Universitat Autònoma de Barcelona

Effect of different fiber sources on the digestive function and development of calves

MEMÒRIA PRESENTADA PER LLORENÇ CASTELLS DOMINGO
DIRIGIDA PER MARTA TERRÉ TRULLÀ
TUTORITZADA PER ALFRED FERRET QUESADA

PER ACCEDIR AL GRAU DE DOCTOR DINS EL PROGRAMA DE DOCTORAT DE PRODUCCIÓ ANIMAL DEL DEPARTAMENT DE CIÈNCIA ANIMAL I DELS ALIMENTS

BELLATERRA, MAIG 2013

FACULTAT DE VETERINÀRIA DE BARCELONA

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Certifiquen:

Que la memòria titulada **"Effect of different fiber sources on the digestive function and development of calves"** presentada per **Llorenç Castells Domingo** per optar al grau de Doctor per la Universitat Autònoma de Barcelona, ha estat realitzada sota la direcció de la Dra. Marta Terré Trullà i, considerant-la acabada, autoritza la seva presentació per què sigui jutjada per la comissió corresponent.

I per tal que consti els efectes que corresponen, signa la present a Caldes de Montbui, 8 d'Abril de 2013.

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Per analitzar l'efecte de la inclusió de fonts de fibra en la dieta dels vedells abans del deslletament sobre el rendiment productiu, comportament, i el desenvolupament i funció del tracte gastrointestinal es van realitzar cinc estudis. En el primer dels estudi es va realitzar una sèrie de proves per tal de trobar les fonts de fibra que poguessin afavorir el creixement i desenvolupament dels vedells. Com a resultat d'aquest estudi, es va trobar que els vedells que rebien un farratge de gramínies, milloraven els paràmetres productius. Cal destacar la palla d'ordi, el fenc de civada i l'ensitjat de triticale. Contràriament, el fenc d'alfals no va millorar els paràmetres productius. De la mateixa manera, en un segon estudi, l'oferta de fonts fibroses no farratgeres tampoc va millorar el creixement i la ingestió de concentrat. Veien la disparitat de resultats entre gramínies i lleguminoses, es va triar el fenc picat de civada i el d'alfals per tal d'intentar dilucidar els mecanismes que poden explicar els diferents resultats. En aquest tercer experiment es va observar que els vedells alimentats amb farratge a la dieta presentaven un pH ruminal més elevat i una menor concentració d'àcids grassos volàtils que els vedells que no rebien farratge. A més, en els vedells alimentats amb fenc de civada es va poder observar un increment en el ritme de pas del marcador en el rumen, un menor temps de retenció del marcador en el tracte gastrointestinal i una major expressió de proteïnes transportadores d'àcids grassos volàtils del rumen en comparació amb els vedells sense farratge a la dieta. Aquests resultats ens informen sobre com el fenc de civada estimula el consum de pinso, i conseqüentment el creixement d'aquests animals. Per altra banda, l'ús de concentrats texturitzats s'ha postulat com una alternativa a l'ús de farratge en vedells lactants per tal d'estimular el correcte desenvolupament funcional del rumen. En els resultats obtinguts en el quart estudi, s'ha vist que l'ús de concentrats texturitzats sense farratge no millora els paràmetres productius i comporta un clar descens del pH ruminal. Finalment, es va estudiar l'ús de fenc de civada abans del deslletament en vedelles de reposició. Les vedelles alimentades amb fenc de civada van créixer més i van tenir una major ingestió de concentrat abans de deslletar. Al deslletament, totes les vedelles van rebre fenc de civada, i les diferències en els paràmetres productius van desaparèixer. Tot i el major pes al deslletament de les vedelles alimentades amb fenc de civada, als 10 mesos d'edat no hi havia diferències de pes entre tractaments, i tampoc es van trobar diferències en els paràmetres reproductius a la primera inseminació.

Five studies were conducted to evaluate the effects of the inclusion of different fiber sources on performance parameters, behavior, and the digestive function and the development of pre- and postweaned calves. First of all, a series of experiments were done to assess the fiber sources that better improve performance of young calves fed following a conventional milk feeding program. Performance improvements were generally observed when forage from chopped grasses were offered to young calves. Chopped oat hay, barley straw, and triticale silage were the best grass forage sources that improve calves performance. Conversely, alfalfa hay did not improve calves performance. Similarly, offering non-forage fiber sources in the diet of young calves did not successfully improve animal performance and concentrate intake. Due to the different results between grasses and legumes forages, alfalfa and oat hay were chosen to find out the mechanisms involved in the different results observed. In a third experiment, calves supplemented with forage in the diet showed an increase on ruminal pH and a decrease on rumen volatile fatty acids concentration compared with animals without forage in the diet. Moreover, oat hay fed animals showed an increase on ruminal marker passage rate, a decrease on marker's retention time in the gastrointestinal tract, and a greater expression of volatile fatty acids transporters. All these parameters explained how oat hay stimulate concentrate intake, and consequently performance results of young calves. Recently, offering texturized concentrate feed to preweaned calves has been suggested as other strategy to stimulate rumen development, concentrate intake and performance of preweaned calves. In the forth study presented herein, it was showed that calves fed only a texturized concentrate feed did not improve performance compared with calves fed texturized and pelleted concentrate feed with straw, and decreased ruminal pH, predisposing those animals to suffer ruminal acidosis. Finally, offering oat hay to young female calves during the preweaning period was studied. Calves with access to oat hay during the preweaning period had greater body weight gain and concentrate feed intake, as previously observed. After weaning, all heifers received forage in the diet, and differences on performance between oat hay-fed and non-forage fed calves disappeared. Despite the improvement on body weight at weaning of forage-fed calves, there were no differences on body weight at 10 months of age, and on age at first artificial insemination, age at fertile artificial insemination, and times bred.

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Chapter 1

LITERATURE REVIEW

Introduction

In commercial farms, newborn calves are usually fed limited amounts of milk or milk replacer (around 10% of birth BW). The aim of this feeding program is to encourage young calves to consume starter feed. The type of starter feed used in cattle farms is very similar among farms, being high in starch and low in fiber. Contrary, forage offer in commercial farms is an inconsistent practice. Quality, availability, and physical form of forages highly changes from farm to farm, mainly due to a lack of appropriate assessment. This problem arises from the high variety of scientific results that are available in the literature over the years.

The objective of this literature review is to have an overview of young calf development. Furthermore, the effect of the different type of feed (milk, starter, and forage) offered to calves on the development of those calves will be reviewed. Finally, attention will be paid to the different effects of forages on ruminant, such as feed consumption or differences between type of forages.

1.1. The Newborn Calf

The newborn calf is totally dependent on milk as are the young of the other mammalian species (Radostits and Bell, 1970). One of the most important feedings for the newborn calf is the colostrum. Colostrum is defined as the first secretion produced by the mammary gland of cows after calving. Colostrum is not only important because of its immune protection role, but it is also important as the first source of nutrients for the calf after birth. After colostrum feeding, the diet of young calves is based on milk or high quality milk replacers, because they have a high nutrient demand and there is a limited nature of the nutrients that the young calf can digest. The dependence on milk is basically due to the inability of the newborn calf to digest feed containing fibrous ingredients. Dependence on milk lasts until energy intake coming from dry feed consumption contributes at least to supply the amount of energy required for maintenance. At birth, calf's abomasum is well developed and highly functional (Short, 1964), allowing the digestion of milk. The early development of the abomasum is clearly observed comparing its weight to the total stomach weight. Newborn calf abomasum is the most developed and important compartment of the stomachs, representing around 51 % of total stomach weight (Lyford, 1988).

Furthermore, in newborn calves, forestomachs have a poor development compared with the abomasum. An example of the little development of forestomachs in the newborn is the reticulorumen that representes a 35 % of the total stomach weight compared with the 62 % in adult cattle (Lyford, 1988). Moreover, rumen in the newborn calf is small and flaccid with rudimentary papillae (Lyford, 1988). All these factors together do not allow the digestion of feed containing fibrous ingredients in the rumen in newborn calves. During the firsts weeks of age, the digestive system and metabolism of the calf is more similar to nonruminant animals than that of a mature ruminant (NRC, 2001).

1.2. Rumen development

During the firsts weeks of age, young calf undergoes extensive physiologic and metabolic changes (Toullec and Guilloteau, 1989). These changes are related to the different type of feeds that calves consume during this period of time. From birth until weaning, diet changes from liquid to solid feed. Therefore, in few weeks, calves change from a nonruminant digestion and metabolism, when its diet is based on milk or milk replacer, to a mature ruminant digestion and metabolism, when its diet is based on solid feed. During the first 2-3 wk of life calf's digestive system is immature, but developing rapidly with regard to digestive secretions and enzymatic activity (Toullec and Guilloteau, 1989; Davis and Drackley, 1998). To ensure a successful transition from nonruminant to a mature ruminant calf, it is mandatory to promote an adequate development of forestomachs, especially the rumen. Development of the rumen is the key factor in the transition to solid feed because the solid feed will be fermented in the rumen. For this reason, intake of solid feed should be encouraged at an early age to stimulate the development of a functional rumen.

1.2.1.Rumen development during the first weeks of age

As previously mentioned, rumen of the newborn calves is physically and metabolically underdeveloped (Lyford, 1988). As solid feed intake starts, rumen size increases, and at 4 wk of age, rumen can be 4-8 times bigger than at birth. However, rumen is not completely developed until rumen wall becomes thicker than at birth, and papillae grow in length as in mature animals. Usually at 8 weeks of age rumen approaches the adult proportions relatively to the other digestive organs and to body weight, and rumen papillae are well developed. Finally, in maturing ruminants, there is an increase in rumen capacity and a proliferation of the smooth musculature of the rumen.

1.2.2. Physical and metabolic factors that help to develop the rumen

Harrison et al. (1960) and Heinrichs (2005) pointed out that dietary factors are very important to develop the functional rumen. Growth and development of ruminal papillae is absolutely necessary to enable absorption and utilization of end products of microbial fermentation, as volatile fatty acids (VFA) (Warner et al., 1956; Sutton et al., 1963; Church, 1988; Van Soest, 1994). Moreover, the presence of VFA in the rumen is the triggering factor that develops a functional ruminal epithelial tissue, enabling the absorption of VFA (Flatt et al., 1958; Sander et al., 1959). Different researchers

(Brownlee, 1956; Warner et al., 1956; Flatt et al., 1958; Stobo et al., 1966; Nocek et al., 1984; Greenwood et al., 1997) have shown the importance of dry feed ingestion. Rumen microbiota ferment dry feed in the rumen, and several end products (i.e. mainly VFA, ammonia, lactate) are produced. These end products are needed to stimulate rumen epithelial development. The three most produced VFA during microbial fermentation are acetate, propionate, and butyrate. Even all of them are produced in the rumen, their potential effect stimulating rumen epithelial tissue development is different. Butyrate is the VFA that stimulates the most the differentiation of the ruminal epithelial tissue into its characteristic papillae, followed by propionate (Flatt et al., 1958; Sander et al., 1959; Harrison et al., 1960; Tamate et al., 1962; Sutton et al., 1963; Stobo et al., 1966). Pennington (1952) and Pennington and Pfander (1957) found that, compared with rumen tissue incubated whithout VFA, rumen tissue incubated with butyrate produced much more ketone bodies than incubations with acetate or propionate (20-57, 1-2 and -3 - -4 µmoles of ketone bodies, respectively), suggesting that butyrate was the most preferred VFA to be metabolized by rumen epithelial cells. On the other hand, acetate is less stimulant of epithelial development because of the low activity of the acetyl-CoA synthetase enzyme in rumen epithelium (Ash and Baird, 1973; Harmon et al., 1991).

Finally, to have a fully developed rumen, rumen volume and muscularization must be promoted. These two parameters are not stimulated by the presence of VFA in the rumen, but by the physical structure of the feed (Heinrichs, 2005). Stimulation of rumen motility and muscularization are governed by the same factors, particle size and effective fiber, in the neonatal ruminant as in the adult ruminant (Van Soest, 1994; Beauchemin and Rode, 1997). Actually, bulky material in the rumen, even if those materials are inert material such as sponges (Harrison et al., 1960), can help to develop rumen volume and muscularization. Harrison et al. (1960) found that calves fed only milk but with access to wood shavings (used as bedding material) had minimal development of ruminal papillae, but they had an extensive muscular growth in the rumen. Tamate et al. (1962) also found that the introduction of plastic sponges into the rumen promoted rumen capacity and muscular development.

1.2.3. How the diet affects the rumen development

1.2.3.1. Milk

It has been clearly demonstrated in the literature that diets of calves based only on milk, produce little development of rumen in both, ruminal volume and epithelial tissue. When calves drink milk, a contraction of a serial of muscles from the esophagus to the reticulo-omasal orifice forms a duct known as esophageal groove (Ørskov et al., 1970 i 1972). The formation of the esophageal groove conducts very efficiently the milk and other liquids directly to the abomasum, with little or no spillage into the reticulo-rumen. Without any kind of feed susceptible to be fermented reaching the rumen, it does not exist any stimulation to develop a functional rumen. Partially replacing milk replacer by solid feed to veal calves resulted in an increase of rumen weight, and an increase in epithelial and absorptive surface area (Berends et al., 2012). Similarly, Tamate et al. (1962) found that calves fed only milk had less developed ruminal papillae length compared with calves fed concentrate feed and hay (0.5 vs 2.5 mm, respectively).

1.2.3.2. Starter

Starter feed fermentation is possible thanks to rumen bacteria enzymatic activity. At birth rumen is completely sterilized, but it is rapidly colonized within the first 24 h of life. In fact, Rey et al. (2012) reported a change from a positive to a negative redox potential from 1 to 2 days of age. During the first days of age, rumen microbiota is mainly composed by facultative bacteria, but they start to consume oxygen and at 3 weeks of life, when solid feed intake starts, anaerobic, amylolytic and proteolytic bacteria are well established in the rumen, and they remain fairly constant until 12 weeks of life (Anderson et al., 1987). Similarly, Rey et al. (2012) concluded that rumen enzymatic activities were maximal between 10 and 23 days after birth. Contrary, protozoa establishment has more limitations. Protozoa establishment is highly dependent on the presence of other animals containing protozoa in their rumen, and the ruminal pH of calves (Yokoyama and Johnson, 1988). Protozoa are known to be particularly sensitive to low pH, requiring high rumen pH to be established (i. e. pH 6 or more to establish entodinia or holotrichs). Until third week of age, protozoa are low in number in the rumen, after which they begin to increase in number. Depending on the diet, adults levels of protozoa are reached between 5 and 9 weeks of age (Yokoyama

and Johnson, 1988). The main end products of concentrate fermentation are propionate and butyrate, which enhances rumen epithelium development (Heinrichs et al., 2005). However, as concentrate intake increases, more VFA are produced, and consequently rumen pH decreases, leading the young calf to suffer ruminal acidosis. Another disadvantage of increasing concentrate intake is the possibility of rumen parakeratosis. Parakeratosis occurs when epithelial squamous cells develop a hardened keratin layer due to a diet's inability to continuously remove degenerating epithelial cells (Hinders and Owen, 1965). Factors commonly associated with occurrences of parakeratosis are an increased of VFA production, decreased rumen buffering capacity, and subsequently decreased rumen pH (Anderson et al., 1982). These three factors usually appear when ruminants are fed a starter diet with small particle size and low abrasive value (Greenwood et al., 1997). Although rumen parakeratosis do not appeared in Beharka et al. (1998) study, offering an unground concentrate to preweaning calves increased papillae length compared with ground concentrates.

1.2.3.3. Forage

Contrary to starter feed, when forage is fed to ruminants, the most VFA produced during the ruminal microbial fermentation is acetate, which capacity to stimulate rumen epithelium development to its characteristic papillae is low. Feeding forage maintains a greater rumen pH compared with only feeding concentrate, and supports microbiota associate with forages (i.e. *Fibrobacter succinogenes*, *Ruminococcus spp*.) (Petri et al., 2012). Forage gives to the diet a greater particle size and effective fiber compared with ground starter feeds. As previously mentioned, these two conditions are the primary stimulators of rumen muscularization and to increase rumen volume (Zitnan et al., 1998). It is clearly demonstrated in young calves that large particle size, high effective fiber content, and increased bulk of forages or high fiber sources, increases physical rumen wall stimulation, subsequently increasing rumen motility, muscularization and volume (Warner et al., 1956; Stobo et al., 1966; Zitnan et al., 1998). Stobo et al. (1966) found that the greatest ruminal volume (27 l) belonged to calves fed high forage diets (74% forage), and the ruminal volume decreased as forage allowance in the diet also decreased, being animals fed high concentrate diet (95% concentrate) those with the lowest ruminal volume (22 l).

1.2.4. Rumen pH Homeostasis

As consumption of dry feed becomes important in the diet of the young calf, microbial fermentation of those feeds in the rumen releases several products that alter pH homeostasis. Young calves starter feeds are generally high in carbohydrate content. Microbial fermentation of those carbohydrates produces large quantities of short chain fatty acids (**SCFA**) that readily dissociate and release protons and decrease pH in the rumen (Allen, 1997; Russell and Rychlick, 2001). Thus, acidic disturbance in the rumen is closely associated with high concentrate diets (Plaizier et al., 2008; Lechartier and Peyraud, 2010). It exists several chemical and homeostatic mechanisms to regulate ruminal pH and avoid problems related with ruminal acidosis. This regulation is based on the release of buffer molecules to the rumen, and the removal of protons from the rumen.

1.2.4.1. Buffer molecules

1.2.4.1.1. Short-chain fatty acids

Feed fermentation produces large amounts of SCFA. These weak acids release protons when they dissociate in the ruminal environment (Gäbel and Aschenbach, 2006). Ruminal SCFA behave as a buffer system, releasing protons when pH increases and binding it when pH decreases. The dissociation equilibrium is characterized by the pK_a value of the molecule. In the case of SCFA is set at 4.8. This means that the buffer capacity of SCFA will occur at pH of 4.8. Therefore, the problem of SCFA as buffer is that its effect is useful at pH around 4.8 (Counotte et al., 1979), and this is when animals are suffering clinical ruminal acidosis.

1.2.4.1.2. Phosphate

Phosphate is an important buffer found in the saliva (pK_a = 7.2). Phosphate concentration in saliva is typically described to be of 20-30 mmol/L (Bailey and Balch, 1961). The base nature of the phosphate contributes to capture protons from the rumen, because 80% of its buffering capacity is observed at ruminal pH range between 8.2 and 6.2.

1.2.4.1.3. Bicarbonate

Bicarbonate is one of the most important buffering molecules found in the rumen. Bicarbonate concentration in the saliva has been reported to be around 120 mmol/L (Bailey and Balch, 1961; Erdman, 1988). Furthermore, bicarbonate is also secreted directly by the rumen epithelium in large amounts (Gäbel et al., 1991), comparable

with the bicarbonate secreted by the salivary glands. The ruminal bicarbonate system is more complex than the SCFA and phosphate buffering systems. The bicarbonate buffering system is a double-open system in the rumen (pK_a = 6.1). Once the bicarbonate has fixed a proton, the resulting H_2CO_3 can decay to H_2O and CO_2. Furthermore, only 0.5% of the dissolved CO_2 combines with water to form H_2CO_3, and eventually, dissolved CO_2 may escape into a gas phase and be eructated or absorbed (Kohn and Dunlap, 1998). This system allows a very effective capture of the protons in the rumen. Aschenbach et al. (2011) estimated that more than 90% of the bicarbonate is converted to CO_2 in this double-open buffering system.

1.2.4.1.4. Ammonia

Ammonia is a potent ruminal content buffer. Its concentration generally increases after meals (Reynolds and Kristensen, 2008). However, its concentration rapidly decreases due to ruminal bacteria utilization, omasum efflux, or absorption across the rumen wall (McDonald, 1948; Kennedy and Milligan, 1980; Siddons et al., 1985; Obara et al., 1991). In the liver, ammonia is detoxified to form urea, and this urea can reenter into the rumen through an epithelium secretion, supported by some salivary urea secretion (Harmeyer and Martens, 1980; Marini and Van Amburgh, 2003). Then, urea in the rumen is fractioned by ruminal bacteria to form 2 molecules of NH_3. Ammonia immediately binds a proton forming NH_4^+.

1.2.4.2. Proton removal from the rumen

The first mechanism to remove protons from the rumen is the lipophilic diffusion of protonated SCFA (HSCFA). Proton removal as HSCFA is very efficient because remove one proton every SCFA absorbed (Allen, 1997). However, lipophilic diffusion cannot be the only way to remove protons from the rumen. First of all, HSCFA represent a small fraction in the HSCFA-SCFA acid-base equilibrium at ruminal pH (Aschenbach et al., 2011). And secondly, there is a constraint between the SCFA production rate and its lipophilic permeability. The production of SCFA (acetate>propionate>butyrate) is inversely related with their permeability (butyrate>propionate>acetate). This fact leads to consider a non-diffusional absorption mechanism of SCFA.

Non-diffusional absorption of dissociate anions of SCFA is carried out by different proteins. The main pathway for apical diffusion is the SCFA / HCO_3 exchange (Gäbet et al., 1991; Kramer et al., 1996; Aschenbach et al., 2009). Some studies (Ash and Dobson,

1963; Gäbel et al., 1991; Penner et al., 2009) attributed more than 50% of the SCFA absorption to the SCFA / HCO_3 exchange. Furthermore, this diffusion gets large quantities of HCO_3 into the rumen, with the consequent use as proton buffer. Another related mechanism involved in ruminal pH control is the apical export of SCFA. Monocarboxylate transporter 1 (MCT1) is responsible for the basolateral export of ketone bodies from intracellular metabolism of butyrate and lactate from the metabolism of propionate (Müller et al., 2002; Gäbel and Aschenbach, 2006). The basolateral export of these two metabolites to blood is a determinant factor for the removal of butyrate and proton from the rumen (Gäbel et al., 2001; Penner et al., 2009). Some authors also attributed direct SCFA export via MCT1 (Kirat et al., 2006; Graham et al., 2007). This mechanism allows a continuous import of SCFA from the rumen lumen to the epithelium, and, thereby, it progressively decreases the ruminal SCFA concentration and consequently, increases rumen pH.

