

Gerrits, W.J.J., J. Dijkstra, and J. France. 1997a. Description of a model integrating protein and energy metabolism in preruminant calves. The Journal of Nutrition 127 (6):1229-1242.

Gerrits, W.J.J., J.France, J. Dijkstra, M.W. Bosch, G.H. Tolman, and S. Tamminga. 1997b. Evaluation of a model integrating protein and energy metabolism in preruminant calves. The Journal of Nutrition 127 (6):1243-1252.

Hanson, R. W. and F. J. Ballard. 1967. The relative significance of acetate and glucose as precursors for lipid synthesis in liver and adipose tissue from ruminants. Biochemical Journal 105(2):529-536.

Harrison, H. N., R. G. Warner, E. G. Sander, and J. K. Loosli. 1960. Changes in the tissue and volume of the stomachs of calves following the removal of dry feed or consumption of inert bulk. Journal of Dairy Science 43:1301-1312.

Hostettler-Allen, R., L. Tappy, and J. Blum. 1994. Insulin resistance, hyperglycemia, and glucosuria in intensively milk-fed calves. Journal of Animal Science 72(1):160-173.

Hugi, D., R. M. Bruckmaier, and J. W. Blum. 1997. Insulin resistance, hyperglycemia, glucosuria, and galactosuria in intensively milk-fed calves: dependency on age and effects of high lactose intake. Journal of Animal Science 75(2):469-482.

Hugi, D., L. Tappy, H. Sauerwein, R. M. Bruckmaier, and J. W. Blum. 1998. Insulin-dependent glucose utilization in intensively milk-fed veal calves is modulated by supplemental lactose in an age-dependent manner. The Journal of Nutrition 128(6):1023-1030.

Huntington, G. B. and C. K. Reynolds. 1987. Oxygen consumption and metabolite flux of bovine portal-drained viscera and liver. The Journal of Nutrition 117(6):1167-1173.

Joliffe, I. T. 1986. Principal component analyses. Springer-Verlag, New York.

Khan, M., H. Lee, W. Lee, H. Kim, S. Kim, K. Ki, J. Ha, H. Lee, and Y. Choi. 2007. Pre-and postweaning performance of Holstein female calves fed milk through step-down and conventional methods. Journal of Dairy Science 90(2):876-885.

Mattiello, S., E. Canali, V. Ferrante, M. Caniatti, F. Gottardo, G. Cozzi, I. Andrighetto, and M. Verga. 2002. The provision of solid feeds to veal calves: II. Behavior, physiology, and abomasal damage. Journal of Animal Science 80(2):367-375.

Mbanya, J., M. Anil, and J. Forbes. 1993. The voluntary intake of hay and silage by lactating cows in response to ruminal infusion of acetate or propionate, or both, with or without distension of the rumen by a balloon. British Journal of Nutrition 69(03):713-720.

Merchen, N. R. and E. C. Titgemeyer. 1992. Manipulation of amino acid supply to the growing ruminant. Journal of Animal Science 70(10):3238-3247.

Miltenburg, G., T. Wensing, F. Smulders, and H. Breukink. 1992. Relationship between blood hemoglobin, plasma and tissue iron, muscle heme pigment, and carcass color of veal. Journal of Animal Science 70(9):2766-2772.

NRC. 2001. Nutrient requirements of dairy cattle. 7th rev. ed. Natl. Acad. Press, Washington, DC.

Palmquist, D., J. Doppenberg, K. Roehrig, and D. Kinsey. 1992. Glucose and insulin metabolism in ruminating and veal calves fed high and low fat diets. Domestic Animal Endocrinology 9(3):233-241.

Prevedello, P., L. Moro, M. Brscic, F. Gottardo, and A.-L. Stefani. 2010. Trend overtime of total haemoglobin, iron metabolism and trace minerals in veal calves fed high amounts of two different solid feeds. Italian Journal of Animal Science 8(3):184-186.

Sander, E. G., R. G. Warner, H. N. Harrison, and J. K. Loosli. 1959. The stimulatory effect of sodium butyrate and sodium propionate on the development of rumen mucosa in the young calf. Journal of Dairy Science 42(9):1600-1605.

Seo, S., L. Tedeschi, C. Lanzas, C. Schwab, and D. Fox. 2006a. Development and evaluation of empirical equations to predict feed passage rate in cattle. Animal Feed Science and Technology 128(1):67-83.

Seo, S., L. Tedeschi, C. Schwab, B. Garthwaite, and D. Fox. 2006b. Evaluation of the passage rate equations in the 2001 Dairy NRC Model. Journal of Dairy Science 89(6):2327-2342.

Sharifi, K., W. Grünberg, S. Soroori, M. Mohri, and M. S. Ahrari-Khafi. 2009. Assessment of the acetaminophen absorption test as a diagnostic tool for the evaluation of the reticular groove reflex in lambs. American Journal of Veterinary Research 70(7):820-825.

Stobo, I. J., J. H. Roy, and H. J. Gaston. 1966a. Rumen development in the calf. 1. The effect of diets containing different proportions of concentrates to hay on rumen development. British Journal of Nutrition 20(2):171-188.

Stobo, I. J., J. H. Roy, and H. J. Gaston. 1966b. Rumen development in the calf. 2. The effect of diets containing different proportions of concentrates to hay on digestive efficiency. British Journal of Nutrition 20(2):189-215.

Suárez, B., C. Van Reenen, W. Gerrits, N. Stockhofe-Zurwieden, A. Van Vuuren, and J. Dijkstra. 2006a. Effects of supplementing concentrates differing in carbohydrate composition in veal calf diets: II. Rumen development. Journal of Dairy Science 89(11):4376-4386.

Suárez, B. J., C. G. Van Reenen, G. Beldman, J. Van Delen, J. Dijkstra, and W. J. J. Gerrits. 2006b. Effects of supplementing concentrates differing in carbohydrate composition in veal calf diets: I. Animal performance and rumen fermentation characteristics. Journal of Dairy Science 89(11):4365-4375.

Suárez, B. J., C. G. Van Reenen, N. Stockhofe-Zurwieden, J. Dijkstra, and W. J. J. Gerrits. 2007. Effect of roughage source and roughage to concentrate ratio on animal performance and rumen development in veal calves. Journal of Dairy Science 90(5):2390-2403.

Tamate, H., A. D. McGilliard, N. L. Jacobson, and R. Getty. 1962. Effect of various dietaries on the anatomical development of the stomach in the calf. Journal of Dairy Science 45(3):408-420.

