Influence of feed characteristics and sensorial perception on solid feed consumption of young calves around weaning

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RESUM

El consum de concentrat al voltant del deslletament dels vedells pot ser determinant per assegurar un correcte desenvolupament de l'animal i no comprometre la seva viabilitat. Durant aquesta tesis es varen realitzar sis estudis en vedells joves amb l'objectiu de conèixer estratègies per incrementar el consum de concentrat al voltant del deslletament. En primer lloc, es va realitzar un estudi per desenvolupar un mètode per determinar preferències oro-sensorials en vedells deslletats, aquest mètode va consistir en registrar el consum d'un grup mínim de 20 animals individualitzats, als quals se'ls ofertà una doble opció de dos ingredients o concentrats durant un període de 6 h. Amb aquest mètode es va realitzar el segon estudi, on es van determinar les preferències oro-sensorials entre 8 ingredients utilitzats com a font energètica (arròs, blat, blat de moro, *corn gluten feed*, civada, melca, ordi i segones de blat) i 6 ingredients proteics (colza, *DDG* de blat, *corn gluten meal*, girasol, pèsol i soja) habitualment utilitzats per formular concentrat. El blat i la soja van ser els ingredients preferits per vedells deslletats, mentre que el *corn gluten feed*, l'arròs i el *corn gluten meal* van ser els ingredients menys preferits a curt termini. En el tercer estudi, es testà l'efecte d'addicionar un edulcorant amb aroma al lactoremplaçant i al concentrat. En aquest estudi s'observà que al addicionar l'aroma es pot incrementar el consum de concentrat en aquells vedells que presenten un baix consum al moment del deslletament. Al quart estudi s'avaluà la capacitat de regulació dels requeriments nutricionals que poden presentar el remugants en vedells nounats. En aquest estudi un grup de vedells van rebre un concentrat convencional i es van comparar amb un altre grup als quals se'ls ofertà una bateria de diferents ingredients que composaven el concentrat. Els vedells que tenien accés a la bateria d'ingredients van descriure un consum total i creixement similar als vedells que rebien concentrat. Però en canvi van descriure un major consum de proteïna i greix, i un menor consum de carbohidrats que els que rebien concentrat. Aquestes diferències en consum de nutrients es van deure principalment per la predilecció cap a la soja i la soja grassa que van mostrar els animals que podien escollir entre diversos ingredients. El cinquè estudi va analitzar el paper de la mida de partícula del farratge en vedells lactants. En aquest estudi es comparaven dos tipus de dietes, composades per un concentrat convencional al qual se li addicionava un 10% de farratge. Aquestes dues dietes diferien en la mida de partícula del farratge, molturat (2mm) o trinxat (3-4cm). Es va observar que els animals que rebien el farratge més groller (3-4 cm) incrementaven

més el seu consum la setmana després del deslletament, millorava la seva digestibilitat de matèria seca, proteïna, fibra neutra detergent i àcid detergent, i també reduïa la realització de conductes orals no-nutritives. Es va realitzar un sisè estudi on es va avaluar el paper que tenen els opioides en la regulació de la ingesta mitjançant l'estimulació o inhibició del plaer. En aquest estudi es va provar l'efecte de la naloxona, un antagonista opioide, sobre l'elecció de concentrats preferibles en vedells deslletats, i les seves possibles interaccions amb altres metabòlits relacionats amb la regulació de la ingesta. Es va observar que en animals saciats la naloxona podria reduir el consum, indicant que els opioides intervenen en la regulació de la ingesta total, però a més es va observar redueix la preferència per concentrats amb edulcorants, que prèviament s'havien observat altament preferibles. Pel que fa als metabòlits analitzats només es va observar una interacció entre la naloxona i els nivells de *glucagon-like peptide-1*. Resumint, els vedells lactants van mostrar preferència pels edulcorants al voltant del deslletament, tant a curt termini com a llarg termini. La soja va ser un ingredient d'elecció, mentre que la civada no va ser desitjada, tant a curt termini com a llarg termini. El fet d'addicionar un mateix aroma amb edulcorant al concentrat com a la llet va incrementar el consum d'aquells animals que presentaven un consum baix prèviament al deslletament. La mida de partícula del farratge va condicionar el creixement, consum, digestibilitat i comportament dels vedells al voltant del deslletament. Finalment, es va observar que els opioides juguen un paper important en la regulació de la ingesta mitjançant l'hedonisme o el plaer.

SUMMARY

The concentrate intake around weaning can be crucial to ensure proper animal development of the animal and not compromise their viability. In this thesis, six studies in young calves were performed in order to find strategies to increase concentrate intake around weaning. The first study was conducted to develop a method to determine oro-sensory preferences in weaned calves. This method consist on measure the solid feed consumption of a minimum group of 20 calves, which recieve two options of concentrates or ingredients during a period of 6 h. Using this method, the second study was performed, where the oro-sensory preferences between 8 energetic ingredients (rice, wheat, corn, corn gluten feed, oats, sorghum, barley and second wheat) and 6 protein ingredients (canola, corn DDG, corn gluten meal, sunflower, soybean and pea) were determined. Wheat and soybean meal were the favorite ingredients for weaned calves, while the corn gluten feed, rice and corn gluten meal ingredients were less preferred in this short term study. The third study was conducted to evaluate the effect of flavoring a starter concentrate in a same manner as a milk replacer on intake and performance of young calves. This study claims, that offering a starter concentrate flavored as the milk replacer enhances solid feed consumption of those calves that have a low intake around weaning. The fourth study evaluated the ability of calves to meet their nutrient requirements when different ingredients were offered indepently in newborn calves. In this study a group of calves received a conventional concentrate whereas another group received a battery of different ingredients. The calves that had access to the free-choice of ingredients described a similar total consumption and growth compared to calves that received concentrate. However, calves that had access to all ingredients described a higher consumption of protein and fat, and lower consumption of carbohydrates that those receiving concentrate. These differences in nutrient intake were mainly due to the preference toward soybean meal and soybean full fat described by the animals that had access to all ingredients. The fifth study analyzed the role of particle size of forage in lactating cattle. This study compared two diets, composed of a conventional concentrate with a 10% of forage added. These two diets were different in the particle size of forage, ground (2 mm) or choped (3-4cm). It was observed that animals receiving chopped forage (3-4 cm) increased solid feed consumption the week after weaning, improved the apparent digestibility of dry matter, crude protein, neutral detergent fibre and acid detergent fibre, and reduce undesired

behaviors, such as non-nutritive oral behaviors. The sixth study was conducted to evaluate the role of opioids in the regulation of food intake by stimulation or inhibition of pleasure. This study tested the effect of naloxone, an opioid antagonist, on preferred concentrates consumption in weaned calves, and their possible interactions with other metabolites related to the regulation of food intake. It was observed that satiated animals treated with naloxone reduced solid feed consumption, indicating that opioids are involved in the regulation of total intake. Furthermore, calves treated with naloxone reduced their preference for concentrate with sweetener, which had been previously observed as a preferred concentrate. Regarding the analyzed metabolites, GLP-1 was influences by naloxone administration. In summary, calves showed preference for sweeteners around weaning, either in short term or long term assays. Soybean meal was a preferred ingredient, while oats was not desired, both in short term and long term. Adding a sweetener with the same aroma in concentrate and in milk replacer increased consumption of animals that had a low intake prior to weaning. The particle size of forage influenced the growth, consumption, digestibility and performance of calves around weaning. Finally, we observed that opioids play an important role in the regulation of food intake by hedonism or pleasure.

ABBREVIATIONS USED

AA: Amino acid

ADF: acid detergent fibre

ADG: average daily gain

AOAC: Association of official analytical chemists

BW: body weight

CCK: cholecystokinin

CI: conversion index

CP: crude protein

DDG: distillers dried grains

DM: dry matter

DMI: dry matter intake

EE: ether extract

GLP-1: glucagon like peptide-1

ME: metabolize energy

MR: milk replacer

NDF: neutral detergent fibre

NNOB: non-nutritive oral behavior

NRC: National Research Council

SE: standard error

TMR: total mixed ration

USDA: United States Department of Agriculture

VFA: volatile fatty acids

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Chapter 1

LITERATURE REVIEW

Introduction

In artificial rearing of calves, the period around weaning is critical to the calf for completing the transition from a preruminant animal to a functioning ruminant. This transition from liquid feed (milk or MR) to solid feed (grains and forage) is key to minimize mortality and morbidity losses related to diseases (Curtis et al., 1988; Svensson et al., 2003) and increase daily weights (Drackley, 2008). Research during the last decade has shown that feeding calves with high amounts of MR enhances growth rates and improves feed efficiency (Diaz et al., 2001; Jasper and Weary, 2002; Brown et al., 2005). Nevertheless, increased milk intake decreases intake of solid feed, and this lag in the initiation of solid feed intake could delay rumen development and retard growth at weaning (Jasper and Weary, 2002). Solid feed intake stimulates rumen microbial proliferation and VFA production, and thus initiates rumen development (Suárez et al., 2006).

This review will cover different aspects that influence performance and solid feed intake of calves around weaning, and propose strategies that may improve solid feed consumption of calves around weaning period.

1.1. Weaning

Weaning is the time when milk or MR is removed form the diet of a calf forcing the transition into solid feed as sole source of nutrients. The process of transitioning calves from their neonatal reliance on nutrients supplied from milk to nutrients supplied from solid feed (cereal grains and hay) is of substantial economic importance to the producer. Furthermore, labor is greatly reduced when calves are fed starter rations when compared to feeding milk or MR (NRC, 2001). For this reason, decreasing weaning age is an economic strategy to reduce costs. But calves at this age are most at risk for diarrhea (Svensson et al., 2003; Lundborg et al., 2005) and it is necessary to stimulate early solid feed intake to ensure an early reticulorumen development without compromising animal health. Moreover, regrouping of calves from different locations, as it is routinely done when buying calves for fattening, poses and especially great risk for these young animals, which do not yet have a complete functional immune system (Radostits, 2001).

1.1.1. Changes around weaning

The change from a liquid to a solid diet induces some changes in the gastro-intestinal tract, mainly in the rumen. The transition from "pseudo-monogastric" digestion of MR or milk to ruminant digestion of solid feed is a delicate process for the young calf, and mechanisms controlling ruminal differentiation are not entirely understood (Baldwin et al., 2004). In addition to a change of diet, calves are often regrouped and have to adjust to a new social environment.

1.1.1.1. Changes induced by diet

Digestive tract of neonatal calf undergoes an important change during the first weeks of age. At birth reticulo-rumen, omasum, and abomasum represent about 38, 13, and 49 % of stomach compartments, but at 12-14 weeks of age these stomach compartments represent 67, 18, and 15 %, respectively (Warner and Flatt, 1965). Rumen development is known to be greatly affected by diet (Tamate, et al., 1962). In contrast the abomasum is not affected greatly by diet and its growth is roughly proportional to the body proper (Stobo et al., 1966; Huber, 1969). Furthermore, another unique feature of the pre-ruminant digestive system is the esophageal groove. The esophageal groove leads from the base of esophagus (cardia) to the reticulo-omasal orifice (Ørskov et al., 1970; Ørskov, 1972). Contraction of muscles in this fold of tissues forms a tube through which milk and other liquids pass directly to the abomasum with little or no spillage into the reticulo-rumen. As solid feed consumption increases, the spillage of MR or milk into the reticulo-rumen also increases.

Solid feed consumption has an important role on rumen development. In the pre-weaned dairy calf, solid feed intake, especially high carbohydrate diets, stimulates rumen microbial proliferation and VFA production, and subsequently stimulates papillae development (Sander et al., 1959; Warner, 1991). On the other hand, forage intake stimulates rumen muscular layer, but not rumen papillae development (Tamate et al., 1962). Rumen microbial development also occurs early in life. During the first 3 weeks of life total anaerobic bacteria counts increase and remain fairly constant until 12 weeks of age (Anderson et al., 1987b; Beharka et al., 1998). However, the type of microbial population changes according to calf age. Early supply of moderate fermentable dry feed is necessary for the timely establishment of amylolytic, fibrolytic,

and proteolytic capacities of the reticulo-rumen (Sander et al., 1959; Maiga et al., 1994; Van Soest, 1994). Amylolytic, proteolytic, cellulolytic and methanogenic bacteria increase linearly in early ages (Anderson et al., 1987b; Beharka et al., 1998), at the expense of lactate-utilizing and coliform bacteria that reduce their presence gradually during the first weeks of age (Anderson et al., 1987b; Agarwal et al., 2002). Furthermore, the physical form of the diet can influence ruminal microbiota, ground diets stimulated the presence of amylolytic and reduced the amount of cellulolytic bacteria compared with feeding unground diets (Beharka et al., 1998).

1.1.1.2. Changes induced by regrouping animals

The new social environment could affect young calves, specially the low-ranking animals. Reduced access to feed in the postweaning period, due to competition, may increase distress associated with weaning and may represent a welfare problem for low-ranking animals. Recent studies on social behavior and competition in groups of newly weaned calves have been carried out in age-homogeneous groups. In this situation, aggression is rare and calves mainly engage in non-agonistic social interaction with their pen mates (Færevik et al., 2007). However, low-ranking animals are found to be displaced from limited resources such as feed and attractive resting places (Færevik et al., 2007, 2008). In practice, age differences within groups of calves may be larger than in aforementioned studies, and other studies illustrate that age heterogeneity may increase competition within groups. For instance, Hindhede et al. (1999) found that lightweight heifers had fewer lying periods and performed more abnormal oral behavior that did heavy heifers in weight-heterogeneous groups. Recently, Færevik et al. (2010) reported that age-heterogeneity leads to increase displacement from the manger and reduce weight gain, and housing calves in age-heterogeneous groups may reduce performance of young calves.

1.1.2. Milk feeding and weaning methods

Weaning is conditioned by the preweaning feeding plan. Conventional feeding provides a limited supply of milk (typically 10 % of BW/d) to calves with the aim of encouraging starter intake and promoting early rumen development and weaning. However, recent studies (Jasper and Weary, 2002; Rincker et al., 2006; Khan et al., 2007a) show benefits of feeding calves larger amounts of milk than the traditional 10 to

12 % of BW/d, such as increasing growth, improved mammary development, accelerated age at first calving and increased milk production during the first lactation. However, there is evidence that calves suffer from hunger with a restrictive diet (De Paula Vieira et al., 2008; Borderas et al., 2009). However, under field conditions, most calves are fed insufficient amounts of milk. A Canadian survey (Vasseur et al., 2010) reported that the feeding plan used in the surveyed herds was a median of 4 L of milk or MR per day given in 2 meals during the first week, 5.5 L in 2 meals between the first and the last week before weaning, and 3 L in 2 meals during the last week of milk feeding. Similarly, Pettersson et al. (2001) reported that on Swedish herds, calves generally received 5 L per day in 2 meals during the pre-weaning period.

1.1.2.1. Milk feeding

Some studies show advantages of whole milk as compared with MR: lower mortality and morbidity (Godden et al., 2005), higher energy content, and better balance of nutrients (Davis and Drackley, 1998). Also, a variety of hormones and growth factors in milk are not incorporated into MR. Nevertheless, Davis and Drackley (1998) reported similar growth rates of calves fed whole milk compared to calves fed with MR, with the benefit of lower cost per unit gain. In the US (USDA, 2007) 69 % of dairy calves are fed MR during the pre-weaning period.

There are different kinds of MR used to feed calves. The main differences among them are the protein and the lipid sources. There are two main protein sources: milk (dried skim milk, whey protein concentrate, dried whey, and sodium caseinate) or vegetable (soy protein, wheat protein, and potato protein). There are other alternative sources of protein, such as liquid or spray-dried whole egg. Identifying a high-quality and low-cost protein source has been challenging. Several authors (Scott et al., 1999; Catherman, 2002; Quigley, 2002) replaced milk protein for spray-dried whole egg as a MR protein source, and reported that BW gains of calves fed with MR containing spray-dried whole egg were less than that of calves fed an all milk protein replacer. Moreover, soy protein preparations are commonly incorporated in MR, and it has been reported (Nitsan et al., 1971; Lallès et al., 1995; Xu et al., 1997) that growth performance of calves fed a MR containing soy protein is inferior to calves fed milk proteins. Regarding the fat content, as a consequence of the high cost of milk fat its use on MR formulation is scarce. Animal fat, such as tallow or lard, and vegetable oils, such as coconut or palm oil, are

commonly used as lipid MR source. Similar results have been found in calf performance and fat digestibility when using coconut and palm oil instead of tallow (Jenkins et al., 1985; Huuskonen et al., 2005) as lipid MR source.

Much research has been conducted evaluating effects of varying MR protein and energy content (Jaster et al., 1992; Tikofsky et al., 2001; Blome et al., 2003; Bartlett et al., 2006; Bascom et al., 2007). The MR protein content varies form 18 to 28 %. The content of protein for MR manufactured with alternative proteins should be greater than in all-milk protein MR, to compensate the lower protein digestibility in alternatives protein MR (Davis and Drackley, 1998) than in milk proteins MR. Referring to energy content, carbohydrate is a more readily available form of energy than fat for lean tissue growth (Donnelly, 1983; Tikofsky et al., 2001). Increased lean gain has been observed with increased MR protein content when energy was not limiting, and increased fat gain has been reported when protein was limiting (Tikofsky et al., 2001; Blome et al., 2003; Bartlett et al., 2006). Blome et al. (2003) fed Holstein heifers MR with 16.1, 18.5, 22.9, or 25.8 % CP and measured linear increases in BW gain, gain:feed ratio, absorbed N, and retained N. All these studies have claimed that the balance among protein and energy received with MR has to be determined to optimize the efficiency of protein utilization and avoid excess of fat deposition. The NRC (2001) recommendations for CP content vary in function of BW in young calves. When calves were fed only with milk or MR, to achieve the maximum growth for 25 kg calves is recommended a 28.8% of CP content, whereas for calves of 50 kg of BW it is recommended 23.7% of CP. However, when calves fed starter and milk or MR this range of CP recommendation is between 25.1 and 23.7 % CP in function of BW (30 kg or 50 kg respectively). Furthermore, NRC recommendations for the protein to energy ratio (g of CP/ Mcal of ME) vary in function of BW and if calves receive starter or nor. To achieve the maximum growth calves fed only with milk or MR need 60 g of CP/Mcal of ME for calves of 25 kg, but calves of 50kg need 50 g of CP/Mcal of ME. However, when calves fed milk or MR with starter the range of protein to energy ratio is among 60 to 57 g of CP/Mcal of ME in function of BW (30 kg or 50 kg respectively).

1.1.2.2. Weaning method

All changes around weaning are conditioned by the weaning method, because amount of milk or MR, the quality of this milk or MR and the age of animal at weaning influence calves development. Data from one study of management practices of pre-weaned calves in the US set by the Center for Animal Health Monitoring (1993) indicated three primary methods of weaning calves: weaning according to BW, age, or when intake reached a predetermined amount of dry feed intake. When age was used as a criterion for weaning, 32.9 % of producers weaned calves at 8 wk of age, although some (2.3 %) weaned calves as early as 3 wk, and others (14.2 %) delayed weaning for 12 wk. Similarly to the majority of producers in the previous study, National Dairy Herd Evaluation Project in US (Heinrichs et al., 1995) reported an average age at weaning of 7.9 weeks. A typical recommendation is to wean large breed calves when intake of calf starter reached 700 to 1000 g/d for 3 consecutive d (Morril, 1992). In a survey performed in Sweden (Pettersson et al., 2001), the average age at weaning was 8 weeks. In this study, 46 % of the herds were weaned using calf age as weaning criterion, 18 % of the herds the concentrate consumption (the average consumption at weaning was 1 kg), 7 % of the herds used calf BW as criterion (the average weight at weaning was 70 kg), and 29 % of the herds used combinations of these alternatives to determine the appropriate weaning time. Recently, Vasseur et al. (2010) reported a survey performed in Quebec (Canada) where age was used by 66.7 % of farms as the main criteria for weaning, whereas concentrate intake was used by 43.9 %. The median age at weaning was 7 weeks, the median BW was 82 kg, and the median concentrate intake was 2 kg. The average age at weaning is higher in the United States, at 8.2 weeks (USDA, 2008).

Despite the fact that weaning in function of age reduces aggression or undesirable social interactions (Færevik et al., 2007), solid feed intake is key to minimize morbidity losses related to diseases, and increase daily weight gains (Drackley, 2008). Greenwood et al. (1997a) reported that using dry feed intake at 1 % of birth weight as a weaning criterion reduced days to weaning, increased dry feed intake from birth to 8 wk, decreased variation in weaning age, and had no apparent negative effect on growth at 20 weeks of age compared to calves weaned with starter intake of 1.5 % or 2 % of birth weight. Greenwood et al. (1997a) weaned calves at 31.7, 42.9, and 45.2 days of age

with starter intake of 1, 1.5, and 2 % of birth weight. Similarly, several authors (Anderson et al., 1987a; Klein et al., 1987) described that early-weaned calves had a higher feed intake than conventional weaned calves. Although removal of liquid diet is the major stimulus for dry feed consumption (Appleman and Owen, 1975), higher intake of starter before weaning helps to ensure intake and sustain a desirable growth rate after weaning (Kertz et al., 1979).