1.3. Starter feed

As soon as young calves begin to show interest towards dry feed, a good quality calf starter must be offered to the animals. As solid feed consumption increases, the source of nutrients available to calf changes. During the firsts weeks of age, nutrition of calves relies exclusively on products absorbed directly from digestion of milk or milk replacer. Once dry feed becomes a part of the young calf diet (with little impact on calf nutrition at the beginning, but with an increasing importance as animal grows) not only products absorbed directly from digestion of milk are important to calf nutrition. At this point and due to the ingestion of solid feed, end products of microbial fermentation become an important part of the calf nutrition. These end products are basically VFA and microbial protein. To ensure an adequate transition to solid feeds, chemical composition and physical form of the starter must be appropriate for young calves (Warner, 1991). Drackley (2008) defined as a good calf starter, a starter that allows high rates of fermentation and microbial protein synthesis, yet still provides some undegraded or bypass protein and starch to be digested in the lower tract.

Another parameter to consider in the formulation of starter feed is the palatability of feeds. It has been recommended to offer concentrates highly palatable to calves around weaning to foster consumption of solid feed (Morril and Dayton, 1978). In various trials testing different feedstuffs in young calves, it was clearly established the preferences of young calves towards certain ingredients commonly used in starter feed formulation (Montoro, 2012). Therefore, it would be reasonable to use the most preferred feedstuff to encourage starter intake in young calves.

1.3.1. Energy sources

Starter feeds must be high in energy to cover maintenance needs and allow growth of the calf once diet is based only on dry feed. Energy content of starters mostly comes from carbohydrates. The ingredients most widely used to obtain carbohydrates and, consequently energy, are cereal grains (Drackley, 2008). Fat could be thought to be an appropriate source of energy in starters, but it is not included at a high rate to the starter's formulation due to the detrimental effect on starter intake, and decreased or unchanged growth performance (Fallon et al., 1986; Caffrey et al., 1988; Doppenberg and Palmquist, 1991; Kuehn et al., 1994; Bunting et al., 1996). Kuehn et al. (1994) found that calves fed high fat starter (7.3% fat) consumed less starter feed and grew

less than those calves fed low fat starter (3.7% fat). However, some benefits at weaning were reported in calves fed 6 l/d of milk replacer, not at 4 l/d of milk replacer, when a high (9.5% fat) versus a low (3.5% fat) concentrate was offered (Araujo et al., 2013, unpublished).

1.3.2. Protein sources

As fermentation of dry feed becomes established, the nature of protein that calves absorb changes from milk protein to a combination supply of rumen microbial protein and undegraded dietary protein. Therefore, protein content in the diet is an important component to be considered. The NRC established the CP requirement for calf starter about 20% of DM. Akayezu et al. (1994) studied the effect of increasing CP content in the calf starter on performance. This study showed a response up to 20% CP but no further increases in starter intake and growth of calves when starter CP exceeded 20%. Soybean meal is the most used protein source in starter feeds formulation. However, when soybean meal is substituted by lupines or roasted soybean grains, similar performance results have been reported (Wright et al., 1989; Abdelgadir et al., 1996a).

1.4. Forage

1.4.1. Analytical methods to evaluate fiber content

1.4.1.1. Crude fiber

Crude fiber (CF) is the oldest method to measure the amount of cell wall in feeds, and is also known as Weende method (Henneberg and Stohman, 1859). It has been used for over a century to evaluate the fiber content of the feeds. This fraction was designed to include the materials in a feed that were low in digestibility. Cellulose, certain hemicellulose, and lignin are included here.

This method consists on treating the feed sample with sulfuric acid, and a posterior treatment with sodium hydroxide.

Crude fiber analysis limitations:

- Crude fiber is not a chemically uniform substance but a variable mixture. As mentioned before, the major components of CF are cellulose, hemicellulose, and lignin. During this procedure, some of the hemicellulose and lignin are extracted from the samples, and showing up in the Nitrogen-Free Extract (NFE).

- The CF value would not reflect all of the most indigestible portion of the feed, and NFE would be larger and would have lower digestibility than would be true if it included only sugars and starches.

- The limitation in evaluation of fiber content of feeds detected in the CF method is only a problem with the most fibrous feeds. In concentrate feeds, where the content of fiber is low, the bias in fiber analysis is not very important. However, in forage crop or high fiber feeds evaluation, where the content in fiber is high, this error in the determination of fiber content entails a problem in the quality evaluation.

1.4.1.2. Van Soest analysis method

This method was developed due to the limitations of the CF content. This procedure involves the separation of feed dry matter into two fractions:

· High digestibility fraction

· Low digestibility fraction

This method consists on extract forage with a neutral detergent solution and/or an acid detergent solution.

The more soluble, and accordingly more digestible nutrients come out in the filtrate of the neutral detergent solution, and are known as neutral detergent solubles (NDS). NDS fraction is composed for the most part of the cell contents. They are composed of

lipids, sugars, starches, and protein and are all highly digestible. The neutral detergent insolubles are usually referred as neutral detergent fiber (NDF). This fiber fraction is composed for the plant cell wall. It basically consists of hemicellulose, cellulose, lignin, silica, and some protein.

The insolubles after the treatment with the acid detergent solution are known as acid detergent fiber (ADF), and are composed of cellulose, lignin, and variable amounts of silica. The difference in the amount of NDF and ADF is an estimate of the hemicellulose in the feed.

Advantage compared with Weende method:

- In the Van Soest procedure all the lignin and hemicellulose are included in the NDF fraction, whereas with the Weende method, variable amount of these two low digestible components are lost from the CF to the NFE.
- NDF represents better the total fiber fraction of a forage feed than does CF.
- NDF is a more effective measure for evaluating a feed material, from a roughage standpoint, than CF.

1.4.2. Voluntary Intake of Forages

How forage intake interferes in other feeds intake is an important parameter to be aware in cattle feeding management. In this section, overview of the different factors affecting ruminant voluntary intake (VI) of forages will be done.

Forages are usually used as a source of bulkiness in cattle diets. However, bulkiness of forage could be a negative factor affecting VI. It has been observed that intake of forages varies inversely with the filling capacity of forages, which is represented by fiber mass (Balch and Campling, 1962). In ruminants, forage VI is more clearly related to NDF content of the forage than other chemical measurements (Van Soest, 1965). Moreover, Waldo (1986) suggested that NDF is the best single chemical predictor of ruminants VI of forage. However, other authors (Mertens, 1987; Mertens, 1994) pointed out that using NDF alone is inadequate to predict filling effects of forages. Filling effects of forages are not only based on the chemical composition of the fiber. Filling effects vary depending on the different initial particle size, particle fragility and rate, and extent of NDF digestion.

Voluntary intake is also modulated by the physical distention of the gastrointestinal tract (Campling, 1970; Baile and Forbes, 1974; Grovum, 1987; Forbes, 1995).

Furthermore, distention of the reticulorumen has also been reported to limit intake with high fill diets (Campling, 1970; Baile and Forbes, 1974). To determine the effects of distention on feed intake, Anil et al. (1993) inflated ballons with warm water in the rumen of hay and silage fed cows and found a depression of feed intake of 66 and 28 g DM/l of warm water, respectively. Another factor involved in the modulation of VI of forages is their particle size. Reduction of particle size of forages generally increases VI (Minson, 1963) as a result of a reduction initial volume and retention time in the rumen (Moore, 1964). However, reducing particle size of forages does not affect all kind of forages at the same extent. An example is that pelleting low quality forages resulted in a greater increase of forage intake than pelleting high quality forages (NDF < 40%) (Minson, 1963). Finally, an inverse relationship between retention time in the reticulorumen and forage VI has been noted in many experiments (Campling, 1961; Freer and Campling, 1963; Thornton and Minson, 1972, 1973; Laredo and Minson, 1975).

Digestibility of forages also plays a role in the VI. It is well established that the more digestible the forage is, VI of forage is less affected (Blaxter et al., 1961; Blaxter and Wilson, 1962; Van Soest, 1965). Voluntary intake of low digestibility feeds is thought to be limited by the physical distention of the gastrointestinal tract (Allen, 1996). Conrad et al. (1964) suggested that there is a breakpoint in digestibility at which limitations of forage VI because of physical fill in the GI tract is replaced by the limitation to satisfy energy demands. However, this breakpoint is more likely a mathematical simplification because VI is thought to be controlled by a multiple and complex inputs to the brain (Forbes, 1986), but it is clear that the effect of fill on VI gradually diminishes as digestibility of forages increases (Allen, 1996).

As mentioned before, rumen retention time of forage also affects forage VI. Therefore, factors related to feed flow in the rumen must be reviewed. Riewe and Lippke (1970) demonstrated an increase in the reticulorumen fractional passage rate as DM intake increases. Furthermore, an increase in DM intake also produces a greater fecal particle size (Van Soest, 1966; Luginbuhl et al., 1990; Okine and Mathison 1991a), a greater fill in the reticulorumen (Okine and Mathison, 1991b), and an increased proportion of large particles in the reticulorumen (Okine and Mathison, 1991a). Another factor related to reticulorumen feed flow is the particle size. For almost a century, it is known

that a requisite for feed flow from the reticulorumen is the reduction of particle size, especially forage particles (Ewing and Wright, 1918). Furthermore, large particle size of feeds increases the resistance to flow from the reticulorumen to abomasum (Poppi et al., 1980; Dixon and Milligan, 1985). Therefore, small particle size is necessary to promote flow of feed from the rumen to abomasum. Kennedy (1985) reported that particle breakdown in the reticulorumen is primarily a result of mastication, having digestion and detrition of feeds little impact on it (McLeod and Minson, 1988). Even a very important factor affecting reticulorumen flow is the particle size reduction, it may not be always a constraint. Most of the particulate matter in the reticulorumen is smaller than the maximum particle size observed in feces (Evans et al., 1973; Welch and Smith, 1978; Poppi et al., 1981; Ulyatt et al., 1986). Moreover, the diameter of the reticulo-omasal orifice is much larger than those fecal particles (Bueno and Ruckebusch, 1974; McBride et al., 1983).

Allen and Mertens (1988) exposed that reticulorumen flow must depend on the quantity of particles eligible to pass from the reticulorumen that are in close proximity to the reticulo-omasal orifice at the second phase of reticular contraction, which is clearly dependent on particle density. In experiments using inert particles of different densities, it was reported a negative, nearly linear relationship between retention time of particles in the reticulorumen and the density of the particles (Lechner-Doll et al., 1991). Wyburn (1980) showed that small and dense particles fall to the ventral rumen where they flow with the ventral currents cranially to the reticulum. Increase of density of feed particles depends on fermentation. Particle density is determined by the retention of carbon dioxide and methane produced by microbes within the void spaces of forages. As fermentation proceeds, amount of OM susceptible to be fermented decreases, resulting in a lower production of gas and an increase in density. Density is then a function of rate of fermentation of potentially fermentable fiber and the fraction of fiber that is potentially fermentable (Jung and Allen, 1995). A combination of mechanism produced during digestion could better explain how reticulorumen feed flow works. When a reduction of forage particle size by chewing is produced, then increases the rate of fermentation by increasing surface area (Cherney et al., 1988) and decreases the ability of the particles to retain gases, both mechanisms affecting particle density.

1.4.3.Physically Effective Fiber

Evaluation of fiber content of feeds is extensively done using quantitative analytical methods. These methods include different analytic systems as crude fiber, neutral detergent fiber, or acid detergent fiber. The problem of these quantitative analytical procedures is that are only based on the chemical characteristics of the feed, and do not account for the properties of fiber related with kinetics of digestion and passage or with physical characteristics, such as particle size and density (Mertens, 1997). Physical characteristics of fiber can influence animal health, rumination fermentation and utilization, animal metabolism, and milk production independently of the amount or composition of chemically measured NDF. An example of that is when the forage included in the diet of dairy cows is finely ground or chopped (without changes in the forage:concentrate ratio), an alteration of the fermentation in the rumen by decreasing ruminal pH and a depression in milk fat percentage is produced, indicating that the effectiveness of fiber is the primary cause of problems (Sudweeks et al., 1981; Woodford et al., 1986; Woodford et al., 1988; Grant et al., 1990a,b).

1.4.4.Types of Forages: Grasses vs Legumes

Grasses and legumes plants belong to two families of the phylogenetic division of the Angiosperms. Grasses are from the Graminaceae family and legumes are from the Leguminosae family. Generally, grasses contain much lignified structural matter in their leaves, particularly the midrib, and in leaves sheaths. Legumes use to be treelike, with relatively unstructured leaves held on the ends of woody stems. These general aspects are important to consider because the direct impact on their use animals nutrition.

From the nutritional point of view, one important aspect that can modify the value of a forage is the lignin content of the plant. Lignin deposition in legumes is more restricted and localized in the stems, whereas in grasses is spread over the entire plant. Stems and leaves of legumes are of similar digestibility in their immature state. However, as legumes mature, stems digestibility is depressed, while that of leaves is maintained. In general, legumes are more digestible than grasses because of the lower cell wall content of the former, but the cell wall of legumes is less degradable than that of grasses, because of the greater lignin content in legumes cell wall compared with grasses cell wall (Galyean and Goetsch, 1993). Further differences between legumes

and grasses are in the nutrient content of the plants. Generally, legumes have a greater content in soluble substrates, and store starch (Jones and Wilson, 1987) compared with grasses. Furthermore, legume plants have greater content in fermentable OM, and more synchronous availability of N and energy compared with grasses (MacRae and Ulyatt, 1974; Beever et al., 1986a; Jones and Wilson, 1987). All these factors together promote a greater rumen microbial growth when legumes are fed compared with feeding grasses.

Differences between grasses and legumes have more implications in their feeding properties. The low cell wall content of legume plants is responsible of a greater particle breakdown during mastication (Minson, 1990). Furthermore, legumes and grasses particles are different in shape. While legumes particles are described to be cubical, grasses particles are long and slender (Moseley and Jones, 1984; McLeod et al., 1990). Cubical legumes particles pass through the rumen more easily than grasses particles do (Moseley and Jones, 1984; McLeod et al., 1990). The greater particle breakdown of legumes particles and the easiest passage of those particles from the rumen entail a greater outflow of legume digesta from rumen to abomasum (Galyean and Goetsch, 1993). Finally, the greater outflow of legumes particles from the rumen compared with that of grass particles, makes possible to increase the intake of legumes in ruminants. Consequently, ruminants fed a legume-based diet perform better than those fed a grass-based diet (Thorton and Minson, 1973; MacRae and Ulyatt, 1974; Moseley and Jones, 1979; Cruickshank et al., 1985; Rooke et al., 1985,1987; Beever et al., 1986a,b; Jones and Wilson, 1987; Reid et al., 1990). Finally, grasses generally have greater fractions of potentially fermentable fiber, and lower fermentation rates (Smith et al., 1972), tending to make them buoyant for a longer time, due to the particles retention of carbon dioxide and methane produced by particle-associated microbes. Therefore, retention time of grasses in the reticulorumen is usually longer than of legumes. All these factors related with rumen outflow and retention time in the rumen have a direct effect in the rumen fill. It is known that rumen fill of legumes is generally lesser than of grasses (Thorton and Minson, 1973; Thomson et al., 1985). The lesser rumen fill of legumes is due to the greater rate of digesta outflow from the rumen of legume particles that is related to its physical characteristics.

1.4.5. Use of Forages in Preweaned Calves

In nature conditions, cattle calved in the spring when the grass is immature with high content in sugars and relatively low in fiber (NRC, 2001), leading to great proportion of propionate and butyrate during its fermentation in the rumen. However, in farm conditions, forage fed to calves is usually hay or straw, and it is low in sugars and high in less digestible fiber (Drackley, 2008). As mentioned before, rumen forage fermentation produces mainly acetate, and its ability to stimulate rumen epithelial development is limited. Moreover, use of fibrous feeds in young dairy calves has been discouraged due to the limited use of cellulose during the preweaning period, and because the accumulation of undigested forage material in the rumen could decrease voluntary intake of concentrate (Drackley, 2008). On the other hand, improvements in performance of young calves offered forage have been reported. Thomas and Hinks (1982) described improvements in performance when barley straw was offered to preweaned calves. Furthermore, in a more recent study (Khan et al., 2011) with calves fed with orchard grass, it was observed that the inclusion of forage in the diet did not reduce starter intake but increased total DM intake, physical development of the reticulorumen, and rumen pH.

Therefore, there is controversy in the literature about the effect of offering forage to young calves. Due to the disagreement between authors that are in favor of feeding forage in young calves and the authors that totally refused the idea of offering forage to young calves, there exists the necessity of clarify the effect of offering forage to young calves to be able to properly advise farmers in the management of solid feed diet for young calves.

Chapter 2

OBJECTIVES

This research has been done because of the appearance of some articles that discouraged the use of forage in the diet of preweaned calves because of the inability of these animals to properly digest fiber, even though other articles showed improvements in calves performance. The main objective of this thesis was to evaluate the positive and negative effects of the inclusion of different types of forage in the diet of preweaned calves. The specific objectives were:

1. To evaluate the effects on performance and feeding behavior of the inclusion of different sources of chopped forage in the diet of pre- and postweaned Holstein calves. And to select the ones that improve calves performance the best for further research.
2. To evaluate the inclusion of non-forage fiber sources, soybean hulls and dried citrus pulp, in the diet of pre- and postweaned calves on animal performance and feeding behavior.
3. To investigate some potential factors related to rumen and gastrointestinal tract development that may be involved in intake regulation of pre- and postweaned calves when a grass or legume forage is offered.
4. To evaluate the possibility to use a texturized concentrate feed instead of feeding forage to young calves to stimulate concentrate intake around weaning.
5. To compare the short- and long-term effects on performance and reproductive parameters of feeding female Holstein calves during the preweaning period with oat hay or without hay availability.

To achieve these objectives, five studies were conducted:

- Study 1: Comparison of performance parameters, feed digestibility at weaning, and animal behavior among pre- and postweaned calves supplemented or not with different forage sources (chopped alfalfa hay, chopped oat hay, chopped barley straw, chopped rye-grass hay, triticale silage, corn silage).
- Study 2: Comparison of performance parameters and animal behavior among pre- and postweaned calves supplemented or not with a non-forage fiber sources (soybean hulls and dried citrus pulp).

- Study 3: This study was performed to try to explain the differences observed on performance and concentrate intake in pre- and postweaned Holstein calves when oat hay or alfalfa hay was offered in its diet. To assess this objective, it was evaluated some metabolic and microbiological ruminal parameters, the digestive tract development, and the kinetics of an external marker in animals fed with or without access to oat and alfalfa hay in the diet.
- Study 4: Comparison of performance and ruminal pH among calves fed a texturized concentrate with or without straw supplementation, and calves fed pelleted concentrate with straw availability.
- Study 5: Investigate short and long-term benefits of the improvements observed on performance of female Holstein calves supplemented with oat hay during the preweaning period. The short-term parameters determined were: performance and digestibility at weaning when forage was supplemented to all treatments, and the long-term parameters evaluated were: reproductive parameters at first breeding and milk yield at first lactation (data not shown in the present thesis).

Chapter 3

EFFECT OF DIFFERENT FORAGE SOURCES ON PERFORMANCE AND FEEDING BEHAVIOR OF HOLSTEIN CALVES

A fraction of this research has been published in:

Journal of Dairy Science, 2012. 95:286-293.

1

3.1. Introduction

There exists some controversy about the kind of solid feed that should be included in calf diets during the pre-weaning period. Feeding only starter feed to pre-weaning calves may reduce ruminal pH (Beharka et al., 1998), decrease rumen motility (Clarke and Reid, 1974), and cause hyperkeratinization and clumping of ruminal papillae (Bull et al., 1965); decreasing the ability of rumen mucosa to absorb nutrients (Hinders and Owen, 1965), especially if the starter feed is finely ground (Greenwood et al., 1997). On the other hand, forages stimulate the muscular layer of the rumen (Tamate et al., 1962), promote rumination (Hodgson, 1971; Phillips, 2004), maintain the integrity and healthiness of rumen wall (Haskins, 1969; Suárez et al., 2007), and reduce behavioral problems (Phillips, 2004).

Provision of forage to young calves has not being recommended because it has been shown to reduce starter feed intake in individually-housed calves (Phillips, 2004), impair rumen papillae development (Nocek and Kessler, 1980), and decrease BW and DM digestibility (Leibholz, 1975). Interestingly, studies that provided a source of forage (chopped or long hay or straw) to calves younger than 2 wk of age reported improvements in performance (Thomas and Hinks, 1982; Phillips, 2004). In contrast, studies that limited starter feed intake, or established a fixed forage to concentrate ratio in a pelleted diet reported different results depending on the ratio, the forage sources provided, and the ADF content of the diet (Stobo et al., 1966; Bartley, 1973; Leibholz, 1975; Block and Shellenberger, 1980; Beharka et al., 1998; Coverdale et al., 2004; Suárez et al., 2007).

Nowadays, starter feeds with relatively low NDF contents (16-18%) are commonly used (Franklin et al., 2003; Lesmeister and Heinrichs, 2004; Kehoe et al., 2007) to optimize digestibility. In the literature, there is an array of forage sources (alfalfa hay, straw, grass hay, fresh grass, corn silage), chopped lengths, and concentrate to forage ratios that have been used, but no study compares more than 2 forage sources under the same management conditions. Considering the need to optimize solid feed consumption of young calves, the objectives of this study were to evaluate the potential benefits of providing different forage sources, in a chopped form and offered separately from the starter feed, and determine the forage source that could best improve calf performance and welfare.

3.2. Materials and methods

3.2.1. Animals and treatments

A total of 179 Holstein male calves were used in a series of 3 experiments. The first experiment was conducted with 60 calves (43.8 ± 5.7 kg of BW and 7.9 ± 5.2 d of age), the second involved 59 calves (46.1 ± 5.0 kg of BW and 7.0 ± 3.5 d of age) and the third involved 60 calves (44.1 ± 5.4 kg of BW and 10.0 ± 3.8 d of age). Calves were purchased from commercial farms, raised in the facilities of Torre Marimon (Caldes de Montbui, Spain), and managed according to the recommendations of the Animal Care Committee of IRTA. After arrival, calves were given a broad-spectrum antibiotic (Draxxin, tulathromycin, Pfizer Animal Health, Spain) to prevent respiratory disease. Furthermore, calves were vaccinated against respiratory syncytial virus (Rispoval RS, Pfizer Animal Health, Madrid, Spain) 3 d after arrival. Calves were allowed a 6-d adaptation period to the new facilities, milk replacer (MR), and calf starter feed. Increasing levels of milk replacer DM concentration, starting at 10% DM, were offered until reaching the desired 12.5% DM the 6th d after arrival. Thus, the study was initiated when calves were 14.1 ± 4.2 d old. Calves were housed in individual pens (1.6 x 1.0 m), and bedded with sawdust. The 3 experiments followed the same management scheme. After the adaptation period, animals were weighed, and randomly distributed according to BW and age in 3 groups. Calves in the control (CTR) group were fed a starter feed (Table 3.1) without any forage supplementation. Calves in the other 2 treatments had access to the same starter feed in one bucket as those in CTR treatment, plus an additional bucket containing a forage source, which depending on the experiment was chopped alfalfa (AH) and rye-grass (RH) hay (Experiment 1), chopped oat hay (OH) and chopped barley (BS) straw (Experiment 2), and corn silage (CS) and triticale silage (TS) in Experiment 3.