Tikofsky, J. N., M. Van Amburgh, and D. Ross. 2001. Effect of varying carbohydrate and fat content of milk replacer on body composition of Holstein bull calves. Journal of Animal Science 79(9):2260-2267.

Van den Borne, J., M. W. A. Verstegen, S. J. J. Alferink, R. M. M. Giebels, and W. J. J. Gerrits. 2006. Effects of feeding frequency and feeding level on nutrient utilization in heavy preruminant calves. Journal of Dairy Science 89(9):3578-3586.

Webb, L. E., E. A. M. Bokkers, B. Engel, W. J. J. Gerrits, H. Berends, and C. G. van Reenen. 2012. Behavior and welfare of veal calves fed different amounts of solid feed supplemented to a milk replacer ration adjusted for similar growth. Applied Animal Behavioral Science 136(2-4):108-116.

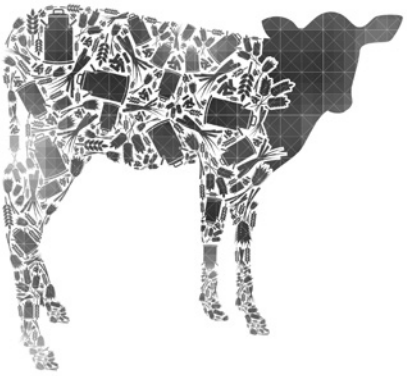
Welchman, D. D. and G. N. Baust. 1987. A survey of abomasal ulceration in veal calves. Veterinary Record 121(25-26):586-590.

Zitnan, R. 1998. Influence of dietary concentrate to forage ratio on the development of rumen mucosa in calves. Archives of Animal Nutrition 51(4):279-291.

Zitnan, R. 1999. Morphological and functional development of the rumen in the calf: Influence of the time of weaning 1. Morphological development of rumen mucosa. Archives of Animal Nutrition 52(4):351-362.

Zitnan, R., J. Voigt, U. Schönhusen, J. Wegner, M. Kokardová, H. Hagemeister, M. Levkut, S. Kuhla, and A. Sommer. 1998. Influence of dietary concentrate to forage ratio on the development of rumen mucosa in calves. Archives of Animal Nutrition 51(4):279-291.

Zitnan, R., J. Voigt, J. Wegner, G. Breves, B. Schröder, C. Winckler, M. Levkut, M. Kokardová, U. Schönhusen, S. Kuhla, H. Hagemeister, and A. Sommer. 1999. Morphological and functional development of the rumen in the calf: Influence of the time of weaning 1. Morphological development of rumen mucosa. Archives of Animal Nutrition 52(4):351-362.



SUMMARY

The diet of veal calves consists primarily of milk replacer (**MR**). In addition, some fibrous solid feed (**SF**) is included. For animal welfare as well as economic performance, increasing the proportion of SF at the expense of MR in veal calf diets is beneficial.

The aim of this thesis was to provide a scientific basis for the development of novel feeding strategies combining MR and SF. Effects of combining SF and MR on feeding values, growth performance, and health aspects were studied in a series of experiments. Especially effects of interactions between MR and SF occurring either at the level of digestion or post-absorption, and the potential age-dependency of these interactions, on the prediction of the nutritional value of SF components were considered. In addition, effects on health aspects were studied, including rumen development, ruminal drinking (**RD**), and abomasal lesions.

The experiment described in **Chapter 2** was designed to study the importance of early rumen development and composition of SF intake on growth performance and abomasal health in veal calves. A total of 106 Holstein-Friesian male calves was included to study effects of partially replacing MR by SF during the first half of the fattening period and partially replacing roughages by concentrate during the second half of the fattening period were tested. Calves that consumed more SF and less MR during the first period, reached identical carcass weight gains at 25 wk, and utilization of the SF provided for growth appeared similar to that of MR. Furthermore, calves with early rumen development (due to increased SF allowance) had less large scars in the abomasum, which may indicate protection against abomasal lesions. In conclusion, early compared with late rumen development may benefit SF utilization and abomasal health in veal calves.

In **Chapter 3 and 4**, the contribution of low-protein SF intake, consisting of 50% concentrates, 25% corn silage, and 25% straw, to protein and energy metabolism was quantified at two stages of the fattening period. Holstein-Friesian male calves were provided with an identical amount of MR and four incremental amounts of SF (n = 12 per treatment). The incremental efficiency of energy retention was 41% at 108 kg of BW and 54% at 164 kg of BW, and was accompanied by an increase in apparent NDF digestibility and an increase in methane emissions. On average, 5.5% of gross energy from SF was released as methane, which is similar to reported values in cattle fed only SF. Moreover, low-protein SF intake increased the gross efficiency of N retention. This effect was more pronounced at 164 kg BW (+ 12.3% / kg DM SF per day) than at 108 kg BW (+ 6.7% / kg DM SF per day). Provision of low-protein SF increased N utilization for protein gain in calves, particularly towards the end of the fattening period, as evidenced by the marginal efficiency of N utilization of 77%. This can be explained by the re-use of urea originating from MR protein. It was concluded from measurements with labelled ($^{15}N^{15}N$) urea that urea recycling accounted for approximately 20% of the observed increase in N retention with increasing SF intake. Furthermore, the up-regulation of ruminal urea transporter-B

and aquaporin-3 expression with SF intake may indicate that these transporters are associated to urea recycling.

In **Chapter 5,** the long-term dietary preference and intake of calves, and the associations between dietary preferences and measures of health and performance were studied. In total, 23 Holstein-Friesian male calves were given ad libitum access to MR, water, hay, barley straw, concentrate, and corn silage from 2 wk to 6 months of age. This study is the first to investigate long-term preferences of calves for ration components including MR. Free diet selection resulted in large individual variation in dietary preferences, and a high nutrient intake and average daily gain. At 3 months of age, gross energy intake averaged 37 MJ per day, including 52% from MR, 27% from concentrate, and 21% from roughages. At 6 months of age, gross energy intake averaged 84 MJ per day, including 31% from MR, 46% from concentrate, and 23% from roughages. Despite the large variation in choices for dietary components, calves chose a constant ratio of digestible energy to digestible crude protein (Chapter 10). Voluntary feed intake was not related to bulkiness of the diet, proportion of concentrates, or proportion of MR. Dietary preferences changed with age, and results indicate that feed intake pattern is associated to health measures. An additional experiment was designed to study the effect of free selection in comparison to mixed provision of SF components on performance and intake. Providing SF sources separately compared with a mixture of the same SF sources lead to a slight increase in SF intake but did not affect growth performance (Chapter 10).