1.1.3. Solid feed intake around weaning

The intake of solid feed is vital to the calf for making the transition from preruminant to a functional ruminant animal. The solid feed intake around weaning can be affected by the milk feeding received during the preweaning period, the weaning method, and the solid diet characteristics.

1.1.3.1. Effects of Milk feeding and weaning methods on solid feed intake

Preweaning feeding plan and weaning method influence solid feed intake at weaning. Calves start consuming measurable amounts of solid feed at about 14 d of age (Khan et al., 2008). Around weaning, solid feed intake increases when milk or MR are reduced (Khan et al., 2007 a,b) or milk is withdrawn (Jasper and Weary, 2002). Several authors (Kertz et al., 1979; Terré et al., 2007; Raeth-Knight et al., 2009) described an inverse relationship between MR and solid feed intake. It has been reported (Jasper and Weary, 2002; Cowles et al., 2006; Raeth-Knight et al., 2009) that calves fed following the conventional feeding program with a limited supply of milk or MR (typically 10 % of BW) consumed almost twice as much starter as calves fed with higher amounts of milk or MR in the weeks before weaning. Conventional feeding program is performed with the aim of encouraging starter concentrate intake and promoting early rumen development and weaning. These calves fed limited amounts of milk showed more behavior indicatives of chronic hunger (Thomas et al., 2001; Jensen and Holm, 2003; De Paula Vieira et al., 2008). On the other hand, calves fed with higher amounts of milk reduced solid feed intake because these calves likely felt less hungry, as a consequence of increased satiety associated to the chemical (higher blood glucose and insulin) and mechanical factors (continuous gut-filling because of curd formation). Nevertheless, when calves were fed with unlimited amounts of milk or MR started to chew solid food about 2 wk of age (Forbes, 1971). Diaz et al., (2001) reported that calves consumed

bedding material in the absence of solid feed, suggesting a growing hunger for solids as age of calves.

1.1.3.2. Solid feed characteristics before weaning

The solid feed offered before weaning also has an effect on solid feed intake after weaning. The particle size, nutrient composition and forage supplement (if any) could influence on rumen development and consequently on solid feed intake. The physical form of diet influences solid feed intake. It has been reported that pelleted starter reduces starter feed intake compared to texturized or mash starters (Franklin et al., 2003). Similar performance and starter intake were reported when calves were fed with texturized, coarse or ground starters, and ground or pelleted starters (Franklin et al., 2003; Coverdale et al., 2004). Bach et al. (2007) compared pelleted starter to multiparticle starter and it was observed that the efficiency was increased with pelleted starter, because calves reduced dry feed intake of pelleted starters compared to multiparticle starter, but calves had a similar growth. Ration particle size influences ruminal environment, VFA production, and papillae structure and function. Diets that are chopped or ground to fine particle size decrease rumen pH and cellulolytic bacteria populations (Beharka et al., 1998). Furthermore, ruminal papillae of animals receiving small forage particles have increased keratinization (McGavin and Morrill, 1976). This decrease in active tissue results in decreased VFA absorption (Hinders and Owen, 1965; Nocek et al., 1980; Greenwood et al., 1997b). This fact could induce post-ingestive negative effects reducing consumption in a long-term. Providing chopped diets or forage increases chewing activity, and consequently increases ruminal pH, and helps to maintain the integrity and healthiness of the rumen wall (Krause et al., 2002; Yansari et al., 2004).

The nutrient composition of starter concentrates influences digestibility, rumen development, and animal growth; consequently influences future solid feed consumption. The levels of CP during the firsts weeks of age are crucial to optimize growth. The NRC recommendations (2001) for protein content in calf starter are about 18 %. But calf starter intake and acceptance to dry feeds is highly variable early in life (Jenny et al., 1991; Kertz and Chester-Jones, 2004), and it is difficult to specify which level of CP is more appropriate, because it is the amount of CP ingested (determined in part by the level of DM intake) and not the CP content per se, what determines growth.

Limited content of CP could induce an excessive accumulation of body fat, whereas an excessive CP content is expensive and inefficient. Furthermore, excessive nitrogen to energy ratios could cause ammonia toxicity and decrease solid feed consumption (Lobley and Milano, 1997). On the other hand, other components such as fiber could also affect solid feed intake. Previous studies (Porter et al., 2007; Zanton and Heinrichs, 2009) reported that high-fiber diets and high DMI (Zanton and Heinrichs, 2008) compromise diet digestibility. Porter et al. (2007) reported that DM digestibility in animals fed high-fiber diets (27% NDF) was lower than calves offered low-fiber diets (20% NDF). Therefore, nutrient composition of starter concentrates must be balanced to avoid inefficient use of protein, excess of fat deposition, and lower digestibility.

Introducing forage during the milk feeding is a controversial issue. It has been discouraged because forage was thought to displace concentrate intake and shift rumen fermentation in favor of acetate rather than butyrate production, delaying rumen papillae development (Tamate et al., 1962; Zintan et al., 1998). But recently, it has been reported that introducing forage during the milk feeding period improves ADG, total DM intake (Castells et al., 2012) and feed efficiency (Coverdale et al., 2004).

After weaning, voluntary feed intake in calves depends on calves' digestive capacity (rumen volume, feed fermentation patterns, absorption and metabolic activities of rumen epithelium, rumen motility and digesta flow), physio-chemical attributes of solid feed (carbohydrate nature, feed processing; protein level and quality; feed particle size and length) and changes in post-absorptive metabolites (Lesmeister and Heinrichs, 2004; Khan et al., 2007a, 2008; Porter et al., 2007). Feeding larger amounts of milk before weaning may delay physical and metabolic rumen development depressing solid feed consumption around weaning (Hill et al., 2010; Sweeney et al., 2010). For this reason it seems necessary to encourage solid feed consumption during the preweaning period without compromise calf's viability.

1.2. Feed intake regulation

As described in the previous section, solid feed intake is required to ensure rumen development. Solid diet plays an important role around weaning, because calf's digestive system is not completely mature and is developing rapidly with regard to digestive secretions and enzymatic activity (Toullec and Guilloteau, 1989; Davis and Drackley, 1998). To ensure an adequate rumen development nutrient composition is crucial, because starter concentrate composition could contain excess of nutrients, nutrient imbalances, and toxins that could adversely influence future consumption, digestibility, and consequently feed efficiency. The ingredients used to achieve these nutrient requirements are also crucial, because aspects such as digestibility, nutrient availability, and potential toxins of each ingredient have to be considered.

The regulation of feed intake is mediated by complex interactions between animal and feed characteristics, including large variety of factors, such as feed availability and quality, circulating concentrations of hormones and metabolites, as well as physical responses of the gastrointestinal system, all integrated by central nervous system to yield the final physical act of eating.

1.2.1. Sources of nutrients

Ingredients with high content on carbohydrates are the main source of energy. Cereal grains are the primary source of carbohydrates in ruminant diets. Corn, rice, barley, wheat, oat and sorghum are commonly used as carbohydrates sources in animal feeds (Huntington, 1997). The selection of one of these ingredients to formulate solid feed is not always following nutrient or digestive parameters. In most of the United States corn is the least expensive grain, consequently is commonly used in calves starter. Oats are also a commonly used ingredient in calves' starter because of bulkiness and because it is thought that oats increase palatability. But, other aspects should be considered in this election. Nutrient digestibility and grain availability to microbes are affected by the physical form of starch and the cellular integrity of starch-containing units. Small grains (wheat, barley, or oats) are more rapidly fermented than corn and sorghum (Huntington, 1997) because the distribution of starch granules within the kernel varies with cereal type (Kotarski et al., 1992; Swan et al., 2006). The ratio of amylose:amylopectin also varies in cereal grains and was negatively correlated with starch digestion (Svihus et al.,

2005). Differences in starch granule size, granule shape (lenticular, polyhedric, or spherical), and interactions between amylose and surface compounds such as fatty acids and protein could alter the rate of enzymatic digestion of corn, barley, oat, and wheat starch (Nocek and Tamminga, 1991; Kotarski et al., 1992; Svihus et al., 2005). Khan et al. (2007c) reported than an equal amount of starch supplied by corn supports more ADG in calves than starch from oats, barley, or wheat, when these ingredients were offered finely ground with free-choice hay. However, Hill et al. (2008) reported that replacing corn in a calf starter with whole oats did not reduce ADG and appeared to be an acceptance substitute.

Apart of cereal grains, other ingredients are also used as carbohydrates source, for example cane molasses. Molasses are high in sugar (sucrose, a ruminally fermented carbohydrate; NRC, 2001), are perceived to be to a palatability enhancer, and it are used to reduce the appearance of fine particles in calf starters (Morales et al., 1989). However, a high concentration of molasses (12 vs. 6 %) has been shown to decrease starter intake, may have possibly palatability problems, reduce ADG and increase the incidence of scouring in calves (Lesmeister and Heinrichs, 2005). The NRC (2001) recommended that the starter should be relatively high in readily fermentable carbohydrates but adequate in digestible fiber to support the fermentation necessary for proper ruminal tissue growth (Brownlee, 1956; Flatt et al., 1958; Greenwood et al., 1997b). The source of fiber also influences solid feed intake. Recently, Castells et al. (2012) reported that providing chopped oat hay, chopped barley straw, or triticale silage ad libitum with concentrate starter improves solid feed consumption and ADG compared to forage-deprived calves. However, these benefits were not observed when chopped alfalfa hay was offered.

The levels of CP during the firsts weeks of age are important to optimize growth. The most common protein source used in calf starter is soybean meal. Other plants such as rapeseed meal and sunflower meal are also used. In addition, other products like distillers grains could be used as a protein source. The source of protein used to achieve the appropriate level of CP could differ on the amount of digestible protein from rumen microbes, rumen undegradable protein or amino acid (AA) profile. Furthermore, digestibility could also influence the protein content of diet. It has been reported that inclusion of 20 % rapeseed meal in calf starters for rearing male calves in substitution

for soybean meal decreased organic matter digestibility (Fiems et al., 1985). Prestløkken and Rise (2003) observed that AA digestibility varies among feedstuffs. Recently, Mjoun et al. (2010) reported that distillers grains products were more resistant to ruminal degradation compared with soybean products. Consequently, more protein escaped the rumen as rumen undegradable protein in dried distiller grains sources. However, intestinal digestibility of Lys was higher in soybean products (97.3 %) compared with distillers grains (84.6 %).

In the artificial rearing of dairy calves, the same feeding plan is applied to all animals during the milk-feeding period, with individual differences attributable to development or health status rarely considered. After weaning, calves are usually grouped and all animals receive the same concentrate, despite animals could be different on BW or age. The diets applied during the first weeks of age are usually based on the NRC (2001) recommendations. These diets usually are composed with high level of CP balanced with an adequate level of energy to achieve the optimum growth in function of weight. These nutrients requirements are based on the "average" response of a group of animals in a treatment. However, each animal may have different individual feed preferences, nutrient tolerances (Provenza et al., 1996; Villalba and Provenza, 1996; Scott and Provenza, 1999) and nutrient needs (Provenza et al., 2003). Differences among individuals occur because each animal has particular differences in morphology and physiology, which are influenced by their experiences beginning in uterus (Provenza et al., 2003). Without the ability to choose, individuals may be forced either to over- or under-consume particular nutrients. If a nutrient in a pellet or a mixed ration does not set a limit on intake, individuals may over-consume that nutrient in the process of meeting needs for other nutrients. Conversely, if a nutrient limits intake of the mixed ration, individuals may not meet needs for other nutrients. Consequently, animals could use nutrients inefficiently. Offering the possibility to choose among different ingredients, ruminants have demonstrated the capability to select a diet balanced for macronutrients in response to their needs (Kyriazakis and Oldham, 1997; Villalba and Provenza, 1999; Scott and Provenza, 2000), and in some animals reaching their nutritional needs more accurately than feeding animals in a non-selecting feeding system (Provenza, 1996; Early and Provenza, 1998).

1.2.2. Mechanisms controlling feed intake in ruminants

Appetite control is a complex process that results from the interaction of multiple factors, such as quality of feed, circulating hormones, and physical response of the gastrointestinal system. All of these factors are integrated in the central nervous system.

The hypothalamus integrates various signals, resulting in activation of feeding or inhibition of feeding responses. Electrical stimulation of ventromedial nucleus of sheep and goats produced a satiety response, whereas stimulation of lateral hypothalamic area in satiated sheep and goats increase feeding (Baile and Forbes, 1974). In addition, feeding in sheep increased neurons activation of the supraoptic nucleus, whereas fasting increased neurons activation of paraventricular nucleus and dorsomedial nucleus (Chaillou et al., 2000). The arcuate nucleus, located at the base of the hypothalamus, is the major location for the processing of these appetite regulatory signals (Sartin et al., 2010). In addition to the hypothalamus, the brain stem contains pathways that are thought to control satiety or meal termination, such as the nucleus tractus solitarius. The hypothalamus and nucleus tractus solitarius act in concert to integrate signals leading to increased or decreased feed intake (Allen et al., 2009).

Neuropeptide Y has been demonstrated to be a potent appetite-stimulating neurotransmitter whose site of synthesis is localized to the arcuate nucleus in cattle (Bahar and Sweeney, 2008). The neuropeptide Y gen expression increases after short-term fasting or longer-term undernutition (Henry et al., 2000; Polkowska and Gladysz, 2001; Adam et al., 2002; Archer et al., 2002). It seems that neuropeptide Y is strongly coordinated with energy balance of the animal. Other orexigenic neurotransmitter is the agouti-related protein, which is localized in neuron cell bodies at arcuate nucleus, co-localized with neuropeptide Y neurons (Henry et al., 2001; Adam et al., 2002; Archer et al., 2002; Wagner et al., 2004). Injection of agouti-related protein into the lateral ventricle was found to increase feed intake in sheep allowed ad libitum consumption (Wagner et al., 2004).

Opiates have also been implicated in the control of feed intake in rodent species, although these molecules have been much less studied in ruminants. Opiate receptors have been found in the hypothalamus (Hokfelt et al., 1977) as well as in limbic structures involved in the control of food reward and eating behavior (Berridge et al.,

2009). Injection of the opiate antagonist naloxone was found to inhibit feed intake in sheep, suggesting a stimulatory role for opiate receptors in regulating feed intake (Alavi, et al., 1991; Obese et al., 2007). This inhibitory effect of naloxone on feed intake was more pronounced in obese sheep (Alavi et al., 1993). However, it has been reported that opiate agonists increase intake (Glass et al., 1999; Sörderplam and Berridge, 2000), and they participate in short-term regulation of feed intake promoting oro-sensory reward mechanisms. Intravenous injection of an opiate receptor agonist (syndyphalin) increases feed intake in sheep (Obese, et al. 2007). Opiate peptides may thereby influence feed intake by mediating hedonism and incentive motivation upon feed consumption (LeMagnen, 1990; Berridge, 2009), and it has been suggested that blocking their activity may render palatable feeds less rewarding (Drewnowski et al., 1992; Giraudo et al., 1993). Barbano and Cador (2006) have showed that the effects of opiates are only visible in well-fed animals because under feed-deprivation states the effect of other metabolic hormones overruns the feed control by opiates. However, it seems to be some evidence that blocking the effects of opiates in sheep may affect blood insulin concentrations (Burgwald-Balstad et al., 1995) suggesting a potential link between opiates and energy-modulating hormones.

The central nervous system regulates solid feed intake, mediating energy homeostasis, but also influencing the pleasure related to feed consumption. This regulation of feed intake is influenced by peripheral signals that depend on status of adipose tissue reserves, presentation and ingestion of feed and products of digestion. One of the hormones related to energy homeostasis that influences solid feed intake is leptin. Plasma concentrations of leptin were highly correlated with body fat mass in cattle (Ehrhardt et al., 2000), because it is secreted by adipocytes (Cowley et al., 2001). Circulating concentrations of leptin seems to serve as a signal of fat stores and energy intake of animals. Central administration of leptin suppressed feed intake in ovarioectomized ewes (Henry et al., 1999; Morrison et al., 2001). Leptin inhibits the orexigenic neurons while stimulate anorexigenic ones (Cowley et al., 2001).

Insulin is a hormone involved in the regulation of energy homeostasis and intake. Insulin is secreted by pancreas with another peptide called amylin. Amylin has a direct effect on the brain promoting satiety, depresses gastric emptying, and inhibits glucagon secretion (Riediger et al., 2004). High plasma insulin concentration has a negative

impact on feed intake. However, plasma insulin concentration could be modified for external aspects, such as the amount of milk received before weaning. Hugi et al. (1997) reported that young calves fed with large amounts of milk develop insulin resistance. This insulin resistance is developed age-dependently and is primarily a postpandrial phenomenon.

Cholecystokinin (CCK) and glucagon-like peptide 1 (GLP-1) are two peptides released by the gut that participates on the regulation of feed intake. The CCK is secreted by endocrine cells of the upper small intestine as a response to the presence of fat and protein in the duodenum (Liddle et al., 1985). In sheep it has been described that feeding fat increases plasma CCK concentration (Relling et al., 2010). This secretion of CCK has an impact in the hypothalamus, where CCK acts as a neuropeptide decreasing intake (Della Fera and Baile, 1979). Jugular infusion of CCK decreased intake in sheep (Grovum, 1981). Furthermore, CCK regulates gastric motility as well as energy intake (Nguyen et al., 2007). Referring to GLP-1, this peptide is secreted in response to the ingestion meal for the distal portion of the small intestine. Suominen et al. (1998) reported that plasma GLP-1 concentration decreased during feed deprivation in Holstein heifers. Moreover, GLP-1 reduces gastro intestinal motility and acts as a satiety signal.

Nutrients, such as glucose, exert both direct and indirect actions on the hypothalamus and on peripheral activation of nerves to the nucleus tractus solitarius to control appetite in laboratory animals (Schwartz et al., 2000; Minokoshi et al., 2004). However, intestinal, intravenous, and peritoneal glucose infusions do not decrease intake in ruminants (Faverdin, 1999; Allen, 2000). On the contrary, circulating VFA concentrations (Trenkle, 1970; Leuvenink et al., 1997; DiCostanzo et al., 1999), AA (Kuhara et al., 1991), and lipids (Choi and Palmquist, 1996; Faverdin, 1999; Allen, 2000) seem to be the nutrients stimulating regulatory mechanisms in ruminants.

1.3. Palatability of solid feed

Because fostering consumption of solid feed is a key objective around weaning, offering concentrates that are highly palatable is useful. However, information regarding oro-sensorial preferences of calves and the palatability of different feeds used to formulate starter feeds for young calves is scarce. Nowadays, oats are used in starter composition as a carbohydrate source, and it is though that oats enhance palatability (Hill et al., 2008). Molasses are also perceived as a palatability enhancer, but increasing molasses concentration on solid feed decreases starter intake and reduce ADG in calves (Lesmeister and Heinrichs, 2005). Several authors (Matthews and Temple, 1979; Arave, 1997; Spörndly and Åsberg, 2006) evaluated the preference for common ingredients in ruminants, but in each of these studies preference was evaluated with a different method. It is not clear how to measure feed palatability.

Palatability is a controversial term and it has been argued during long term. Church (1979) defined palatability as the dietary characteristics or conditions that stimulate a selective response by animal. This definition is based on the consideration that palatability is an inherent characteristic of the feed (Hodgson, 1979). Matthews (1983) suggested that palatability of a feed is interchangeable with preference for the feed, it is determined by the taste, smell, appearance, temperature and texture of the feed. However, Forbes (1986) claims that palatability cannot be considered solely as a quality of the feed, because palatability depends also on the experience and metabolic status of the animal. Kissileff (1990) suggested the use of two concepts: “intrinsic palatability”, which refers to the characteristics of a feed, and “learned palatability”, which refers to the response that is based on previous feed experience of the animal. Finally, it has been recommended that the term palatability should not be used because of the limited possibilities to define the exact nature of palatability (Ramirez, 1990). Therefore, feed preference is more appropriate than palatability to describe a desired feed under concrete conditions.

1.3.1. Measurement of feed preferences

Behavioral or intake measurements are used to determine feed preference. But, these measurements could present drawbacks that have to be controlled.