Hay and straw forages were chopped before being offered to calves with a forage-chopper machine (Seko, Curtarolo, Italy). Nutrient composition and particle size distribution of the forages studied are depicted in Table 3.2. Particle size distribution of the forage sources was determined using 2 sieves with screen sizes of 20 and 8 mm diameter (Table 2). The CTR treatment was repeated in each of the 3 experiments to account for the period effect and allowing comparison among all treatments. A commercial MR (25% CP and 19.2% fat, Sprayfo Excellent 60, Sloten BV, Holland) was

offered in 2-L feeding bottles twice daily at 0700 and 1700 h. Calves received 4 L/d of MR at 12.5% DM dilution rate until 50 d of age. From 50 to 56 d of age, calves received only the morning feeding of 2 L at 12.5% DM. Animals were weaned at 57 d of age, and the study completed when calves were 71 d old. Starter feed and forage were offered ad libitum 1 h after MR was consumed in two separate buckets throughout the study.

Table 3.1. Ingredient and chemical composition of experimental starter feed.

Ingredient,	Composition, %DM
Wheat	20.0
Corn	15.0
Barley	11.2
Sorghum	12.0
Soybean meal	23.0
Wheat middling	12.0
Soybean hulls	5.0
Premix[1]	0.2
Calcium carbonate	0.5
Dicalcium phosphate	0.3
Sodium chloride	0.8
Nutrient composition, %DM	
CP	19.5
NDF	17.7
ADF	8.0
Ash	5.6

[1] Premix composition: vitamin A 2,007,000 IU/kg; vitamin D3 433,000 IU/kg; vitamin E 3,685 mg/kg; vitamin B1 52 mg/kg; vitamin B2 197 mg/kg; vitamin B6 98 mg/kg; vitamin B12 0.76 mg/kg; vitamin K3 52 mg/kg; nicotinic acid 656 mg/kg; pantothenic acid 394 mg/kg; Mn 5,877 mg/kg; Fe 7,093 mg/kg; Cu 2,026 mg/kg; Co 46 mg/kg; Zn 8,112 mg/kg; I 304 mg/kg; Se 46 mg/kg

3.2.2. Measurements and sample collection

Starter feed, MR, and forage intakes were recorded daily on an individual basis. Calves were weighed weekly. The week after weaning (64 d of age) plastic bags were glued to 8 animals (randomly chosen) per treatment to determine apparent DM, OM, CP, and

NDF digestibility of the diet as described elsewhere (Terré et al., 2007a). During five consecutive days, all feces were collected and weighed. Bags were changed four times a day. Then, a subsample equivalent to 30% of total daily feces was obtained and dried at 60°C for 72 h, and subsamples of the 5 d were composited by animal proportionally to the dry weight of the feces produced each day. Samples were ground to pass through a 1-mm screen (Cyclotech 1093 mill, Tecator, Sweden), and analyzed for DM, OM, CP, and NDF. Daily orts from forages were collected, composited for the 5-d period, and processed similarly to fecal samples. Apparent nutrient digestibility was calculated as the quantity of nutrient consumed minus the quantity of nutrient defecated, divided by the quantity of nutrient consumed.

Table 3.2. Chemical composition and particle size of forages.

	Treatment[1]					
Item	AH	RH	OH	BS	CS	TS
DM, %	88.7	92.1	90.9	93.3	28.6	25.0
Composition, %DM						
CP	16.6	6.8	8.4	4.2	8.6	7.5
NDF	40.2	59.3	59.6	74.0	41.9	64.7
ADF	30.2	35.1	31.8	42.5	25.2	42.3
Ash	10.4	13.4	8.5	8.8	7.8	5.5
Particle size, %						
>20mm	39.4	50.3	28.4	31.1	8.4	1.7
8-20mm	17.1	24.5	27.2	33.4	31.6	50.5
<8mm	43.5	25.2	44.4	35.5	60.0	47.8

[1]AH=alfalfa hay; RH=rye-grass hay; OH=oat hay; BS=barley straw; TS=triticale silage; CS=corn silage

3.2.3. Chemical analysis

Samples of MR were analyzed for DM (24 h at 103ºC), ash (4 h at 550ºC), N content using the AOAC (1990) method (988.05) adapted for an automatic distiller Kjeldahl (Kjeltec Auto 1030 Analyser, Tecator, Sweden) with copper sulphate/selenium as a catalyst instead of copper sulphate/titanium dioxide, and ether extract using the AOAC

method (920.39) using petroleum ether for distillation instead of diethyl ether (AOAC, 1990). Samples of starter feed and forages were analyzed for DM, ash, and CP following the same methods described above, plus NDF with sodium sulphite and heat-stable alpha-amylase (Van Soest et al., 1991), and ADF following the AOAC (1990) method (973.18).

To determine apparent digestibility of nutrients, samples of feces, and starter feed and forage orts were analyzed for DM, ash, CP and NDF as described above.

3.2.4. Animal behavior

Behavior was monitored by direct observations for 10 animals randomly chosen within treatment once a week during 2 wk before and 2 wk after weaning. Animals were observed for 1 h immediately following the morning MR feeding and one additional hour after solid feed was weighed and offered during the two pre-weaning weeks. After weaning, calves were observed for two consecutive hours after offering the solid feed. Therefore, total observation time per animal was 8 h for the entire monitoring period. Calves were observed every minute, and the observer recorded the occurrence of the following behaviors: lying (no chewing activity), standing (no chewing activity), eating starter feed, eating forage, ruminating (either lying or standing), and non-nutritive oral behaviors (when the animal licked any surface, tongue rolled, or consumed wood shavings).

3.2.5. Statistical analysis

First, an analysis of variance among CTR treatments from the three different experiments was performed to confirm that there were no differences in responses to CTR among periods, thus ensuring that data from the 3 experiments could be safely analyzed together.

Performance data were analyzed with a mixed-effects model for repeated measures. The statistical model included initial BW as a covariate, treatment, week of study and their interaction as fixed effects, and animal and period as random effects. Due to the lack of normality, data from starter feed, forage and total DM intake (TDMI) were root-square transformed. Least square means presented herein for these three parameters

correspond to non-transformed data, and SEM and P-values correspond to the results from the mixed-effects model using root-square transformed data.

Apparent nutrient digestibility was analyzed with the same mixed-effects model described above but without the effect of week of study and the BW covariate.

Behavior data were summarized individually as the total time (min) devoted to each monitored behavior before and after weaning (2 h per wk and animal during 2 wk before and 2 wk after weaning; therefore 4 h per animal before and 4 h per animal after weaning). After that, a mixed-effects Poisson regression analysis including calf and period as random effects, and treatment and time relative to weaning (before or after) as fixed effects was performed.

3.3. RESULTS AND DISCUSSION

3.3.1. Performance

Animal performance and feed consumption are presented in Table 3.3. Increases ($P < 0.001$) equivalent to 33, 29 and 20 % in starter feed intake were observed in TS, OH, and BS calves compared to those offered CTR. However, calves in AH treatment had similar starter feed intake to CTR calves and consumed less starter feed than those offered other forage treatments. Starter feed intake of RH and CS calves was similar to CTR, OH, and BS treatments, but greater than intake recorded on AH calves. Starter feed intake was increased ($P < 0.001$) when a forage source was offered (with the exception of AH) compared with CTR calves beginning at 29, 36, 43 and 50 d of age throughout the end of the study for TS, OH, BS and RH, respectively (Figure 3.1).

Forage intake was greatest ($P < 0.001$) for calves fed AH and OH compared with the other treatments. Interestingly, AH and OH calves consumed similar amounts of forage, but the consumption of AH did not stimulate starter feed intake, whereas OH promoted a significant increase in starter feed intake (Table 3.3). In spite of the high forage intake of the AH treatment, TDMI of AH calves was similar to that of CTR calves. Calves fed the OH, TS and BS had the greatest ($P < 0.001$) TDMI. A large intake of AH was expected because voluntary intake of legumes is consistently reported to be greater (at equivalent stages of maturity) than that of grass forages (Colburn et al., 1968; Moseley and Jones, 1979). A relatively low silage consumption could be expected because of potential negative effects on intake of some fermentation end-

products such as amines (Van Soest, 1982). On the other hand, the high NDF and ADF contents of BS and RH may have potentially limited forage intake. In the current study, a different response between animals offered grass and legume forages was observed. Offering a legume (AH) failed to improve TDMI and calf performance, whereas calves fed a grass forage showed an increased TDMI and thus performance. The small forage consumption in calves offered grasses may have improved the rumen environment, which in turn may have contributed to stimulation of starter feed intake, TDMI, and increased calf performance. Probably, these observations are due to differences in some properties between grasses and legumes such as a greater flow rate from the rumen for legumes compared to grasses (Moseley and Jones, 1979). In addition, a greater pectin and lesser hemicelluloses content in legumes than in grasses might be responsible for the contrasting results obtained with these two forage types.

Table 3.3. Performance and feed intake of calves supplemented or not with different forage sources.

	Treatment[1]								P-value[2]	
	CTR	AH	RH	OH	BS	TS	CS	SEM[3]	T	Txt
Initial BW, kg	45.2	43.6	43.3	46.7	46.2	45.0	44.8	1.13	0.40	-
Final BW, kg	84.5^{d}	86.4^{cd}	91.6^{ab}	96.1^{a}	93.2^{ab}	93.6^{ab}	89.8^{bc}	2.03	<0.001	-
ADG, kg/d	0.72^{c}	0.76b^{c}	0.84^{ab}	0.93^{a}	0.88^{a}	0.88^{a}	0.82^{ab}	0.038	<0.001	<0.001
Intake, kg DM/d										
Starter	0.88^{cd}	0.76^{d}	0.99^{abc}	1.14^{ab}	1.06^{ab}	1.17^{a}	0.98^{bc}	0.028	<0.001	<0.001
Forage	-	0.120^{a}	0.046^{b}	0.101^{a}	0.060^{b}	0.048^{b}	0.051^{b}	0.0198	<0.001	<0.001
TDMI [4]	1.29^{e}	1.37^{de}	1.46^{cd}	1.67^{a}	1.55^{abc}	1.64a^{b}	1.48^{bcd}	0.024	<0.001	<0.001
DMI, % of BW	2.14^{d}	2.26^{cd}	2.36^{bc}	2.55^{a}	2.42^{abc}	2.54^{ab}	2.35^{c}	0.0004	<0.001	<0.001
Gain to feed[5]	0.55	0.54	0.56	0.55	0.56	0.54	0.55	0.013	0.82	<0.001

[1]CTR=control; AH=alfalfa hay; RH=rye-grass hay; OH=oat hay; BS=barley straw; TS=triticale silage; CS=corn silage

[2]T=treatment effect; Txt=interaction between treatment and time

[3]SEM=Standard error of the mean

[4]TDMI=Total dry matter intake (milk replacer, starter feed, and forage)

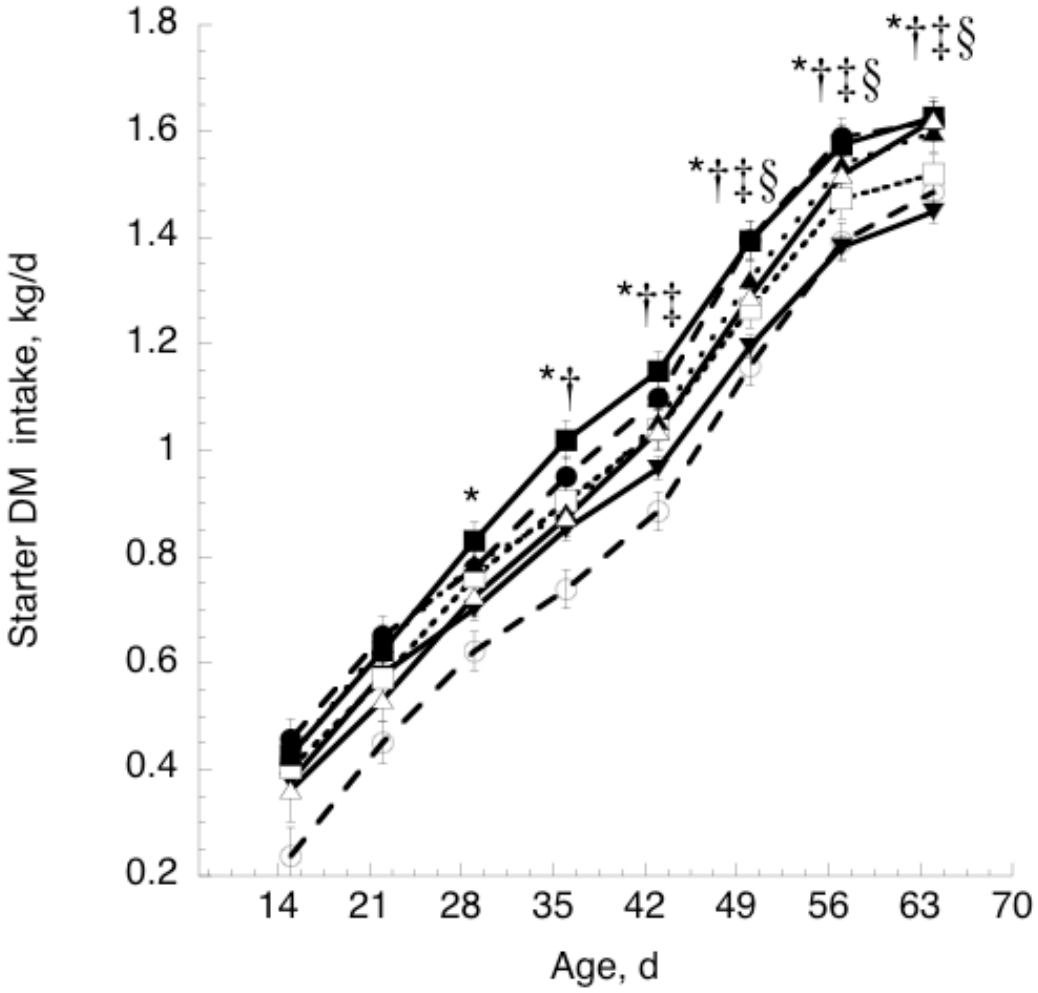
[5] kg BW gain/kg TDMI

[abcde]Means within row with different superscripts differ ($P < 0.05$)

Providing a chopped forage source, other than AH, to pre-weaning calves improved ADG ($P < 0.001$) compared to calves on the CTR treatment. The greatest ADG were observed in animals offered OH, TS, and BS, with ADG ranging from 21 to 28% over those observed with CTR calves. Calves in RH and CS treatments had greater ADG than CTR, and similar to AH calves. Similar to the patterns observed in starter feed intake, ADG was affected ($P < 0.001$) by forage source (Figure 3.2). Calves receiving TS reached a greater ADG than CTR starting at 29 d of study, those offered OH or CS at 43 d, and calves offered BS or RH after 50 d of study. Despite the forages tested in the present study being chopped, they had different particle sizes, and these differences might have contributed to elicit the observed responses among treatments. To our knowledge, the only study evaluating the potential impact of particle size of forage in calves was conducted by Hodgson (1971), who concluded that the most influential factor promoting solid consumption was the ease with which the diet could be eaten by calves.

Despite the changes observed in intake and performance with the provision of forages, no differences were observed in gain to feed ratio among treatments. This result would suggest that, contrary to common thought, forage consumption within the levels reported herein does not compromise nutrient utilization in young calves. However, several authors (Coverdale et al., 2004; Suárez et al., 2007) did not observe differences in TDMI when part of the starter feed was substituted by roughage during the pre-weaning period. But, Suárez et al. (2007) established different forage to concentrate ratios (i.e., 70 concentrate:30 straw; 70 concentrate:30 corn silage; 40 concentrate:60 corn silage), where the forage proportion was far greater than the ratio "chosen" by the calves in the present study when forage and starter feed were offered free-choice. For instance, in the current study, when forages were offered ad libitum and separately from the starter feed, calves in OH, BS, TS, RH, and CS groups consumed a diet with a final forage:concentrate ratio of 8:92, 5:95, 4:96, 4:96, and 5:95, respectively. Therefore, the greater forage content in the diets studied by Suárez et al. (2007) compared with forage consumed voluntarily by calves in the current study, may have masked the positive effect on stimulating starter feed intake when a grass forage source is provided to young calves.

Figure 3.1. Evolution of starter feed intake as affected by forage supplementation. Control (no forage supplementation;), alfalfa hay ( ), rye-grass hay ( ), oat hay ( ), barley straw ( ), corn silage ( ), and triticale silage ( ). *means that starter feed intake was different (P < 0.05) between triticale silage and control treatments, †means that starter feed intake was different (P < 0.05) between oat hay and control treatments, ‡means that starter feed intake was different (P < 0.05) between barley straw vs control treatments, and §means that starter feed intake was different (P < 0.05) between rye-grass hay vs control treatments.



On the other hand, Coverdale et al. (2004) fed a forage to concentrate ratio similar to that observed with the OH treatment herein, but the total amount of solid feed offered during the pre-weaning period was limited. This probably hampered the potential positive effect of roughage to stimulate TDMI. Hill et al. (2008) found that animals fed increasing percentages of hay in the starter feed linearly declined starter

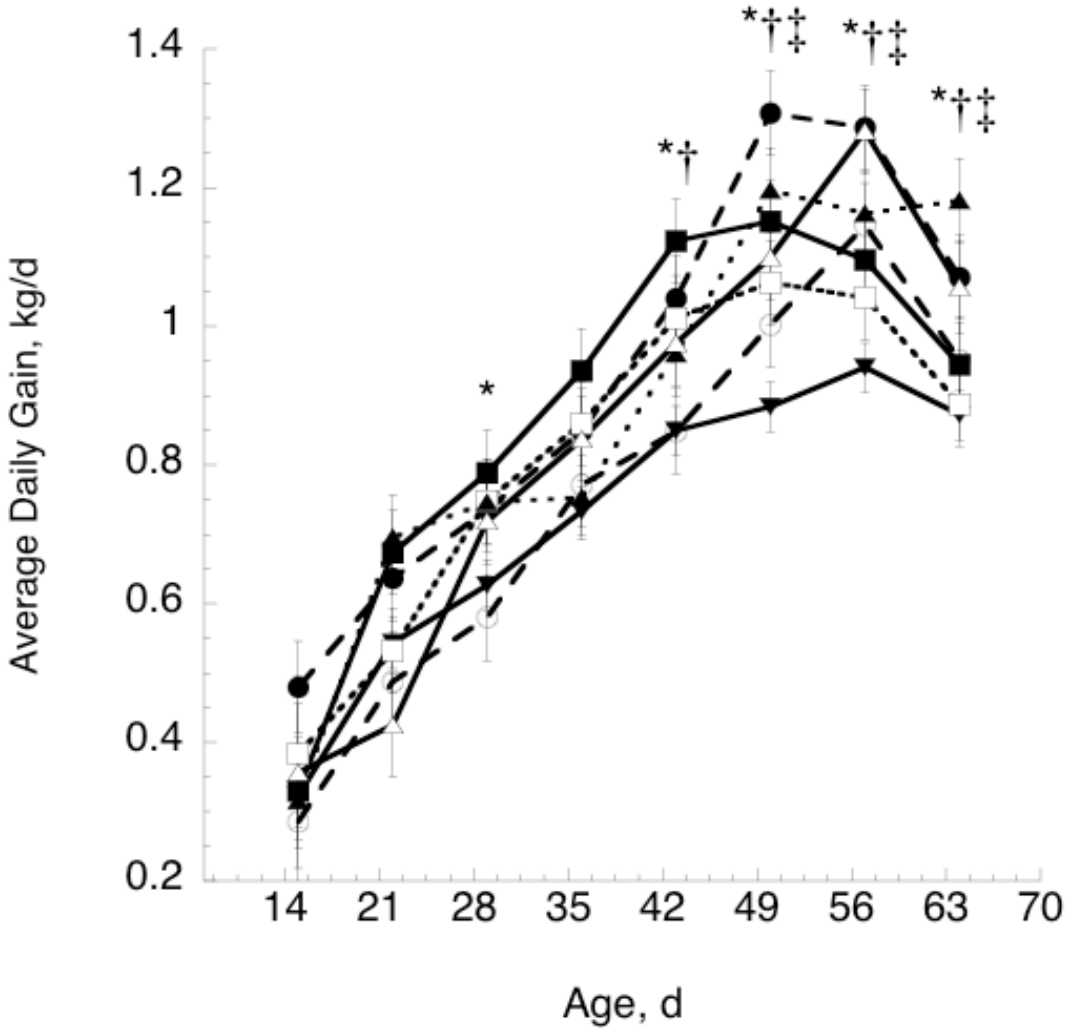
feed intake, ADG, and feed efficiency. However, Hill et al. (2008) mixed the forage source with the starter feed and thus calves were also forced to consume a predetermined fixed forage to concentrate ratio that was also greater than the ratio freely-chosen by calves herein. The incorporation between 10 and 25% of ground or chopped hay or straw into a complete starter feed is generally recommended for calves because it has been reported to increase DMI and ADG (Thomas and Hinks, 1982; Davis and Drackley, 1998). However, the current study illustrates that when forage and starter feed are offered separately, calves choose a proportion less than 10-25% of chopped forage, which improved calf performance.