From literature and the results of Chapter 2, subclinical RD (i.e. leakage of MR into the rumen) appears to be quantitatively important in veal calves. Rumen fermentation of MR may increase the incidence of bloat, reduce post-absorptive availability of nutrients from MR, and increase variation in growth rate. In addition, RD can cause health problems. To date, however, the measurement of RD can only be performed at slaughter by assessing rumen recovery of a marker ingested with the MR. In **Chapter 6**, several non-invasive, repeatable methods to quantify RD were evaluated in three consecutive experiments. Plasma acetaminophen kinetics and recovery of non-metabolizable monosaccharides in urine, both following a pulsed dose in MR, were indicative for RD but not quantitatively accurate. Measuring changes in abomasal volume by ultrasonography in response to a MR meal was considered as an accurate indirect indicator of RD, as compared with recovery of an indigestible marker measured in the rumen at slaughter. Finally, a dual tracer method with ^{13}C and $^{15}N_2$ urea was developed and tested (Chapter 10), but this method needs further refinement and validation.

A meta-analysis was conducted (Chapter 10), using data on RD of 328 calves from seven experiments, including three from this thesis. No associations were found between RD and any SF of MR related variable or variation in average daily gain. The considerable between-animal and unknown within-animal variation in RD needs to be assessed, as well as other factors (a.o. age, stress, feeding frequency, MR composition) that may influence RD. Studying interactions between MR for SF is characterized by complexity in terms of

experimental design. Either SF is dosed in addition to a constant quantity of MR (Chapter 3, 4), or SF is exchanged for MR. Interactions between MR and SF complicate prior assumptions in feeding values, and inaccurate assumptions may result in large differences in growth performance of calves. Therefore, in **Chapter 7**, a novel paired-gain setting was applied to evaluate the feeding values of MR, roughage, and concentrates for veal calves. To this end, 160 calves were assigned to one of four incremental pre-defined levels of SF and one of two roughage-to-concentrate (**R:C**) ratios (50:50 vs. 20:80). MR allowance was adjusted throughout the experiment to realize equal carcass gain for all SF levels. When adjusted for realized rates of carcass gain, calves fed an R:C ratio of 20:80 required 10% less MR than calves fed an R:C ratio of 50:50, indicating that the utilization of SF for gain increased with concentrate inclusion. Averaged over the 16-wk experimental period, the feeding value of MR relative to that of concentrates and roughages was close to that predicted based on their respective digestible energy contents. Nevertheless, the feeding value of SF relative to that of MR increased substantially with age. Therefore, additivity in feeding values of these ration components cannot be assumed.

Like in all ruminants, in veal calves, feeding values of SF components are dependent on their passage and degradation kinetics. These, however, have not been established for veal calves and may be affected by MR. In **Chapter 8**, the effects of SF level and R:C ratio on RD and passage kinetics of concentrate, straw, and MR were assessed. Thirty-two Holstein-Friesian calves were assigned to one of two SF levels at each of two R:C ratios. The recovery of indigestible markers for MR (CoEDTA; 4 h before slaughter), concentrate (hexatriacontane; 24 h before slaughter), and straw (Cr-NDF; 48 h before slaughter) was measured in four gastrointestinal compartments (rumen, abomasum, small and large intestine). RD averaged 20 ± 4.0% of the ingested MR and was unaffected by SF level and R:C ratio. Fractional rumen passage rate of straw increased from 1.3%/h to 1.7%/h with increasing SF level. Fractional rumen passage rate of concentrate increased from 3.3%/h to 4.9%/h with increasing SF level. The rumen contents also increased in response to increasing SF level. Abomasal damage and rumen development score increased with SF level, but were not affected by R:C ratio. It was concluded that SF level, but not R:C ratio, increases fresh and dry rumen contents and rumen fractional passage rates of SF components.

From Chapter 3 and 4, it appeared that the nitrogen economy of calves could be improved by combining feeding of a low-protein SF with a high-protein MR. It remained, however, unclear whether urea recycling was capable to completely compensate for a protein deficiency in the rumen. Therefore, in **Chapter 9**, the effect of the route administration of dietary protein (high-protein SF, low-protein MR vs. low-protein SF, high-protein MR, the latter at two levels of SF intake) on urea recycling and N retention was studied. Increasing low-protein SF intake at the expense of protein intake from MR did not affect N retention efficiency. The high-protein SF, low-protein MR combination maximized N retention and N efficiency, and coincided with improved fibre degradation. Therefore, these results

suggest that low N availability in the rumen limits microbial growth in calves fed low-protein SF (93 g crude protein/kg DM), and this effect cannot be compensated for by recycling of urea originating from MR.

The studies reported in this thesis are among the first ones extensively dealing with the nutritional and health consequences of combining SF and MR in feeding strategies for veal calves. They highlight how combinations of ration components differentially affect energy and nitrogen metabolism, methane emissions, ruminal passage rates, ruminal drinking, rumen development and (abomasal) health. These concepts provide a solid basis for the development of growth simulation models. For the development of novel feeding strategies, the impact on animal welfare, farm economy, and environmental impact should be considered.