Behavioral measurements allow the evaluation of the motivation of the animal for the feed rather than the result of this motivation, which is intake. Two different types of behavior can be measured: associated with eating freely-accessible feeds or behavior that will gain access to elected feeds. Under grazing conditions eating time is much easier to measure than intake. The time spent grazing on different feeds is considered to reflect the motivation for these feeds (Newman et al., 1992). Monitoring grazing time allows an analysis of the temporal pattern of preference at pasture (Parsons et al., 1994). However, time spent grazing varies not only with the palatability of plants but also with the plants structure (height, density, etc.). Other mechanisms used to measure behavioral response is operant conditioning procedures. In farm animals a frequently used device consists of a nose-plate press. It can be used to measure preference, using these nose-plates animals have to carry out a defined behavioral sequence to be rewarded by feed. The relation between the occurrences of the responses (pushes) in the sequence and the feed deliveries is called the schedule of reinforcement (Matthews, 1983). One of the advantages of this technique is that measured behaviors and could estimate intake. However, the effect of learning and previous experience with these devices could influence animal response (Forbes and Kyriazakis, 1995; Arave, 1997).

Differences in voluntary intake cannot be attributed only to feed preference, because other aspects, such as digestive, metabolic and hormonal effects could affect animal response. For this reason, post-ingestive effects have to be considered in long-term studies. Greenhalgh and Reid (1971) developed a method to isolate feed preference from post-ingestive effects. Voluntary intake of straw or dried grass was measured, and then the same amount of the other feed was introduced directly into the rumen through a fistula. Consequently, diet composition and digestibility are kept constant, and the difference in voluntary intake between the two feeds could be attributed to feed preference. In a later experiment, Grovum and Chapman (1988) designed another technique called sham-feeding, in which the ingested feed is diverted from the digestive tract through an esophageal fistula. However, it has been described that digestion of hay is not the same if it is ingested or introduced through the fistula, influencing post-ingestive effects (Van Niekerk et al., 1973). Furthermore, Forbes (1995a) suggested that esophageal-fistulated sheep could lose saliva through the fistula and become sodium-deficient, thereby developing preferences for salty feeds. It seems that to assess feed preference avoiding post-ingestive effects it is necessary to conduct short-term

experiments. Some researchers (Gherardi and Black, 1991) have proposed short-term experiments using previously trained animals. However, pre-conditioning animals to short-term experiments may modify behavior and oro-sensorial preferences (Forbes and Kyriazakis, 1995; Arave, 1997). Consequently, short term experiments with animals that had not earlier feed experiences with the tested feeds seems to be the optimum method to asses feed preference.

1.3.2. Feed characteristics

It has been described that DM content, particle size and resistance to fracture or height and density of pasture affect ease of apprehension and mastication, and thus intake rate (Jarrige et al., 1995). In dairy cows drying the grass increases voluntary intake when the DM content of the fresh grass is lower than 15 % (Vérité and Journet, 1970). When the mean particle size of the forage decreases voluntary intake increases, eating time decreases and thus intake rate increases (Colebrook et al., 1985). Furthermore, comparing different starter feeds it has been observed that mash starters are more consumed than pelleted starter feeds (Franklin et al., 2003). In summary, physical characteristics of feeds, such as DM content or particle size could influence feed preference; consequently these parameters have to be controlled when different feeds were compared.

The taste and odor of feed are determined by the chemical characteristics of the feed. These chemicals compounds allow grazing animals to detect toxins through undesirable taste or odor. The effects of the primary tastes (sweet, salty, bitter and sour) were studied with water solutions (Goatcher and Church, 1970a,b), resulting sweet taste the most desirable taste. Accordingly, Nombekela (1995) reported preference for sweet feeds in dairy cow. Furthermore, Thomas et al. (2007) reported that calves seem to have preference for orange smell of water. These results suggested that chemical compounds affects animal feed perception and consequently modify their intake.

1.3.3. Animal characteristics

Each animal may have different individual food preferences (Provenza et al., 1996; Villalba and Provenza, 1996), because each animal has particular differences in morphology and physiology, which are influenced by their experiences beginning in uterus (Provenza et al., 2003). Furthermore, different feeds or nutrients are offered to each animal during their life, and this fact induces post-ingestive effects that could condition future intake. The affective system integrates the taste of a feed with post-ingestive feedback and the cognitive system integrates the odor and sight of the feed with its taste. Learned feed aversions against toxic plants or feeds experimentally laced with several compounds that cause malaise have been established in ruminants (Provenza and Balph, 1987; Burrit and Provenza, 1989; Ralphs and Cheney, 1993; Provenza, 1995). Moreover, Simitzis et al. (2008) described that lambs selected in favor of feeds with flavors to which they had been exposed to early in life, indicating that the flavor familiarity may influence feed preferences. These studies suggested that feed preference could be clearly marked by previous experiences.

Considering the important role of postingestive feedback on intake regulation and consequently on feed preferences, it is necessary to control that previous contact with tested feed is minimized to avoid this effect on feed preferences.

Chapter 2

OBJECTIVES

This thesis was conceived from the necessity to foster consumption of solid feed around weaning. The main objective of this work was to promote solid feed intake around weaning through palatability of feed, applying new strategies to encourage solid consumption, and amplifying the knowledge about some of the hormonal mechanisms that participate in the regulation of solid feed intake. The specific objectives were:

1. Determine the oro-sensorial preferences for common ingredients used to formulate solid feed concentrates for young calves.
2. Evaluate the importance of feed preference on solid feed consumption around weaning
3. Determine the importance of opioid-mediated hedonism on feed intake regulation.

To achieve these objectives, six studies were conducted:

- Study 1: A method was developed to evaluate oro-sensory preferences for different feeds in weaned.
- Study 2: This study was conducted to determine the oro-sensorial preferences for eight energetic and six protein ingredients commonly used to formulate starter concentrates for young calves.
- Study 3: Possible influences of previous experience were evaluated in this study, where milk replacer was flavored in a same manner as starter concentrate to encourage solid feed consumption in young calves.
- Study 4: Individual characteristics of young calves could condition feed preferences and nutrient requirements. In this study a voluntary selection of starter ingredients are offered separately to nursing calves.
- Study 5: The provision of forage could increase starter feed intake. In this study the physical form of forage on performance, feeding behavior, and digestibility of Holstein calves were evaluated.
- Study 6: This study assessed the role of opiates on oro-sensorial preference, based on the opiates role in intake regulation, and on hedonism or pleasure behavior.

Chapter 3

STUDY 1:

DEVELOPMENT OF A METHOD TO EVALUATE ORO-SENSORY PREFERENCES IN WEANED CALVES

3.1. Introduction

Information regarding oro-sensory preferences of calves and the degree of palatability of different feeds used to formulate concentrates is scarce. There have been several experimental methods used to assess oro-sensory preferences in young animals, but to our knowledge, there is no clear method to evaluate oro-sensory preferences in young ruminants. Some researchers have proposed short-term experiments (Gherardi and Black, 1991) using previously trained animals, and others have used long-term assays (Greenhalgh and Reid, 1974). However, pre-conditioning animals to short-term assays may modify behavior and oro-sensory preferences (Forbes and Kyriazakis, 1995; Arave, 1997), and long-term assays may result in interferences between oro-sensory signals and post-ingestive feedback mechanisms (Provenza, 1995). Regarding the outcome variable to determine oro-sensory preference, some researchers have proposed total feed consumption (Baumont et al., 1990; Faverdin et al., 1995); whereas others have suggested to monitor eating behavior (Matthews, 1983; Newman et al., 1992). Based on the lack of a consistent method to determine oro-sensory preferences for young calves, the objective of this study was to develop such as an experimental method.

3.2. Materials and methods

3.2.1 Animals and housing

A total of 135 recently weaned female Holstein calves (65 ± 0.3 d of age) were involved in five experiments conducted to develop a method to determine oro-sensory preferences in young calves. Calves were housed in individual hutches (1 x 1.55 m) with access to solid feed through two open areas (40 x 45 cm) onto two plastic buckets (capacity: 8 L). These buckets were subjected by a metal surface (35 x 100 cm) with two holes, and were located in front of the hutches at 60 cm to the floor and horizontally separated by 50 cm. Before weaning, calves received MR in bottles twice daily (2 L each time) at 0700 and 1900 since arrival day to one week before weaning (55 d of age), and once daily at 0700 during the last week before weaning (mean weaning age = 62 d of age). All calves participated in each of these five different experiments 3 d after weaning (65 ± 0.3 d of age; range of 55 to 72 d of age). The same concentrate (Table 3.1) was offered ad libitum in one bucket, 24h a day (except during experiments 2, 3, 4

and 5 were performed) during the first 90 days of age. Hutches were bedded with straw every 3 d and calves were managed under the guidelines and approval of the Animal Care Committee of IRTA.

Table 3.1. Ingredient composition of the concentrate diet.

Ingredient[1]	
Wheat middlings	29.0
Corn	20.0
Oats	15.0
Barley	7.0
Corn gluten meal	7.0
Soybean meal	5.1
Corn gluten feed	5.0
Distillers dried grains	5.0
Wheat	3.1
Rapeseed meal	2.1
Calcium carbonate	0.8
Sodium chloride	0.5
Vitamin and mineral premix	0.4

[1] Percentage as fed

3.2.2. Experiment 1: *Eating pattern*

The eating pattern of 35 calves (65 ± 0.7 d of age) that were weaned at 62 d of life was monitored every 60 min during a 24-h period individually. During this period, calves had *ad libitum* access to a starter feed (Table 3.1), and feed consumption was recorded at 60-min intervals weighing manually the buckets that contained solid feed. Results were used to determine the optimum time to conduct oro-sensory preference tests in recently weaned calves.

3.2.3. Experiments 2 and 3: *Sweetness preference tests*

Considering the results from Exp. 1, an experimental method was designed to test oro-sensory preferences of sweet feeds in young calves. The experimental method

consisted of offering *ad libitum* two different feeds simultaneously 1 h after the morning peak of feed consumption took place (0700). The two feeds were presented in two different buckets to each animal, in alternative location to minimize location effect, during 6 h, and feed consumption was measured every hour starting at 0800.

Experiments 2 and 3 involved two groups of 30 naive weaned calves (65 ± 0.5 d of age), that were offered a double choice of ingredients in two separate buckets: a familiar concentrate (CF; i.e., previously consumed by these calves; Table 3.1) and the same concentrate with added sucrose (SF; 10% of sucrose in Experiment 2; and 5% of sucrose in Experiment 3). Every calf only participated in one experiment conducted only one day in order to avoid the effect of previous experiences. The addition of sucrose was used to validate the method, as it is known that sucrose improves palatability and stimulates feed consumption in ruminants (Goatcher and Church, 1970a; Nombekela and Murphy, 1995).

3.2.4. Experiment 4 and 5*: Novel feeds.*

The feasibility of using the proposed experimental method to depict differences in oro-sensory preferences of calves offered novel feeds was evaluated. Novel feeds may elicit neophobia (Herskin and Munksgraad, 2000), and thus calves may be reluctant to consume a novel feed when it is first presented potentially leading to erroneous assessments of oro-sensory preferences. Experiment 4 and 5 were conducted following the same procedure described in Exp. 2 and 3, except for number of animals and the kind of feed offer. In Experiment 4, a group of 20 calves (65 ± 1.1 d of age) that were weaned at 62 d of life were offered simultaneous double choice of ground barley or ground oats grains in two separate buckets replacing starter concentrate, located in different position for each calf to minimize lateralization effect, during 6 hours, and feed consumption was measured every hour starting at 0800. In Experiment 5, to evaluate the repeatability of outcomes obtained from the use of the proposed experimental method, another group of 20 calves (65 ± 0.9 d of age) weaned 3 d before the onset of the preference assay, were also presented with a double choice of ground barley or ground oats grains following the same scheme described above.

3.2.5. Statistical Analyses

In Experiment 1, feed consumptions inferior to 20 g/d were discarded and considered null. Data were log-transformed to achieve a normal distribution and fitted to a mixed-effects model with time as a fixed effect and animal as a random effect. Oro-sensory preference of Experiments 2, 3, 4 and 5 was calculated as a percentage [(cumulative consumption of one ingredient / total cumulative solid feed consumption) x 100].

Concentrate or ingredient consumption data from each experiment (Exp. 2, 3, 4 and 5) were square root transformed to achieve a normal distribution and were analyzed using a mixed-effects model for repeated measures that accounted for the random effect of calf, and the fixed effects of feed (either sweet or plain concentrate for Exp. 2 and 3, or barley and oats for Exp. 4 and 5), time (hour), bucket location (right or left), their 2-way and 3-way interactions. Furthermore, hourly consumption data were also analyzed using a mixed-effects model that accounted random effect of calf, and the fixed effects of feed, bucket location and their 2-way interaction. Time was included in the model as a repeated measure. Oro-sensory preference data were analyzed using a mixed-effects model for repeated measures that accounted for the random effect of calf, and the fixed effects of time (hour), bucket location (right or left) and their 2-way interactions. Furthermore, oro-sensory preference data of each hour were subjected to one-sample comparison t-test using 50% as reference value (i.e., lack of preference).

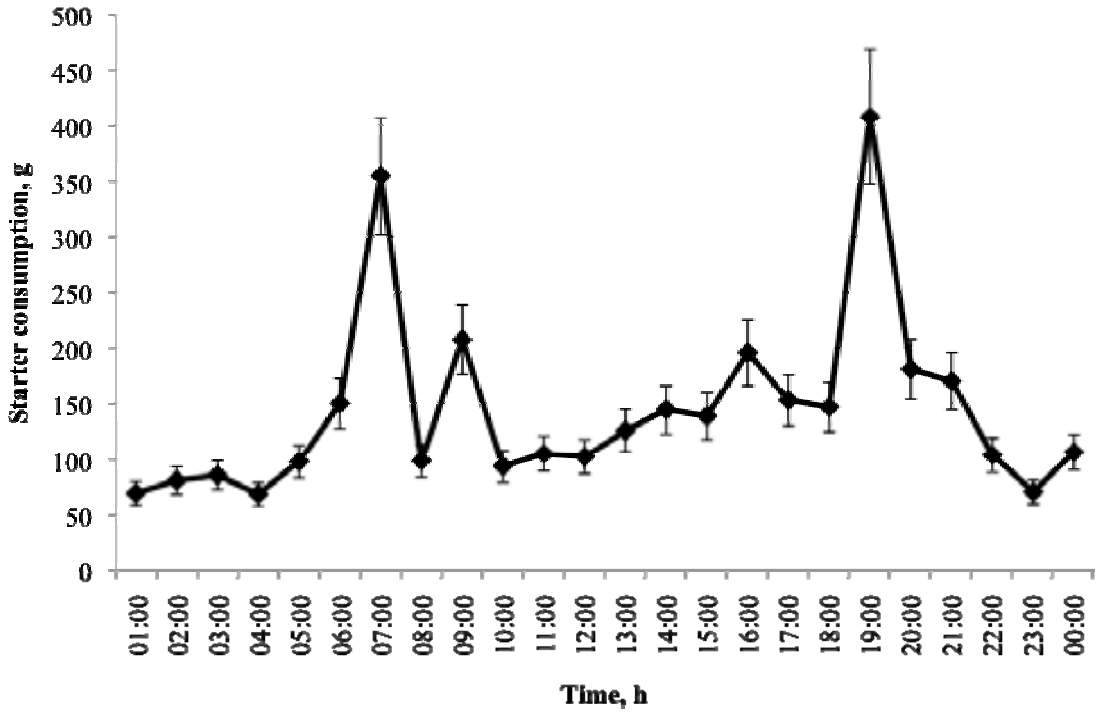
In addition, a power analysis for one sample proportion was performed using an alpha of 0.05 and a power of 0.80 with a baseline proportion of 0.5 and a minimum difference to detect of 0.25 to determine the minimum sample size required for detecting significant preferences for the sweetened concentrate. Furthermore, using oro-sensory preference data from sweetened concentrate experiments and novel feeds experiments, calves were sorted in descending order according to the preference shown for the sweetened concentrate or barley, respectively. Then, animals showing the greatest preference were sequentially removed from the dataset until the difference in consumption was no longer significant.

3.3. Results and discussion

3.3.1. Experiment 1: *Eating pattern*

Calves concentrated their starter feeding activity early in the morning and late in the afternoon (Fig. 3.1). Peaks of starter consumption occurred at 0700 and 1900 (355 g/h and 409 g/h, respectively) coinciding with times at which MR was offered before animals were weaned. Ray and Roubicek (1971) described a similar eating pattern. Metabolic and physiological states should be considered when implementing preference tests because they can modify eating behavior (Forbes, 2007).

Figure 3.1. Eating pattern (hourly consumption of starter, g/h) of recently weaned calves (Exp. 1). Bars denote standard errors.



During peaks of feed consumption, hunger is expected to reach a maximum and, consequently it may void oro-sensory preferences. For this reason, conducting preference assays during periods at which the amount of feed eaten is maximum was avoided. Palatable ingredients are commonly used with the aim of fostering solid feed intake, and thus, using animals that are partly satiated seems to be the most appropriate method to assess oro-sensory preferences. Therefore, it was decided that the optimal

time to conduct an oro-sensory preference assay would be 1 h (at 0800) following the morning peak of consumption (0700). The duration of the preference assays is a controversial issue. Short-term studies with naive animals may be blunted by neophobia against novel feeds; whereas long-term assays may be influenced by post-ingestive effects (Forbes and Kyriazakis, 1995; Arave, 1997). Because mean of fluid ruminal retention time around weaning is about 8 h (Vazquez-Añon et al., 1993), conducting preference assays for 6 h would allow achieving a good level of consumption and yet minimize post-ingestive effects as a consequence of nutrient absorption in gut. Digestion and absorption of VFA in the rumen is faster than 6 h (López et al., 1994), but it has been described (Zahorik et al., 1990) that ruminants can learn to form taste aversions, but not if the onset of illness occurs with as little as 30 min delay, and this aversion for feed was described in 18h fasted animals. In the present study calves were able to eat starter concentrate until few minutes before the experiment began, then part of the rumen content of previous consumption remains during the experiment influencing on post-ingestive effect perception of calves. Furthermore, offering a choice that makes it more difficult for the animal to associate a consequence with the food responsible (Duncan and Young 2002; Favreau et al., 2010). Consequently, it was decided that a good oro-sensory preference test should be conducted 1 h after morning feed consumption (at 0800) and run it for a 6-h period.

3.3.2. Experiments 2 and 3: *Sweetness preference tests*

When calves (n=30) were presented with a choice of plain or sweetened concentrate, bucket location has no effect ($P > 0.1$) on starter feed consumption, whereas feed and time had and effect on solid feed consumption. The interactions between these effects were not significant in experiment 2 and 3. The average of SF consumption was greater ($P < 0.01$) than CF in experiment 2 and 3 (141 vs 55 g/h and 131 vs 56 g/h, respectively). In the experiment 2, calves consumed more ($P < 0.01$) solid feed during the first two hours of assay compared to the fifth hour. In the experiment 3, calves focused their consumption basically on the first hour consuming more ($P < 0.01$) solid feed than during the rest of assay. The hourly consumption of SF was greater ($P < 0.01$) than that of CF at all times in both experiments, whereas bucket location has no effect on solid feed consumption analyzed hourly (Table 3.2). Furthermore, during the 6-h preference assay, no decrease in the consumption of SF relative to CF was observed

(Table 3.2), confirming the initial hypothesis that a 6-h preference assay would prevent or minimize potential negative post-ingestive effects. Oro-sensory preference (%) was not affected by bucket location or time ($P > 0.1$). Calves also displayed a preference ($P < 0.01$) for the SF every hour during the 6-h assay (Table 3.3). These data agree with the notion that calves appreciate the sweet taste of sucrose (Hellekant et al., 1994) and validate the use of the proposed method for studying such behavioral responses.

The minimum number of animals needed to detect differences performing the t-test comparing the oro-sensory preference to 50% (lack of preference) during the first hour of the assay was 21 and 28 calves in Exp. 2 and 3, respectively (Table 3.4). In contrast, to detect statistical differences after 6 h, only 9 and 17 calves were sufficient in Exp. 2 and 3, respectively. Considering that after 6h feed preference for SF did not changed, and calves could be reluctant to eat new feed during short term assays, 6 hours could be a reasonable time to study feed preferences. Therefore, considering that 17 calves are necessary to detect significant differences in Exp. 3, it is concluded that to conduct experiments with new feed which could compromise solid feed intake, a minimum of 20 calves is required to detect significant differences in oro-sensory preferences when the proposed experimental method is used.

Table 3.2. Hourly (g/h) and cumulative (g) feed consumption of a plain concentrate (Control) or the same concentrate containing 10% (Exp. 2) or a 5% (Exp. 3) sucrose during a 6-h oro-sensory preference assay.