Compared to CTR, NDF consumption (156 g/d) was greater ($P < 0.05$) in OH (293 g/d), BS (258 g/d), TS (248 g/d), CS (208 g/d), and AH and RH (173 g/d) calves. These differences in NDF consumption were mainly due to the inclusion of a forage source in the supplemented treatments. On the other hand, CP intake was similar among CTR, AH and RH treatments (173 g/d). However, a greater CP intake was observed in calves fed OH, BS, and TS treatments compared to CTR (235 vs. 171 g/d, respectively). Differences in CP intake among treatments were mainly due to variations in starter feed intake. The proportion of total NDF relative to the total diet consumed when a forage source was provided relative to CTR was 3.4, 1.96, 3.26, 2.92, 0.92 and 2.29 percentage units for AH, RH, OH, BS, CS, and TS calves, respectively. In contrast, CP content of the total diet consumed relative to CTR decreased 0.2, 0.5, 1, 0.9, 0.4 and 0.6 percentage units for AH, RH, OH, BS, CS, and TS treatments, respectively.

3.3.2. Apparent nutrient digestibility

There were no differences in total tract apparent DM, OM, and NDF digestibilities (Table 3.4). This may explain the absence of differences in feed efficiency among treatments (Table 3). However, CP digestibility was greater ($P < 0.05$) in AH, RH, and CS treatments compared to CTR, OH, and BS, with TS being in an intermediate position similar to all treatments. Apparent nutrient digestibilities observed herein are in the range previously reported in weaned calves (Terré et al., 2007a; Hill et al., 2010).

Figure 3.2. Evolution of average daily gain (ADG) as affected by forage supplementation. Control (no forage supplementation; —▼—), alfalfa hay (—⊖-), rye-grass hay (—△—), oat hay (—●-), barley straw (-·▲·-), corn silage (--□--), and triticale silage (—■—). *means that ADG was different (P < 0.05) between triticale silage and control treatments, †means that ADG was different (P < 0.05) between oat hay and control treatments, and ‡means that ADG was different (P < 0.05) between barley straw and rye-grass hay vs control treatments.



Generally, high-fiber diets (Porter et al., 2007; Zanton and Heinrichs, 2009) and high DMI (Zanton and Heinrichs, 2008) compromise diet digestibility. However, in the present study, DM, OM, and NDF digestibilities observed in calves supplemented with a forage source were similar to those observed in forage-deprived calves, and CP digestibility of free-choice forage treatments was greater or similar, but never lower, to that observed in CTR animals. In contrast to the current study, Porter et al., (2007)

reported that DM digestibility in animals fed high-fiber diets (27% NDF) was lower than that of calves offered low-fiber diets (20% NDF). It is possible that the decrease in NDF digestibility reported by Porter et al. (2007) was due to greater NDF contents of the total diet consumed by calves than those used in the current study. In the current study, the lack of a decrease in NDF apparent digestibility when offering chopped forages to calves could be associated with a potential improvement of the rumen environment. On the other hand, the improvements of CP digestibility observed with RH, CS, and AH treatments, might be related to the lower TDMI compared to OH, BS and TS treatments. In fact, CP apparent digestibility relative to CTR calves did not improve in OH, BS, and TS calves, probably because a potentially increased passage rate.

Table 3.4. Total tract nutrient apparent digestibility of calves supplemented or not with different forage sources.

	Treatment[1]								
	CTR	AH	RH	OH	BS	TS	CS	SEM[2]	P-value
Digestibility, %									
DM	80.0	81.3	83.0	80.7	80.0	81.2	81.2	1.03	0.43
OM	80.5	82.1	83.5	81.1	80.4	81.5	81.6	1.02	0.42
NDF	50.0	55.8	57.6	54.5	52.8	58.0	52.5	2.85	0.21
CP	76.4^{b}	79.8^{a}	81.0^{a}	76.3^{b}	75.8^{b}	79.1^{ab}	80.7^{a}	1.30	0.01

[1]CTR=control; AH=alfalfa hay; RH=rye-grass hay; OH=oat hay; BS=barley straw; TS=triticale silage; CS=corn silage

[2]SEM=Standard error of the mean

[ab]Means within row with different superscripts differ ($P < 0.05$)

3.3.3. Animal behavior

Table 3.5 depicts the length of time and the odds ratio for each behavioral occurrence (compared to CTR treatment) during 8 h of observations conducted during 2 wk before and 2 wk after weaning. No differences were found in time standing and time devoted consuming forage among treatments. However, AH calves spent less ($P < 0.001$) time eating starter feed and lying without chewing activity ($P < 0.05$) than CTR animals. The differences in lying behavior between AH and CTR treatments could be explained by the fact that AH calves spent more time ruminating, which mainly occurs while lying, and eating forage, which did not occur in CTR calves. Calves in AH and RH treatments devoted more ($P < 0.001$) time to ruminate compared with CTR calves. A reduction of the ruminating activity has been previously observed when forage is not offered (Hodgson, 1971; Phillips, 2004; Martin et al., 2006). Therefore, the increase in time devoted to rumination in AH and RH treatments relative to CTR may be due to having access to a forage source; however, no differences were found in the other forage treatments. It is probable that, the observation sessions were excessively short to allow detecting differences among all treatments relative to CTR.

Calves in the AH and RH groups devoted less ($P < 0.01$) time to perform non-nutritive oral behaviors, and TS calves tended ($P = 0.06$) to perform less non-nutritive oral behaviors than CTR calves. The reduction of non-nutritive oral behavior when AH, RH, and TS were offered to calves is in accordance to the observations by Redbo and Nordblad (1997) and Phillips (2004) who reported that a restrictive allowance of roughage has a considerable effect on the number of bouts and length of oral stereotypies in heifers.

Although there were no differences in starter feed intake between CTR and AH calves, AH animals devoted less time eating starter feed during the observation sessions. This may be explained either because AH calves divided their eating behavior between consuming starter feed and consuming forage, or by a hypothetical increase in eating rate for AH compared to CTR calves.

Table 3.5. Total time (in minutes) devoted to perform different behaviors during 8 h of observations[1,2].

	Treatment[3]						
	CTR	AH	RH	OH	BS	CS	TS
Standing, min	155.6	181.5 (0.85)	154.3 (0.88)	184.3 (0.98)	176.9 (1.15)	192.4 (1.30)	192.7 (1.17)
Lying, min	272.3	200.7 (0.74)**	255.3 (0.91)	230.1 (0.73)	224.9 (0.76)	205.7 (0.77)	221.6 (0.86)
Eating starter, min	15.3	16.9 (0.38)**	11.9 (0.77)	12.4 (1.10)	9.0 (0.94)	14.3 (1.39)	17.2 (0.86)
Eating forage, min	-	23.1 (1.00)	9.2 (0.97)	8.8 (0.75)	12.7 (1.70)	11.4 (1.93)	8.1 (0.96)
Ruminating, min	15.7	45.0 (5.24)**	35.0 (5.40)**	32.7 (2.92)	49.8 (1.40)	43.2 (1.52)	22.9 (2.32)
NNOB4, min	21.1	12.8 (0.38)**	14.4 (0.34)**	11.7 (0.79)	6.8 (1.34)	13.0 (0.58)	17.5 (0.21)*

[1] Observations were conducted between 2 wk before and 2 wk after weaning by calves offered a free choice of forage

[2] The respective probability of each behavior performed by each forage-supplemented calves relative to those performed by forage-deprived calves, odds ratio, is shown in parenthesis

[3] CTR=control; AH=alfalfa hay; RH=rye-grass hay; OH=oat hay; BS=barley straw; TS=triticale silage; CS=corn silage

[4] Non-nutritive oral behavior

*P<0.10

**P<0.05

3.4. Conclusions

Generally, the provision of chopped grass hays or grass silages improved average daily gain and total dry matter intake in pre-weaned calves without impairing nutrient digestibility and gain to feed ratio. However, these benefits were not observed when chopped alfalfa hay was offered. Consequently, the greatest starter feed intake and best performances were obtained when chopped oat hay, chopped barley straw, or triticale silage was offered ad libitum to young calves from 2 wk of life to weaning.

Chapter 4

EFFECT OF NON-FORAGE FIBER SOURCES ON PERFORMANCE AND FEEDING BEHAVIOR OF HOLSTEIN CALVES

4.1. Introduction

Fiber inclusion in the diet of preweaned calves has been studied during the last years (Suárez et al., 2007; Castells et al., 2012; Khan et al., 2011). Forage inclusion in the diets of young calves was believed to be negative for calf's development, due to its detrimental effect on intake and consequently to growth. However, several studies have shown benefits when forage was included in the diet of young calves (Thomas and Hinks, 1982; Khan et al., 2011). These authors observed an increase in starter feed and total dry matter intake, and greater ADG when forage was offered ad libitum to young calves, without any effect on the gain to feed ratio of those calves. Some authors (Thomas and Hinks, 1982; Castells et al., 2013) attributed the greater starter feed intake to a better rumen environment, greater rumen pH compared with non-forage supplemented calves, which allowed to increase starter intake of forage supplemented calves. Therefore, forage supplementation in the diet of preweaned calves can be a good strategy to foster starter concentrate intake at weaning to avoid a slump in ADG from the pre- to postweaning period. However, there is a wide variety of industry by-products (distiller's dried grains, soybean hulls, dried citrus pulp, cotton seed hulls,...) that are commonly used in the formulation of cattle feeds, which are rich in fiber and they could be use as forage substitute to improve rumen environment of young calves, and consequently stimulate starter intake. Some industry dry feeds by-products have been studied to increase coarseness of calf starters (Hill et al., 2008) or to improve welfare, especially in veal calves. Morisse et al. (2000) concluded that a pellet concentrate based on ground barley and straw, with 50% NDF improved veal calf welfare without compromise its performance. The objective of this study was to assess the viability of using industry by-products rich in fiber to promote starter feed intake and animal growth of pre- and postweaned calves.

4.2. Materials and methods

4.2.1. Animals and Treatments

Fifty-two Holstein male calves (initial BW = 44.5 ± 5.47 kg, initial age = 17.5 ± 6.04 d) were used in the present study. Calves were purchased from commercial farms, raised in the facilities of Torre Marimon (Caldes de Montbui, Spain), and managed according to the recommendations of the Animal Care Committee of IRTA. Calves were housed in

individual pens (1.6 x 1.0 m), and bedded with wood shavings. Calves were randomly assigned, according to weight and age, to one of three different dietary treatments that consisted on a pellet starter concentrate (Table 4.1) plus a free access to soybean hulls (**SBH**), or to dried citrus pulp (**DCP**), or without any access to an extra fiber source (**CTR**). The nutrient composition of the fiber sources studied is depicted in Table 1. A commercial milk replacer (MR) (25% CP and 19.2% fat, Sprayfo Excellent 60, Sloten BV, Holland) was offered in 2-L feeding bottles twice daily at 0700 and 1700 h. Calves received 4 L/d of MR at 12.5% DM dilution rate until 50 d of age. From 50 to 56 d of age, calves received only the morning feeding of 2 L at 12.5% DM. Animals were weaned at 57 d of age. Study ended at 70 days of age. Starter feed, fiber source, and water were offered ad libitum in separate buckets throughout the study.

4.2.2. Measurements and Sample Collection

Starter feed, MR, and fiber source intakes were recorded daily on an individual basis. Calves were weighed weekly.

4.2.3. Chemical Analysis

Samples of MR were analyzed for DM (24 h at 103ºC), ash (4 h at 550ºC), N content using the AOAC (1990) method (988.05) adapted for an automatic distiller Kjeldhal (Kjeltec Auto 1030 Analyser, Tecator, Sweden) with CuSO4/Se as a catalyst instead of CuSO4/TiO2, and ether extract using the AOAC method (920.39) using petroleum ether for distillation instead of diethyl ether (AOAC, 1990). Samples of starter feed and fiber sources were analyzed for DM, ash, and CP following the same methods described above, plus NDF with sodium sulphite and heat-stable alpha-amylase (Van Soest et al., 1991), and ADF following the AOAC (1990) method (973.18).

Table 4.1. Ingredients and chemical composition of the experimental concentrate feed and fiber sources.

	Concentrate feed	Dried Citrus Pulp	Soy Bean Hulls
Ingredient composition, % DM			
Wheat	20.0	-	-
Corn	15.0	-	-
Barley	11.2	-	-
Sorghum	12.0	-	-
Soybean meal	23.0	-	-
Wheat middling	12.0	-	-
Soybean hulls	5.0	-	-
Premix[1]	0.2	-	-
Calcium carbonate	0.5	-	-
Dicalcium phosphate	0.3	-	-
Sodium chloride	0.8	-	-
Nutrient composition, % DM			
CP	21.0	7.1	12.0
NDF	14.4	29.8	63.8
ADF	6.1	23.1	46.5
Ash	5.6	3.1	5.5

[1] Premix composition: Vitamin A 2,007,000 IU/kg; Vitamin D_3 433,000 IU/kg; Vitamin E 3,685 mg/kg; Vitamin B_1 52 mg/kg; Vitamin B_2 197 mg/kg; Vitamin B_6 98 mg/kg; Vitamin B_{12} 0.76 mg/kg; Vitamin K_3 52 mg/kg; nicotinic acid 656 mg/kg; pantothenic acid 394 mg/kg; Mn 5,877 mg/kg; Fe 7,093 mg/kg; Cu 2,026 mg/kg; Co 46 mg/kg; Zn 8,112 mg/kg; I 304 mg/kg; Se 46 mg/kg

4.2.4. Animal Behavior

Behavior was monitored by direct observations for 10 animals within treatment once a week during 2 wk before and 2 wk after weaning. The weeks before weaning, calves were observed for 1 h immediately after the morning MR feeding, and for 1 h after concentrate was offered. After weaning, calves were observed for 1 h before offering the solid feed and 1 h after it. Therefore, total observation time per animal was 8 h for the entire monitoring period. Calves were observed every minute, and the observer

recorded the occurrence of the following behaviors: lying, standing, eating starter feed, eating forage, ruminating (either lying or standing), and non-nutritive oral behaviors (when the animal licked any surface, tongue rolled, or consumed wood shavings).

4.2.5. Statistical Analysis

Performance and intake data were analyzed with a mixed-effects model for repeated measures. The statistical model included initial BW as a covariate, treatment, week of study and their 2-way interaction as fixed effects, and animal as random effects. Due to the lack of normality, data from starter feed and total DM intake (TDMI) were root-square transformed, and data from forage intake was natural log transformed. Least square means presented herein for these parameters correspond to non-transformed data, and SEM and P-values correspond to the results from the mixed-effects model using root-square and natural log transformed data.

Behavior data were summarized individually as the time (min) devoted to each monitored behavior before and after weaning (2 h per wk and animal during 2 wk before and 2 wk after weaning; therefore 4 h per animal before and 4 h per animal after weaning). After that, a mixed-effects Poisson regression analysis including calf and period as random effects, and treatment and time relative to weaning (before or after) as fixed effects was performed.

4.3. Results and discussion

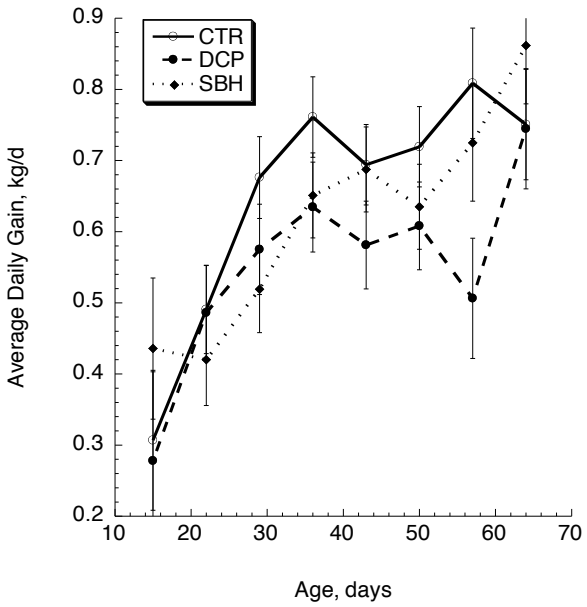
4.3.1. Performance

Results of animal performance and feed intake during the pre- and postweaning period are presented in Table 4.2. During the preweaning period, animals without access to any fiber source consumed more ($P < 0.01$) starter than those animals in SBH and DCP treatments. Intake of NDF was not different among treatments, probably due to the low intake of the supplemental fiber source and concentrate feed of SBH and DCP calves. However, CP intake was greater ($P < 0.05$) in CTR compared with DCP animals, but not different to SBH animals. This result could be explained due to the greater content in CP of SBH (12%) compared with DCP (7.1%). Even animals fed non-forage fiber source showed a decrease in starter feed intake, no differences among

treatments were observed in ADG and gain to feed ratio. Finally, TDMI of CTR animals expressed as percentage of BW tended (P = 0.08) to be greater than DCP animals.

During the postweaning period, concentrate intake of CTR and SBH animals was greater (P < 0.01) than those of DCP animals. Fiber source intake tended (P = 0.08) to be greater in DCP animals than in SBH animals. Even fiber source intake of DCP animals was more than two times greater than that of SBH animals, it did not compensate the low starter intake of DCP animals. Total DM intake of CTR was greater (P < 0.05) than that of DCP animals, but not different than that of SBH animals after weaning. Total DM intake expressed as percentage of BW was greater (P < 0.05) in CTR and SBH compared with DCP animals. Intake of NDF was not different between treatments, but CTR and SBH animals had a greater (P < 0.01) intake of CP compared with DCP animals. Finally, CTR and SBH animals tended (P = 0.07) to grow more than DCP calves the first week after weaning (Figure 4.1), but differences in the gain to feed ratio were not observed among treatments.

Figure 4.1. Evolution of average daily gain (ADG) as affected by non-forage fiber source supplementation.* ADG tended to be greater (P = 0.07) in control and soybean hulls fed animals compared with dried citrus pulp fed animals.



Greater intake of DCP than SBH was expected because of the different content of NDF in both non-forage fiber sources. Generally, in forages, the greater the cell wall content the lower the voluntary intake of that forage (Colburn et al., 1968).

Similar to the present study, Hill et al. (2008) did not find any positive effect in a serie of 2 studies comparing the inclusion of cottonseed hulls, a non-forage fiber source, at the rate of 5% or 10% in a texturized concentrate feed. On the other hand, several authors (Thomas and Hinks, 1982; Khan et al., 2011; Castells et al., 2012) have shown an increase of the intake of a pelleted concentrate when a forage source was offered in the diet ad libitum, probably due to a better rumen environment.

Intake regulation in ruminants can be affected by several factors, such as ruminal fill, or metabolic and rumen fermentation parameters. Although rumen VFA concentrations were not determined in the present study, fermentation of DCP and SBH usually results in an increase of the acetic acid, and a decrease in propionic acid (Ipharraguerre and Clark, 2003; Bampidis and Robinson, 2006). Therefore, the hypophagic effects of propionate (Allen et al., 2009) could not be the cause of depressing DM intake of DCP and SBH treatments. Voelker and Allen (2003) observed a decrease in DMI in dairy cows when high-moisture corn was replaced by beet pulp, a byproduct rich in pectin as DCP and SBH are. They attributed this intake depression to the filling effects of beet pulp in the rumen, since they observed a tendency for apparent increase in digesta wet weight. The filling effects hypothesis may explain the depression in starter intake of DCP and SBH in preweaned calves. Although calves consumed more DCP than SBH, pectin content of SBH (25%) is greater than that of DCP (15%), and roughly they result in similar daily pectin intake, estimated in 7.5 and 5 g/d in DCP and SBH, respectively. Even there were not improvement in starter feed intake when non-forage fiber sources were offered in the diet, animals fed with SBH in the diet show similar performance results after weaning than CTR animals.

The low concentrate feed intake observed in DCP calves during the preweaning period may have caused the decrease in the ADG at weaning. Similarly, when calves were fed an enhanced-growth feeding program low concentrate fed intake during the preweaning period were reported (Terré et al., 2006), causing a low rate of growth after weaning.

Table 4.2. Performance and feed intake of calves supplemented or not with different fiber sources during the pre- and postweaning period.

	Treatment[1]				P-value[2]	
	CTR	DCP	SBH	SEM[3]	T	Txt
Initial BW, kg	44.3	45.0	44.3	1.34	0.92	-
Final BW, kg	80.9	76.4	78.2	2.55	0.45	-
Preweaning, from 15 to 56d of age						
ADG, kg/d	0.61	0.53	0.66	0.042	0.40	0.61
Feed intake, kg DM/d						
Milk	0.44	0.44	0.44	0.0003	0.56	0.71
Starter	0.56[a]	0.27[b]	0.36[b]	0.046	<0.01	0.13
Fiber source	-	0.05	0.02	0.343	0.13	0.14
TDMI [4]	1.07	0.94	0.96	0.030	0.25	0.28
NDF	0.08	0.08	0.09	0.020	0.66	0.63
CP	0.12[a]	0.07[b]	0.09[ab]	0.019	<0.05	0.13
DMI, %BW	2.0	1.7	1.8	0.08	0.09	0.08
Gain to feed[5]	0.54	0.54	0.57	0.019	0.69	0.09
Postweaning, from 57 to 70 d of age						
ADG, kg/d	0.78	0.63	0.79	0.068	0.18	0.07
Feed intake, kg DM/d						
Starter	1.97 [a]	1.24[b]	1.74[a]	0.024	<0.01	0.27
Fiber source	-	0.09	0.03	0.438	0.08	0.87
TDMI [4]	1.97[a]	1.58[b]	1.89[ab]	0.042	<0.05	0.14
NDF	0.29	0.27	0.33	0.021	0.21	0.25
CP	0.41[a]	0.29[b]	0.38[a]	0.022	<0.01	0.19
DMI, %BW	2.6[a]	2.3[b]	2.6[a]	0.11	<0.05	0.06
Gain to feed[5]	0.39	0.43	0.43	0.024	0.38	0.66

[1]CTR=calves without access to a fiber source; DCP=calves with free access to dried citrus pulp; SBH=calves with free access to soybean hulls

[2]T=treatment effect; Txt=interaction between treatment and time

[3]SEM=Standard error of the mean

[4]TDMI=Total dry matter intake (milk replacer, starter feed, and fiber)

[5] kg BW gain/kg TDMI

[ab]Means within row with different superscripts differ (P < 0.05)

4.3.2. Animal behavior

Odds ratio of behaviors observed during the observation sessions in the present study are presented in Table 4.3. Animals in DCP treatment dedicated less ($P < 0.01$) time eating starter feed than CTR animals (odds ratio 0.17). Moreover, DCP animals spent less ($P < 0.05$) time eating the fiber source than SBH animals (odds ratio 0.06). Results in time dedicated to eat starter feed could be expected since DCP animals showed a lower starter feed intake compared with CTR animals. The reduction in time eating fiber in DCP animals compared with SBH animals, even though DCP showed a greater intake of fiber source, could be hypothesized because of an increase in the rate of intake of DCP compared with SBH. Time dedicated to ruminate during the observation sessions was greater ($P < 0.05$) in SBH animals compared with CTR animals. The exclusion of fiber in the diet of ruminants has been shown to entail a reduction in the rumination (Hodgson, 1971; Phillips, 2004; Martin et al., 2006; Faleiro et al., 2011). Citrus pulp did not stimulate rumination at the same level as SBH probably due to the different proportion of fiber (NDF and ADF). Due to the greater time dedicated to ruminate by SBH animals, these animals also tended ($P < 0.10$) to show a reduced time spent lying. This is, generally, because animals ruminate while are lying. Finally, animals consuming DCP devoted less ($P < 0.05$) non-nutritive oral behaviors compared with CTR animals.