Samenvatting

De voeding van witvleeskalveren bestaat voornamelijk uit melkvervangers (MV). Daarnaast dient in het rantsoen enig vezelhoudend droogvoer (DV), bestaande uit kracht- en ruwvoer, te worden opgenomen. Ten behoeve van dierwelzijn is het gewenst om het aandeel DV in het rantsoen te vergroten. Dit kan ook vanuit economisch oogpunt interessant zijn, afhankelijk van prijzen van grondstoffen voor MV en DV.
Het doel van dit proefschrift was een wetenschappelijke onderbouwing te leveren voor voerstrategieën waarin MV en DV gecombineerd worden. Effecten van combinaties van DV en MV op de voederwaarde, de groei en de gezondheid van kalveren werden onderzocht in groot- en kleinschalige dierproeven. Hierbij lag de nadruk op het bestuderen van interacties tussen MV en DV op het niveau van vertering en na absorptie, alsmede op de leeftijdsafhankelijkheid van deze interacties, die voor een betrouwbare inschatting van de groeiprestatie van kalveren van belang zijn. Daarnaast is bijzondere aandacht besteed aan gezondheidsaspecten die bij de opfok van vleeskalveren van belang zijn zoals pensontwikkeling, pensdrinken en het voorkomen van lebmaagzweren.
In **Hoofdstuk 2** wordt onderzoek naar het effect van DV en MV op de groei en pensontwikkeling van vleeskalveren beschreven. In een groep van 106 stierkalveren werd in de eerste 12 weken van de mestperiode MV gedeeltelijk vervangen door DV. Na deze 12 weken kregen de kalveren eenzelfde combinatie van DV en MV. Kalveren met een hoog aandeel DV in het rantsoen tijdens de eerste helft van de mestperiode hadden na 25 weken hetzelfde karkasgewicht als kalveren met een hoog aandeel MV, terwijl ze over het hele groeitraject beduidend meer DV, en minder MV hadden opgenomen. De kalveren met een hoog aandeel DV hadden na 12 weken een beter ontwikkelde pens en hadden ook minder grote littekens in de lebmaag. Hieruit werd geconcludeerd dat een vroege pensontwikkeling als gevolg van een hoger aandeel DV in de voeding gunstig is voor de voederwaarde van DV in een later stadium en voor de lebmaaggezondheid van het kalf.
Hoofdstuk 3 en 4 beschrijven studies waar het effect van een relatief eiwitarm DV, bestaande uit krachtvoer, maïs en stro, op de eiwit- en energiestofwisseling van vleeskalveren werd gekwantificeerd. Alle kalveren kregen dezelfde hoeveelheid MV maar de hoeveelheid DV verschilde per behandeling (4 DV niveaus met n = 12 per niveau). De verteerde energie die met extra DV werd opgenomen, werd met een efficiëntie van 41% omgezet in groei bij een lichaamsgewicht (LG) van 108 kg. Bij een LG van 164 kg was deze efficiëntie 54%. Een groter aandeel DV in het rantsoen ging gepaard met een toename van de schijnbare verteerbaarheid van de vezelfractie van DV en een toename van de methaanuitstoot. Gemiddeld ging 5.5% van de bruto energieopname uit DV verloren als methaan. Deze waarde komt overeen met data van herkauwers die alleen DV opnemen.
Opvallend was dat een hogere DV-opname gepaard ging met een grote toename van de efficiëntie waarmee stikstof uit het rantsoen wordt omgezet in lichaamseiwit. De bruto

efficiëntie van stikstof aanzet steeg met DV opname; 6.7 en 12.3% / kg DV per dag op 108 en 164 kg LG. Meer DV in het rantsoen leidde tot een meer dan evenredige toename van de N-benutting voor eiwitaanzet. Het bleek dat stikstof in de vorm van ureum, afkomstig van MV eiwit, werd hergebruikt. Uit metingen met gelabeld ($^{15}N^{15}N$) ureum bleek dat ureumrecycling voor ongeveer 20% verantwoordelijk was voor de betere benutting van stikstof bij een hogere DV-opname. Deze conclusie werd ondersteund door analyse van expressie van ureumtransporters in de penswand. Het bleek dat de genexpressie van ureumtransporter-B en aquaporine-3 in de penswand met toenemende DV-opname hoger is. Deze transporters spelen waarschijnlijk een rol bij ureumrecycling.

In **Hoofdstuk 5** worden de resultaten gepresenteerd van onderzoek naar lange termijn voorkeur voor MV en individuele DV-componenten en het effect daarvan op de gezondheid en groei van vleeskalveren. Vanaf een leeftijd van 2 weken tot 6 maanden kregen 23 stierkalveren vrije keuze en onbeperkte toegang tot MV, water, hooi, gerstestro, krachtvoer en maïs. De onbeperkte vrije keuze uit voercomponenten resulteerde in een grote individuele variatie, een hoge nutriëntopname en een hoge gemiddelde groei. Op 3 maanden leeftijd was de gemiddelde vrije opname in bruto energie 37 MJ per dag met een bijdrage van MV, krachtvoer en ruwvoer van respectievelijk 52, 27 en 21%. Op 6 maanden leeftijd steeg de gemiddelde vrije opname naar 84 MJ per dag met een bijdrage van MV, krachtvoer en ruwvoer van respectievelijk 31, 46 en 23%. Ondanks de grote individuele variatie in de samenstelling van de gekozen voercomponenten, was de verhouding van opgenomen verteerbare energie ten opzichte van verteerbare eiwitopname (Hoofdstuk 10) vrijwel constant. De fysieke structuur, het aandeel krachtvoer en het aandeel MV waren niet van invloed op de vrijwillige opname. In Hoofdstuk 5 worden ook verschillende relaties gelegd tussen de gekozen voersamenstelling en gezondheidsparameters. In een afzonderlijk experiment met 50 kalveren werd vrije selectie uit afzonderlijke DV-componenten vergeleken met het een vrij aanbod van een mengsel van DV-componenten. Het aanbieden van afzonderlijke DV-componenten leidde tot een iets hogere voeropname maar dat had geen effect op de groei van de kalveren (Hoofdstuk 10).

Hoofdstuk 6 beschrijft onderzoek naar technieken om (subklinisch) pensdrinken te kwantificeren. Uit literatuurgegevens en het onderzoek gerapporteerd in Hoofdstuk 2 bleek dat door het zogenoemde pensdrinken bij vleeskalveren een substantieel deel van de MV in de pens terechtkomt. Fermentatie van MV kan leiden tot gasvorming in de pens (oplopen), verminderde post-absorptieve beschikbaarheid van nutriënten en daardoor verminderde groei. Daarnaast kan het ook een gezondheidsprobleem vormen. Kwantificeren van pensdrinken is mogelijk door kalveren kort na inname van MV en een markeerstof te slachten en de hoeveelheid van de markeerstof in de pens te meten. Hoofdstuk 6 beschrijft een studie waar gezocht werd naar een alternatieve methode om pensdrinken op een minder invasieve en herhaalbare manier te kwantificeren. In drie opeenvolgende experimenten zijn enkele alternatieve methoden geëvalueerd. Geconcludeerd wordt dat de kinetiek van plasma acetominofen en de recovery van monosacchariden in

urine, beide toegediend als markeerstof in de MV, een indicatie geven van de mate van pensdrinken. Beide metingen bleken echter onvoldoende nauwkeurig voor een kwantitatieve evaluatie. Het meten van veranderingen in het volume van de lebmaag door middel van echografie direct na de inname van MV is qua nauwkeurigheid vergelijkbaar met het meten van een markeerstof in de pens na slachten. Naast bovengenoemde metingen is ook een tracermethode met ^{13}C- en $^{15}N_2$-ureum getest (beschreven in Hoofdstuk 10). Hoewel de rationale van deze methode veelbelovend is, is verdere verfijning en validatie nodig om de toepasbaarheid ervan goed te kunnen beoordelen.