	Feed consumption (g/h)		*P*-value		Cumulative feed consumption (g)		*P*-value	
Time (h)	Control	Sweet	Feed	Location	Control	Sweet	Feed	Location
Exp. 2								
1	81 ± 19.5	174 ± 25.8	<0.01	0.91	81 ± 19.5	174 ± 25.8	<0.01	0.91
2	56 ± 10.1	171 ± 29.6	<0.01	0.84	137 ± 25.6	346 ± 40.2	<0.01	0.84
3	57 ± 10.9	132 ± 19.7	<0.01	0.71	194 ± 27.6	477 ± 47.1	<0.01	0.92
4	49 ± 8.4	127 ± 17.7	<0.01	0.45	243 ± 27.0	604 ± 47.1	<0.01	0.88
5	42 ± 10.6	98 ± 21.2	<0.05	0.17	285 ± 33.0	702 ± 51.6	<0.01	0.77
6	44 ± 7.1	141 ± 15.4	<0.01	0.83	328 ± 37.4	843 ± 55.2	<0.01	0.76
Exp. 3								
1	201 ± 32.2	366 ± 43.9	<0.05	0.80	201 ± 32.2	366 ± 43.9	<0.05	0.80
2	25 ± 15.6	68 ± 16.5	<0.01	0.39	226 ± 32.0	434 ± 44.2	<0.01	0.63
3	20 ± 4.3	73 ± 20.4	<0.01	0.53	246 ± 33.2	507 ± 45.1	<0.01	0.86
4	25 ± 8.3	56 ± 13.2	<0.01	0.66	271 ± 34.1	563 ± 44.6	<0.01	0.99
5	26 ± 7.6	65 ± 14.7	<0.05	0.93	298 ± 35.8	628 ± 50.2	<0.01	0.96
6	41 ± 5.4	158 ± 26.6	<0.01	0.14	338 ± 38.3	786 ± 58.3	<0.01	0.77

Table 3.3. Preference scores calculated based on relative hourly or cumulative feed consumption of a sweetened starter (10 or 5% sucrose in Exp 2 and 3, respectively).

	Oro-sensory preference (%)[1]			
Time, h	Hourly feed consumption	*P*-Value[3]	Cumulative consumption	*P*-Value[3]
Exp. 2				
1	70.6 ± 3.97	<0.001	70.6 ± 3.97	<0.001
2	71.3 ± 3.46	<0.001	74.1 ± 3.05	<0.001
3	64.5 ± 4.62	<0.01	71.1 ± 2.37	<0.001
4	71.7 ± 3.52	<0.001	69.9 ± 2.39	<0.001
5	62.4 ± 5.41	<0.05	69.6 ± 2.64	<0.001
6	74.9 ± 3.31	<0.001	72.5 ± 2.23	<0.001
Exp. 3				
1	64.1 ± 5.06	<0.01	64.1 ± 5.06	<0.01
2	74.1 ± 4.50	<0.001	64.4 ± 4.88	<0.01
3	65.4 ± 4.16	<0.01	66.4 ± 4.26	<0.001
4	67.0 ± 4.34	<0.01	67.1 ± 3.99	<0.001
5	63.3 ± 4.70	<0.05	67.2 ± 3.83	<0.001
6	70.4 ± 4.65	<0.001	69.2 ± 3.19	<0.001

[1] Percentage of consumption of sweetened concentrate, % of hourly or total cumulative feed consumption

[2] SE = Standard Error

[3] Tests whether consumption of sweetened concentrate differs from 50%.

Table 3.4. Minimum number of calves required to detect significant differences in oro-sensory preferences (based on cumulative feed consumption) for a sweetened concentrate (10 or 5% sucrose in Exp 2 and 3, respectively) at each hour of an oro-sensory preference test.

Time, h	Calves required[1]	Oro-sensory preference (%)[2]	SE[3]	*P*-value[4]
Exp. 2				
1	21	61.5	4.83	0.028
2	15	59.5	4.04	0.035
3	10	57.6	3.19	0.042
4	11	57.5	3.06	0.035
5	13	58.2	3.54	0.040
6	9	58.4	3.15	0.028
Exp. 3				
1	28	61.4	5.31	0.040
2	27	60.6	4.89	0.040
3	24	59.5	4.25	0.035
4	22	58.3	3.94	0.048
5	22	58.7	3.81	0.033
6	17	56.4	2.64	0.028

[1]Calves showing a marked preference for sweetened concentrate were progressively removed until significance was lost.
[2] Percentage of consumption of sweetened concentrate, % of hourly or total cumulative feed consumption
[3] SE = Standard Error
[4] Tests whether the consumption of sweetened concentrate differs from 50%.

3.3.3. Experiments 4 and 5: *Novel feeds*

In both, Exp. 4 and 5, bucket location has no effect ($P > 0.1$) on starter feed consumption. During Exp. 4 there were no differences on ingredient consumption among different time, whereas in Exp. 5 ingredient consumption was greater ($P < 0.05$) in the first and the last hour compared to the others time. The average of barley consumption was greater ($P < 0.01$) than oats consumption in experiment 4 and 5 (147 vs 62 g/h and 46 vs 9 g/h, respectively). Barley was clearly consumed in greater amounts ($P < 0.01$) than oats (Table 3.5) every hour during the 6 h test in both experiments. The difference on total ingredient consumption between Exp. 4 and Exp. 5 could be explained as a consequence of weather during Exp. 5, because during the 6 h of experiment it was raining all time and calves showed less activity, and consequently less consumption, but animal age and characteristics were similar. Oro-sensory

preference (expressed as a percentage of barley consumption relative to total consumption) was also greater ($P < 0.01$) for each hour of both Exp. 4 and 5. This is in agreement with the results of Spörndly and Asberg (2006), who found that barley was one of the preferred ingredients by heifers. On the other hand, oats grains have been described as unpalatable feed in short term studies in lambs (Cannas et al., 2009), accordingly with the present results.

In addition, the repeatability of the outcome of the assay was high at all hours (Table 3.6). Although total amount of feed consumed by calves in the two assays was substantially different, both the pattern of feed consumption and the oro-sensory preference (percentage of total feed intake) were equivalent in the two experiments.

Similarly to the procedure used for the analysis of data from Exp. 2 and 3, 15 and 16 calves were required to detect differences performing the t-test comparing the oro-sensory preference to 50% (lack of preference) 1 h after the onset of the tests, in experiment 4 and 5 respectively (Table 3.7). In contrast only 9 and 12 calves were needed to detect differences after the 6-h assay, in Exp. 4 and 5 respectively. It can be concluded that the proposed experimental method allows finding differences in calves feed preferences and its oro-sensory preference outcomes are reproducible despite variability in total feed intake among assays.

The initial results would suggest that an experimental method involving less than 6 h of exposure would suffice. However, during Exp. 5, three calves did not eat until 5 h had elapsed from the onset of the experiment. This delay in feed consumption may be more pronounced when testing ingredients with poor palatability. Therefore, the experimental method to assess oro-sensory preferences in calves would consist of presenting calves with a two-bucket preference assay for a minimum of 6 h starting at one hour after MR was given before weaning, coinciding to peak of consumption (in the present study starting at 0800 and finishing at 1400). A sample size as small as 10 could be used when expected differences between ingredients are pronounced, but a larger samples size (20 calves) is recommended when small differences between ingredients are anticipated.

Table 3.5. Hourly (g/h) and cumulative (g) feed consumption of barley or oats ground grains during two 6-h oro-sensory preference assays involving 20 calves each (Exp. 4 and 5).

	Feed consumption (g/h)		*P*-value		Cumulative feed consumption (g)		*P*-value	
Time (h)	Barley	Oats	Feed	Location	Barley	Oats	Feed	Location
Exp. 4								
1	186 ± 54.4	51 ± 45.3	< 0.01	0.67	186 ± 54.4	51 ± 45.3	< 0.01	0.67
2	185 ± 31.9	32 ± 19.7	< 0.01	0.84	371 ± 68.2	82 ± 47.7	< 0.01	0.68
3	111 ± 23.4	7 ± 3.6	< 0.01	0.30	481 ± 73.9	89 ± 48.4	< 0.01	0.91
4	141 ± 28.0	15 ± 3.8	< 0.01	0.42	622 ± 76.2	103 ± 49.6	< 0.01	0.79
5	165 ± 43.5	13 ± 5.3	< 0.01	0.54	787 ± 105.5	116 ± 52.6	< 0.01	0.98
6	151 ± 30.5	29 ± 17.3	< 0.01	0.82	938 ± 113.3	145 ± 54.6	< 0.01	0.92
Exp. 5								
1	64 ± 13.1	15 ± 2.8	< 0.01	0.93	64 ± 13.1	15 ± 2.8	< 0.01	0.93
2	28 ± 8.3	6 ± 3.1	< 0.01	0.44	92 ± 16.9	20 ± 4.6	< 0.01	0.48
3	56 ± 13.4	9 ± 2.6	< 0.01	0.37	148 ± 25.2	29 ± 6.2	< 0.01	0.87
4	36 ± 8.3	5 ± 2.0	< 0.01	0.50	184 ± 28.7	34 ± 6.7	< 0.01	0.80
5	27 ± 6.5	7 ± 2.0	< 0.05	0.59	212 ± 31.6	42 ± 8.3	< 0.01	0.87
6	67 ± 15.2	8 ± 2.5	< 0.01	0.74	279 ± 34.2	50 ± 9.7	< 0.01	0.57

Table 3.6. Evolution of the relative cumulative feed consumption of barley as a proportion of total intake (barley plus oats ground grains) in Experiments 4 and 5.

	Oro-sensory preference (%)[1]			
Time, h	Hourly feed consumption	*P*-Value[3]	Cumulative consumption	*P*-Value[3]
Exp. 4				
1	84.6 ± 5.03	<0.001	84.6 ± 5.03	<0.001
2	83.2 ± 5.04	<0.001	82.6 ± 5.43	<0.001
3	84.4 ± 5.74	<0.001	85.3 ± 5.34	<0.001
4	77.2 ± 6.72	<0.01	84.8 ± 5.27	<0.001
5	87.7 ± 3.10	<0.001	85.9 ± 5.14	<0.001
6	82.3 ± 5.96	<0.001	86.4 ± 4.58	<0.001
Exp. 5				
1	68.5 ± 4.42	<0.001	68.5 ± 4.42	<0.001
2	71.5 ± 3.96	<0.001	71.4 ± 4.34	<0.001
3	70.5 ± 5.16	<0.01	74.6 ± 4.76	<0.001
4	72.5 ± 4.53	<0.001	75.8 ± 4.52	<0.001
5	67.1 ± 4.95	<0.01	75.4 ± 4.55	<0.001
6	79.4 ± 3.50	<0.001	80.3 ± 3.72	<0.001

[1] Percentage of barley consumption, % of hourly or total cumulative feed consumption (barley + oats)
[2] SE = Standard Error
[3] Tests whether the consumption of barley differs from 50%.

Table 3.7. Minimum number of calves required to detect significant differences in oro-sensory preferences (based on cumulative feed consumption) for barley at each hour of two oro-sensory preference assays (Exp. 4 and 5).

Time, h	Calves required[1]	Oro-sensory preference (%)[2]	SE[3]	*P*-value[4]
Exp. 4				
1	15	82.9	10.10	0.023
2	10	74.9	9.30	0.037
3	11	73.7	9.41	0.033
4	10	72.0	8.96	0.037
5	10	74.0	8.92	0.025
6	9	72.9	8.29	0.025
Exp. 5				
1	16	78.6	2.98	0.030
2	18	73.4	6.50	0.027
3	17	70.2	7.50	0.031
4	14	68.2	6.45	0.042
5	14	66.3	6.30	0.035
6	12	66.6	6.08	0.025

[1]Calves showing a marked preference for barley were progressively removed until significance was lost.
[2] Percentage of cumulative consumption of barley , % of total cumulative consumption (barley + oats consumption)
[3] SE = Standard Error
[4] Tests whether the consumption of barley differs from 50%.

3.4. Conclusion

In summary, the proposed experimental method for assessing oro-sensory preference in calves consists of conducting 1 h after the morning peak of feed consumption, a two-bucket preference assay in which 2 feeds are simultaneously offered *ad libitum* to a minimum of 20 naive calves and feed consumption is measured during 6 h after the onset of the test.

Chapter 4

STUDY 2:

ORO-SENSORY PREFERENCES FOR ENERGY AND PROTEIN INGREDIENTS IN WEANED CALVES.

4.1. Introduction

Because fostering consumption of solid feed is a key objective around weaning, offering concentrates that are highly palatable is commonly recommended (Morrill and Dayton, 1978). However, information regarding oro-sensorial preferences of calves and the palatability of different feeds used to formulate starter feeds for young calves is scarce. For instance, oats are commonly included in starter feeds for calves because it is believed they are highly palatable. Oats are preferred over corn, barley, wheat meal and rye in ponies (Hawkes et al., 2010), but to our knowledge, there is no evidence that oats are perceived as palatable by young calves. In fact, Bush (1989) conducted an experiment in young calves (3 to 17 wk of age) and reported maximum preferences for barley and no differences among corn, oats and wheat meal. There are several studies (Arave, 1997; Matthews and Temple, 1979; Spörndly and Åsberg, 2006) that have evaluated the preference for common ingredients in ruminants, but none of these studies involved young calves.

Therefore, the objectives of this study were to determine oro-sensorial preferences of calves for several energy and protein sources commonly used to formulate starter feeds.

4.2. Materials and Methods

This study involved a total of 49 feedstuff preference assays and 500 young female Holstein calves (65 ± 0.2 d of age; 81 ± 0.3 kg of BW) distributed in 2 separate experiments. In both experiments, each feedstuff preference assay involved 20 calves that were offered a choice of two different feeds in separate buckets ad libitum. Calves were housed in individual hutches (1 x 1.55 m) with access to solid feed through two open areas (40 x 45 cm) to two plastic buckets (capacity: 8 L). These buckets were suspended by a metal surface (35 x 100 cm) with two holes, and were located in front of the hutches 60 cm from the floor and horizontally separated from each other by 50 cm. Hutches were bedded with straw every 3 d and calves were managed under the guidelines and approval of the Animal Care Committee of Institute for Research and Technology in Agrifood (IRTA, Barcelona, Spain).

An animal model specifically developed to assess oro-sensorial preferences of calves was used in both experiments. Briefly, the model consisted of offering a choice of two

different feeds ad libitum to a group of 20 calves that had been weaned for either 3 or 5 d. The two feeds were offered ad libitum (2 kg) for 6 h. The peak of consumption of solid feed coincides with MR offer (Ray and Roubicek, 1971). Therefore, the test was conducted 1 h after the peak of consumption of solid feed under the assumption that calves would then be partly satiated, and thus they would be more sensible to oro-sensorial preferences than if calves were consuming feed driven by hunger. Furthermore, the preference assays were limited to 6 h to minimize post-ingestive effects on oro-sensorial preferences, as mean ruminal retention time of fluids around weaning age is about 8 h (Vazquez-Añon et al., 1993).

All calves were fed the same starter feed, containing most of the ingredients that were evaluated in this study (Table 4.1), from birth to the moment the feedstuff preference assays were conducted (around 65 d of age). The day that the feedstuff preference assays were performed, the starter feed was removed 1 h before the beginning of the assays, substituted by the two ingredients to be evaluated, and it was not provided again until the preference assays were completed 6 h later. Feed consumption during the 6-h preference assays was determined for each of the two feeds offered.

4.2.1.Experiment 1: *Oro-sensorial preferences for energy and protein feedstuffs commonly used in starter feeds*

The experimental model to assess oro-sensorial preferences described above was used to assess the palatability of eight energy and six protein ingredients commonly used to formulate starter feeds for calves. The energy ingredients were: barley meal, corn meal, corn gluten feed (CGF), oats meal, rice meal, sorghum meal, wheat meal, and wheat middlings. The selected protein sources were: corn gluten meal (CGM), pea meal, rapeseed meal (RSM), soybean meal (SBM), sunflower meal (SFM) and wheat distillers dried grains (DDG). To avoid potential interference of particle size on oro-sensorial preferences, all ingredients were ground through a 3-mm mesh using a hammer mill (Nöel-Guy, Bugey, France).

A total of 28 feedstuff preference assays (as described above) involving 280 calves (65 ± 0.4 d of age) were performed to conduct 28 pair-wise comparisons among all selected energy ingredients. Each preference assay involved 20 calves, and each calf participated in two different preference assays, which were conducted 3 and 5 d after

weaning. To perform the 15 pair-wise comparisons needed to evaluate oro-sensorial preferences among the selected protein sources, 15 preference assays involving 160 calves (65 ± 0.7 d of age) were conducted with 20 calves per preference assay. Each calf participated in two different preference assays, at 3 and 5 d after weaning, except the last group of 20 calves that only participated in one preference assay conducted 3 d after weaning. No calf was presented twice with the same ingredient in any of the feedstuff preference assays performed.

Table 4.1. Ingredient composition of the starter feed.

Ingredient	Concentration, % as fed
Wheat middlings	25.0
Corn meal	20.0
Oats meal	12.0
Barley meal	7.0
Corn gluten meal	7.0
Soybean meal	5.0
Corn gluten feed	5.0
Distillers dried grains	5.0
Wheat meal	3.1
Sunflower meal	3.1
Sorghum meal	3.1
Rice meal	3.0
Calcium carbonate	0.8
Sodium chloride	0.5
Vitamin and mineral premix	0.4

4.2.2. Experiment 2: *Oro-sensorial preferences for corn gluten feed, wheat meal, corn gluten meal, and soybean meal mixtures*

Four combinations of the most and least preferred energy and protein ingredients from experiment one were mixed to assess whether calves were able to maintain oro-sensorial preferences for specific ingredients within a concentrate mixture. The mixtures

(50:50) consisted of: CGM:CGF, CGM:wheat meal, SBM:CGF, and SBM:wheat meal. All ingredients were ground at 3 mm. Six preference assays and a total of 60 calves (62 ± 1.3 d of age) were needed to perform 6 pair-wise comparisons among all concentrate mixes, following the same procedure as described for experiment one. Twenty calves were involved in each test, and each calf participated in two different preference assays at 3 and 5 d following weaning without being presented twice with the same mixture.

4.2.3. Chemical and physical analyses

All ingredients were analyzed for DM (24 h at 103ºC), ash (4h at 550ºC), N content (AOAC, 2000), ether extract (EE; AOAC, 2000), neutral detergent fiber (NDF) and acid detergent fiber (ADF) (Van Soest et al., 1991). Furthermore, 100 g of each ingredient were sieved through a 2- and 1-mm mesh to determine the particle size distribution.

4.2.4. Calculations and statistical analyses

In both experiments, oro-sensorial preference was measured as the consumption of a feed expressed as a percentage of total feed consumed [(consumption of feed A / consumption of feed A + consumption of feed B) x 100]. To define the particle size distribution, the weight of the different fractions (<1mm, 1-2mm, and >2mm) was expressed as a proportion of total sample weight (g of fraction/ g of total sample).

Oro-sensorial preference was analyzed using a one-sample comparison t-test using SAS (SAS Inst. Inc., Cary, NC) to assess whether the relative consumption of a feed (as a percentage of total intake) differed from 50% (i.e., lack of preference). All statistical tests were performed using the accumulated feed consumption over the 6-h duration of each preference assay.

4.3. Results and discussion

4.3.1. Experiment 1: *Oro-sensorial preferences for energy and protein feedstuffs commonly used in starter feeds*

Energy sources. Wheat meal was preferred ($P < 0.05$) in six out of seven preference assays (Table 4.2). When confronted with wheat meal and other energy ingredients, calves consumed (or preferred) more wheat meal than barley meal, CGF, oats meal, rice

meal, sorghum meal, and wheat middlings. However, calves showed no clear preference between wheat meal and corn meal.

Table 4.2. Average consumption over a 6-h period and oro-sensorial preferences (ingredient consumption as a proportion of total feed intake) by weaned calves participating in 28 pair-wise comparisons between eight selected energy ingredients.