Table 4.3. Probability (odds-ratio) of calves offered a free choice of fiber sources relative to control of standing, lying, eating starter feed, eating fiber, ruminating and performing non-nutritive oral behaviors.

	Treatment[1]	
	DCP	SBH
Standing	1.01	1.16
Lying	1.01	0.87*
Eating starter	0.17**	0.97
Eating fiber	0.06**	-
Ruminating	0.27	13.74**
NNOB[2]	0.11**	0.90

[1]DCP=dried citrus pulp; SBH=soybean hulls

[2]Non-nutritive oral behavior

*P < 0.10

**P < 0.05

4.4. Conclusions

In conclusion, the inclusion of non-forage fiber source in the diet of young calves, reduces starter intake, specially dried citrus pulp, without impairing ADG during the preweaning period, but impairing ADG after weaning in DCP calves. The supplementation of DCP decreases non-nutritive oral behaviors, and the inclusion of SBH in the diet of young calves stimulates rumination.

Chapter 5

EFFECTS OF FORAGE PROVISION TO YOUNG CALVES ON RUMEN FERMENTATION AND DEVELOPMENT OF THE GASTROINTESTINAL TRACT

A fraction of this research has been published in:

Journal of Dairy Science, 2013. *in press*

5.1. Introduction

During recent years several studies have attempted to define the most appropriate solid diet for calves during the preweaning period (Bach et al., 2007; Khan et al., 2011; Castells et al., 2012). However, there is no agreement about the optimum solid feed strategies for preweaned calves. Use of fibrous feeds has been discouraged due to the limited use of cellulose during the preweaning period, and because the accumulation of undigested forage material in the rumen could decrease voluntary intake of concentrate (Drackley, 2008). On the other hand, when chopped grass hay or grass silages were offered to young dairy calves during the pre and postweaning period separately from the starter feed, calves consumed a low amount of forage, but it was sufficient to foster an increase in concentrate intake and improving ADG (Castells et al. 2012). Similarly, Thomas and Hinks (1982) have shown improvements in performance when offering barley straw and Khan et al. (2011) reported increases in total solid feed intake and physical development of the reticulorumen when orchard grass hay was fed. In contrast, when the forage fed is alfalfa hay, these benefits have not been observed (Castells et al., 2012). Although there is a positive effect of the inclusion of forage (other than alfalfa) in the diet of young calves, the potential mechanisms that elicit improvements of DMI and ADG are not clear. Castells et al. (2012) hypothesized that the increased intake observed in calves offered forage could be due to an improvement of the rumen environment. Furthermore, Khan et al. (2011) found that calves offered forage presented greater rumen liquid pH compared with those calves fed no forage. Similarly, Thomas and Hinks (1982) reported a positive relationship between postweaning intake and rumen liquid pH, and highlighted the importance of rumen liquid pH on explaining variations in concentrate intake.

The objective of this study was to investigate some potential factors related to rumen and gastrointestinal tract (GIT) development that may be involved in intake regulation of young calves when a grass or legume forage is offered.

5.2. Materials and methods

5.2.1. Animals and treatments

Fifteen Holstein male calves were purchased from commercial farms and raised at the facilities of IRTA (El Prat, Spain). Calves were managed according to the

recommendations of the Animal Care Committee of IRTA, and had the approval of national animal care committee (number 6271). After arrival, calves were given a broad-spectrum antibiotic (Draxxin, tulathromycin, Pfizer Animal Health, Spain) to minimize respiratory disease. Also, calves were vaccinated against respiratory syncytial virus (Rispoval RS, Pfizer Animal Health, Spain) 3 d after arrival. Calves were housed in individual wooden pens (2.0 x 1.55 m) and bedded with sawdust in a barn with forced ventilation. The study began when calves were 8.3 ± 2.96 d old and 43.7 ± 4.39 kg of BW. Animals were stratified according to BW and age in 3 groups. Calves in the control (CON) group were fed a starter concentrate (pelleted) without any forage supplementation until the end of the study. The other 2 treatments were offered the same starter concentrate as the CON group plus a source of chopped forage fed separately (Table 5.1). Forages were either chopped alfalfa (AH) or chopped oat hay (OH). Forages were chopped (particle size distribution measured with two sieves with openings of 20 and 8 mm: 38.4% >20 mm, 16.9% between 8-20 mm and 44.7% <8 mm for alfalfa hay; and 27.5% >20 mm, 26.3% between 8-20 mm and 46.2% <8 mm for oat hay) with a forage-chopper machine (Seko, Curtarolo, Italy). Calves were fed milk replacer (MR) (25% CP and 19.2% fat, Sprayfo Excellent 60, Sloten BV, Holland) at 12.5% DM dilution rate. Calves received 4 L of MR at 12.5% DM dilution rate until 50 d of age. From 50 to 56 d of age, calves received only the morning feeding of 2 L at 12.5%. Animals were weaned at 57 d of age and the study ended 3 wk after weaning.

5.2.2. Measurements and sample collection

Starter feed, MR, and forage intakes were recorded daily on an individual basis. Body weight was recorded weekly. A sample of rumen liquid was taken using an oral tube every week. A 50-mL subsample was immediately frozen to -80ºC for subsequent bacteria population analyses. The remainder of the sample was filtered through 2 layers of cheesecloth, and the pH was immediately measured with a pH meter (Crison pH25, Spain). Also, 4 mL of the filtered rumen liquid were mixed with 1 mL of solution containing 2 g/L of mercuric chloride, 20 mL/L orthophosphoric acid, and 2 g/L of 4-methylvaleric (internal standard) in distilled water and stored at -20ºC to analyze concentrations of volatile fatty acids (VFA).

Table 5.1. Ingredient and chemical composition (as % of DM) of experimental starter feed and forages.

	Concentrate	Alfalfa hay	Oat hay
Ingredient			
Wheat	20.0	-	-
Corn	15.0	-	-
Barley	11.2	-	-
Sorghum	12.0	-	-
Soybean meal	23.0	-	-
Wheat middlings	12.0	-	-
Soybean hulls	5.0	-	-
Premix[1]	0.2	-	-
Calcium carbonate	0.5	-	-
Dicalcium phosphate	0.3	-	-
Sodium chloride	0.8	-	-
Nutrient composition			
CP	20.4	17.0	9.7
NDF	21.1	47.9	72.1
ADF	9.8	41.8	46.2
Ash	5.0	7.1	7.1

[1]Premix composition: vitamin A 2,007,000 IU/kg; vitamin D3 433,000 IU/kg; vitamin E3 685 mg/kg; vitamin B1 52 mg/kg; vitamin B2 197 mg/kg; vitamin B6 98 mg/kg; vitamin B12 0.76 mg/kg; vitamin K3 52 mg/kg; nicotinic acid 656 mg/kg; pantothenic acid 394 mg/kg; Mn 5,877 mg/kg; Fe 7,093 mg/kg; Cu 2,026 mg/kg; Co 46 mg/kg; Zn 8,112 mg/kg; I 304 mg/kg; Se 46 mg/kg.

Total GIT passage rate was estimated 2 wk after weaning using chromic oxide as an external marker. Animals were orally dosed before the morning concentrate offer with a single dose of 2 g chromic oxide in a gelatin capsule and fecal grab samples were taken at 4, 8, 12, 16, 20, 24, 28, 32, 48, 56, 72, 80, 96 and 120 h after dosing. Fecal samples were frozen immediately after collection for subsequent chromium determination.

Finally, 3 wk after weaning, between 0800 and 1100, calves were euthanized following the European Guidelines for Animal Welfare (Directive 86/609 EEC) with an

intravenous injection (200 mg/kg of BW) of pentobarbital (Dolethal, Vetoquinol, France). Animals were abdomen opened, and each individual anatomical part (rumen, reticulum, omasum, abomasum, duodenum, jejunum, ileum, cecum, colon, rectum) of the GIT was separated and weighed. Then, the contents of each section were collected and weighed, and the respective GIT parts were weighed again to determine mucosal empty weight. The pH of the content of each individual part of the GIT was measured. Samples from the dorsal sac of the rumen and parotid gland tissue were taken after death and preserved in RNAlater® solution (Ambion, USA) for subsequent analysis of mRNA gene expression. Additionally, a sample of rumen content was obtained to determine DM and buffering capacity. Furthermore, homogenized samples of rumen and cecum contents were obtained to analyze viscosity and bacterial populations. Samples taken for the analysis of bacterial population were immediately frozen to -80ºC and stored until subsequent analysis. Moreover, 4-mL samples from the cecum contents were immediately acidified with 1 mL of a solution containing 2 g/L mercuric chloride, 20 mL/L orthophosphoric acid, and 2 g/L 4-methylvaleric (internal standard) in distilled water, and stored at -20ºC for subsequent VFA analyses. Tissue samples from the cranio-dorsal rumen wall were excised and preserved by immersion in 10% (v:v) phosphate-buffered formalin for histomorphometric analysis.

5.2.3. Analytical procedures

Samples of starter, forage and MR were analyzed for DM (24h at 103ºC), ash (4 h at 550ºC), N content, ether extract, NDF, and ADF. N content was measured using the AOAC (1990) method (988.05) adapted for an automatic distiller Kjeldahl (Kjeltec Auto 1030 Analyser, Tecator, Sweden) with copper sulphate/selenium as a catalyst instead of copper sulphate/titanium dioxide. Ether extract was measured using the AOAC method (920.39) using petroleum ether for distillation instead of diethyl ether (AOAC, 1990). NDF was analyzed with sodium sulphite and heat-stable alpha-amylase (Van Soest et al., 1991), and ADF following the AOAC (1990) method (973.18). The content of VFA in the ruminal fluid was analyzed by gas chromatography (HP-6890 Series II Agilent, Hewlett Packard, Palo Alto, CA) using a capillary column (10 m x 0.10 mm x 100 µm; DB5, Agilent, Palo Alto, CA). The determination of chrome concentration in feces was

conducted by atomic absorption spectrophotometry according to the method described by Williams et al. (1962).

Total RNA from ruminal and salivary gland tissue samples was extracted using Trizol (Invitrogen, Paisley, Scotland). One microgram of RNA was retrotranscribed to cDNA using IScript cDNA synthesis kit (Bio-Rad, California, USA) following manufacturer's instructions. Real time PCR was performed using specific primers. Primers were designed either using the Vector NTI Advance software package or using primers previously reported in the literature (Table 5.2). The target genes were: monocarboxylate transporter 1 (MCT1), monocarboxylate transporter 4 (MCT4), down regulated in adenoma (DRA), Na+/H+ exchange 1 and 3 (NHE1 and NHE3), and SPC25 for rumen tissue, and aquaporin-5 (AQ5), and calcium activated chloride channel (TMEM16) for parotid gland tissue. A total reaction volume of 20 µL was used, containing 100 ng of cDNA, 10 µL of SYBER Green Fluorescent (Bio-Rad, California, USA), and the optimized primer concentration for each gene (see Table 5.2). The PCR reactions were conducted using a thermocycler iCycler (Bio-Rad, California, USA) and cycled as follows: an initial denaturing step of 10 min at 95°C, followed by 40 cycles of 10 s at 95°C, 15 s at optimized annealing temperature for each gene, 30 s at 72°C and a final extension of 5 min at 72°C. Gene expression values were evaluated using the ΔCt method with β-actin as the housekeeping gene.

To study rumen microbiota, samples of ruminal content were thawed and centrifuged at 6,654 g for 15 min. Supernatant was discarded, the pellet homogenized and a subsample of 0.25 g was weighed. From each sample, DNA was extracted by bead-beating in the presence of high concentrations of sodium dodecyl sulfate, salt, and EDTA, and with subsequent DNA purification by QIAmp DNA Stool Mini Kit columns (Qiagen, Hilden, Germany) (Yu and Morrison, 2004). Real time PCR was performed using specific primers. Primers were designed using the Vector NTI Advance software package (Table 5.2). Microorganisms evaluated were Streptococcus bovis and Ruminococcus albus. A total reaction volume of 20 µL containing 100 ng of cDNA, 10 µL of SYBER Green Fluorescent (Bio-Rad, California, USA), and the optimized primer concentration for each gene (see Table 5.2) was used, the PCR reactions were conducted using a thermocycler iCycler (Bio-Rad, California, USA) and cycled as follows: an initial denaturing step of 10 min at 95°C, followed by 40 cycles of 30 s at

95°C, 10 s at optimized annealing temperature for each gene, 30 s at 72°C, and a final extension of 5 min at 72°C.

Table 5.2. Gene names, primer sequences, annealing temperature, primer concentration, and references of the used genes.

Gene name	Primer sequence		Tm	µM	Reference
β-actin (ACTB)	Fw	CTGGACTTCGAGCAGGAGAT	57ºC	0.125	-
	Rv	CCCGTCAGGAAGCTCGTAG			
Monocarboxylate transporter 1 (MCT1)	Fw	CAATGCCACCAGCAGTTG	55ºC	0.5	Graham, 2007
	Rv	GCAAGCCCAAGACCTCCAAT			
Monocarboxylate transporter 4 (MCT4)	Fw	AGCGTCTGAGCCCAGGGAGG	55ºC	0.5	-
	Rv	ACCTCGCGGCTTGGCTTCAC			
SPC25	Fw	TAGGAAAGCTGGCTTGGCTGC	62ºC	0.5	-
	Rv	GACAAGGCTTCTCCGGCAGGC			
Down regulated in adenoma (DRA)	Fw	TGCACAAAGGGCCAAGAAA	60ºC	0.5	Laarman et al., 2012
	Rv	GCTGGCAACCAAGATGCTATG			
Sodium/proton exchanger 1 (NHE1)	Fw	GAAAGACAAGCTCAACCGGTTT	60ºC	0.5	Laarman et al., 2012
	Rv	GGAGCGCTCACCGGCTAT			
Sodium/proton exchanger 3 (NHE3)	Fw	AGCCTTCGTGCTCCTGACA	60ºC	0.5	Laarman et al., 2012
	Rv	TGACCCCTATGGCCCTGTAC			
Aquaporin 5 (AQ5)	Fw	GCCTGTCCGTCACACTGGGC	57ºC	0.5	-
	Rv	CCCAGTCCTCGTCCGGCTCA			
Calcium activated chloride channel (TMEM16)	Fw	ACTTCGCCTGGCTTGGCGTC	58ºC	0.5	-
	Rv	CTTGTCGCAGAGCGGGCACA			
Streptococcus bovis	Fw	ATTCTTAGAGATAGGGTTTCTCTT	60ºC	0.5	-
	Rv	ACCTTATGATGGCAACTAACAATA			
Ruminococcus albus	Fw	CCCTAAAAGCAGTCTTAGTTCG	60ºC	0.5	Wang et al., 1997
	Rv	CCTCCTTGCGGTTAGAACA			

Buffering capacity was measured according to Tucker et al. (1992). Viscosity was measured in ruminal and cecal samples after centrifugation at 12,000 g for 10 min. The supernatant was withdrawn and the viscosity was determined using a Brookfield Digital DV-II cone/plate viscometer (Brookfield Engineering Laboratories, Inc, MA, USA)

maintained at 30º C and a shear rate of 12-60 s^{-1}. Absolute viscosity at a shear rate of 60 s^{-1} is presented.

Tissue samples for the histological morphometric study were dehydrated and embedded in paraffin wax, sectioned at 4 mm and stained with haematoxylin and eosin. Morphometric measurements were performed with an optical microscope (BHS, Olympus, Hamburg, Germany) using a grid ocular (Olympus, Microplanet, Hamburg, Germany). Rumen papillae height and width, keratin layer, and muscular width were measured on all well-oriented rumen papillae. All morphometric measurements were performed by the same person, who had no prior knowledge of the correspondence between samples and treatments.

5.2.4. Statistical analysis

Data from performance and rumen parameters were analyzed using a mixed-effects model for repeated measures. The statistical model included treatment, time (week of study), and their interaction as fixed effects, and animal within treatment as a random effect. Compound symmetry was used as variance-covariance structure. All statistical analyses were performed with SAS (Version 9.2; SAS Inst. Inc, Cary, NC).

Fractional rate of passage in the rumen (k_r) and cecum (k_c), transit time (TT) in the tubular compartment of the GIT, and total mean retention time (TMRT) of the marker in the GIT were calculated according to the multicompartmental model proposed by Dhanoa et al. (1985). Fractional rate of passage, TT, TMRT, and data obtained from slaughtered animals were analyzed using an ANOVA with treatment as main effect.

5.3. Results and discussion

5.3.1. Performance

Animal performance and feed consumption are presented in Table 5.3. During the preweaning period, there were no differences in starter concentrate intake among treatments. Forage intake was greater (P = 0.01) in AH compared with OH calves. In spite of the additional forage consumption of AH and OH animals, there were no differences in total dry matter intake (TDMI) among treatments. Similarly to concentrate intake, there were no differences in ADG and the gain to feed ratio was also similar among treatments. During the postweaning period, intake results were

similar to those observed during the preweaning period. Calves had similar starter intake among treatments, and AH calves consumed more forage than calves in OH treatment. However, during the postweaning period, OH animals tended (*P* = 0.07) to grew more than AH and CON animals.

Table 5.3. Performance and feed intake of calves supplemented with alfalfa (AH) or oat hay (OH), or not supplemented (CON).

	Treatments[1]				P-value[2]		
Item	CON	AH	OH	SEM[3]	F	T	F × t
Initial BW, kg	44.7	43.2	44.1	1.96	0.86	-	-
Final BW, kg	89.8	92.3	98.6	5.99	0.58	-	-
Preweaning (from 8 to 56 d of age)							
ADG, kg/d	0.53	0.62	0.66	0.085	0.54	<0.001	0.16
Intake, kg DM/d							
Starter	0.54	0.62	0.66	0.089	0.64	<0.001	0.74
Forage	-	0.11[a]	0.02[b]	0.021	0.01	<0.001	<0.01
TDMI[4]	0.99	1.17	1.13	0.090	0.35	<0.001	0.74
Gain to feed5	0.61	0.60	0.60	0.034	0.95	<0.001	0.11
Postweaning (from 57 to 70 d of age)							
ADG, kg/d	0.79	0.82	1.29	0.149	0.07	0.09	0.36
Intake, kg DM/d							
Starter	1.90	2.08	2.61	0.271	0.20	<0.001	0.13
Forage	-	0.29[a]	0.13[b]	0.046	<0.01	0.45	0.85
TDMI[4]	1.90	2.37	2.74	0.299	0.18	<0.001	0.16
Gain to feed[5]	0.45	0.37	0.48	0.059	0.42	0.62	0.73

[a,b]Numerical values within a row with different superscripts differ (P<0.05)

[1]CON = control; AH = alfalfa hay; OH = oat hay.

[2]F = forage supplementation effect; t= effect of time; F × t = interaction between forage supplementation and time.

[3]SEM= Standard error of the mean

[4]TDMI = total DMI (milk replacer, starter feed, and forage).

[5]Kilograms of BW gain/kg of TDMI

Previous studies (Thomas and Hinks, 1982; Castells et al., 2012) have shown an increase in concentrate intake and growth performance in young calves supplemented

with forage. In the present study, even no differences were observed among treatments, animals fed forage had a numerically greater concentrate intake and BW gain compared with animals without access to a forage source. The lack of significant results was probably due to the low number of animals used in this study (the objective was not to evaluate differences in performance but in GIT parameters). On the other hand, the greater consumption of AH was expected because voluntary intake of legumes is usually greater than that of grasses (Colburn et al., 1968; Moseley and Jones, 1979).

5.3.2. Rumen fermentation parameters

Total VFA concentration and individual proportion of VFA, as well as pH of ruminal liquid are presented in Table 4. Animals supplemented with a forage source presented a greater ($P < 0.01$) rumen liquid pH compared with CON calves. On the other hand, CON animals showed the greatest ($P < 0.05$) total rumen VFA concentration from the week prior to the weaning to the end of the study. Calves receiving the AH treatment presented a different VFA fermentation profile compared with the other treatments. Rumen acetate proportions in AH calves increased ($P < 0.01$) from wk 3 to 6 at the expenses of valerate which was lesser in the rumen liquid of AH calves than in that of calves in other treatments throughout the study. Furthermore, the acetate:propionate ratio tended ($P = 0.09$) to be greater in CON animals the first week of the study, and in AH calves at 6 wk compared with the other treatments.

There were no differences in the abundance of Streptococcus bovis in the rumen among treatments throughout the study, whereas Ruminococcus albus population increased ($P < 0.05$) in AH compared with OH animals at wk 6 of study (Table 5.4).

Similar to the current study, an increase of rumen pH when forage is included in the diet of young calves has been previously reported in the literature (Thomas and Hinks 1982; Khan et al., 2011). In contrast to these studies, Suárez et al. (2007) did not find any difference in rumen pH when part of the concentrate was replaced by forage. Values for ruminal VFA concentrations in the current experiment are in the range previously reported in the literature (Coverdale et al., 2004; Suárez et al., 2007), and a negative correlation between rumen VFA concentration and rumen liquid pH was observed ($R^2=0.50$, $P < 0.05$). Results reported herein are in accordance with Coverdale

et al. (2004) who found an increase in ruminal VFA concentration (and decrease in rumen pH) in animals fed only concentrate compared with those fed concentrate plus a fixed proportion of hay. In contrast, Suárez et al. (2007) and Thomas and Hinks (1982), who offered different forage presentations to young calves, did not observe differences in total rumen VFA concentrations compared with non-forage fed calves. Furthermore, rumen valerate molar concentrations in the current study were lowest in AH calves. Cline et al. (1958) described a positive relationship between numbers of cellulolytic rumen microbes, and the rate of valerate utilization in the rumen. Although differences in R. albus, a cellulolytic bacteria, could not be described in the present study, the lower valerate proportion in AH might suggest an improved growth of cellulolytic bacteria in calves supplemented with AH.