Op basis van een meta-analyse met data van 328 kalveren uit 7 experimenten (Hoofdstuk 10) bleek dat gemiddeld 17% van de MV in de pens van vleeskalveren terecht komt. In alle kalveren werd pensdrinken gemeten na de slacht. Er werden geen correlaties gevonden tussen pensdrinken en DV- en MV-gerelateerde variabelen of groei, wat betekent dat pensdrinken niet afhankelijk is van hoeveelheid MV en DV of de samenstelling van DV. Nader onderzoek naar de grote variatie in pensdrinken tussen kalveren en de variatie binnen in individuele kalveren (geen data beschikbaar) kan naar verwachting inzicht geven in factoren die pensdrinken beïnvloeden (bijvoorbeeld leeftijd, stress, wijze van toedienen van MV, MV samenstelling).

In **Hoofdstuk 7** wordt onderzoek beschreven naar het effect van verschillende combinaties van MV en DV op de groei van vleeskalveren. Inzicht in dit effect is van belang omdat interacties tussen DV en MV kunnen optreden waarmee bij het bepalen van voer-strategieën rekening gehouden moet worden. Gedurende een periode van 16 weken zijn 8 groepen van elk 20 kalveren gevoerd met één van vier vooraf vastgestelde niveaus van DV en één van twee ruwvoer:krachtvoer-verhoudingen (RK-verhouding, 50:50 vs. 20:80). Door aanpassing van de MV-opname gedurende het experiment werd gestreefd de karkasgroei voor alle 8 proefgroepen gelijk te houden. Uit de resultaten blijkt dat kalveren voor eenzelfde karkasgroei bij een RK-verhouding van 20:80 gemiddeld 10% minder MV nodig hadden dan bij een RK-verhouding van 50:50. Dit betekent dat de benutting van DV, ofwel de voederwaarde van DV, hoger is bij een groter aandeel krachtvoer in het DV. Gemiddeld over de periode van 16 weken was de voederwaarde van MV in verhouding tot de voederwaarde van DV vergelijkbaar met de uitwisseling van verteerbare energie van beide componenten. De voederwaarde van DV werd echter groter met de (leef)tijd, en daarom kan additiviteit niet worden aangenomen.

Hoofdstuk 8 is een verslag van onderzoek naar de effecten van de hoeveelheid DV en de RK-verhouding op pensdrinken en de passage van MV en DV-componenten door het maagdarmkanaal in kalveren. In herkauwers is de voederwaarde van DV-componenten afhankelijk van de afbraak- en passagekinetiek in de pens. Deze processen zijn in vleeskalveren niet eerder gekwantificeerd en kunnen worden beïnvloed door de hoeveelheid DV en MV, en de samenstelling van het DV. In totaal werden 32 kalveren toegewezen aan één van twee RK-verhoudingen en één van twee DV-niveaus. Onverteerbare markeerstoffen werden

toegevoegd aan MV (CoEDTA; 4 u voor slacht), krachtvoer (hexatriacontaan; 24 u voor slacht) en stro (Cr-NDF; 48 u voor slacht) en recovery daarvan werd bepaald in de pens, lebmaag, dunne en dikke darm. Gemiddeld kwam 20 ± 4.0% van de opgenomen MV in de pens terecht. Dit was onafhankelijk van DV-niveau of RK-verhouding. De fractionele passagesnelheid van stro uit de pens was 1.3%/uur bij het lage en 1.7%/uur bij het hoge DV-niveau. De fractionele passagesnelheid van krachtvoer was 3.3%/uur bij het lage en 4.9%/uur bij het hoge DV-niveau. De hogere DV-opname resulteerde ook in een grotere pensinhoud en betere pensontwikkeling, maar ook in meer lebmaagschade. Op grond van de resultaten wordt geconcludeerd dat een hogere DV-opname, ongeacht RK-verhouding, leidt tot meer pensinhoud en een hogere fractionele passage van DV-componenten.

In **Hoofdstuk 9** is het effect van de route van eiwitopname (via DV of MV) op de eiwitstofwisseling en vertering in kalveren onderzocht. Op basis van Hoofdstuk 3 en 4 werd geconcludeerd dat stikstof uit MV beter wordt benut bij een voerstrategie met eiwitarm DV. Dat effect kon deels worden verklaard door ureumrecycling, maar het was niet duidelijk of hierdoor een eiwittekort in de pens volledig kan worden gecompenseerd. Om dit te onderzoeken werden kalveren toegewezen aan i) een eiwitrijk DV + een eiwitarm MV (n=10), ii) een eiwitarm DV + een eiwitrijk MV (n=10), beiden bij een hoge DV-opname, of iii) een eiwitarm DV + een eiwitrijk MV bij een lage DV-opname (n=10). De totale N-opname was hetzelfde voor alle behandelingen. Het verhogen van de eiwitarme DV-opname ten opzichte van eiwitopname via MV had geen effect op de efficiëntie van N-aanzet. De combinatie van een eiwitrijk DV met een eiwitarme MV leidde tot de hoogste N-aanzet en efficiëntie in kalveren, en ging bovendien gepaard met een verhoogde vezelafbraak in de pens. Hieruit werd geconcludeerd dat een laag-eiwit DV microbiële groei in de pens kan beperken, omdat de lagere N-beschikbaarheid niet kon worden gecompenseerd door een hogere ureumrecycling.

De studies die in dit proefschrift staan beschreven, zijn voornamelijk gericht op de nutritionele en gezondheidsaspecten van het combineren van MV en DV in voerstrategieën voor vleeskalveren. De nadruk ligt hierbij op energie- en eiwitmetabolisme, methaan emissies, passage kinetiek, pensdrinken, pensontwikkeling en (lebmaag)gezondheid. De ontwikkelde concepten bieden een solide basis voor de ontwikkeling van groeisimulatie modellen. Voor een verdere ontwikkeling van voerstrategieën, zullen ook aspecten op het gebied van dierwelzijn, bedrijfseconomie en milieu-impact moeten worden geëvalueerd.