Ingredient A	Ingredient B	Consumption Ingredient A, g	Consumption Ingredient B, g	Oro-sensorial preference[1] A/(A + B) x 100	SE	P-value[2]
Barley meal	Corn meal	684.1 ± 75.9	410.7 ± 69.3	62.8	9.89	0.01
Barley meal	Corn gluten feed	249.0 ± 21.3	130.0 ± 52.7	55.5	13.61	0.41
Barley meal	Oats meal	469.0 ± 56.7	72.0 ± 27.3	86.4	9.6	<0.01
Barley meal	Rice meal	550.3 ± 71.3	194.8 ± 46.0	72.3	13.17	<0.01
Corn meal	Corn gluten feed	609.5 ± 115.6	41.0 ± 8.3	88.5	6.01	<0.01
Corn meal	Oats meal	656.5 ± 129.4	122.5 ± 24.4	77.8	7.77	<0.01
Corn meal	Rice meal	400.5 ± 60.5	107.3 ± 28.7	78.8	9.78	<0.01
Corn meal	Wheat middlings	301.1 ± 63.0	80.0 ± 19.5	72.2	11.65	<0.01
Corn gluten feed	Oats meal	162.0 ± 25.0	142.8 ± 28.4	53.8	12.28	0.53
Oats meal	Rice meal	287.8 ± 70.0	255.0 ± 64.2	53.2	15.41	0.67
Oats meal	Wheat middlings	427.5 ± 44.9	95.8 ± 21.9	79.8	8.94	<0.01
Rice meal	Corn gluten feed	219.4 ± 37.9	142.1 ± 28.0	52.8	14.89	0.69
Sorghum meal	Barley meal	530.5 ± 124.6	239.4 ± 55.3	65.8	13.44	0.02
Sorghum meal	Corn meal	487.8 ± 93.7	302.6 ± 84.2	66.1	14.53	0.03
Sorghum meal	Corn gluten feed	354.4 ± 77.8	160.0 ± 51.3	68.9	13.59	<0.01
Sorghum meal	Oats meal	428.0 ± 63.6	174.5 ± 29.8	67.8	8.93	<0.01
Sorghum meal	Rice meal	466.0 ± 130.6	65.5 ± 19.2	74.8	10.73	<0.01
Sorghum meal	Wheat middlings	385.5 ± 84.5	290.5 ± 64.0	53.8	13.62	0.56
Wheat meal	Barley meal	441.5 ± 19.0	124.5 ± 8.3	69.4	16.28	0.02
Wheat meal	Corn meal	411.5 ± 72.9	422.0 ± 80.9	52.3	16.5	0.77
Wheat meal	Corn gluten feed	385.0 ± 76.8	121.0 ± 23.0	66.1	14.13	0.03
Wheat meal	Oats meal	413.8 ± 68.8	58.3 ± 18.2	80.3	13.07	<0.01
Wheat meal	Rice meal	633.4 ± 120.9	141.3 ± 93.1	88.4	11.26	<0.01
Wheat meal	Sorghum	484.1 ± 81.0	125.9 ± 30.6	70.5	11.39	<0.01
Wheat meal	Wheat middlings	630.9 ± 132.1	160.9 ± 33.8	68.1	12.84	<0.01
Wheat middlings	Barley meal	415.0 ± 73.7	330.0 ± 62.8	51.6	14.17	0.82
Wheat middlings	Corn gluten feed	412.9 ± 71.6	160.4 ± 42.6	65	11.62	0.01
Wheat middlings	Rice meal	377.6 ± 59.4	127.9 ± 41.8	73.7	13.47	<0.01

[1]Relative consumption of ingredient A as a proportion of total feed intake over a 6-h period.
[2]Tests whether relative consumption of ingredient A differs from 50%.

Sorghum meal was preferred ($P < 0.05$) over barley meal, corn meal, CGF, oats meal, and rice meal (Table 4.2), and no clear preference was detected between sorghum meal and wheat midlings. Corn meal was preferred ($P < 0.05$) over CGF, oats meal, rice meal, and wheat midlings, and barley was preferred ($P < 0.05$) over corn meal, oats meal, and rice meal. The greatest feed consumption over the 6-h preference assays was obtained with wheat meal (487 ± 36.1 g), corn meal (437 ± 34.2 g), barley meal (389 ± 27.8 g), and sorghum meal (385 ± 35.1 g). A previous study conducted in young Holstein calves (Bush, 1989) reported a preference for barley over corn, oats, and wheat, but in that study calves had continuous access to the different ingredients from 3 to 17 wk of age, and thus preferences might not be directly linked to oro-sensorial characteristics of the feeds but to post-ingestive effects among other factors. However, Spörndly and Åsberg (2006) found no differences in preferences (assessed as a function of eating rate) among wheat, oats, and barley in a short-term study involving heifers. Barley was used as a control feed and was offered repeatedly throughout the experiment. This factor in conjunction with differences in age and gut development might be responsible for the discrepancies found between the study by Spörndly and Åsberg (2006) and the results presented herein.

Calves consumed less rice meal and CGF compared to the other ingredients in all preference assays. These two ingredients were not preferred in any test, and no differences in oro-sensorial preferences by calves were detected between them, indicating that rice meal and CGF have undesirable characteristics for young calves. Interestingly, in piglets, rice is considered a highly palatable ingredient (Solà-Oriol et al., 2009), but to our knowledge, no information is available for calves. Regarding CGF, contrary to the results presented herein, Schroeder (2003) did not observe changes in DMI when comparing four TMR for cows containing 0, 15, 30 or 45% of the total dietary DM as wet CGF. Also, another study (Armentano and Dentine, 1988) involving dairy cattle described that wet CGF up to 36% of dietary DM had no negative effects on DMI. Again, the lack of consistent results among studies could be attributed to differences in age and nutritional needs of the animals considered in each experiment (adult cows vs calves).

Protein sources. Soybean meal was the most preferred feed among the six selected protein ingredients (Table 4.3) as it was preferred in all five preference assays

conducted. Overall, SBM consumption was greater (664 ± 43.6 g) than that of the remaining protein ingredients (average intake = 220 ± 14.0 g). Dried distillers grains were preferred over CGM, RSM, and SFM, suggesting that DDG was one of the most desirable ingredient among the protein sources after SBM.

Table 4.3. Average consumption over a 6-h period and oro-sensorial preferences (ingredient consumption as a proportion of total feed intake) by weaned calves participating in 15 pair-wise comparisons between six selected protein ingredients.

Ingredient A	Ingredient B	Consumption Ingredient A, g	Consumption Ingredient B, g	Oro-sensorial preference[1] A/(A + B) x 100	SE	P-value[2]
DDG[3]	Corn gluten meal	407.5 ± 51.1	69.5 ± 25.3	86.0	11.05	<0.001
DDG	Pea meal	644.8 ± 85.2	490.8 ± 123.3	60.9	15.88	0.17
DDG	Rapeseed meal	625.8 ± 80.4	176.1 ± 44.1	77.0	11.63	<0.001
DDG	Sunflower meal	355.0 ± 31.5	174.4 ± 44.0	73.3	10.89	<0.001
Pea meal	Corn gluten meal	497.1 ± 85.4	40.1 ± 24.1	88.3	12.27	<0.001
Rapeseed meal	Corn gluten meal	286.8 ± 50.4	29.1 ± 15.2	90.5	5.24	<0.001
Rapeseed meal	Pea meal	125.6 ± 56.2	101.3 ± 37.4	53.1	13.24	0.63
Soybean meal	Corn gluten meal	637.8 ± 49.5	8.3 ± 0.9	98.6	0.39	<0.001
Soybean meal	DDG	666.1 ± 17.0	48.9 ± 4.3	97.4	1.46	<0.001
Soybean meal	Pea meal	796.3 ± 180.0	28.2 ± 4.3	87.8	7.99	<0.001
Soybean meal	Rapeseed meal	618.8 ± 95.5	11.9 ± 1.7	94.2	4.6	<0.001
Soybean meal	Sunflower meal	602.3 ± 49.1	103.5 ± 23.1	84.9	6.18	<0.001
Sunflower meal	Corn gluten meal	580.8 ± 57.3	14.5 ± 5.7	97.7	1.46	<0.001
Sunflower meal	Pea meal	161.8 ± 44.0	105.4 ± 36.8	59.7	20.19	0.32
Sunflower meal	Rapeseed meal	320.5 ± 56.0	21.4 ± 5.3	88.3	7.33	<0.001

[1]Relative consumption of ingredient A as a proportion of total feed consumption over a 6-h period.
[2]Tests whether relative consumption of ingredient A differs from 50%.
[3]DDG: wheat distillers dried grains

In contrast, RSM and CGM were the two least preferred ingredients by calves, with CGM being refused in all preference assays. Furthermore, total consumption of CGM (32 ± 7.7 g) by calves was clearly the least ($P < 0.05$) among all protein feeds evaluated. Lardy and Kerley (1994) suggested that, when compared with SBM, rapeseed products decrease feed intake in 200-kg steers due to the presence of glucosinolates in RSM. In contrast, Bertilsson et al. (1994) described a preference for RSM over SBM in a long-term experiment conducted with adult cows. Simlarly, Spörndly and Åsberg (2006) reported that 550-kg pregnant heifers preferred RSM (heat-treated) over SBM.

However, in the latter study, heifers were repeatedly exposed to different ingredients for 2 h twice a day during four consecutive days and consequently post-ingestive effects might have influenced the results (Forbes and Kyriazakis, 1995; Provenza, 1995). In any case, animals in the current experiment were much younger than those used in previous studies, and thus, with limited previous oro-sensorial experiences that could have influenced eating behavior and oro-sensorial preferences.

Oro-sensorial preferences for feeds are influenced by the interaction among physicochemical characteristics and factors related to the animal and context in which feeds are consumed. More precisely, chemical senses (i.e., smell, taste,...) aid ruminants to select and ingest feeds by generating information in the form of sensory inputs about the nutritional and anti-nutritional properties of feeds (Provenza, 1995). Within this context, nutrients are thought to elicit tastes that trigger hedonism, like sweet by carbohydrate (energy) sources, umami by protein sources, and salty by mineral sources. In contrast, the stimulation of sour and bitter receptors appears to cause unpleasant sensations that serve to warn the animal about deteriorated feed or toxic compounds, respectively. In addition, ingredients are characterized by a particular volatile profile (i.e., odor) that can be distinguished by the olfactory system of mammals (Zhou et al., 1999; Sides et al., 2001). Although hundreds of volatiles are synthesized at different stages of plant development, only a small subset helps animals recognize ingredients and avoid consumption of poor or dangerous feeds (Goff and Klee, 2006). Studies on flavor preferences and aversions indicate that flavor perception is also linked to the nutritional value of feeds (Provenza, 1995, 1996; Myers et al., 2005). Interestingly, CGF, oats meal, and wheat middlings had the greatest content of NDF and were among the least preferred ingredients, but rice meal, which was the least preferred energy source, had a low NDF content (Table 4.4). In the current study, the potential impact of the nutritive value of a feed on palatability was likely minimal due to the relative short duration of the test, and the oro-sensorial preferences shown by calves were most likely affected by their basal nutritive status. (i.e. it is likely that oro-sensorial preferences shown herein would be different that those shown by heifers at more advanced developmental stages).

Physical texture can also alter oro-sensorial selection of feeds (Colebrook, et al., 1985). In the present study, all ingredients were ground through a 3-mm mesh to

minimize this potential inference, but nevertheless, the resulting particle size distribution was slightly different for each ingredient (Table 4.5). Rice meal (the least preferred energy feed) was the ingredient with the greatest proportion of particles between 1 and 2 mm and the least proportion of fines (< 1 mm), but CGM, the least preferred ingredient among the protein sources, had the greatest proportion of fines. Contrary, SBM was the most preferred protein feed and had the greatest proportion of particles larger than 2 mm. Again, it could be concluded that with the experimental model used herein, factors that could potentially affect palatability, such as nutritive value, post-ingestive effects, and particle size distribution had a minimal impact on the results, and thus oro-sensorial preferences reported herein are largely a function of the inherent characteristics of the evaluated feeds and the physiological status of the calves.

Table 4.4. Nutrient composition (% of DM) of eight energy and six protein ingredients used to determined oro-sensorial preferences in weaned calves.

Ingredient	CP	EE	NDF	ADF	Ash
Barley meal	11.5	2.3	24.6	9.5	5.5
Corn meal	9.4	4.3	14.5	3.9	2.3
Corn gluten feed	22.2	2.3	44.3	11.0	8.3
Oats meal	12.7	4.0	30.8	12.6	3.4
Rice meal	8.3	1.1	3.4	1.0	0.9
Sorghum meal	12.5	3.5	13.5	6.3	2.5
Wheat meal	14.1	3.1	15.3	4.7	2.4
Wheat middlings	16.7	3.3	35.8	12.2	5.6
Corn gluten meal	72.6	0.9	2.4	0.6	3.4
Distillers dried grains	31.2	5.3	24.7	9.7	4.6
Pea meal	21.8	1.5	17.9	6.7	2.8
Rapeseed meal	43.1	2.5	31.1	20.0	9.2
Soybean meal	49.2	2.2	25.4	9.1	7.3
Sunflower meal	31.6	1.7	46.2	30.6	6.6

Table 4.5. Particle size distribution of eight energy and six protein ingredients used to determine oro-sensorial preferences in weaned calves.

	Particle size, %		
Ingredient	< 1 mm	1 mm- 2 mm	> 2 mm
Barley meal	59.58	39.52	0.21
Corn meal	74.44	24.55	0.11
Corn gluten feed	56.81	38.25	4.29
Oats meal	70.64	28.20	0.58
Rice meal	15.69	81.08	2.65
Sorghum meal	70.33	29.20	0.04
Wheat meal	68.20	29.94	0.93
Wheat middlings	77.70	21.06	0.51
Corn gluten meal	94.21	4.77	0.21
Distillers dried grains	61.17	28.47	9.72
Pea meal	82.93	16.19	0.23
Rapeseed meal	64.57	31.21	3.45
Soybean meal	21.32	44.24	33.49
Sunflower meal	69.23	25.94	3.96

4.3.2. Experiment 2: *Oro-sensorial preferences for corn gluten feed, wheat meal, corn gluten meal, and soybean meal mixtures*

Concentrate mixtures containing SBM were preferred over those containing CGM except for the mixture CGM:wheat meal (Table 4.6), with the mixture containing SBM and wheat being the most preferred. These two ingredients were the most preferred by calves in experiment one. Interestingly, when two mixtures containing one palatable and one unpalatable ingredient (SBM:CGF vs CGM:wheat meal) were compared, no differences in preferences were found. However, calves showed a clear preference ($P < 0.05$) when either of these two mixtures were compared with mixes composed of two unpalatable ingredients (CGM:CGF). These results suggest that preferences for highly

palatable ingredients (i.e. wheat meal or SBM) can be maintained even when these are part of a mixture containing a high proportion of unpalatable feedstuffs (i.e. CGM or CGF). These results are in agreement with those reported elsewhere (Armentano and Dentine, 1988; Schroeder, 2003) describing no differences in DMI with increasing proportions (up to 33 to 45%) of wet CGF in the diet of dairy cows.

Table 4.6. Average consumption over a 6-h period and oro-sensorial preferences (ingredient consumption as a proportion of total feed intake) by weaned calves submitted to 6 pair-wise comparisons between four mixtures (50:50).

Mixture A	Mixture B	Consumption Mixture A, g	Consumption Mixture B, g	Oro-sensorial preference[2]	SE	P-value[3]
SBM:Wheat meal	CGM:CGF	713.3 ± 59.3	30.6 ± 9.8	95.7	2.66	<0.001
SBM:Wheat meal	CGM:Wheat meal	472.4 ± 81.7	50.0 ± 14.4	83.1	11.37	<0.001
SBM:Wheat meal	SBM:CGF	500.9 ± 95.1	90.9 ± 24.7	80.2	11.42	<0.001
SBM:CGF	CGM:CGF	223.3 ± 35.4	47.6 ± 10.6	81.5	5.82	<0.001
SBM:CGF	CGM:Wheat meal	258.6 ± 38.5	206.9 ± 43.2	58.6	11.34	0.130
CGM:Wheat meal	CGM:CGF	439.5 ± 101.3	106.7 ± 31.3	81.8	8.1	<0.001

[1] CGF: corn gluten feed; CGM: Corn gluten meal; SBM: soybean meal.
[2]Relative consumption of mixture A as a proportion of total consumption of mixture A plus mixture B over a 6-h period.
[3]Tests whether relative consumption of ingredient A differs from 50%.

4.4. Conclusions

Wheat meal was the most preferred, and corn gluten feed and rice meal the least preferred energy ingredients by young calves among the eight feeds evaluated in this study. Soybean meal was the most, and corn gluten meal the least preferred ingredient among the six protein sources assessed herein. Oro-sensorial preferences for highly palatable ingredients are maintained when these represent a large proportion (50%) of a compound feed. Therefore, if the aim is improving oro-sensorial characteristics of starter feeds for young calves, the inclusion of wheat meal, sorghum meal, corn meal, and soybean meal, should be prioritized over the inclusion of barley meal, wheat middlings, oats meal, corn gluten feed, rice meal, distillers dried grains, sunflower meal, pea meal, rapeseed meal, and corn gluten meal.

Chapter 5

STUDY 3:

EFFECT OF FLAVORING A STARTER IN A SAME MANNER AS A MILK REPLACER ON INTAKE AND PERFORMANCE OF CALVES.

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5.1. Introduction

Recently, Thomas et al. (2007) reported that calves seem to have a preference for orange smell of water, and other authors have reported a preference for sweet products on feeds for ruminants (Goatcher and Church, 1970a; Nombekela and Murphy, 1995). In addition, it has been hypothesized that feeding behavior of ruminants is influenced by previous experience with different feed types (Provenza and Balph, 1987). This hypothesis has been reinforced by Simitzis et al. (2008), who described that lambs selected in favor of feeds with flavors to which they had been exposed to early in life. Therefore, the objective of the current study was to determine whether flavoring a MR and a subsequent starter with an orange flavor combined with a sweetener would increase solid feed intake around weaning.

5.2. Materials and methods

5.2.1. Animals and design

Twenty-two male Holstein calves (22±1.6 d old and 51.2±0.82 kg of BW) were purchased from commercial farms and raised in the facilities of IRTA (Prat, Spain) under the guidelines of the IRTA Animal Care Committee. Calves were individually-housed in wooden pens (1m×1.55m) bedded with sawdust. These pens were in a barn with forced ventilation. Starting the first day of study, all calves received the same MR (Sprayfo, Slote, Holland) flavored with a sweetener completed with orange smell (Luctarom® SFS-R, Lucta, Spain), was added to the MR at a rate of 1 g/kg just before mixing it with water (Table 5.1). All animals were offered the same unflavored starter (Table 5.1). Water and starter were offered ad libitum until the end of study at 56 d. Water was offered in individual buckets, and the starter was offered in individual metal troughs. MR was fed in buckets twice daily (2 L each time) at 0730 and 1630, at 125 g/L DM during the first 35 d of study. The experimental period of the study started at day 35, when MR was reduced to 2 L per day offered at 07:30. Each treatment group was composed of 11 calves. One treatment group continued to receive the unflavored starter (S0) whereas the other group (SF) was switched to the starter supplemented with the same flavor that was added to the MR at the rate of 1.3 g/kg before pelleting (Table 5.1). The change of starter was applied at this moment rather than at the beginning of the study to allow the assessment of a direct effect of flavor on an hypothetical increase

in solid feed consumption during weaning and avoid potential confounding effects due to previous exposure. Calves were completely weaned 7 d after MR was reduced to one daily offer, at 42 d of study, and the experiment was concluded 14 d later.

Table 5.1. Ingredient and nutrient composition (% of DM) of starters and milk replacers.

	Starter 0	Starter F	Milk replacer
Ingredients			
Barley	5	5	-
Corn	22.4	22.3	-
Oats	18	18	-
Wheat	5	5	-
Soybean	21	21	-
Wheat middlings	28	28	-
Dry sweet whey	-	-	18.9
Salt	0.09	0.09	-
Premix	0.4	0.4	-
Calcium carbonate	0.08	0.08	0.1
Dry skim milk	-	-	56
Palm oil	-	-	10
Wheat starch	-	-	8
Coconut oil	-	-	7
Flavor	-	0.13	-
Nutrients			
DM	89.8	89.7	95.7
NDF	21.9	22	-
ADF	7.5	8	-
CP	13.4	13.2	16.9
Ether extract	3.7	3.6	18
Ash	4.5	4.7	6.7

5.2.2. Measurements and chemical analyses

Consumption of MR was recorded daily for each calf. Individual starter intake was recorded daily and body weight (BW) weekly. Samples of MR and starter were analysed for DM (24 h at 103 ◦C), ash (4 h at 550 ◦C), N content using the AOAC (2000) 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, EE following the AOAC method (920.39) using petroleum ether for distillation instead

of diethyl ether (AOAC, 2000), aNDFom, with sodium sulphite and heat-stable alpha-amylase (Van Soest et al., 1991), and ADFom following the AOAC (2000) method (973.18).