Table 5.4. Ruminal pH, total VFA concentration, individual molar proportion of VFA, and bacterial populations from weekly samples of rumen liquid from calves supplemented with alfalfa hay (AH), oat hay (OH) or not supplemented (CON).

	Treatments[1]				P-value[2]		
Item	CON	AH	OH	SEM[3]	F	T	F × t
pH	5.50[b]	6.36[a]	6.00[a]	0.160	<0.01	<0.05	0.61
Total VFA, mM	139.6[a]	109.4[b]	101.5[b]	4.98	<0.001	<0.001	<0.05
VFA, mol/100mol							
Acetate	46.7[ab]	51.8[a]	46.1[b]	1.91	0.10	<0.001	<0.01
Propionate	31.7	34.0	34.0	2.09	0.68	<0.001	0.13
Butyrate	15.1	9.9	14.4	1.77	0.12	<0.001	0.18
Isobutyrate	0.9	0.9	0.9	0.15	0.98	<0.001	0.51
Valerate	5.5[a]	2.8[b]	4.4a[b]	0. 62	<0.05	0.05	0.13
Isovalerate	0.9	0.9	0.7	0.15	0.75	<0.001	0.42
C2:C3	1.8	1.7	1.5	0.16	0.47	<0.001	0.09
S. bovis[4]	32.5	32.1	32.4	0.33	0.64	0.69	0.78
R. albus[4]	28.8	25.6	28.1	1.97	0.49	0.31	<0.05

[a,b]Numerical values within a row with different superscripts differ (P<0.05) or tend to differ (P<0.10)

[1]CON = control; AH = alfalfa hay; OH = oat hay.

[2]F = forage supplementation effect; t= effect of time; F × t = interaction between forage supplementation and time.

[3]SEM= Standard error of the mean

[4]Ct (Cycle treshold) values (smaller numerical values indicates greater abundance)

Results about rumen bacteria populations herein are in agreement with those reported by Petri et al. (2012), who did not find differences in Streptococcus bovis and Ruminococcus spp. abundance between animals fed a high concentrate diet with or without forage inclusion, probably because Ruminococcus spp. were able to use starch as a substrate.

5.3.3. Gastrointestinal passage rate

Results pertaining to the external marker GIT passage rate are shown in Table 5.5. The marker of animals in the OH treatment had the greatest ($P < 0.05$) ruminal fractional passage rate (k_r). There were no differences in the marker fractional rate of passage in the cecum (k_c) and TT in the tubular compartment of the GIT. However, TMRT of the marker in the GIT tended ($P = 0.07$) to be lesser in OH than in CON animals.

Table 5.5. Fractional rates of passage, transit time, and total mean retention time of the external marker in the gastrointestinal tract of calves supplemented with alfalfa hay (AH), oat hay (OH) or not supplemented (CON)

	Treatments[1]				
Item	CON	AH	OH	SEM[2]	P-value
k_r[3], h^{-1}	0.070[b]	0.082[b]	0.147[a]	0.0187	<0.05
k_c[4], h^{-1}	0.667	1.030	1.009	0.3558	0.73
TT[5], h	10.25	7.42	9.57	1.029	0.18
TMRT[6], h	28.36[a]	22.76[ab]	18.84[b]	2.703	0.07

[a,b]Numerical values within a row with different superscripts differ (P<0.05)

[1]CON = control; AH = alfalfa hay; OH = oat hay.

[2] SEM= Standard error of the mean

[3]kr= fractional rate of passage in the rumen

[4]kc= fractional rate of passage in the cecum

[5]TT= transit time in the tubular compartment of the gastrointestinal tract

[6]TMRT= total mean retention time of marker in the gastrointestinal tract

Differences in the marker GIT passage rate among treatments observed herein may be explained, in part, by the numerically greater concentrate intake observed in OH calves, since, generally, greater DM intake results in a greater passage rate (Huhtanen

and Kukkonen, 1995). Although ruminal passage rate of legumes is described to be usually greater than that of grasses, because particle breakdown of legumes makes legumes to pass easily through the rumen (Moseley and Jones, 1984; McLeod et al., 1990), it also contributes to increase legume forage intake, as observed in the present study. Therefore, the greater forage consumption in AH compared with OH treatments may explain the differences between these two treatments (and the similarity between AH and CON calves), since rate of passage of high versus low forage diets entails a decrease in fractional passage rate from the rumen (Voelker-Linton and Allen, 2007).

5.3.4. Slaughter data

Empty body weight (EBW) expressed as percentage of live BW tended (P = 0.08) to be greater in OH and CON compared with AH calves (Table 5.6). Full total gastrointestinal tract (TGIT) weight tended (P = 0.08) to be greater in AH than in OH animals, but no differences were observed in TGIT tissue weight among treatments. Rumen tissue of CON animals tended (P = 0.08) to be heavier and tended (P = 0.07) to represent a greater proportion of the TGIT than in OH calves. On the other hand, the weight of the abomasum tissue and its proportion relative to total BW and EBW, or TGIT weight of OH animals was greater (P < 0.05) than in CON animals. Finally, there were no differences in midgut or hindgut weights among treatments (data not shown). Spleen of OH animals tended (P = 0.08) to weight more and tended (P = 0.09) to represent a greater proportion of BW than that of AH animals, and liver of AH calves tended (P = 0.10) to represent a greater proportion of BW than that of CON animals.

Table 5.6. Body weight and viscera weights at slaughter of calves supplemented with alfalfa hay (AH), oat hay (OH) or not supplemented (CON).

	Treatments[1]				
Item	CON	AH	OH	SEM[2]	P-value
Slaughter BW, kg	89.8	92.3	98.6	5.99	0.58
EBW[3]	68.8	68.8	81.5	4.79	0.19
EBW, % of BW	76.7[a]	74.5[b]	76.9[a]	0.75	0.08
Liver, kg	2.23	2.50	2.48	0.087	0.15
Liver, % of BW	2.38[b]	2.69[a]	2.64[ab]	0.097	0.10
Spleen, kg	0.47[ab]	0.40[b]	0.53[a]	0.038	0.08
Spleen, % of BW	0.51[ab]	0.42[b]	0.57[a]	0.043	0.09
TGIT full, kg	19.5[ab]	21.2[a]	18.3[b]	0.77	0.08
TGIT full, % of BW	20.5[ab]	22.5[a]	19.6[b]	0.82	0.08
TGIT empty, kg	7.7	7.8	7.7	0.29	0.96
TGIT empty, % of BW	8.0	8.3	8.1	0.29	0.83
Rumen full, kg	10.0	10.2	9.0	1.10	0.74
Rumen full, % of BW	10.6	10.8	9.8	1.16	0.83
Rumen empty, kg	2.80[a]	2.37[ab]	2.08[b]	0.205	0.08
Rumen empty, % of BW	2.9	2.5	2.2	0.22	0.15
Abomasum, full, kg	1.11	1.62	1.43	0.215	0.23
Abomasum full, % of BW	1.1	1.7	1.5	0.22	0.13
Abomasum empty, kg	0.52[b]	0.58[ab]	0.63[a]	0.027	<0.05
Abomasum empty, % of BW	0.5[b]	0.6[ab]	0.7[a]	0.03	<0.05
%TGIT					
Rumen full	52.6	47.8	42.2	3.93	0.29
Rumen empty	35.9[a]	30.4[ab]	27.3[b]	2.34	0.07
Abomasum full	5.4	8.0	7.6	1.2	0.26
Abomasum empty	6.8[b]	7.3[ab]	8.2[a]	0.37	0.05
%EBW					
Rumen full	14.0	14.6	10.9	1.45	0.26
Rumen empty	3.7	3.4	2.8	0.31	0.17
Abomasum full	1.3[b]	2.3[a]	2.2[ab]	0.32	0.10
Abomasum empty	0.70[b]	0.83[a]	0.86[a]	0.042	<0.05

[a,b]Numerical values within a row with different superscripts differ (P<0.05) or tend to differ (P<0.10)

[1]CON = control; AH = alfalfa hay; OH = oat hay.

[2]SEM= Standard error of the mean [3]Empty Body Weight (EBW)= BW-TGIT-Liver-Spleen

The rumen liquid pH for the different GIT contents and the VFA concentrations of the cecum at slaughter are presented in Table 5.7. There were no differences in rumen liquid pH at slaughter. However, animals in CON treatment tended (P = 0.07) to present a greater jejunum liquid pH compared with OH animals. Furthermore, ileum pH tended (P = 0.09) to be greatest in AH calves, and rectum pH was least ($P < 0.05$) in OH animals. Although no differences in cecum pH were observed, cecum VFA concentrations tended (P = 0.08) to be greater in forage-fed than in CON animals, and cecum pH and VFA concentrations were moderately correlated (R^2 = 0.72, $P < 0.05$). Cecum individual VFA molar proportions were similar among treatments, with the exception of acetate that was greatest in CON calves. On the other hand, there were no differences among treatments in rumen DM content, rumen buffering capacity, and rumen and cecum viscosity.

Results from rumen histology are presented in Table 5.8. Calves on the CON treatment presented the rumen papillae with the greatest ($P < 0.05$) length, but no further differences were observed for the rest of performed histological measures. Another parameter recorded was the formation of plaque in the rumen, defined as feed and cell debris sticking to the rumen wall (Suárez et al., 2007). In the present study, the percentage of plaque incidence was 80%, 0%, and 0% for CON, AH, and OH animals, respectively. Similar to Suárez et al. (2007), animals fed an all-concentrate diet had an increase of rumen tissue weight. The authors attributed this increase to a greater plaque formation in those calves fed an all-concentrate diet.

Gene expression of rumen epithelia and quantification of bacterial populations in the rumen and cecum are presented in Table 5.9. Animals offered forages tended (P = 0.10) to show a greater MCT1 transporter expression than CON animals, and the expression of NHE1 protein tended (P = 0.10) to be greater in AH than in OH animals. At slaughter, abundance of S. bovis and R. albus in the rumen and cecum did not differ among treatments (Table 5.9).

Table 5.7. Gastrointestinal pH, and cecum VFA concentrations and proportions at slaughter of calves supplemented with alfalfa hay (AH), oat hay (OH) or not supplemented (CON).

	Treatments[1]				
Item	CON	AH	OH	SEM[2]	P-value
pH					
Rumen	5.10	5.20	5.59	0.159	0.11
Abomasum	3.42	3.11	2.75	0.278	0.28
Duodenum	5.31	5.83	5.81	0.246	0.28
Jejunum	6.90[a]	6.75[ab]	6.56[b]	0.091	0.07
Ileum	7.03[b]	7.78[a]	7.09[b]	0.200	0.09
Cecum	6.55	6.30	6.26	0.124	0.24
Colon	6.45	6.35	6.14	0.144	0.33
Rectum	6.40[a]	6.48[a]	5.90[b]	0.112	<0.01
Total cecum VFA, mM	88.7[b]	156.1[a]	159.1[a]	23.03	0.08
Cecum VFA, mol/100mol					
Acetate	72.0[a]	67.0[b]	68.7[ab]	1.31	0.05
Propionate	18.9	21.5	20.2	1.24	0.34
Butyrate	7.7	10.2	10.2	1.02	0.17
Isobutyrate	0.57	0.47	0.31	0.078	0.14
Valerate	1.01	0.88	0.72	0.228	0.69
Isovalerate	0.36	0.32	0.21	0.083	0.45
C2:C3	3.82	3.22	3.44	0.246	0.24
Rumen DM, %	16.8	15.7	16.6	1.18	0.78
Rumen buffer capacity, meqL-1	83.1	82.7	75.9	4.05	0.43
Rumen viscosity, cP	2.0	2.1	2.0	0.07	0.25
Cecum viscosity, cP	3.1	3.8	3.4	0.37	0.45

[a,b]Numerical values within a row with different superscripts differ ($P < 0.05$) or tend to differ ($P < 0.10$)

[1]CON = control; AH = alfalfa hay; OH = oat hay.

[2]SEM= Standard error of the mean

In this study, EBW of OH calves was about 13 kg greater than that of CON calves, although this difference did not reach significance (Table 5.6). Furthermore, OH calves (that ended the study also with the greatest numerical BW) tended to have the lowest weight of full TGIT and similar empty TGIT than the other treatments, and the EBW as a percentage of total live weight tended to be greatest for OH and CON compared with AH calves. These results indicate that BW gain in OH calves fed ad libitum was due to an increase in carcass weight rather than an accumulation of digesta in the gastrointestinal tract and thus an increased gut fill as it has been previously hypothesized in the literature (Kertz, 2007; Khan et al., 2012). On the other hand, the numerical increase of BW gain observed in AH compared with CON calves, was mainly due to a greater weight of the TGIT rather than an increase of carcass weight as occurred in OH animals, which would suggest an increased gut fill in calves receiving AH. Similarly, a study involving calves fed high volumes of milk and provided with or without long-particle orchard grass hay, showed no differences in EBW between treatments, but the rumen digesta content was greater in forage than in non-forage supplemented calves (Khan et al., 2011). Several authors (Jahn et al., 1970; Jahn et al., 1976; Hill et al., 2008) have reported a decrease in EBW as the level of fiber in the diet increases. These authors attributed the difference between live BW and EBW to the accumulation of digesta in the gastrointestinal tract (gut fill). Differences among experiments, and between OH and AH treatments in the present study can be explained by the amount and physical form of forage included in the diet. In the present study, animals were offered chopped forage ad libitum, and forage intake was 14 and 4% of total solid feed intake for AH and OH calves, respectively. Most of the studies comparing different percentage of forage inclusion in the diet of young calves are above 10% (Coverdale et al., 2004; Suárez et al., 2007) or they lack a treatment where calves are deprived of forage (Stobo et al., 1966; Jahn et al., 1970), therefore it is usually concluded that differences in BW when forage is provided to young calves are as a consequence of a gut fill effect. However, from the present study it can be envisaged that forage inclusion at the rate of 4% (as the ones achieved when providing OH) improves rumen environment, tends to improve ADG over time, without incurring in increases in gut fill.

Table 5.8. Rumen muscular width, papillae length and width, and keratin layer of calves supplemented with alfalfa hay (AH), oat hay (OH) or not supplemented (CON).

	Treatments[1]				
Item	CON	AH	OH	SEM[2]	P-value
Muscular width, µm	1141	1031	1286	113.0	0.31
Papillae length, µm	4119[a]	3358[ab]	2784[b]	317.5	0.04
Papillae width, µm	289	327	260	49.3	0.63
Keratin layer, µm	14.2	13.2	13.7	1.36	0.87
Papillae surface, mm^2	1.4	1.3	0.8	0.31	0.45

[a,b]Means within a row with different superscripts differ (P<0.05)

[1]CON = control; AH = alfalfa hay; OH = oat hay.

[2]SEM= Standard error of the mean

Last, differences in abomasum weights among treatments may be attributed to the differences observed in ruminal fractional passage rate. The increase of rumen passage rate in OH calves should result in more undigested feed reaching the abomasum and might induce a greater development of the abomasum tissue. On the other hand, similar to Kosiorowska et al. (2011) who compared two concentrates with different fiber levels, differences in the weight of the small intestine were not observed in the current study.

The results reported in the present study suggest two possible mechanisms that could be involved in the changes observed in rumen fermentation parameters when forage is included in the diet of young calves. First, differences in rumen epithelia gene expression could explain the lower rumen VFA concentration in forage fed calves. Animals fed forage tended to show an increased expression of MCT1 in the ruminal wall. This transporter is located in the basolateral membrane of rumen epithelium, and it is involved in the transport of lactate, acetate, and protons from the rumen epithelium into the bloodstream (Müller et al., 2002; Gäbel and Aschenbach, 2006; Kirat et al., 2006; Graham et al., 2007). Therefore, it could be speculated that due to the enhanced proton export, intracellular pH probably increased, and it may have heighten absorption of short-chain fatty acids (SCFA) from ruminal lumen into epithelium by both simple diffusion of protonated SCFA and SCFA-/ HCO3- exchange.

Despite differences in NHE1 protein expression between AH and OH animals were observed, overall expression of this gene was low, and thus the role of this protein in the control of intracellular pH homeostasis might not be very relevant.

Table 5.9. Relative mRNA expression of selected genes in the rumen epithelium and salivary gland, and abundance of Streptococcus bovis and Ruminococcus albus in ruminal and cecum contents at the slaughter of calves supplemented with alfalfa hay (AH), oat hay (OH) or not supplemented (CON).

	Treatments[1]				
Item	CON	AH	OH	SEM[2]	P-value
Rumen,					
MCT1	0.08[b]	0.36[a]	0.36[a]	0.097	0.10
MCT4	0.01	0.01	0.02	0.011	0.85
SPC25	0.01	0.01	0.02	0.005	0.52
NHE1	0.03[ab]	0.04[a]	0.02[b]	0.006	0.08
NHE3	0.20	0.55	0.25	0.130	0.15
DRA	0.05	0.17	0.14	0.059	0.33
Parotid gland,					
AQ5	20.2	22.6	23.6	6.49	0.93
TMEM16	0.69	0.44	0.88	3.623	0.28
Rumen population,					
S. bovis[3]	32.1	30.5	31.8	1.02	0.56
R. albus[3]	27.7	27.2	22.2	1.89	0.11
Cecum population,					
S. bovis[3]	33.0	31.9	30.9	0.96	0.26
R. albus[3]	33.4	35.3	32.0	1.67	0.41

[a,b]Means within a row with different superscripts differ (P<0.05) or tend to differ (P <0.10)

[1]CON = control; AH = alfalfa hay; OH = oat hay

[2]SEM= Standard error of the mean

[3]Ct (Cycle treshold) values (smaller numerical values indicates greater abundance)

An additional mechanism that could be responsible for the increased rumen liquid pH and decreased VFA concentrations in OH compared with CON calves may be related to the increase in the fractional rate of passage in the rumen observed in calves fed OH compared with CON and also AH. If rumen passage rate increases, the diet will have less time to be fermented, and, consequently, less VFA will be produced, leading to an increase of ruminal pH as it would be the case for OH calves. Moreover, consequence of the increased ruminal fractional passage rate more digesta could reach the cecum, and this could explain the greater VFA concentration in the cecum of animals with access to a forage source compared with calves offered no forage.

Last, calves with access to a forage source have been shown to increase rumination time (Hodgson, 1971; Phillips, 2004; Martin et al., 2006; Castells et al., 2012). Therefore, a potential increase of saliva production associated with a hypothetical increased rumination could also explain the improvement in rumen pH observed in calves that had access to forage. However, in the current study neither the rumen buffering capacity determined at slaughter, nor the expression of genes from the parotid gland related to saliva production supported this hypothesis.

5.4. Conclusions

The present study shows that the inclusion of chopped forage in the diet of young calves leads to a better rumen environment, because rumen pH increases and the expression of MCT1 transporter tends to increase. This improvement in rumen environment together with an increase of ruminal passage rate could allow greater intake of solid feed, and consequently an improvement in calf performance not linked with an increase in gut fill when consumption of forage is about 4% of total solid feed intake, as it occurs when offering chopped oat hay.

Chapter 6

SHORT AND LONG-TERM EFFECTS OF FORAGE SUPPLEMENTATION DURING THE PREWEANING PERIOD ON HEIFER PERFORMANCE

6.1. Introduction

Obtaining high milk production cows is one of the most important goals for dairy producers. Although nutrition is probably the most important factor to achieve high milk production, there are other factors that could improve milk yield. Several studies (Bach and Ahedo, 2008; Moallem et al., 2010; Soberon et al., 2012) have demonstrated that good performance during the preweaning period can result in greater milk yield at first lactation. Soberon et al. (2012) estimated that in calves fed high volumes of milk for every kilogram of gain during the preweaning period, heifers produced 850 kg more milk during their first lactation. During the preweaning period, ADG can be improved by feeding high levels of milk or milk replacer (**MR**) to calves (Terré et al., 2007a; Raeth-Knight et al., 2009), but the supplementation of forage in diets at low levels of MR also resulted in greater performance during this period (Castells et al., 2012). Therefore, some benefits of forage supplementation early in life may be envisaged later in life. The objective of the present study was to evaluate if improvements on performance during the preweaning period of calves fed low volumes of MR, supplemented with forage, could affect breeding parameters and milk yield at first lactation.

6.2. Materials and methods

6.2.1. Animals and treatments

Sixty female Holstein calves (initial BW 39.5 ± 3.76 kg and 6.7 ± 2.12 d of age) were individually housed in the facilities of the contract heifer operation Recria Segle XXI (Figueres, Spain). Calves were randomly assigned to one of the two dietary treatments according to age and BW. Dietary treatments consisted on a ground starter concentrate (19%CP, 19% NDF, and 3.53 Mcal of estimated ME/kg on a DM basis) without any forage supplementation during the preweaning period, and chopped oats hay (8%CP, 68%NDF) supplementation after weaning (**CON**), and the same starter concentrate with oats hay supplementation during the pre- and postweaning period (**OAT**). Calves received 3 L of MR (23.7 % CP and 20.1% fat, Celtilait, France) twice daily until 28 d of age. From 29 to 44 d of age, calves were fed 1.5 L of MR twice daily and from day 45 to 51, 1.5 L of MR were fed once daily. Animals were weaned at 52 d of age. Calves were kept in individual pens until 2 wk after weaning, thereafter animals

were housed in groups of 30 to 40 animals per pen. From 2 wk after weaning to 3 mo of age, heifers were fed the same concentrate feed fed during the pre- and postweaning period plus forage, both offered ad libitum. From 3 mo of age to calving, heifers were fed a TMR based on triticale silage and concentrate. From 3 to 14 mo of age, the TMR contained 15.0% CP and 2.60 Mcal of ME/kg on a DM basis, and from 15 mo of age to calving the TMR contained 14.0% CP and 2.40 Mcal of ME/kg on a DM basis. Hip height was the parameter elected to determine age at first artificial insemination **(AI)**. Animals higher than 130 cm were inseminated. Furthermore, heifers that were not observed in estrus at 13.8 mo of age were synchronized using a PRID.