Curriculum Vitae

Harma Berends was born on July 6, 1986 and grew up in Einighausen, the Netherlands. She finished secondary school (Gymnasium Trevianum, Sittard, the Netherlands) in 2004 whereafter she studied at Wageningen University and obtained her BSc degree in Animal Sciences in 2007. During her MSc in Animal Sciences, Harma followed the specialization in Animal Nutrition. Her major thesis was concerned with the measurement of methane emissions from dairy cows and resulted in two scientific publications. During her MSc internship, Harma worked at DSM Nutritional Products (Basel, Switzerland) on the development of new business models for feed additives. In 2009, she graduated cum laude for her MSc degree in Animal Sciences at Wageningen University. During the last four years, Harma was a PhD scholar at the Animal Nutrition group of Wageningen University resulting in this thesis. In 2011, Harma was Board member of the graduate school Wageningen Institute of Animal Science, and chair of its associated Student Council. From 2011 onwards, Harma took up a position in the Supervisory Board of Idealis, a housing corporation in Wageningen. In 2012, she was team captain of the Wageningen UR team joining the Big Challenge - Alpe d'HuZes (fund raising for cancer research by cycling Alpe d'Huez, France). In March 2013, Harma joined the Ruminant Research Centre of Nutreco.

Peer reviewed scientific publications

Berends, H., van Reenen, C.G., Stockhofe-Zurwieden, N., Gerrits, W.J.J. (2012) Effects of early rumen development and solid feed composition on growth performance and abomasal health in veal calves. Journal of Dairy Science 95: 3190-3199.

Berends, H., van den Borne, J. J. G. C., Alferink, S. J. J., van Reenen, C.G., Bokkers, E.A.M., Gerrits, W.J.J. (2012) Low-protein solid feed improves the utilization of milk replacer for protein gain in veal calves. Journal of Dairy Science 95: 6654-6664.

Berends, H., van den Borne, J.J.G.C., Røjen, B.A., van Baal, J., Gerrits, W.J.J. (2014) Urea recycling contributes to nitrogen retention in calves fed milk replacer and low-protein solid feed. Accepted for publication in Journal of Nutrition.

Labussière, E., Berends, H., Gilbert, M.S., van den Borne, J.J.G.C., Gerrits, W.J.J. (2014) Estimation of milk leakage into the rumen of milk-fed calves through an indirect and repeatable method. Accepted for publication in Animal.

Berends, H., Gerrits, W.J.J., Webb, L.E., Bokkers, E.A.M., Gilbert, M. S., Engel, B., van Reenen, C.G. Effects of free diet selection on dietary preferences, health, and performance of Holstein-Friesian calves. To be submitted.

Berends, H., van den Borne, J.J.G.C., Mollenhorst, H., van Reenen, C.G., Bokkers, E.A.M., Gerrits, W.J.J. The utilization of roughages and concentrates relative to that of milk replacer increases strongly with age in veal calves. Accepted for publicaion in Journal of Dairy Science.

Berends, H., van den Borne, J.J.G.C., Røjen, B.A., Hendriks, W.H., Gerrits, W.J.J. Effect of route of administration of protein on N metabolism in veal calves fed milk replacer and solid feeds. Submitted to Journal of Dairy Science.

Berends, H., van den Borne, J.J.G.C., Stockhofe-Zurwieden, N., Gilbert, M.S., Zandstra, T. , Pellikaan, W.F., van Reenen, C.G., Bokkers, E.A.M., Gerrits, W.J.J. Effects of solid feed level and roughage-to-concentrate ratio on ruminal drinking and passage kinetics of concentrate, straw, and milk replacer in veal calves. To be submitted.

Dijkstra, J., van Zijderveld, S.M., Apajalahtic, J.A., Bannink, A., Gerrits, W.J.J., Newbold, J.R., Perdok, H.B., Berends, H. (2011) Relationships between methane production and milk fatty acid profiles in dairy cattle. Animal Feed Science and Technology 166-167: 590-595.

Berends, H., Gerrits, W.J.J., France, J., Ellis, J.L., van Zijderveld, S.M., Dijkstra, J. 2014. Evaluation of the SF_6 tracer technique for estimating methane emission rates with reference to dairy cows using a mechanistic model. Journal of Theoretical Biology 353: 1-8.

Webb, L.E., Bokkers, E.A.M., Engel, B., Gerrits, W.J.J., Berends, H., van Reenen, C.G. (2012) Behaviour and welfare of veal calves fed different amounts of solid feed supplemented to a milk replacer ration adjusted for similar growth. Applied Animal Behaviour Science 136: 108-116.

Conference and symposia proceedings

Berends, H., van den Borne, J.J.G.C., Røjen B.A., van Baal, J., Gerrits, W.J.J. (2013) Low protein solid feed enhances nitrogen utilization by urea-N recycling in veal calves. In: Proceedings of the 4th International Symposium on Energy and Protein Metabolism and Nutrition, 9-12 September 2013, Sacramento, California, USA.

Berends, H., van den Borne, J.J.G.C., Gerrits, W.J.J. (2013) Quantifiying subclinical ruminal drinking using a [^{13}C]-[$^{15}N_2$]-urea based method in veal calves In: Proceedings of the 4th International Symposium on Energy and Protein Metabolism and Nutrition, 9-12 September 2013, Sacramento, California, USA.

Bokkers, E.A.M., Webb, L.E., Engel, B., Berends, H., Gerrits, W.J.J., van Reenen, C.G. (2012) How does solid feed provision and display of abnormal oral behaviours affect salivary cortisol in veal calves? In: Book of Abstracts of the 46th Congress of the International Society for Applied Ethology, 31 July - 4 August 2012, Vienna, Austria.

Webb, L.E., van Reenen, C.G., Engel, B., Berends, H., Gerrits, W.J.J., de Boer, I.J.M., Bokkers, E.A.M. (2012) What do calves choose to eat and how do preferences affect calf behaviour and welfare? In: Book of Abstracts of the 46th Congress of the International Society for Applied Ethology, 31 July - 4 August 2012, Vienna, Austria.

Berends, H., van den Borne, J.J.G.C., van Reenen, C.G., Gerrits, W.J.J. (2011) Veal calves deposit nitrogen from solid feed as efficient as nitrogen from milk replacer In: Journal of Animal Science 89: 383 - 383, 10 – 14 July 2011, New Orleans, Louisiana, USA.

Berends, H., van den Borne, J. J. G. C., van Reenen, C.G., Gerrits, W.J.J. (2011) Contribution of solid feed intake to energy and protein metabolism in veal calves. In: Proceedings of 5th International veal conference, 19 – 20 May 2011, Noordwijk, the Netherlands.