5.2.3. Calculations and statistical analyses

ADG was calculated by dividing the weekly increases in BW by seven (assuming a linear increases). Applying this ADG, BW at every day within each week was estimated to determine the ratio DMI/BW on a daily basis. Feed consumption during days 28–35 (i.e., before MR offer was reduced to one daily dose) was used to calculate the daily average feed consumption the week preceding the beginning of the experimental period. This average consumption was later used as a reference to calculate the increase in daily intakes of solid feed following the reduction of MR offer that was expressed as a percentage of the feed intake level during days 28–35 of study. In addition, calves were classified as "low" or "high" intake according to the level of solid feed consumption during the week preceding the reduction of MR offer and start of the experimental period (i.e., during days 28–35 of study). The group of "low intake" included the 5 calves that had intakes below the median solid feed consumption within each treatment group, whereas the group classified as "high intake" included the remaining six calves that had intakes above the median solid feed consumption within each treatment group. Average feed consumption during days 28–35 was 612±54.5 g/d for the "low intake" group, and 1331±110.5 g/d for "high intake" group.

The effect of flavor on DMI, ADG, feed conversion index (CI), and increase in starter intake after reducing MR offer to one daily dose were compared using a mixed-effects model that accounted for the random effect of calf, and the fixed effects of pre-weaning solid feed consumption level, flavor in the starter, day (or time of measurement) and their 2-way and 3-way interactions. Time was included in the model as a repeated measure.

5.3. Results

Average total intake across the 21 d of the experimental period was similar among treatments (Table 5.2). Although not significant, starter intake from days 35 to 56 (i.e., after reducing MR offer) increased more for calves in the SF (148.9%) than for those in the S0 (123.1%) treatment (Fig. 5.1). However, calves classified as "low intake" had a greater ($P<0.001$) DMI increase (184.2±18.94%) than those classified as "high intake"

(87.8±17.30%) when MR was reduced to one daily dose (from 35 to 42 d of study), and totally removed (from 42 to 56 d of study). Within the "low intake" group, feed intake (as g/kg of BW) of calves fed SF was numerically (P=0.11) greater than that of calves fed S0 (Table 5.3). In contrast, there were no differences in the increase in DMI before and after weaning within the group of calves classified as "high intake".

Table 5.2. Effect of flavour addition to a milk replacer (MR) and a starter on body weight (BW), starter consumption, average daily gain (ADG), and conversion index (CI).

Item	Treatment			*P*-value	
	S0	SF	SE	Starter	Time x Starter
Initial age, d	21.9	22.2	2.30	-	-
Initial BW, kg	51.4	50.8	1.20	-	-
Starter intake during the first 5wk, g/d	568.1	486.9	67.65	0.40	0.99
Starter intake during the 5th wk, g/d	996.2	946.8	96.28	0.72	0.80
Starter intake/BW during the first 5 wk, % of BW	0.91	0.80	0.08	0.34	0.98
Starter intake/BW during the 5th wk, % of BW	1.49	1.44	0.10	0.75	0.77
ADG during the first 5 wk, kg/d	0.41	0.44	0.05	0.74	0.45
ADG during the 5th wk, kg/d	0.57	0.69	0.09	0.38	-
CI[†] during the first 5 wk, kg/kg	1.44	2.31	0.54	0.27	0.12
CI during the 5th wk, kg/kg	4.01	2.86	0.85	0.35	-
Experimental period (21 d)					
Initial BW, kg	66.5	66.6	2.97	0.99	-
Final BW, kg	82.5	84.9	4.34	0.70	-
Starter intake, g/d	1945.0	2115.0	122.21	0.33	0.60
Starter intake/BW, % of BW	2.57	2.77	0.09	0.14	0.95
Consumption increase, % of previous consumption (5th wk)	123.1	148.9	18.14	0.32	0.99
ADG, kg/d	0.74	0.87	0.07	0.18	0.60
CI, kg/kg	2.12	2.90	0.59	0.36	0.27
Total intake, g/d	1103.5	1118.8	81.9	0.89	<0.05
Total intake/BW, % of BW	1.55	1.56	0.07	0.93	0.08
Total ADG, kg/d	0.54	0.60	0.05	0.35	0.56
Total CI, kg/kg	1.69	2.52	0.45	0.20	0.13

[†]CI = kg of starter consumption / kg of BW gain.
S0: Starter control. SF: Starter with flavour.

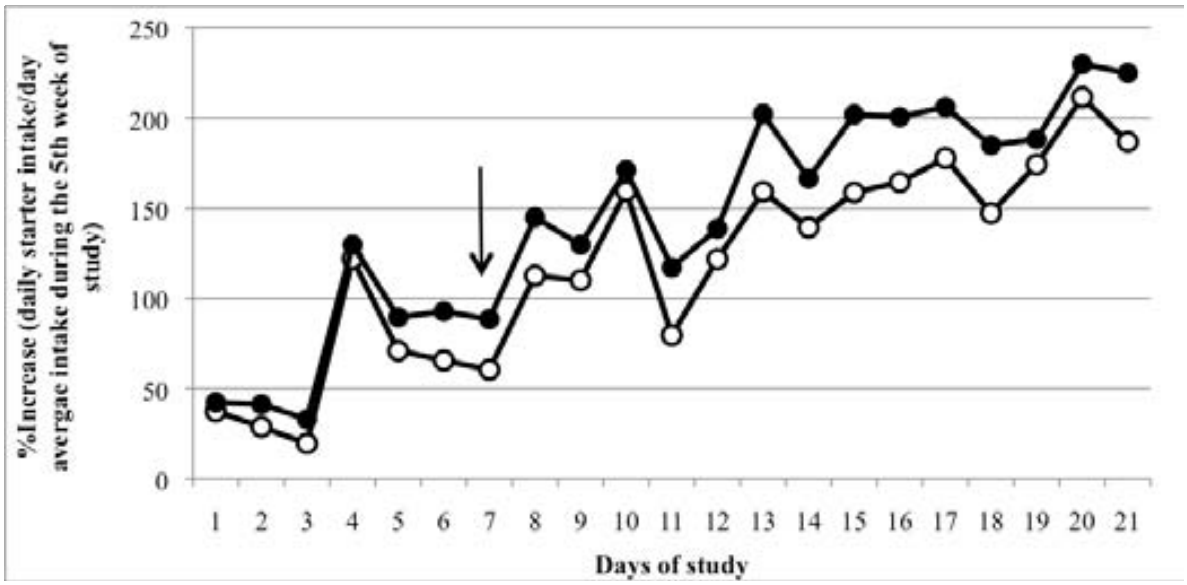
Table 5.3. Effect of flavour addition to a milk replacer (MR) and a starter on starter consumption, average daily gain (ADG), and conversion index (CI) classified as high or low eaters.

	High intake		Low intake			*P*-value				
Item	S0	SF	S0	SF	SE	Starter	Feeding Level (FL)	Starter x FL	Time x Starter	Time x FL
Starter intake from 6 to 8 wk, g/d	2459	2383	1430	1846	172.6	0.33	<0.001	0.15	0.50	0.94
Starter intake/BW from 6 to 8 wk, % of BW	2.93[a]	2.91[a]	2.22[b]	2.62[a]	0.13	0.14	<0.001	0.11	0.91	0.65
Consumption increase, % of previous consumption (5th wk)	83.0	92.5	163.1	205.3	25.63	0.32	<0.001	0.52	0.99	<0.001
ADG (kg/d) from 6 to 8 wk	0.98[a]	0.92[a]	0.50[b]	0.82[a]	0.10	0.18	<0.05	0.06	0.60	0.91
CI from 6 to 8 wk[†]	2.68	2.83	1.57	2.97	0.84	0.36	0.57	0.46	0.27	0.46

[†]CI = kg of starter consumption / kg of BW gain.
M0-S0: Milk replacer control and starter control. M0-SF: Milk replacer control and starter with flavour. MF-S0: Milk replacer with flavour and starter control. MF-SF: Milk replacer with flavour and starter with flavour.

However, calves on the SF treatment, showed a numerically lower feed consumption than S0 calves (2383 g/d vs 2459±172.6 g/d). Flavor addition to the starter did not influence overall ADG during the experimental period (Table 5.2).

Figure 5.1. Increase of intake (daily intake/daily average intake during the 5th wk of study) of calves as affected by treatments: S0 (○) or SF (●). The arrow marks the weaning day.



S0: Starter control. SF: Starter with flavour.

Within the "low intake" group (Table 5.3); however, SF calves tended (P = 0.06) to grow faster (0.82±0.062 kg/d) than S0 calves (0.50±0.075 kg/d) during the experimental period, and they almost grew as fast as the calves in the "high intake" group (0.95±0.062 kg/d). Contrary, within the "high intake" group, S0 and SF calves showed similar ADG. As a result, the coefficient of variation for ADG from days 35 to 56 of study was numerically reduced from 39 to 17.6% by the addition of flavor in the starter. The CI was unaffected by treatments (flavor or plain) and intake groups (high or low intake) of calves (Tables 5.2 and 5.3).

5.4. Discussion

Overall, the presence of flavor in the MR was not clearly related with the consumption of solid feed. Thomsen and Rindsig (1980) also reported a lack of evidence for an

associative effect between flavored MR and a similarly flavored starter. In contrast, Morrill and Dayton (1978) succeeded in increasing the amount of starter consumed by calves fed the same flavor in the milk and in the starter compared to those that did not. Differences between studies could be attributed to the type of MR or the type of flavor used in the starter, but probably the main factor was that in the present study, calves were exposed to flavored MR for the first time at the age of 23 d, whereas Morrill and Dayton (1978) presented the flavor at the age of 2 d. In addition, Morrill and Dayton (1978) registered feed consumption from 21 to 42 d of life, but in the current study calves were offered flavored starter at an approximate age of 58 d (22 d of age plus 36 d of study). Furthermore, Morril and Dayton (1978) reported a starter consumption of about 905 g/d for the flavor group and 763 g/d for the control (plain) group during the last week of study. Whereas in the current study, calves consumed, on average, 1123 g/d when starters were changed at 35 d (58 d of age) of study, with 8 calves consuming more than 2 kg/d of solid feed at weaning. Several authors have suggested (Galef and Henderson, 1972; Galef and Sherry, 1973) that mother's milk contains gustatory cues reflecting the flavor of her diet that can influence dietary preference of the offspring at weaning time, provided they consume milk since birth. It is possible, therefore, that the expected effects of using the same flavor to facilitate the association between the MR and the starter might have been hampered by the relatively late age of calves when first exposed to the flavor studied, or by the short adaptation period to the flavored starter. On the other hand, the addition of flavor to the starter increased consumption of solid feed by calves classified as "low intake". Based on these observations, it seems reasonable to suggest that the positive effects of flavor in the starter are more pronounced in animals with low, rather than high, solid feed intake preceding weaning. As a result of the greater consumption of solid feed between days 35 and 56 of study observed in the "lower intake" calves within the SF treatment, calves in the SF treatment had a greater ADG than S0 calves within "low intake" group. This result further supports that flavoring MR and starter jointly could encourage animals with poor appetite to consume more solid feed and consequently improve growth rate around weaning. Although non-significant, the coefficient of variation in the SF calves was lower than that observed in the S0, and a reduction in coefficient of variation is always desirable because it makes feeding and management of the animals easier than if large differences exist among calves within the same group.

5.5. Conclusions

In summary, flavoring the starter of calves may have nil effects on animals with average or above-average appetite. However, offering flavored solid feeds around weaning to animals with a low drive to eat (less than about 600 g/d) may stimulate feed intake and increase growth performance after weaning. As a consequence, variations in performance parameters and final BW might be reduced facilitating more homogeneous animal groups.

Chapter 6

STUDY 4:

VOLUNTARY SELECTION OF STARTER FEED INGREDIENTS OFFERED SEPARATELY TO NURSING CALVES

6.1. Introduction

Theoretical nutrient requirements of calves are based on the average response of a group of animals in a specific dietary treatment. However, each animal may have different individual feed preferences, nutrient tolerances (Provenza et al., 1996; Villalba and Provenza, 1996; Scott and Provenza, 1999) and nutrient needs (Provenza et al., 2003). Pelleted or TMRs may force calves to over- or under-consume particular nutrients with respect to their individual needs. Small ruminants have demonstrated the ability to select a diet adequately balanced for macronutrients when offered the opportunity to choose among different ingredients or diets (Kyriazakis and Oldham, 1993; Villalba and Provenza, 1999; Scott and Provenza, 2000; Bach et al., 2012). Furthermore, in some instances, ruminants have been able to meet their nutritional needs more accurately when offered a choice of ingredients than when feeding a single diet (Provenza, 1996; Early and Provenza, 1998). Our hypothesis was that newborn calves offered a choice of different ingredients would be able to meet their nutritional needs by properly selecting different proportions of the available ingredients compared to those calves offered a single solid feed. Thus, the objective of this study was to evaluate the benefits of providing a choice of different ingredients compared to a single concentrate to young calves.

6.2. Materials and methods

6.2.1. Animals, housing and milk feeding

Thirty-eight Holstein male calves (initial live weight 41.0 kg and 7 days of age) were purchased from commercial farms and raised in the facilities of Mas Jonquer (Parets d'Empordà, Spain). After arrival, all calves received 3 ml of a broad-spectrum antibiotic (Draxxin, tulathromycin, Pfizer Animal Health, Spain). Furthermore, calves were vaccinated against respiratory syncytial virus (2 ml of Rispoval RS, Pfizer Animal Health, Spain) 3 days after arrival. Calves were housed in individual hutches (1.10 x 1.40 m) with an open area (1 x 1.25 m) during the 35 days of study. Hutches were bedded with sawdust every 3 days. Calves were managed under the guidelines and approval of the Animal Care Committee of IRTA (Barcelona, Spain). Each hutch had seven places to hold buckets that contained different feeds and water separately. A commercial MR [24% crude protein (CP) and 19.5% fat, Sereno, Celtilait, France] was

offered in a bucket twice daily at 0700 and 1600 h. Calves received 4 l per day of MR at 10% DM dilution rate until 28 days of age (21 days of study). From 28 to 35 days of age, calves received only a morning feeding of 2 l at 10% DM. Animals were weaned at 35 days of age, and the study ended when calves were 42 days old (day 35 of study).

6.2.2. Treatments, feed sampling and chemical analyses

The day after arrival, animals were weighed and randomly distributed according to BW and age in two treatments: control (CTR) or free-choice (CH). Calves in CTR treatment (40 ± 1.2 kg of BW and 7 ± 0.7 days of age) were fed a ground starter feed, whereas each calf in CH treatment (42 ± 1.2 kg of BW and 7 ± 0.8 days of age) received the same ingredients (barley, corn, oats, full fat soybean, soybean hulls, and soybean meal) that composed CTR starter feed offered in separate buckets. The six ingredients were offered in the same location for each calf on CH treatment during all the study. To avoid potential interference of particle size on preference or nutrient selection, all ingredients were ground to 3 mm. Despite this fact, not all ingredients present the same distribution of particle size, for this reason, 100 g of each ingredient were sieved through a 2- and 1-mm mesh to determine the particle size distribution. Throughout the study, individual daily intake of starter and ingredients was recorded. Buckets were checked on a daily basis to ensure ad libitum provision of feed. Calves were weighed at the same time each day on two consecutive days each week during the 5 weeks of study.

To determine DM and nutrient content of the experimental feeds (starter feed or individual ingredients), fresh samples of each feed were taken on a weekly basis and frozen at -20 ºC. After completion of the experiment, these samples were thawed and pooled within feed and sent to Laboratorio de Mouriscade (Pontevedra, Spain) for analysis of DM (24 h at 103ºC), ash (4 h at 550ºC), NDF with heat-stable–amylase and sodium sulfite (Van Soest et al., 1991), nitrogen content using the AOAC (2000) method (988.05), and EE using the AOAC method (920.39) using petroleum ether for distillation instead of diethyl ether (AOAC, 2000).

6.2.3 Calculations and statistical analyses

Ingredient consumption (g/d) of the CTR calves was estimated using the proportion of each ingredient in the starter feed multiplied by the consumption of starter feed. The CP to energy ratio was calculated as the total CP consumption from all ingredients divided by the total metabolizable energy (ME) consumed from all ingredients. For the CTR calves, the CP to energy ratio was fixed at 53 g of CP/Mcal of ME. The ME content of each ingredient was calculated based on NRC (2001). The efficiency of CP utilization was calculated as the ADG divided by the total CP consumption (from MR and starter feed). To define the particle size distribution, the weight of the different fractions (<1 mm, 1-2mm, and >2 mm) was expressed as a proportion of total sample weight (g of fraction/ g of total sample).

All data were summarized by calf and week. Data pertaining to total DMI, nutrient intake, ingredient intake, MR intake, ADG, gain to feed ratio, and CP efficiency were analyzed with a mixed-effects model for repeated measures. The statistical model included initial BW as a covariate, treatment, week of study and the 2-way interaction between treatment and week as fixed effects, and animal as a random effect. Due to the lack of normality, data pertaining to intake of starter feed, individual ingredients, and single nutrients were root square-transformed prior conducting the statistical analysis. Least square means presented herein for intake of total feed, individual ingredient and single nutrients correspond to non-transformed data, and SEM and P-values correspond to the results from mixed effects model using root-square transformed data.

The CP to energy ratio was analyzed using a one-sample comparison t-test to assess whether the ratio of CH group differed from 53 g of CP/Mcal of ME (the ratio offered in the CTR treatment).

Last, individual consumption of each separate ingredient among the CH calves was also analyzed separately with a mixed-effects model for repeated measures. The statistical model included ingredient, week of study, and their 2-way interaction as fixed effects, and animal as a random effect.

6.3. Results

The chemical composition of the starter concentrate and the tested ingredients are presented in Table 6.1. The resulting particle size distribution was slightly different for

each ingredient (Table 6.2), despite all ingredients were ground through a 3-mm mesh to minimize the potencial interference of physical texture.

The effects of providing a choice of different ingredients compared to a single concentrate on total feed intake, performance, nutrient and ingredient intake data are presented in Table 6.3. No differences were observed between CTR and CH calves on solid feed intake, MR consumption, BW, ADG and gain to feed ratio (Table 6.3).

Table 6.1. Ingredients and chemical composition of the starter feed, and chemical composition of the individual ingredients.

		Ingredient[1]					
Item	Starter	CM	SBM	OM	BM	SBH	FFSB
Starter composition, % DM							
	-	47.2	20.0	11.0	10.1	8.0	1.2
Chemical composition							
CP, % of DM	17.7	8.9	49.1	11.9	10.9	13.1	42.6
EE, % of DM	3.9	3.7	2.4	5.6	2.1	3.3	21.3
NDF, % of DM	20.1	14.0	17.9	37.6	23.7	69.4	12.4
NFC, % of DM	52.6	71.8	23.5	39.8	59.9	9.5	17.5
Ash, % of DM	5.1	1.3	6.2	4.5	3.0	4.2	5.5
ME, Mcal/kg of DM	3.3	3.3	3.8	3.1	3.2	2.8	4.8

[1] CM = Corn meal; SBM = Soybean meal; OM = Oats meal; BM = Barley meal; SBH = Soybean hulls; FFSB = Full fat soybean.

Table 6.2. Particle size distribution of ingredients offered to CH calves.

	Particle size, %		
Ingredient	< 1 mm	1 mm- 2 mm	> 2 mm
Barley meal	59.58	39.52	0.21
Corn meal	74.44	24.55	0.11
Oats meal	70.64	28.20	0.58
Soybean hulls	77.70	21.06	0.51
Soybean meal	21.32	44.24	33.49
Soybean full fat	69.23	25.94	3.96

There were no differences in total ME consumption between CH and CTR animals. However, the energy source differed between CTR and CH calves. Calves in the CH treatment consumed more ($P < 0.01$) fat and less ($P < 0.01$) NFC than CTR calves (Table 3), especially during the last 2 weeks of study (Figure 6.1). Calves in CH group consumed more ($P < 0.01$) CP (166.9 ± 8.29 g/d) than CTR calves (132.5 ± 8.29 g CP /d). Therefore, calves on the CH group consumed a greater ($P < 0.01$) dietary CP to energy ratio of (81 g of CP/Mcal of ME) than CTR calves (53g of CP/Mcal of ME). However, the difference in CP consumption did not influence ADG, consequently, CTR calves had a greater ($P < 0.05$) efficiency of CP utilization for growth than CH animals (0.62 vs 0.41 g of ADG / g of CP consumed).

Table 6.3. Performance, dry matter, and nutrient intake of calves fed a starter feed or the same ingredients in separate buckets[1].