6.2.2. Measurements and sample collection

Starter feed and forage intake were recorded daily and BW weekly until 65 d of age, and later at 10 mo of age. Two weeks after weaning, total-tract apparent digestibility was determined in 6 calves per treatment following the method used by Castells et al. (2012). Furthermore, breeding data (age at first AI, age at fertile insemination, and number of AI) and milk yield at the first lactation was also recorded.

Samples of MR, starter and feces were analyzed for DM (24 h at 103 ºC), ash (4 h at 550 ºC), N content using the AOAC (1990) method (988.05) adapted for an automatic distiller Kjeldhal (Kjeltec Auto 1030 Analyser, Tecator, Sweden) and using CuSO4/Se as a catalyst instead of CuSO4/TiO2, NDF and ADF, with sodium sulphite and heat-stable alpha-amylase (Van Soest et al. 1991).

6.2.3. Statistical analysis

Performance data were analyzed using a mixed-effects model with repeated measures. The model included treatment, week of study and their interaction as fixed effect, and the animal as random effect. Auto-regressive was used as variance-covariance structure.

Feed digestibility, weight at 10 mo of age, and breeding data were analyzed with an ANOVA with treatment as main effect.

6.3. Results and discussion

6.3.1. Performance

During the preweaning period, concentrate intake of OAT animals was 20 % greater ($P < 0.05$) than in CON animals (Table 6.1). Furthermore, total DM intake (**TDMI**) was also greater ($P < 0.01$) in OAT compared with CON animals. This increase in TDMI was observed from 31 d of age to weaning. The greater feed intake observed in OAT calves resulted in 24% more ($P < 0.01$) growth in OAT than CON animals during the preweaning period without affecting the gain to feed ratio. Furthermore, feed consumption, expressed as percentage of the BW, was greater ($P < 0.01$) in OAT compared with CON calves. After weaning, once CON calves received forage in the diet, differences in concentrate intake, TDMI, ADG, and gain to feed ratio between treatments disappeared (Table 6.1). The only difference observed after weaning was in forage intake, OAT animals consumed more forage ($P < 0.01$) than CON animals. Finally, 2 wk after weaning calves in OAT treatment weighed 4 kg more ($P < 0.05$) than CON calves. Improvements on concentrate intake, TDMI, and ADG during the preweaning period agreed with the results previous found by Thomas and Hinks (1982) and Castells et al. (2012), who also reported an increased in feed intake and ADG when straw was included in a pelleted ratio to young calves. Although Khan et al. (2011) also reported an increase in TDMI of calves when grass hay was offered in the diet of young calves no differences in ADG were observed. Terré et al. (2012) and Castells et al. (2013) found an increase of rumen pH when a forage source was included in the diet of young calves, suggesting that the increase of rumen pH may stimulate calves to consume more starter concentrate. After weaning, no differences in performance were observed in the present study because all treatments were offered forage. In contrast to Castells et al. (2012) and Terré et al. (2012) that no forage was offered two weeks after weaning, and therefore differences in performance and intake after weaning were maintained in their studies.

Although the greater BW at 65 d of age in OAT compared with CON calves, these differences disappeared at 10 mo of age. Several authors have been studying long-term effects on growth when applying different treatments during the preweaning period, especially in intensive MR feeding programs. Some of them maintained a

significant differences in BW (Raeth-Knight et al., 2009; Moallem et al., 2010), others did maintained the numerical, but not the significant, differences (Terré et al., 2009), and in others the differences disappeared (Davis Rinker et al., 2011) as in the current study.

Table 6.1. Performance and feed intake of calves supplemented with oats hay (OAT) during the pre- and postweaning period or not supplemented with oats hay during the preweaning period and supplemented with oats hay during the postweaning period (CON).

	Treatments[1]			*P*-value[2]		
Item	CON	OAT	SEM[3]	T	t	T × t
Initial BW, kg	39.8	40.3	0.72	0.65	-	-
Final BW at 65 d of age, kg	70.1	74.5	1.49	<0.05	-	-
Preweaning (10 to 51 d of age)						
ADG, kg/d	0.42	0.52	0.023	<0.01	<0.001	0.33
Intake, kg DM/d						
Starter	0.50	0.60	0.040	<0.05	<0.001	0.17
Forage	-	0.04	0.003	-	-	-
TDMI[4]	1.07	1.21	0.041	<0.01	<0.001	<0.05
Gain to feed[5]	0.40	0.43	0.014	0.14	<0.001	0.99
DMI, % BW	2.3	2.5	0.06	<0.01	<0.001	0.20
Postweaning (52 to 65 d of age)						
ADG, kg/d	0.89	0.91	0.039	0.78	0.21	0.89
Intake, kg DM/d						
Starter	2.09	2.12	0.081	0.77	<0.001	0.07
Forage	0.09	0.15	0.031	<0.01	<0.05	0.66
TDMI[4]	2.16	2.25	0.088	0.49	<0.001	0.14
Gain to feed[5]	0.42	0.42	0.018	0.99	0.46	0.83
DMI, % BW	3.4	3.3	0.10	0.53	<0.01	0.03
From 65 d of age to 10 mo,						
BW at 10 mo, kg	304.8	306.4	5.38	0.84	-	-
ADG, kg/d	0.96	0.95	0.022	0.74	-	-

[1]CON = calves fed a concentrate starter without hay supplementation during the preweaning period, and supplemented with oats hay after weaning; OAT = calves fed a concentrate starter supplemented with oats hay throughout the study

[2]T = preweaning forage supplementation effect; t= effect of time; T × t = interaction between preweaning forage supplementation and time.

[3]SEM= Standard error of the mean

[4]TDMI = total DMI (milk replacer, starter feed, and forage).

[5]Kilograms of BW gain/kg of TDMI

6.3.2. Apparent nutrient digestibility

Total-tract apparent nutrient digestibility did not differ between treatments two weeks after weaning (Table 6.2). Results reported herein are in the range previously reported in weaned calves (Terré et al., 2007a; Hill et al., 2010; Castells et al., 2012). After weaning, the voluntary intake of forage in CON and OAT animals was 4.3 and 7.1% of their diet, respectively. These differences did not alter digestibility after weaning, and the previous experience of eating forage did not improve diet digestibility in OAT calves. Digestibility results suggest that young calves fed without forage in the diet during the preweaning period are able to adapt their gastrointestinal tract to digest forage once forage is offered.

Table 6.2. Total-tract apparent nutrient digestibility 2 wk after weaning of calves supplemented with oats hay (OAT) during the pre- and postweaning period or not supplemented with oats hay during the preweaning period and supplemented with oats hay during the post weaning period (CON).

	Treatments[1]			
Item	CON	OAT	SEM[2]	*P*-value
Digestibility, %				
DM	78.3	78.4	1.29	0.96
OM	79.2	79.4	1.24	0.93
CP	75.2	74.8	1.55	0.86
NDF	42.3	43.5	3.03	0.80

[1]CON = calves fed a concentrate starter without hay supplementation during the preweaning period, and supplemented with oats hay after weaning; OAT = calves fed a concentrate starter supplemented with oats hay throughout the study

[2]SEM= Standard error of the mean

6.3.3. Reproductive parameters

Reproductive parameters of heifers in both treatments performed in a similar manner (Table 6.3). Most of the above studies mentioned (Raeth-Knight et al., 2009; Terré et al. 2009; Davis Rincker et al., 2011) also reported breeding and milk yield data. Similar to the present study, similar age at calving was observed between control and calves fed on an intensive MR feeding program, but a numerical increase in milk yield at first lactation was reported in those calves that had greater ADG during the preweaning period.

Milk yield data cannot be shown in this thesis, because heifers from the present study are calving these days, and milk yield data will be available at the beginning of next year.

Table 6.3. Breeding and milk yield at first lactation data of heifers that were supplemented with oats hay (OAT) during the pre- and postweaning period or not supplemented with oats hay during the preweaning period and supplemented with oats hay during the post weaning period (CON).

	Treatments[1]			
Item	CON	OAT	SEM[2]	*P*-value
Age at 1st service, d	405.5	407.1	2.48	0.64
Age at fertile AI, d	422.1	426.4	6.40	0.64
Times bred	1.6	1.7	0.17	0.85

[1]CON = calves fed a concentrate starter without hay supplementation during the preweaning period, and supplemented with oats hay after weaning; OAT = calves fed a concentrate starter supplemented with oats hay throughout the study

[2]SEM= Standard error of the mean

6.4. Conclusions

In conclusion, offering forage to young calves early in life allows improvements in growth before weaning and could help the transitions to mixed diets, but the improvement on growth were not maintained at 10 mo of age, and benefits on breeding data.

Chapter 7

EFFECT OF PHYSICAL FORM OF STARTER WITH OR WITHOUT STRAW ON THE PERFORMANCE, RUMEN pH, AND BLOOD METABOLITES OF HOLSTEIN CALVES

7.1. Introduction

Early weaning and restricted milk feeding programs have been widely used as strategies to reduce feeding cost of rearing young calves, and encourage calves to consume starter concentrate. Feeding forage to young calves has traditionally been discouraged because it could decrease voluntary intake of starter feed due to the accumulation of undigested forage in the rumen (Drackley, 2008). However, some fiber may be necessarily to young calves to maintain an abrasion factor in the rumen to avoid abnormal development of rumen epithelium (Greenwood et al., 1997). Drackley (2008) pointed out that if concentrate feeds contain some "long" particles, such as rolled oats, alfalfa meal, beet pulp, or cottonseed hulls, forage supplementation is not needed, especially if calves are bedded on straw. This kind of starter feeds, known as texturized, mash or multiparticle concentrates, increased starter intake compared with a pelleted starter (Bach et al., 2007; Porter et al., 2007), with improvements on ADG in Porter et al. (2007) study, and without further improvement in ADG in Bach et al. (2007) study. On the other hand, ingredients in the texturized concentrates require to be processed, which increases feed costs compared with a pelleted concentrate. Another strategy to avoid abnormal development in the rumen epithelium without increasing concentrate feed cost could be to offer a pelleted concentrate with a forage supplementation. Several authors (Thomas and Hinks, 1982; Khan et al., 2011; Castells et al., 2012) reported that offering forage in the diet of young calves improved performance and concentrate intake.

This experiment was designed to evaluate performance of calves receiving high volumes of milk and fed concentrates with different physical forms, texturized or pelleted, and supplemented or not with forage.

7.2. Materials and methods

7.2.1. Animals and Treatments

This study was conducted in the facilities of Dairy Education and Research Center of the University of British Columbia in Agassiz (BC). Animals were managed according to the guidelines of the Canadian Council on Animal Care. Thirty-two Holstein calves (23 heifers and 9 bulls) were separated from their dams and moved to individual pens (1.7 x 1.2 m) bedded with wood shaving, and fed 4 L of colostrum within 4 h of birth. At 7 d

of age (initial BW = 46.4 ± 4.91 kg) animals were randomly assigned, according to weight, to one of the three dietary treatments. Dietary treatments consisted on a pelleted concentrate feed plus chopped straw (**PS**), a texturized concentrate plus chopped straw (**TS**), and a texturized concentrate without forage supplementation (**TE**). Pelleted and texturized concentrate feed had the same composition (21% CP, 18% NDF, 4.0 MCal of estimated ME/Kg on DM basis), and only differed in the physical presentation. The straw was chopped using a TMR mixer (Loewen Horizontal Mixer, Loewen Welding & Manufacturing Ltd., Matsqui, BC, Canada) at 19000 rpm for 60s. Animals were bottle-fed and received 4 L of pasteurized whole milk twice daily from 7 to 35 d of age, 2 L of milk twice daily from 36 to 42 d of age, and 2 L of milk from 43 to 49 d of age. Animals were weaned at 50 d of age. Calves had free access to water and solid feed was fed ad libitum in buckets until the end of the study (63 d of age).

7.2.2. Measurements and Sample Collection

Concentrate feed, forage, and milk intakes were recorded daily on an individual basis. Calves were weighed twice weekly. Heart girth, body barrel, and hip height were measured at the beginning and at the end of the study (7 and 63 d of age) to control calves structural growth during the study. Blood samples were collected at 21, 35, 49, and 63 d of age from the jugular vein to evaluate the evolution of β-hydroxybutyrate (**BHBA**) with age. Rumen liquid was collected 3-4 h post-feeding using an oral tube at 35 (preweaning), 49 (weaning), and 63 (postweaning) d of age to measure rumen pH using a digital pH meter. Samples of concentrate feeds, forage, and milk were taken weekly to analyze their composition.

7.2.3. Analytical Procedures

Samples of MR were analyzed for DM (24 h at 103ºC), ash (4 h at 550ºC), N content using the AOAC (1990) method (988.05) adapted for an automatic distiller Kjeldhal (Kjeltec Auto 1030 Analyser, Tecator, Sweden) with CuSO4/Se as a catalyst instead of CuSO4/TiO2, and ether extract using the AOAC method (920.39) using petroleum ether for distillation instead of diethyl ether (AOAC, 1990). Samples of starter feed and fiber sources were analyzed for DM, ash, and CP following the same methods described

above, plus NDF with sodium sulphite and heat-stable alpha-amylase (Van Soest et al., 1991), and ADF following the AOAC (1990) method (973.18).

Blood samples were analyzed for BHBA (Precision Xtra blood ketone kit, Abbott Diabetes Care) using the procedures described by Iwersen et al. (2009) for blood ketone analysis.

7.2.4. Statistical Analysis

Data from performance, growth, blood BHBA, and rumen pH were analyzed using a mixed-effects model for repeated measures. The statistical model included treatment, time (week of study), and their interaction as fixed effects, and animal within treatment as a random effect. The gender of the calves was initially included in the statistical analysis as a block, but it was removed because it was not significant for any of the parameters measured. Compound symmetry was used as variance-covariance structure.

7.3. Results and discussion

7.3.1. Performance

Results of animal performance and feed intake are presented in Table 7.1. There were no differences among treatments in concentrate feed, forage, milk, and total DM intake. In contrast to the present study, other studies (Warner et al., 1973; Franklin et al., 2003; Bach et al., 2007; Porter et al., 2007) reported that animals offered mash, multiparticle, or a texturized starter consumed more concentrate than calves fed a pelleted concentrate feed. Moreover, in the present study the physical form of the starter and the availability of forage did not affect ADG and feed intake as percentage of BW. However, the gain to feed ratio was improved from 28 to 42 d of age in PS compared with TS animals. Porter et al. (2007) reported increases in ADG in a mash concentrate feed compared with a pelleted concentrate feed, but Bach et al. (2007) did not observe differences in ADG between a pelleted and a multiparticle concentrate feed. Moreover, in the study of Franklin et al. (2003) showed an increase in total weight gain in calves fed texturized compared with a pelleted starter feed. The present study differs from the previously cited studies in straw allowance. Probably the

negative effects derived of feeding pelleted concentrate alone observed in the other studies were mitigated when straw was offered.

Similar to BW gain, structural body measures were also not affected by the different diets offered to calves.

7.3.2. Rumen pH and blood BHBA

Animals receiving supplemental straw in their diet, PS and TS treatments, had a greater ($P < 0.001$) rumen pH compared with those without straw supplementation (Table 7.1), suggesting a positive effect of forage supplementation on rumen environment, even when feeding a texturized starter. The increase of rumen pH when forage is included in the calves diet has been previously reported in the literature (Thomas and Hinks, 1982; Khan et al., 2011).

Blood BHBA increased with calf age ($P < 0.001$), but there were no differences in blood BHBA among treatments. The increase in blood BHBA with age has been previously described in the literature (Quigley et al., 1992; Greenwood et al., 1997; Coverdale et al., 2004; Khan et al., 2011). This increase indicates a shift in the source of energy from liquid to solid diets, increasing the consumption of fermentable carbohydrates as concentrate feed intake increases with age. Similar to the present study, the inclusion of forage in the diet of young calves, and physical form of the concentrate has little impact in blood BHBA concentration (Greenwood et al., 1997; Coverdale et al., 2004; Khan et al., 2011). Khan et al. (2011) hypothesized that similar concentrations of BHBA (an indicator of metabolic function of the rumen wall) indicates that the rumen walls were equally efficient in converting butyrate to BHBA.

Table 7.1. Performance, feed intake, rumen pH, serum BHBA concentrations, and structural body measures of calves fed texturized concentrate feed, with (TS) or without (TE) straw, or pelleted concentrate plus straw (PS) from 7 to 63 d of age.

	Treatment[1]				P-value[2]	
	PS	TE	TS	SEM[3]	T	Txt
Initial BW, kg	46.5	46.2	46.4	1.55	0.99	-
Final BW, kg	86.5	87.5	83.4	3.48	0.76	-
ADG, kg/d	0.75	0.74	0.69	0.047	0.63	0.68
Intake,kg/d						
Milk	0.63	0.61	0.62	0.007	0.26	0.58
Starter	0.73	0.69	0.67	0.067	0.84	0.99
Forage	0.050	-	0.070	0.0119	0.23	0.41
TDMI [4]	1.41	1.31	1.37	0.070	0.58	0.95
DMI, %BW	2.16	2.01	2.13	0.115	0.62	0.84
Gain to feed[5]	0.55	0.53	0.51	0.028	0.60	<0.01
Ruminal pH	5.9[a]	5.4[b]	5.8[a]	0.07	<0.001	0.85
Blood BHBA concentration, mmol/L	0.15	0.14	0.16	0.034	0.96	0.88
Structural Body Measures, cm						
Hip Height, 7d	82.4	83.5	84.1	1.20	0.88	0.14
Hip Height, 63d	98.5	98.7	97.1	1.16		
Hearth Girth, 7d	85.0	85.2	85.4	1.48	0.96	0.99
Hearth Girth, 63d	103.7	104.1	104.2	1.41		
Body Barrel, 7d	86.9	86.8	85.7	2.11	0.86	0.53
Body Barrel, 63d	118.6	117.7	121.1	2.00		

[1]PS=Pelleted concentrate plus straw; TE=Texturized concentrate; TS=Texturized concentrate plus straw

[2]T=treatment effect; Txt=interaction between treatment and time

[3]SEM=Standard error of the mean

[4]TDMI=Total dry matter intake (milk replacer, starter feed, and fiber)

[5] kg BW gain/kg TDMI

7.4. Conclusions

Using texturized concentrate feed, with or without straw supplementation, to feed young calves failed as feeding system to improve performance results compared with pelleted concentrate fed with straw supplementation. Moreover, when straw was not available for calves fed a texturized concentrate, a reduction in ruminal pH was observed compared with the same texturized or pelleted feed with straw availability.

Chapter 8

GENERAL DISCUSSION

8. General Discussion

In this chapter, the effect of forage inclusion in the diet of young calves on different productive changes when rearing pre- and postweaned calves will be discussed. First of all, due to the constant rise of feed prices, an economic evaluation of the impact of forage inclusion in the diet of young calves will be done. The objective of this analysis is to identify if there is any economical benefit for farmers when including forages in the diet of young calves. Secondly, using data from the literature, the effect of forage inclusion in the diet will be analyzed to determine its effects on rumen weight and concentrate feed intake.

8.1. Economical analysis of forage provision in the diet of young calves

The impact of feeding forage in the diet of young calves has been showed to be beneficial according to the results presented in the Chapter 3. Results presented in this thesis clearly showed that young calves fed low quantities of milk during the preweaning period with a pelleted concentrate and forage ad libitum improved or did not affect (except for alfalfa hay) performance of calves. Performance improvement not only included an increase in concentrate and total dry matter intake, but it also improved ADG of young calves. Therefore, rearing young calves with forage availability enhances calves growth and allows getting weaned calves with greater BW than when forage is not fed. These benefits were obtained when calves were weaned at 57 days of age. Even though weaning animals in a predetermined age is a common practice in commercial farms, there are other valid methods to wean calves. Another practice commonly used to wean animals is to withdraw milk feeding once animals reach a determined concentrate feed intake for 2 or 3 consecutive days. Doing this weaning method farmers ensure that calves will be able to get enough energy from concentrate feed and, thereby, diminishing the impact of weaning in calves performance.

In order to determine the impact of feeding forage on weaning if concentrate intake would have been the factor to determine the weaning day, data from Chapter 3 was checked, and age when concentrate intake was around 1 kg was calculated. Table 8.1 shows weaning age of calves from Study 1, considering that they would have been weaned according to concentrate intake instead of a predetermined age. Weaning age according to concentrate intake agreed with results previously observed on calves

performance. Animals fed alfalfa hay would have been weaned at the oldest age (49 d of age), followed by control and ryegrass hay fed animals that would have been weaned 5 days before (44 d of age). Conversely, animals fed either, triticale silage or oat hay, would have been weaned 7 and 5 days before control animals, respectively. Weaning calves earlier in life without affecting concentrate intake or BW performance reduces the cost of rearing preweaned calves, due to the minor number of days on milk.

The economical cost of the provision of different forage sources in the diet of young calves is presented in Table 8.1. Feeding costs of animals fed alfalfa hay or without forage provision from 15 to 71 d of age were the lowest among treatments. All the other treatments presented greater feeding costs compared with the two treatments previous mentioned. Extra cost of feeding calves with a forage source was calculated to be between 3 and 9 € for the whole period, being triticale silage and oat hay fed animals the most expensive treatments. The greater feeding costs of rearing forage-supplemented animals were due to the greater concentrate feed intake observed in forage compared with non-forage or alfalfa hay supplemented calves. However, body weights of animals fed with alfalfa hay or without forage were lower than those supplemented with a grass forage. Grass-forage fed calves were between 4 and 13 kg heavier than calves fed with alfalfa hay or without forage provision. Therefore, for the comparison of feeding cost among the different treatments, it is better to calculate the feeding cost per kg of BW gain (Table 8.1). Animals fed alfalfa hay or without forage had the greatest cost per kg of BW gain compared with the other forage fed treatments. Feeding costs savings of forage-fed calves per kg of BW gain ranged between 0.10 and 0.20 €/kg BW gain, being oat hay, barley straw, and triticale silage fed calves the least expensive rearing methods per kg of BW gain. Finally, age when calves reached 80 kg of BW was calculated (Table 8.1). Again, animals fed alfalfa or without forage provision in the diet were found to be the latest to reach 80 kg of BW. Conversely, oat hay and triticale silage fed calves were the earliest animals to reach 80 kg of BW. The cost of growing calves until 80 kg of BW was found to be more expensive in alfalfa and rye-grass hay fed calves than control calves, whereas fed calves with oat hay, barley straw, triticale silage, or corn silage in the diet allowed saving money. Despite the economical savings when forages were included in the diet

of young calves were not very important during the preweaning period, the potential improvement on milk yield described in the literature could be important. Soberon et al. (2012) estimated that for every kilogram of ADG during the preweaning period, milk yield was increased 850 kg during the first lactation.