Training and Supervision Plan[1]

Basic package (3 ECTS[2])	
Introduction Course WIAS, Wageningen, the Netherlands	2009
Course on Philosophy of Science, NWO, Lunteren, the Netherlands	2011
International conferences (3 ECTS)	
International Symposium on Energy and Protein Metabolism and Nutrition, Sacramento, USA	2013
5th International Veal Congress, Noordwijk, the Netherlands	2011
Annual meeting of the American Society of Animal Science, New Orleans, USA	2011
Seminars and workshops (3 ECTS)	
"How to feed the world?", Wageningen, the Netherlands	2011
"Dietary lysine and the importance of processing of food- and feedstuffs", Wageningen, the Netherlands	2010
"Developments in Phosphorus Nutrition in Pigs and Poultry", Wageningen, the Netherlands	2012
Annual WIAS Science Day, Wageningen, the Netherlands	2010-2012
"Waardering van Dierenwelzijn", NWO, Den Haag, the Netherlands	2013
"Nutritional Management in Early Lactation", Wageningen, the Netherlands	2012
"Kiezen voor Dieren", GGL congres KNMvD, Arnhem, the Netherlands	2011
"Developments in Ruminant Nutrition", Wageningen, the Netherlands	2013
Presentations (9 ECTS)	
"Early rumen development promotes growth performance in veal calves", WIAS Science Day, Wageningen, the Netherlands, poster presentation	2011
"Solid feed utilization in veal calves", WIAS Science Day, Wageningen, the Netherlands, oral presentation	2012
"Contribution of solid feed intake to energy and protein metabolism in veal calves", 5th International Veal Congress, Noordwijk, the Netherlands, oral presentation	2011
"Veal calves deposit nitrogen from solid feed as efficient as nitrogen from milk replacer", ASAS annual meeting, New Orleans, USA, poster and oral presentation	2011

1 Completed in the fulfilment of the requirements for the education certificate of the Graduate School WIAS (Wageningen Institute of Animal Science)

2 One ECTS equals a study load of 28 hours

"Quantifiying subclinical ruminal drinking using a [^{13}C]-[$^{15}N_2$]-urea based method in veal calves", International Symposium on Energy and Protein Metabolism and Nutrition, Sacramento, USA, oral presentation	2013
"Low protein solid feed enhances nitrogen utilization by urea-N recycling in veal calves", International Symposium on Energy and Protein Metabolism and Nutrition, Sacramento, USA, oral presentation	2013
"Towards a sustainable feed concept for veal calves", 95th Dies Natalis Wageningen University, Wageningen, the Netherlands, oral presentation	2013
"Betere prestaties door ruwvoergift aan vleeskalveren", GGL congres KNMvD, Arnhem, the Netherlands, oral presentation	2011
Disciplinary and Interdisciplinary Courses (10 ECTS)	
Indirect Calorimetry and Selected Applications, University of California, Davis, USA	2013
Ruminant Nutrition, WBS, Wageningen, the Netherlands	2010
Governance and Policy Advise, NWO, Den Haag, the Netherlands	2013
Sustainable Animal Production, NWO, Lunteren, the Netherlands	2010
Animal Behavior and Society Behavior, NWO, Warder-Zeevang, the Netherlands	2011
Advanced Statistics of Experimental Design, WIAS, Wageningen, the Netherlands	2010
Mixed Model Workshop, ADSA, New Orleans, USA	2011
Laboratory Skills, ANU, Wageningen, the Netherlands	2009
Use of Laboratory Animals, WUR, Wageningen, the Netherlands	2010
Professional Skills Support (3 ECTS)	
Techniques for writing and presenting a scientific paper, Wageningen, the Netherlands	2011
Supervising MSc thesis work, Wageningen, the Netherlands	2011
Working with EndNote X, Wageningen, the Netherlands	2008
Media training & writing, NWO Talent day, Utrecht, the Netherlands	2010
Social media & leadership, NWO Talent day, Utrecht, the Netherlands	2011
ExPectationS Career Day, Wageningen, the Netherlands	2010
Research Skills Training (1 ECTS)	
Isotopomer analyses at Aarhus University, Foulum, Denmark	2012
Didactic Skills Training (15 ECTS)	
WBS course Feed Evaluation Science, guest lecture	2011
Supervising Rumen fermentation practical (course Animal Nutrition and Physiology, WUR)	2010-2011

Supervising Energy metabolism computer simulation practical (course Animal Nutrition and Physiology, WUR)	2011-2013
Supervising Introduction to Animal Science, course project	2010
Supervising Research Master Cluster	2012
Supervising MSc theses (5x)	2010-2014
Supervising BSc theses and internships (4x)	2010-2014
Management Skills Training (10 ECTS)	
Secretary WAPS Council	2010
Chair WAPS Council	2011
Education Committee member	2010-2011
WIAS Board member	2011

TOTAL: 57 ECTS

ACKNOWLEDGEMENTS

All of the support and dedication of everyone involved in the work described in this thesis is gratefully acknowledged. Prof. Dr Wouter Hendriks, promotor, thank you for creating an environment in which I have been able to independently work, think, learn, and explore. I wish to express my utmost gratitude towards my co-promotors, Dr Walter Gerrits and Dr Joost van den Borne. Walter, I thank you for both challenging and supporting me. I value your honesty, dedication, humor, and constructive feedback. I am grateful for the time and effort you have put into this project. Joost, I appreciate your ability of combining quantity and quality of work. I thank you for always being there and helping me develop these skills. Dr Kees van Reenen, I appreciate your ongoing enthusiasm, your statistical wisdom, and the support within the project team. I express my gratitude towards the multidisciplinary project team: Dr Walter Gerrits, Dr Kees van Reenen, Dr Eddie Bokkers, Laura Webb, Dr Erwin Mollenhorst, Dr Norbert Stockhofe, Prof. Dr Imke de Boer, and Dr Paul Berentsen. Working in this team has been a tremendous learning experience for me. The output and impact of our project are great.

The Division for Earth and Life Sciences of the Netherlands Organization for Scientific Research (NWO, The Hague, Netherlands) is gratefully acknowledged for financially supporting this research and taking care of the associated educational program, with special thanks to Prof. Dr Tjard de Cock Buning and Dr Kirsten de Bruijn. The Product Board Animal Feed (The Hague, Netherlands) is gratefully acknowledged for the financial support. Special gratitude goes to Wiebe Mulder (VanDrie Group), Arjan Gobius du Sart and Peter Mölder (Denkavit) and Steffen Rouwers (Sloten), and during the initial phase, Dr Machiel Blok (CVB), Jacques de Groot (VanDrie Group), Johan Lemmen (Nutrifeed), and John Achterstraat (Alpuro), for the constructive feedback during the conduct of this research.