	Treatment[2]			P-value[3]		
	CTR	CH	SE[4]	T	W	T × W
Performance and intake						
Solid feed intake, kg/d	0.38	0.36	0.99	0.83	<0.01	0.20
Milk replacer, kg/d	0.27	0.27	0.02	0.53	<0.01	0.82
BW, kg	46.2	46.5	0.67	0.75	<0.01	0.86
ADG, kg/d	0.31	0.32	0.03	0.92	<0.01	0.49
Gain to feed ratio, kg/kg	0.47	0.47	0.05	0.95	<0.01	0.51
Nutrient intake from solid feed						
CP, g/d	67.3	105.5	0.48	<0.01	<0.01	0.27
NDF, g/d	76.6	84.6	0.47	0.63	<0.01	0.23
NFC, g/d	200.5	131.2	0.71	0.02	<0.01	<0.01
EE, g/d	14.9	22.8	0.24	0.03	<0.01	0.40
ME, Mcal/d	1.25	1.32	0.05	0.66	<0.01	0.32
Ingredient intake, g/d						
Barley	38.6	56.7	0.61	0.59	<0.01	0.95
Corn	180.5	67.1	0.72	<0.01	<0.01	<0.01
Oats	42.1	13.0	0.46	<0.01	<0.01	<0.01
Soybean meal	76.5	118.4	0.94	0.58	<0.01	0.61
Full fat soybean	4.6	67.1	0.56	<0.01	<0.01	0.01
Soybean hulls	30.6	39.1	0.42	0.92	<0.01	0.01

[1]Data are weekly averages from 19 calves on each treatment

[2]CTR = calves offered a starter feed; CH = calves offered all the ingredients that composed the CTR starter feed separately.

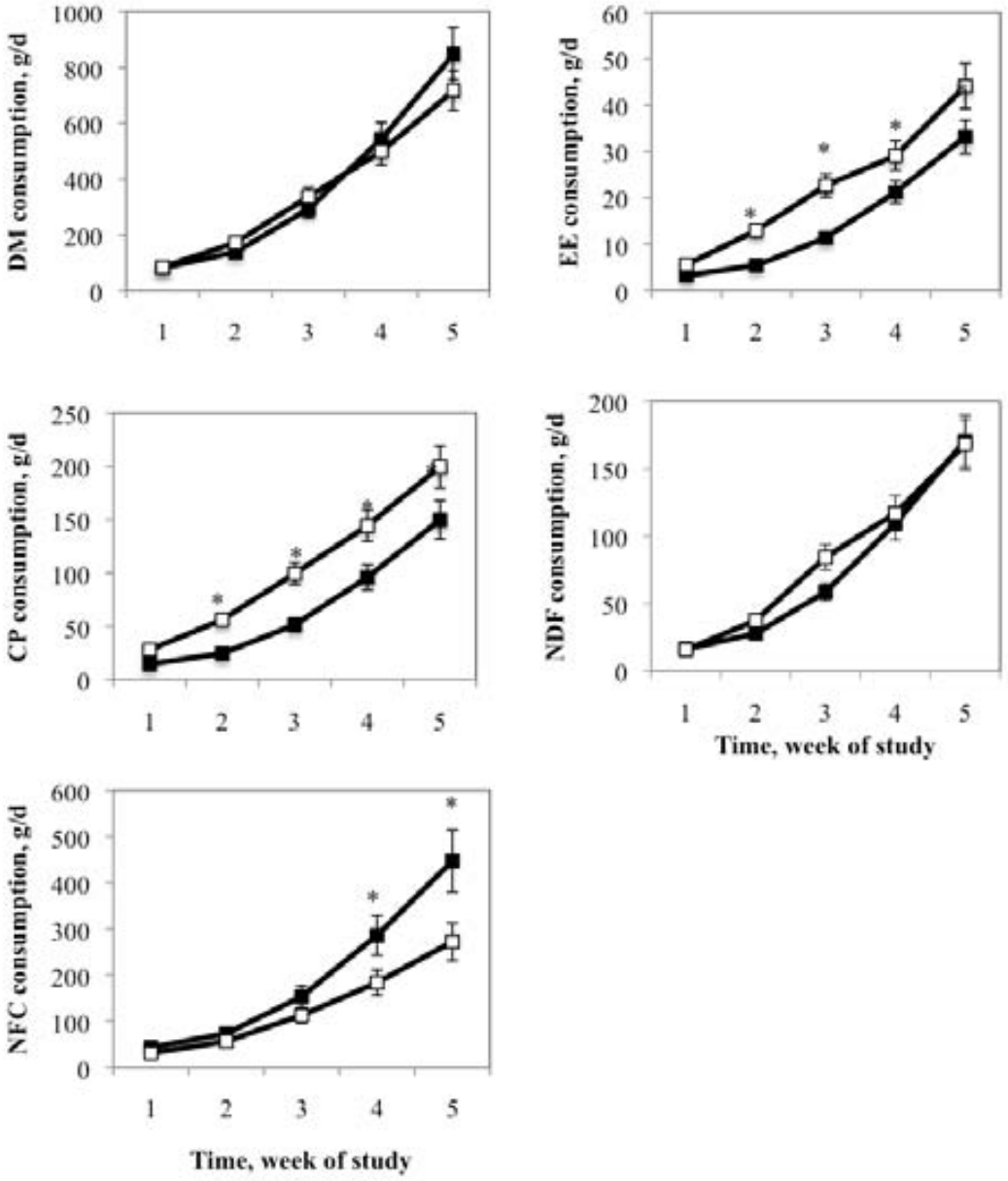
[3] T = effect of feeding either a started feed or its ingredients in different buckets; W = effect of week of study; T × W = interaction of week and treatment.

[4]Standard error. SE of the solid feed intake, nutrient intake and ingredient intake corresponds to root-transformed data, whereas are non-transformed.

The differences in CP, NFC, and EE consumption were mainly a consequence of changes in intake of corn, oats, and full fat soybean between the two treatments (Table 6.3). Calves on the CTR treatment consumed more ($P < 0.01$) corn and oats than CH calves, and therefore more NFC. However, CH calves consumed more ($P < 0.01$) full fat soybean than CTR animals, and therefore more CP and EE (Figure 6.2).

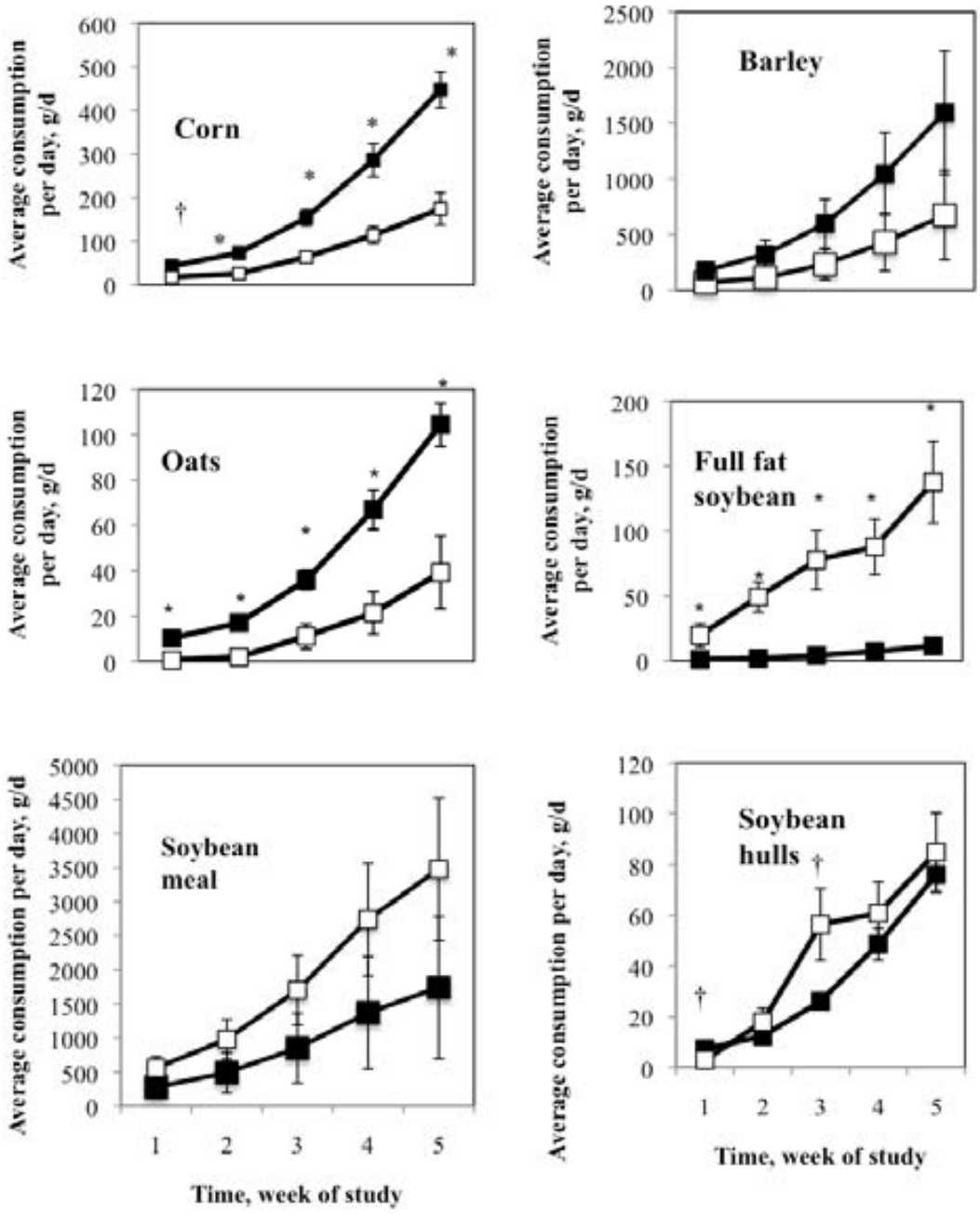
Furthermore, during the first and the third week of study, CH calves tended ($P < 0.10$) to consume more soybean hulls than CTR calves (Figure 6.2), and no differences were observed in barley and soybean meal consumption between treatments (Figure 6.2).

Figure 6.1. Daily consumption of dry matter, crude protein, fat (as ether extract), neutral detergent fiber and non-fiber carbohydrates during the 5 weeks of study in control (■) and free-choice (□) calves.



*CP, EE or NFC consumption (g/d) differs between treatments ($P < 0.05$)

Figure 6.2. Daily consumption of corn, oats, full fat soybean and soybean hulls during the 5 weeks of study in control (■) and free-choice (□) calves.

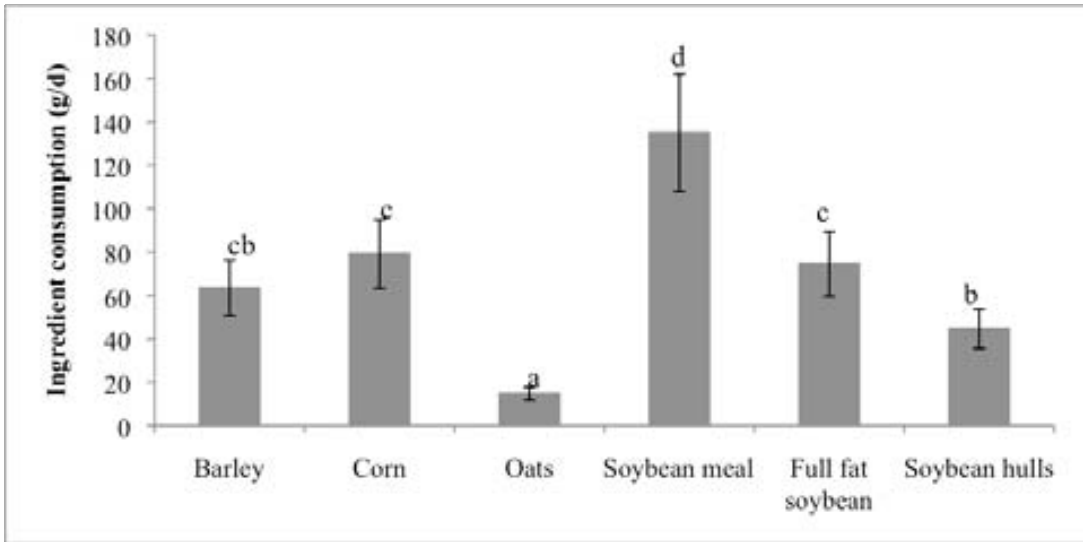


*Average consumption per day (g/d) differs between treatments ($P < 0.05$)

† Average consumption per day (g/d) tended to differ between treatments ($P < 0.10$)

Within the CH calves, soybean meal was consumed the most (P< 0.01) over the other 5 ingredients (Figure 6.3), whereas oats was the least consumed ingredient followed by soybean hulls. Furthermore, observing the variation among days on feed intake, the preference for soybean meal was numerically increasing during the experiment (Figure 6.4).

Figure 6.3. Ingredient consumption of calves offered a choice of six ingredients during the 35 days of study.



[abcd] Bars with uncommon superscripts differ (P < 0.05).

6.4. Discussion

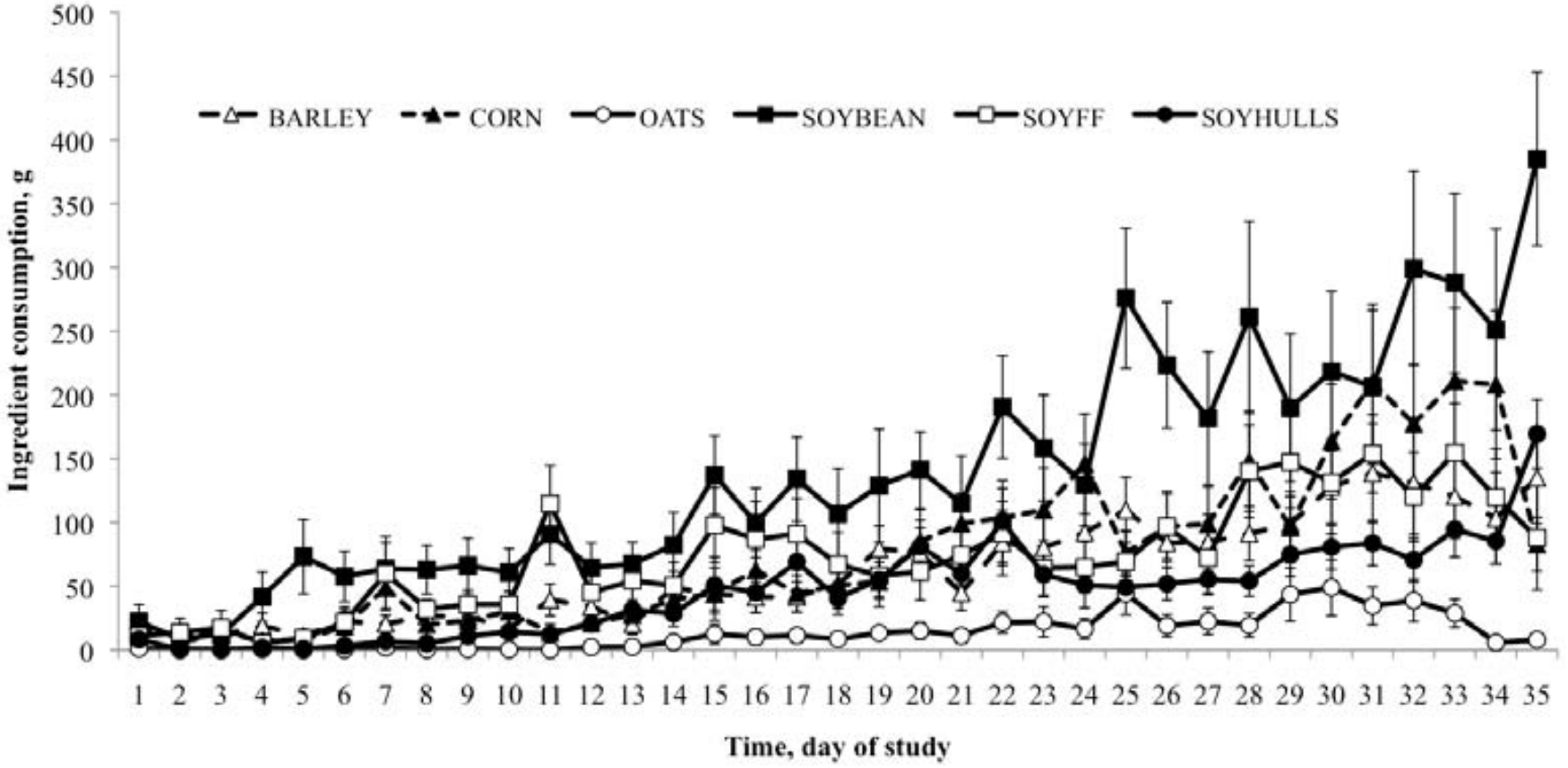
The lack of differences in solid feed intake between the two treatment in the current study is in disagreement with Atwood et al. (2001) who reported that calves offered a choice of different feeds (barley, rolled corn, corn silage and alfalfa hay) tended to eat less than those offered a total mixed-ration. On the other hand, in agreement with the present study, Atwood et al. (2001) reported that the ADG did not differ between animals fed the mixed ration and those allowed to select a diet from separate ingredients. However, the animals in that study were older than the calves in the current study, and these old animals had previous experiences with solid feed. In the current study calves did not have previous experiences with solid feed, in contrast to previous studies (Kyriazakis and Oldham, 1993; Villalba and Provenza, 1999; Scott and Provenza, 2000; Bach et al., 2012) that reported that ruminants are able to meet their

nutrient requirements. During the first days of age calves did not consume greater amounts of solid feed, because their diet is based on MR. This fact taken in conjunction with the fact that CH calves were able to choose among different ingredients, make it more difficult for the animal to associate a post-ingestive effect or a consequence with the ingredient responsible. However, as described in figure 6.4, CH calves increased ingredient consumption of some ingredients, such as soybean meal, whereas other ingredient consumption, such as oats, was reduced. Suggesting that calves perceived some benefits or disadvantages of these different ingredients.

Despite the lack of differences in solid feed intake herein, there were differences in consumption of specific nutrients such as EE, NFC, and CP. The increase in fat consumption showed by CH calves could be potentially explained by early experience, because, during the nursing period, calves obtained most of the dietary energy from the fat present in the MR. Thus, it would seem reasonable to expect that CH calves would have a preference for fatty ingredients in front of ingredients rich in carbohydrates. Although CP consumption was greater in CH than CTR, no differences were observed in DMI, suggesting that there was no negative post-ingestive effect as a consequence of a potential ammonia toxicity as suggested by Lobley and Milano (1997) when offering excessive dietary CP to energy ratios.

The CH calves consumed an unbalanced CP to energy ratio. In contrast to previous studies (Kyriazakis and Oldham, 1997; Villalba and Provenza, 1999) that suggested that herbivores can balance the supply of energy and nitrogen by selecting different ingredients, results herein suggest that CH calves prioritized the consumption of CP without properly balancing it with energy intake. The NRC (2001) recommendations for protein content in calf starter is about 18% CP and this percentage decreases as the BW of animals increases. However, Akayezu et al. (1994) reported improvements in performance when a 19.6 % CP starter feed was offered compared to 15% or 16.8% CP starter feeds, but no advantages were observed when feeding a 22.4 % CP starter feed. In the current study, CTR animals consumed a solid feed containing 18 % of CP, whereas the CP content of the mixture of ingredients freely consumed by CH group was 29%, clearly indicating that CH calves over-consumed protein and failed to adequately select ingredients to meet their needs. The dairy NRC (2001) predicts that calves of 45 kg of BW fed a MR and a starter feed require 125 g/d of CP to achieve an ADG of 300 g/d, respectively.

Figure 6.4. Variation among daily intake of CH calves for the different ingredients offered.



However, in the present study, calves in CH group consumed more CP than CTR calves and gained BW at the same pace (315 g/d), which resulted in a poor efficiency of CP utilization for growth in the CH calves. The poor efficiency of CP utilization was probably due to the fact that ME consumption of CTR and CH calves (2.48 Mcal/d) was within NRC (2001) recommendations for calves of 45 kg of BW growing at 200 g/d (2.31 Mcal/d) or 400 g/d (2.96 Mcal/d), and thus ME was dictating or limiting growth. This indicates that calves failed to select an adequate combination of ingredients to achieve an increased supply of energy, which would potentially allow an improved ADG and efficiency of protein utilization. Furthermore, the lower NFC consumption in the CH compared to the CTR calves, could have impaired microbial protein synthesis (Bach et al., 2005) and this could have further compromised growth of CH calves.

The differences in CP, NFC and EE consumption were mainly a consequence of changes in intake of corn, oats, and full fat soybean between the two treatments. Because corn and oats were two main sources of NFC, and CH calves reduced the consumption of these two ingredients compared to CTR calves (fixed formula), NFC intake was lower in CH than in CTR calves. On the other hand, full fat soybean intake was greater in CH than in CTR calves leading to increased consumptions of CP and EE.