Table 8.1. Estimated age of weaning of calves weaned at 1 kg of starter feed intake, and feeding cost of calves from 15 to 71 of age of calves fed with or without forage supplementation.

	Treatments[1]						
	CTR	AH	RH	OH	BS	TS	CS
Weaning at 1 kg starter intake							
Estimated age at weaning (d)	44	49	44	39	42	37	41
Total feeding cost (€/calf)[2]	71.44	71.43	76.70	80.45	77.55	79.47	74.53
Difference compared with CTR calves	-	-0.01	5.26	9.01	6.10	8.02	3.09
BW at 71 d of age (kg)	85.5	84.5	89.2	98.0	95.0	94.5	90.4
Cost (€) per kg of BW gain	1.77	1.75	1.67	1.57	1.59	1.61	1.64
Difference compared with CTR calves	-	-0.02	-0.10	-0.20	-0.18	-0.16	-0.13
Age at 80 kg of BW (d)	65	67	63	56	59	57	60
Feeding cost at 80 kg of BW (€)	65.50	66.97	66.57	61.62	62.77	63.41	63.23
Difference compared with CTR calves (€)	-	1.47	1.07	-3.88	-2.73	-2.09	-2.27

[1] CTR= control; AH=alfalfa hay; RH=rye-grass hay; OH=oats hay; BS=barley straw; TS=triticale silage; CS=corn silage

[2] Feeding (milk replacer, concentrate and forage) cost of the preweaning period until 71 d of age. Cost of milk replacer 2.46 €/kg DM, concentrate 0.43 €/kg DM, alfalfa hay 0.19 €/kg DM, rye-grass hay 0.14 €/kg DM, corn silage 0.19 €/kg DM, triticale silage 0.18 €/kg DM, oat hay 0.11 €/kg DM, barley straw 0.05 €/kg DM

In conclusion, feeding preweaned calves a source of forage, with the exception of alfalfa hay, allows farmers to wean earlier if the weaning criterion is concentrate feed intake. Even the provision of forage (except alfalfa hay) entails a total increase on feeding cost of young calves, when cost of 1 kg of BW gain is calculated, feeding forage allows saving money. Finally, feeding oat hay, barley straw, triticale silage, or corn silage allows getting calves at 80 kg of BW earlier than non-forage supplemented preweaned calves.

8.2. Full rumen weight

As mentioned in Chapter 3, feeding forage to young calves has been widely discouraged. Authors against using forage in the diet of young calves attributed different drawbacks to forage intake as negative effect on concentrate intake due to an accumulation of undigested materials in the rumen, and decreased BW gain (Warner et al., 1956; Jahn et al., 1970; Drackley, 2008). On the other hand, other authors (Suárez et al., 2007; Khan et al., 2011) did not observed any effect on performance when forage was included in the diet of young calves, and others reported improvements in concentrate intake and body weight gain when forage was included in the diet of preweaned and weaned calves (Thomas and Hinks, 1982; Castells et al., 2012). Detractors on the use of forage in young calves diets (Jahn et al., 1970; Jahn et al., 1976) attributed improvements in BW gain to a greater gut fill of animals fed forage, due to the accumulation of forage in the gastrointestinal tract, rather than carcass weight gain. In the literature, forage to preweaned calves has been fed free choice ad libitum (Thomas and Hinks, 1982; Khan et al., 2011; Castells et al., 2012), or with a predetermined ratio concentrate to forage (Beharka et al., 1998; Coverdale et al., 2004; Suárez et al., 2007) with different results on growth and the rumen fill effects. Therefore, to determine factors related with calves and diet that could affect rumen fill, and consequently modify BW gain, data from different studies, where forage was included in the diet of young calves, were analyzed (Stobo et al., 1966; Suárez et al., 2007; Khan et al., 2008; Khan et al., 2011; Castells et al., 2013). From all the studies found in the literature that analyze forage provision to young calves, the most common parameter reported related to gut fill was full rumen weight. Therefore, this parameter was used to make relationships with young calf starter and forage intake. To avoid the differences in BW at slaughter in the different studies used for this analysis, full rumen weight expressed as percentage of BW was the parameter selected to represent rumen fill effect. Starter intake, starter intake as percentage of BW, forage intake, and forage intake as percentage of BW were assessed if they fit to a linear or quadratic model to explain rumen full weight expressed as a % BW. It was determined that only forage intake expressed as a percentage of BW was a significant parameter in this model. Therefore, after adjusting the data for the random effect of the study, and adding age as a covariate in the model, a quadratic regression analysis

was performed between forage intake expressed as a percentage of BW and full rumen weight. The resulting equation was:

$$\textit{Full rumen weight as \%BW} = 6.2 + 11.1 \cdot \textit{Forage intake as \%BW} - 2.5 \cdot (\textit{Forage intake as \%BW})^2 \quad (R^2=0.74;\ P < 0.001)$$

This equation demonstrates that increasing forage intake entails an increase of full rumen weight (Figure 8.1). Therefore, authors that discouraged forage intake to young calves because observed growth of those calves fed with forage was related to an increase of rumen weight rather than a carcass weight gain, were partially right. However, in Study 1 when calves were offered ad libitum access to forage, forage intake expressed as percentage of BW only represented between 0.15 and 0.32 % of BW depending on the forage source (Table 8.2). The above equation was used to estimate the full rumen weight of calves from Study 1. Then, the estimated rumen full weight was subtracted to final BW of calves in Study 1 (Table 8.2). In Study 1, a numerical difference of 2 kg BW was observed between alfalfa hay and non-forage supplemented calves. However, this difference disappeared when estimated BW corrected by rumen full weight was calculated. Although the differences in BW of grass-forage relative to non-forage supplemented calves were reduced when BW was corrected by rumen full weight, the improvements on BW at the end of the study were maintained, and they were mainly a consequence of carcass weight gain, rather than an accumulation of feed in the rumen. This improvement of BW of grass forage supplemented calves was probably achieved by the increase of concentrate intake, probably assessed by an increase of rumen pH as reported in Study 2.

Figure 8.1. Quadratic relationship between full rumen weight expressed as percentage of BW and forage intake expressed as percentage of BW of young calves fed low quantities of milk.

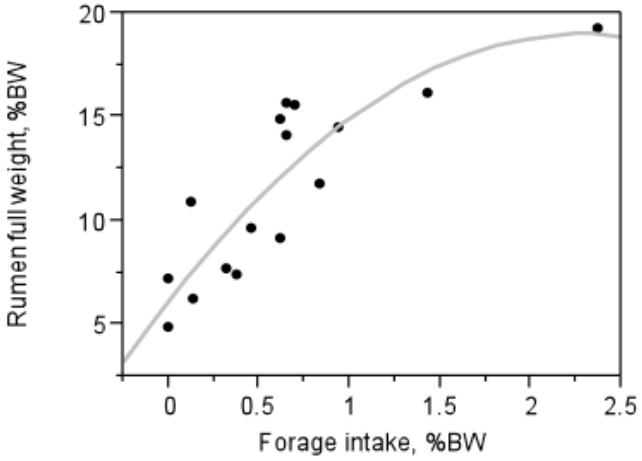


Table 8.2. Body weight, forage intake, and estimated rumen weight of calves from Study 1 supplemented or not with different forages in the diet at 71 d of age (2 wk after weaning).

	Treatments[1]						
	CTR	AH	RH	OH	BS	TS	CS
BW, kg	84.5	86.4	91.6	96.1	93.2	93.6	89.8
Total DM intake, kg	2.1	2.5	2.8	2.8	2.9	2.5	2.8
Forage DM intake, kg	-	0.28	0.14	0.24	0.14	0.14	0.17
Forage intake, % BW	-	0.32	0.15	0.25	0.15	0.15	0.19
Estimated rumen weight, % BW	6.2	9.5	7.8	8.8	7.8	7.8	8.2
Estimated rumen weight, kg	5.2	8.2	7.2	8.5	7.3	7.3	7.4
BW corrected by estimated rumen weight, kg	79.3	78.2	84.4	87.6	85.9	86.3	82.4
Differences in BW relative to CTR	-	1.9	7.1	11.6	8.7	9.1	5.3
Differences in BW relative to CTR corrected by rumen weight	-	-1.1	5.2	8.4	6.7	7.0	3.2

[1] CTR= control; AH=alfalfa hay; RH=rye-grass hay; OH=oats hay; BS=barley straw; TS=triticale silage; CS=corn silage

In conclusion, feeding forage to young calves clearly increase the full rumen weight. However, in the case of calves from Study 1, the low percentage that forage intake represents as percentage of BW in grass forage supplemented calves entails little difference in full rumen weight compared with non-forage fed calves. Therefore, the greater growth observed in animals fed a grass forage is mainly related to a carcass weight gain rather than an increase of rumen weight because of accumulation of digesta in the rumen.

8.3. Individual daily variation of feed intake

Based on literature, improvements on performance were expected when offering forage to young calves. However, a 30% increase of concentrate intake was completely surprising. In the Study 1 discussion section, performance benefits when offering the grass forages studied were attributed to a better rumen environment, allowing greater concentrate feed intake. This hypothesis was later confirmed in Study 3, when animals fed forage were found to have greater rumen pH values. Reviewing the data, another hypothesis to explain the better performance of calves fed free choice grass forages came up. Could it be possible that calves with better rumen environment (forage fed) had a more steady concentrate intake without big fluctuations due to marked rumen pH depressions?

To determine if animals forage fed have less fluctuation on individual daily feed intake, the daily coefficient of variation (**CV**) of concentrate and forage feed intake was calculated by week of age for each calves using data from Study 1. Contrary to the hypothesis formulated, grasses intake did not reduce individual daily variation of concentrate feed intake compared with animals fed without forage availability (Table 8.3). However, when calves were offered alfalfa hay, an increase ($P < 0.05$) in the CV of concentrate intake, but not that of forage, was observed compared with the other treatments (except for RH animals). These data suggested that the greater forage intake observed in alfalfa hay calves compared with the other treatments may be influencing concentrate intake. On the other hand, CV of concentrate and forage feed intake decreased ($P < 0.001$) with calf age in all feeding diets, indicating a high variability in concentrate and forage intake during the first two weeks of age. After the first two weeks, starter feed becomes more important in the diet of the young calves, and individual intake of dry feed becomes more constant. The interaction of forage source with calves age in CV of forage fed calves ($P < 0.001$), it is difficult to discuss because it did not follow a determined pattern. Different treatments were different at a determined week of age, but none was repeatedly observed throughout calf age.

Table 8.3. Daily coefficient of variation of starter feed and forage intake of calves from Study 1 supplemented or not with different forage sources in the diet.

	Treatment[1]								P-value[2]		
	CTR	AH	RH	OH	BS	TS	CS	SEM[3]	T	t	Txt
Starter CV	21.8^{bc}	29.5^{a}	25.6^{ab}	22.1^{bc}	22.5^{bc}	19.0^{c}	23.3^{bc}	1.92	<0.05	<0.001	0.40
Forage CV	-	57.2	65.0	52.3	53.0	49.4	50.2	4.85	0.22	<0.001	<0.001

[1]CTR=control; AH=alfalfa hay; RH=rye-grass hay; OH=oat hay; BS=barley straw; TS=triticale silage; CS=corn silage

[2]T=treatment effect; t=time effect; Txt=interaction between treatment and time

[3]SEM=Standard error of the mean

8.4. How the percentage of forage in the calf diet can influence starter intake

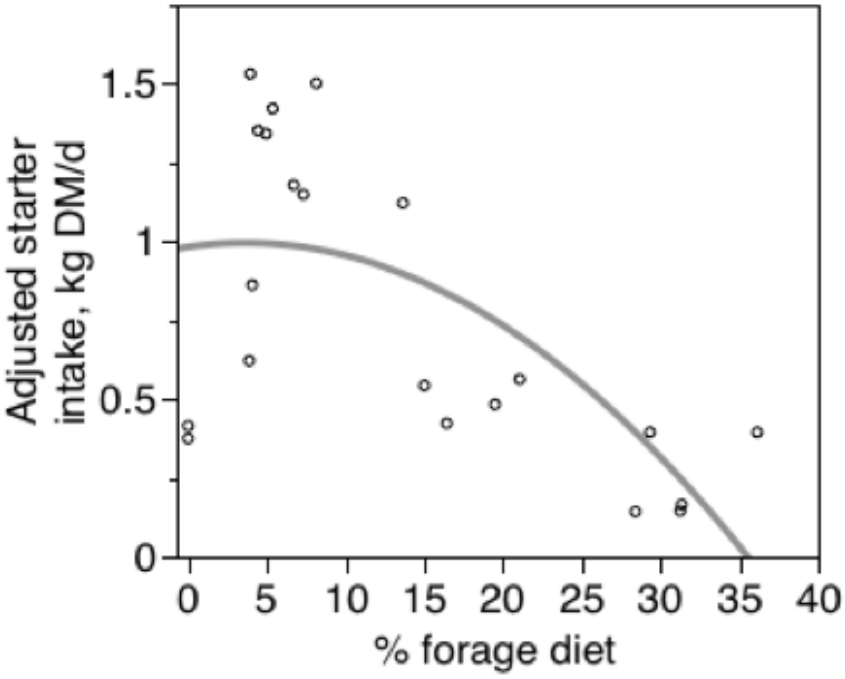
As mentioned before, results in Chapter 3 showed improvements on performance when calves had an ad libitum access to a grass forage. However, these improvements were not observed when the forage included in the diet was alfalfa hay. Curiously, alfalfa hay was the forage consumed at the greatest amounts in the studies performed. As observed in the above section, the CV of starter intake was greater in alfalfa hay fed calves compared with grass forages fed calves, suggesting that alfalfa intake was affecting concentrate feed intake of young calves fed low quantities of milk. In Study 1, when forage consumption was up to 10% of the solid diet, as it was in grass forages supplemented calves, resulted in similar or greater concentrate feed intake compared with animals fed no forage. On the other hand, alfalfa hay intake represented around 14% of the total solid feed intake, and a decrease in concentrate feed intake was observed compared with animals supplemented with grass forages.

In order to determine if there exists a breakpoint where forage intake has a detrimental effect on concentrate feed intake, studies with preweaned calves fed on a conventional feeding program (4 L/d at 12.5%DM) and forage and concentrate feed were offered ad libitum were analyzed (Huuskonen et al., 2005; Khan et al., 2007; Kristensen et al., 2007; Huuskonen et al., 2011; Castells et al., 2012; Terré et al., 2012; unpublished data from IRTA studies). Using these data, a regression analysis was used to test whether percentage of forage intake of young calves fed low quantities of milk could be affecting concentrate feed intake. After adjusting data for the random effect of the study, and including age as a covariate, a quadratic regression analysis was performed. The resulting equation was:

$$\textit{Adjusted starter intake (kg/d)} = 0.982 + 0.007 \cdot \%\textit{Forage} - 0.00098 \cdot (\%\textit{Forage})^2$$

$$(R^2 = 0.45;\ P < 0.01)$$

Figure 8.2. Quadratic relationship between starter feed intake and percentage of forage in the diet of young calves fed low quantities of milk.



The present analysis indicates that the inclusion of forage at 3.75% of the diet maximizes starter feed intake. However, the breakpoint of the above equation at which increasing percentage of forage intake in the diet, has a negative effect on concentrate intake, was visually established around 10% of forage in the diet of young calves.

Calf starters in the diet of preweaned calves have usually low NDF content (from 12 to 20 % NDF) (Hill et al., 2008; Bateman et al., 2009). If the inclusion of forage in the diet, at some point, is beneficial in stimulating starter intake, the question is: is this due to an increase of NDF of the total diet or because the "bulky effect" of forage? To answer this question, data from the preweaning period of a total of 16 studies, which used low levels of milk replacer in their preweaning feeding program, (Abdelgadir et al., 1996b; Huuskonen et al., 2005; Lesmeister and Heinrichs, 2005; Bach et al., 2007; Khan et al., 2007; Kristensen et al., 2007; Porter et al., 2007; Terré, 2007b; Hill et al., 2008; Bateman et al., 2009; Hill et al., 2009; Bach et al., 2010; Huuskonen et al., 2011; Castells et al., 2012; Terré et al., 2012; data unpublished from IRTA commercial studies) were used to analyzed the NDF percentage of the total diet when this percentage was assessed by a forage source or by the concentrate starter. After

adjusting the data by the random effect of the study and considering age as a covariate a linear relationship of NDF content of the diet (%) and starter concentrate intake was obtained grouped by forage provision in the diet. The following equations were obtained

from data of diets with no forage provision to preweaned calves:

$$Adjusted\ starter\ intake\ (kg/d) = -0.31 + 0.029 \cdot \%NDF$$

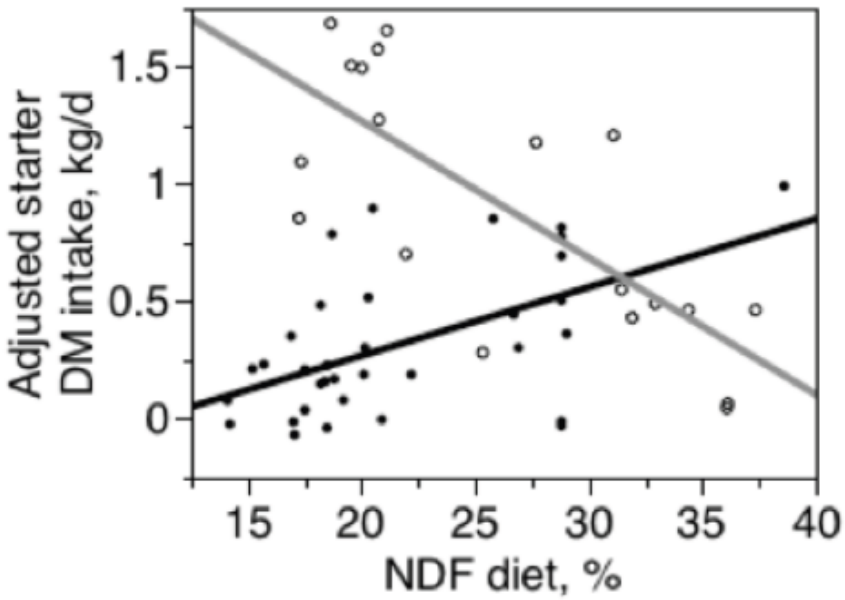
(R^2=0.29; $P < 0.001$)

from data of diets that provided forage to preweaned calves:

$$Adjusted\ starter\ intake\ (kg/d) = 2.44 - 0.058 \cdot \%NDF$$

(R^2=0.57; $P < 0.001$)

Figure 8.3. Linear relationships between starter feed intake and percentage of NDF in the diet of young calves fed low quantities of milk and grouped by forage provision (open circles, line in grey) or not forage supplementation (close circles, line in black).



From the following equations, it can be observed that at the same level of NDF content of the total diet, diets that provide forage present greater starter intake than non-forage supplemented diets. However, as the NDF of the diet in forage-supplemented calves increases, probably because of forage intake, it has a detrimental effect on starter concentrate intake. In contrast, in non-forage supplemented calves an increase

of NDF content of the concentrate stimulates starter concentrate intake. Therefore, the improvement on concentrate intake observed when including low quality of grass forages in the diet of the preweaning calves are probably due to a bulky effect, which helps to stimulate rumination and improve rumen pH of young calves, instead of an increase of the NDF content of the diet.

In conclusion, the inclusion of forage in the diet of preweaning calves below 10% of concentrate intake does not compromise starter concentrate intake, and it seems a better strategy to stimulate concentrate intake rather than increasing the NDF content of the concentrate offered.

Chapter 9

CONCLUSIONS

The results obtained in this thesis allow to conclude that:

1. Feeding pre- and postweaning calves with chopped-grass forages generally increases concentrate feed intake and ADG compared with animals fed without forage availability, without impairing feed digestibility.

2. Chopped oats hay, barley straw, and triticale silage are the best grass forage sources that improve calves performance.

3. Inclusion of alfalfa hay or non-forage fiber sources in the diet of young calves does not entail any improvement in pre- and postweaning calves performance.

4. Chopped-alfalfa or oats hay inclusion in the diet of pre- and postweaning calves directly affects rumen environment by increasing ruminal pH. Furthermore, calves fed alfalfa or oats hay have a lesser concentration of VFA in the rumen probably not due to decrease of VFA production but to an increase in expression of transporters (MCT-1) related to VFA uptake from the rumen.

5. Providing chopped oats hay in the diet of postweaned calves does not increase gastrointestinal weight compared with animals fed without forage in the diet. Therefore, weight gain of oat hay fed animals is more related to carcass weight rather than a gastrointestinal weight gain. However, when alfalfa hay is fed, empty body weight as a percentage of body weight tends to be lesser than that of animals fed oats hay or without forage in the diet. Suggesting that the increase of body weight in alfalfa hay fed calves is partly due to a gut fill effect of the forage.

6. Providing chopped oat hay in the diet of postweaned calves increases and feeding chopped-alfalfa hay results in similar rumen passage rate of an external marker than non-forage supplemented calves.

7. Performance improvements observed in animals supplemented with chopped oats hay compared with animals fed without forage during the preweaning

period disappear once non-forage supplemented calves get forage in the diet after weaning.

8. The improvements on body weight of animals supplemented with chopped oats hay in the diet during the preweaning period compared with non-forage supplemented animals disappear at 10 months of age, and there are no differences in reproductive parameters (age at first breeding, age at fertile artificial insemination, and overall conception rate) of heifers.

9. At high milk feeding rates (8 L/d), texturized concentrate feed fed without straw supplementation does not improve performance parameters compared with texturized and pelleted concentrate feeds supplemented with straw. Furthermore, calves fed texturized starter feed without forage supplementation have lower rumen pH compared with forage-supplemented calves, either on texturized or pelleted starter feeds.

Chapter 10

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