There would be no results without trials, laboratory, and statistical analyses. I have to confess that there is not a person in the ANU laboratory has *not* been bothered with my samples. Thank you Saskia van Laar, Léon de Jonge, Xuan Huong van der Schans-Lé, Erika Beukers-van Laar, Michel Breuer, Jane-Martine Muijlaert, Joswita van der Velde, and Adriaan Wissink. Ták Dr Betina Røjen, Birgit Løth, and Anne Krustrup, for having me in Foulum. I experienced valuable cooperation and collaboration which resulted in two chapters of this thesis. Dr Etienne Labussière, merci for being with us, showing dedication and enthusiasm. We set up great trials exploring novel techniques while French solids and liquids really contributed to our dedication and creativity. Sven Alferink, Tamme Zandstra, Joswita van der Velde, and Marcel Heetkamp, thank you for the technical support during trials and respiration chamber measurements. Bert Beukers, Ries Verkerk, André Jansen, Tonnie van Omme, Willem van Ommeren, and Leen Bijl (Carus experimental facilities, Wageningen University), thank you for the dedicated care for the calves during experiments. The largest experiment, a.k.a. 'all-in-one', was conducted at VanDrie Group in Scherpenzeel.

This huge effort resulted in a valuable outcome. I like to thank Klaas Boeder, Bart Evers, and Ewart van Voorst for their great help. In addition to the scientific value, I enjoyed the working atmosphere. Thanks guys! Thanks as well to Ralph Kok and Pieter Roskam (CVI, Lelystad, the Netherlands) for sharing pathological expertise. Dr Astrid de Greeff and Dr Norbert Stockhofe (CVI, Lelystad, the Netherlands) shared their expertise on pathology and physiology. I hope we manage to publish our findings in the near future. To Dr Jurgen van Baal and Leo Kruijt, thank you for introducing me to the world of nutrigenomics. There is still much to be learned, and calves may be the best ruminant models! Dr Bas Engel and Willem Buist, thanks for the support on statistics. Dian Harjanti, thanks for your contribution and work on gene expression during rumen development. Mehdi Mirzaee, thank you for the pleasant cooperation during your stay in Wageningen. Myrthe S. Gilbert, thank you for your support and help. Dian, Mehdi, and Myrthe, lots of success with the completion of your PhD!

I am very grateful to all students involved for their enthusiastic and dedicated help, whether it was digging into literature, performing laboratory analyses or getting up early and rolling up their sleeves to mix feed or to collect manure or urine samples. My thanks to Janneke Snijders, Maike Pegge, Sabrina Podesta, Laurie Poussier, Teun Keizer, Carène Bulte, Pierre-Alain Renaud, Marijke Schop, Laure Hygonnet, Marit Cornelissen, Anne Raats, and Kitty Elsinghorst.

I will always remember the nice atmosphere at ANU, sense and nonsense discussions during coffee breaks and other events, and especially the international flavor to it. Thank you, PhD colleagues: Lotte, Myrthe, Sonja, Mubarak, Bayissa, Attje-Rieke, Wouter, Daniel, Kasper, Henk, Geronda, Sanne, Sandra, Tetske, Mascha, Antoon, Judith, Yvonne, Omid, Shafqat, Sabrina, Esther, Sergio, Hsuan, Hoa Ly, Huyen, Genet, Jacqueline, Christiaan, Nazir, Melkamu, Syafwan, Felicidade, Pierre, Sandra, Francine, Carol, Anton, Anne, Patricia. A special note of appreciation goes to Sonja, Lotte, and Myrthe: thank you for the day-to-day distraction (keywords: euro-bets, planking, donkeys, conferences, pasta, style and class) and friendship! Also the ANU staff members and Prof. Dr Martin and Mariet Verstegen are acknowledged for their collegiality. Betty Looijen and Yvonne van Holland, thank you for your support. Dr Wilbert Pellikaan and Dr Jan Dijkstra, thank you for exchanging thoughts on fully ruminants; veal calves are getting closer! Tamme and Sonja, great that the two of you are my paranymphs!

One of the highlights of my PhD was the Big Challenge Alpe d'HuZes; cycling Alpe d'Huez (France) to raise funding for cancer research. Team WUR: Johan van Arendonk, Piter Bijma, Henk Bovenhuis, Christiaan Kapper, Jennifer Ellis (St-Pierre), Daniel Warner, Wilbert Pellikaan, Lotte van Rooijen, Marit van Arendonk and Jan van Geel; thanks for the great time we had together. Let's keep on rolling!

Dr Javier Martín-Tereso and Prof. Dr Leo den Hartog, thank you for providing the opportunity to join Nutreco while finishing my PhD. I look forward to our further cooperation.

To my dear family, my close friends, and my biggest-friend Julius, thank you for being there. Writing this booklet is just doing my job, what really matters in life is you.

Harma

LIST OF ABBREVIATIONS

ADG	average daily gain
AMV	abomasal milk volume
AUC	area under the curve
bUTB	bovine urea transporter-B
BW	bodyweight
CF	crude fat
CONC	inclusion of concentrate in the solid feed mixture
CP	crude protein
DCP	digestible crude protein
DE	digestible energy
DM	dry matter
DMI	dry matter intake
ERD	early rumen development
FCR	feed conversion ratio
GE	gross energy
GER	gastrointestinal entry rate
GLM	generalized linear model
HP	heat production
HPHS	high protein high solid feed level
HSF	high solid feed level
LPHS	low protein high solid feed level
LPLS	low protein low solid feed level
LSF	low solid feed level
ME	metabolizable energy
MR	milk replacer
MSC	mixture of maize silage, straw, and concentrate
N	nitrogen
NDF	neutral detergent fiber
NS	not significant
PCA	principal component analysis
PC	principal component
PCR	principal component regression
R:C	roughage-to-concentrate (ratio)
RD	ruminal drinking
RMV	ruminal milk volume
RMSL	ratio of mucosa length to serosa length
ROC	return to the ornithine cycle
RSD	residual standard deviation
SE	standard error
SEM	standard error of the mean
SF	solid feed
TMR	total mixed ration
UER	urea production
UFE	urea-N to fecal excretion
UUA	urea-N utilized for anabolism
UUE	urea-N to urinary excretion
VFA	volatile fatty acids

COLOPHON

Design & Lay-out	Promotie In Zicht
Printing	Wöhrmann Print Service
Support research	Product Board Animal Feedstuffs
	Netherlands Organization for Scientific Research
Support printing	VanDrie Group
	Sloten - a Nutreco company
	Denkavit
	Verveka Veevoeder
	Klaremelk