Regarding the ingredient consumption or preferences within CH calves, soybean meal (32.8% of solid feed intake) was the ingredient consumed the most, followed by corn (19.3%), full fat soybean (18.1%), barley (15.4%), soybean hulls (10.8%), and oats (3.5%). Therefore, 50.9% of the CH solid diet corresponded to soybean meal and full fat soybean. Montoro et al. (2010) reported that young calves had oro-sensorial preference for soybean meal in short-term assays. It appears that in the current study, calves selected separate ingredients based on oro-sensorial preference or palatability rather than nutrient balance.

6.5. Conclusions

In summary, young calves given a choice among six separate ingredients selected a diet high in CP and fat, and low in NFC compared to calves that received a fixed mixture of the same six ingredients. Furthermore, calves offered a choice showed a marked preference for soybean meal and rejected oats. Despite these differences in ingredient and nutrient intake between a free-choice and a fixed diet, no differences

were observed in total intake of solid feed, ADG, and gain to feed ratio. It appears that calves between 7 and 42 days of age select ingredients based on aspects different than nutrient supply.

Chapter 7

STUDY 5:

EFFECT OF PHYSICAL FORM OF FORAGE ON PERFORMANCE, FEEDING BEHAVIOR, AND DIGESTIBILITY OF HOLSTEIN CALVES

7.1. Introduction

Ingestion of solid feed is necessary to stimulate rumen development in the young calf and facilitate the transition from a pre-ruminant to functioning ruminant. This transition involves changes to the intestinal tract, as tissues must convert from reliance on glucose supplied from milk to the use of short-chain fatty acids as primary energy substrates (Baldwin et al., 2004). In the pre-weaned dairy calf, the intake of solid feed, particularly that high in carbohydrates, stimulates rumen microbial proliferation and VFA production, which subsequently stimulates development of rumen papillae (Sander et al., 1959; Warner, 1991). Forage intake contributes less to rumen papillae development (Tamate et al., 1962), forage stimulates development of the rumen muscular layer. Furthermore, forage intake promotes rumination (Hodgson, 1971; Phillips, 2004) and maintains the integrity and health of the rumen wall (Haskins, 1969; Suárez et al., 2007). Introducing forage during the milk feeding period has been found to improve ADG, total DMI (Castells et al., 2012; Khan et al., 2011), and feed efficiency (Coverdale et al., 2004).

In addition to forage content of the diet, particle size also influences the ruminal environment. Feeding diets that are chopped or ground to fine particle sizes results in decreased rumen pH and cellulolytic bacteria populations (Beharka et al., 1998). While particle size clearly affects rumen environment, it is unclear whether fiber particle size or total fiber content of pre-weaned calf diets has the greatest influence on early rumen development . The objective of this study was to evaluate the effects of particle size of forage mixed with a starter concentrate on calf performance, digestibility and feeding behavior.

7.2. Materials and methods

7.2.1. Animals and housing

Twenty Holstein male calves (46.8 ± 1.2 kg) participated in this study. Calves received colostrum (at least 4 L within the first 12 h of life) and were enrolled in the study within 48 h of birth. All calves received 2 mL of a vitamin supplement containing vitamins A, D, and E (E-Master, Vétoquinol Canada Inc., Lavaltrie, QC, Canada), 1 mL of selenium (Dystosel, Pfizer Animal Health, Kirkland, QC, Canada), and 1 mL of a

broad-spectrum antibiotic (Draxxin, tulathromycin, Pfizer Animal Health, Kirkland, QC, Canada) i.m. on the day they were placed on the study. One calf experienced scours for more than 1 d and was therefore treated with electrolytes; however, none of this scouring occurred past the first 2 wk of life, and no further cases of illness requiring treatment occurred.

Calves were housed in individual pens (1.2 m wide × 1.8 m deep) at the University of Guelph Kemptville Campus Dairy Education and Research Centre (Kemptville, ON, Canada) and were managed according to the standard operating procedures of this research station, in accordance with the guidelines set by the Canadian Council on Animal Care (CCAC, 2009). The pens prevented physical contact between calves but allowed for them to be in visual and auditory range. Pens were bedded with straw during the first 5 d of life, and then bedding was replaced with wood shavings. Bedding was replenished and replaced as needed, with fresh bedding added weekly at minimum. The insides of the pens were cleaned biweekly before weaning and weekly after weaning. Pens were located under a 3-sided, roofed shelter to protect from excessive exposure to the weather.

7.2.2. Milk Feeding Procedure

During the milk feeding period, calves were fed acidified MR by teat. The feeding setup involved a rubber teat protruding into the side of the pen, attached to a tube fitted with a 1-way valve running into buckets placed adjacent to the pens. All buckets, lines, and teats were removed for cleaning before each feeding. Calves were fed 22% CP and 18% fat (Shur-Gain High-Performance Milk replacer, Nutreco Canada Inc., Guelph, ON, Canada). Each liter of prepared MR contained 150 g of powder as indicated; the powder was combined with hot water at a rate of 150 g of powder per 1/4 L, with additional cold water added to reach the final volume. MR was mixed daily in sufficient volume to feed all calves. At the time of preparation, a pre-diluted form of formic acid (acidified milk solution, 9.8% formic acid; NOD Apiary Products Ltd., Frankford, ON, Canada) was added to acidify the MR to a target pH between 4.0 and 4.3. All calves were fed twice daily at 0800 and 1600 h a fixed amount of 4 L (total 1.2 kg/d of DM) for the first 35 d (5 wks). The amount of MR offered was decreased to 6 L/d (0.9 kg/d of DM) during days 36 to 38, 4 L/d (0.6 kg/d of DM) during days 39 to 41, and incrementally decreased daily during week 7 to facilitate weaning by day 49.

7.2.3. Treatments

Calves were randomly assigned to a mixed ration containing (on a DM basis) either: 1) 90% crumb starter concentrate and 10% chopped (3-4 cm) grass hay (COARSE; n = 10) or 2) 90% crumb starter concentrate and 10% ground (2 mm) grass hay (FINE; n = 10). Birth weights were similar for both COARSE calves and FINE calves (45.8 ± 1.6 kg vs. 47.9 ± 1.8 kg; P = 0.7). Calves received their respective solid feed type (Table 7.1) ad libitum in circular buckets (diameter = 47.0 cm, height = 21.6 cm) attached to the front of the hutch, beginning on day 5 of the study.

Table 7.1. Chemical composition of feed components and mixed ration (mean ± SD; DM basis)

Item	Concentrate[1]	Hay[2]	Coarse feed[3]	Fine feed[4]
DM, %	89.2 ± 0.75	88.5 ± 1.13	89.3 ± 0.30	89.4 ± 0.41
CP, % of DM	24.7 ± 0.60	12.3 ± 0.30	23.6 ± 0.72	23.3 ± 0.53
Soluble Protein, % of DM	4.8 ± 0.15	3.0 ± 0.17	4.7 ± 0.48	4.6 ± 0.31
ADF, % of DM	11.1 ± 0.37	44.3 ± 1.38	13.6 ± 0.75	14.4 ± 0.56
NDF, % of DM	21.0 ± 0.90	64.8 ± 1.27	25.9 ± 1.73	25.5 ± 0.86
Ash, % of DM	9.6 ± 0.21	14.2 ± 2.03	9.7 ± 0.17	9.7 ± 0.10

[1] Concentrate was a dairy calf rum crumb starter (Shur-Gain, Nutreco Canada Inc., Guelph, ON, Canada)
[2]Hay was a second-cut of rye-grass hay.
[3]Coarse feed contained 90% concentrate and 10% hay chopped at about 3-4 cm.
[4]Fine feed contained 90% concentrate and 10% hay ground at about 2 mm.

7.2.4. Measurements, sample collection and analyses

Calves were weighed at the same time each day on two consecutive days each week. Repeated measurements were made to obtain an accurate weekly weight and to account for day-to-day variability. Throughout the study, MR, water and TMR (starter feed + grass hay) consumption were recorded daily on an individual basis. As a consequence of the low temperatures during the first 30 d of study the water was often frozen. Water consumption during the first 35 d was below 1 L/d, possibly due to the quantity of MR provided. Therefore, water consumption data refer only to measurements obtained between days 31 to 56. To determine DMI, fresh concentrate, fresh forage, fresh mixed rations, and orts from all calves were sampled twice weekly throughout the study. In addition, during weeks 7 and 8, fresh concentrate, fresh forage, fresh mixed rations, and

orts samples from all calves were obtained daily. All samples were immediately frozen at -20ºC until subsequent analyses.

Feed samples obtained for DM and chemical analyses were oven-dried at 55ºC for 48 h. Fresh feed samples taken to determine nutrient contents were pooled by week, and orts samples from weeks 7 and 8 were pooled by animal and week. The week after weaning (49 d of age), plastic bags were glued to each calf to determine apparent DM, CP, NDF, and ADF digestibilities of the diets. This procedure has been described elsewhere (Terré et al., 2007). During wk 8 of the study, all feces were collected and weighed. Bags were changed at least four times per day. For each calf, a daily subsample equivalent to 30% of total feces collected was obtained and dried at 60ºC for 72 h. Subsamples of the 7 d were composited by animal proportionally to the dry weight of feces produced each daily. Feed and fecal samples collected to determine nutrient content were ground to pass through a 1-mm screen (Wiley Mill, Arthur H. Thomas Co., Philadelphia, PA), and sent to Cumberland Valley Analytical Servces Inc. (Maugansville, MD) for analysis of DM (135ºC; AOAC, 2000: method 930.15), ash (535ºC; AOAC, 2000: method 942.05), ADF (AOAC, 2000: method 973.18), NDF with heat-stable α-amylase and sodium sulfite (Van Soest et al., 1991), and CP (N × 6.25; AOAC 2000: method 990.03; Leco FP-528 nitrogen analyzer, Leco, St. Joseph, MI).

7.2.5. Animal Behavior

Behavior was monitored by direct observations of all calves during 2 periods of 7 d, 2 wk before weaning (wk 6) and in the week after weaning (wk 8). Calves were observed 1 h immediately following the morning MR feeding (at 0830 h) and 1 h after solid feed was weighed and offered (at 1030 h) during the pre-weaning week. Although no MR was provided in the second observation week, calves were observed at the same times as in wk 6. Total observation time per animal was 28 h for the entire monitoring period. Scans of each calf were performed every minute. The observer recorded the occurrence of the following behaviors: lying or standing; and the activity: eating, drinking, ruminating, performing non-nutritive oral behaviors (NNOB; licking any surface, tongue rolling, or consuming wood shavings), head butting (when the animal pressed any surface with the head), or idling (when none of the above behaviors were being performed).

7.2.6. Calculations and Statistical Analyses

Weekly ADG was calculated as the difference between weights taken 1 wk apart divided by seven. Gain to feed ratio was calculated for each week by dividing BW gained in that week by total DMI in that week. Apparent nutrient digestibility was calculated as the difference between the quantity of a nutrient consumed and the quantity of that nutrient defecated, divided by the quantity of that nutrient consumed.

Sorting activity during weeks 7 and 8 for indivivdual nutrients was calculated as the actual intake of each nutrient expressed as a percentage of the predicted intake of that nutrient. The actual intake of each nutrient was calculated as the difference between the amount of each nutrient in the feed offered and that in the feed refused. The predicted intake for an individual nutrient was calculated as the product of DMI of the total diet times the percentage of that nutrient in the offered mixed feed. Values equal to 100% indicate no sorting, <100% indicate selective refusals (sorting against), and >100% indicate preferential consumption (sorting for).

Data for DMI, ADG, and gain to feed ratio were summarized for each calf by week. Data for nutrient intakes, and sorting activity for individual nutrients were analyzed for weeks 7 and 8. Sorting activity for each nutrient was tested for a difference from 100 using TTEST procedure of SAS. All data were analyzed with a mixed-effects model for repeated measures, except apparent nutrient digestibility data. The statistical model included the fixed effects of week, treatment, and week by treatment interaction, and the random effect of calf within treatment. Differences in apparent nutrient digestibility between treatments were analyzed using a simple t-test.

Behavior data were summarized individually as the total time (min) devoted to each monitored behavior per day. Data for standing, lying, idling, eating, and performance of NNOB were analyzed with a mixed-effects model for repeated measures. The statistical model included the fixed effects of day, treatment, their 2-way interaction, and the random effect of calf within treatment. Due to the lack of normality, data from NNOB were root-square transformed. Least square means presented for NNOB correspond to non-transformed data, and SEM and P-values correspond to the results from the mixed-effects model using root-square data. Ruminating, drinking, and heading butting behavior data were analyzed with a mixed-effects poisson regression model including

calf as random effect, and treatment, time (day) and the interaction between treatment and day as fixed effects. Least square means presented for ruminating, drinking, and press heading behaviors correspond to non-transformed data, and SEM and P-values correspond to the results from the mixed-effects logistic regression data.

7.3. Results and discussion

7.3.1. Performance and sorting

Intakes and performance data are reported in Table 7.2. No treatment differences were observed in water and MR consumption as well as solid feed intake and total DMI. However, during the last week of the study (wk 8), after all calves were weaned, those receiving COARSE had greater DMI ($P < 0.05$) than FINE calves (Figure 7.1). Both treatments had similar ADG during the study (Table 7.2). Interestingly, COARSE calves tended to be more efficient than FINE calves. It is possible that the presence of chopped hay in the COARSE diet improved the rumen environment of those calves, which in turn may have contributed to the stimulation of solid feed intake and improved feed efficiency. Several researchers (Thomas and Hinks, 1982; Davis and Drackley, 1998) have reported that DMI increased when incorporating between 10 and 25% of ground or chopped hay or straw into a complete diet. Coverdale et al. (2004) reported that the addition of hay to diets appeared to favorably alter rumen environment, resulting in increased intake and improved efficiency. Furthermore, Castells et al. (2012) reported that the provision of chopped grass hay improved total DMI in young calves. However, in all former studies (Thomas and Hinks, 1982; Coverdale et al., 2004; Castells et al., 2012) nutrient composition was not controlled across treatment diets. In the present study, all calves received a diet with the same nutrient composition; thus differences in total DMI and feed efficiency can be solely attributed to the physical form of forage present in the diet.

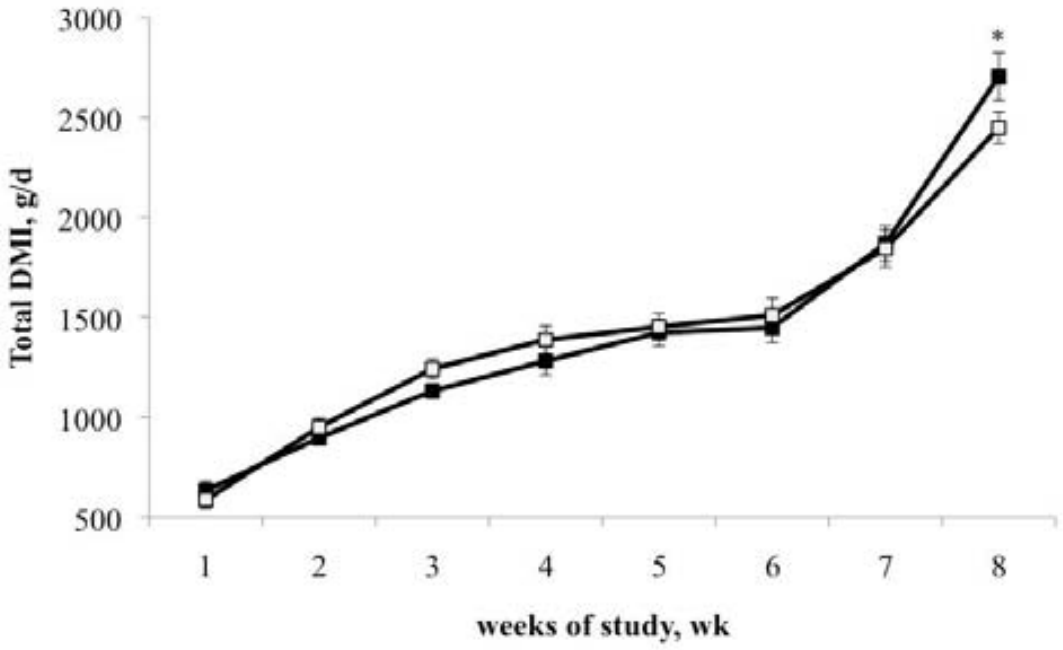
No differences were observed in CP, soluble protein, and ADF consumption between COARSE and FINE calves during the last 2 wk of the study (weeks 7 and 8; Table 7.3). However, COARSE calves had greater NDF consumption than FINE calves. Furthermore, COARSE calves tended to consume more NDF than FINE calves (719.2 vs 610.5 ± 25.84; respectively) during the last week of study (week 8).

Table 7.2. Intakes and weight gain of calves fed mixed rations containing chopped hay at different particle sizes[1].

	Treatment			P-value[2]		
	COARSE	FINE	SE	T	W	W x T
Water intake[3], L/d	3.51	3.34	0.30	0.68	<0.01	0.11
BW, kg	64.7	66.3	1.83	0.53	<0.01	0.88
ADG, kg/d	0.94	0.89	0.03	0.34	<0.01	0.17
Feed DMI, kg/d	0.76	0.76	0.05	0.90	<0.01	0.01
Milk replacer, kg/d	0.66	0.66	0.01	0.70	<0.01	0.86
Total DMI, kg/d	1.42	1.43	0.05	0.95	<0.01	0.03
Gain to feed ratio[4], kg/kg	0.68	0.63	0.02	0.09	<0.01	0.47

[1]Data are averaged by week for 10 calves on each treatment.
[2]T = Treatment effect; W = week effect; T x W = Treatment x week interaction.
[3]Water consumption obtained from measurements only during weeks 5 to 8.
[4]Gain to feed ratio = Gain of BW, kg / Total DMI, kg

Figure 7.1. Total DMI (g/d) of calves fed fed mixed rations containing chopped hay at different particle size: COARSE (■) and FINE (□).



*Total DMI between treatments differs at $P < 0.05$.

Table 7.3. Nutrient intakes during the last week of the milk-feeding stage (wk 7) and the first week after weaning (wk 8) for calves fed mixed rations containing chopped hay at different particle size. [1]

	Treatment			P-value[2]		
Item	COARSE	FINE	SE	T	W	T x W
CP, g/d	486.8	466.0	21.25	0.50	<0.01	0.18
Soluble Protein, g/d	85.5	85.6	3.99	0.98	<0.01	0.33
ADF, g/d	295.1	284.0	11.87	0.52	<0.01	0.20
NDF, g/d	587.6	507.6	21.87	0.02	<0.01	0.09

[1]Data are averaged by week for 10 calves on each treatment.
[2]T = Treatment effect; W = week effect; T x W = Treatment x week interaction.

Although differences in nutrient intake were only observed for NDF consumption, sorting of nutrients differed between treatments for both NDF and CP. Calves on the COARSE treatment sorted to a greater extent (for NDF than FINE calves, whereas FINE calves sorted to a greater extent for CP (Table 7.4) than COARSE. Given the nutrient composition of hay and concentrate (Table 7.1), these results suggest that COARSE calves were sorting for hay instead of concentrate, whereas FINE calves were sorting for concentrate instead of hay. This result is interesting, given that previous studies have shown both cattle (e.g. DeVries et al., 2007) and calves (eg. Miller-Cushon and DeVries, 2011) sort against NDF. The present result suggests that preference for the forage component of a mixed ration may depend on the particle size.

Table 7.4. Sorting (%) of nutrients during the last week of the milk-feeding stage (wk 7) and the week after weaning (wk 8) for calves fed mixed rations containing chopped hay at different particle size.

	Week 7			Week 8			P-value[2]		
Item	COARSE	FINE	SE	COARSE	FINE	SE	T	W	T x W
CP, %	99.98	101.96 *	0.52	101.01	102.59**	0.64	0.01	0.17	0.74
Soluble Protein, %	94.84*	94.65	2.40	95.42**	97.66	0.86	0.55	0.36	0.53
ADF, %	101.11	99.51	1.56	98.26*	97.13	1.43	0.46	0.03	0.83
NDF, %	104.97**	99.27	1.48	102.17**	98.70	1.14	0.01	0.15	0.33

[2]T = Effect of treatment. W = week effect. T x W = Treatment x week interaction.
*Sorting values differ at $P < 0.05$ from 100%.
** Sorting values differ at $P < 0.01$ from 100%.

7.3.2. Apparent nutrient digestibility

Total tract apparent digestibility of DM, CP, ADF, and NDF were greater COARSE than for FINE calves (Table 7.5). This may also explain the tendency for an improved feed efficiency in COARSE compared to FINE calves (Table 7.2). No differences were observed in apparent digestibility of soluble protein between treatments. Apparent nutrient digestibilities observed herein are in the range previously reported in weaned calves (Terré et al., 2007; Hill et al., 2010; Castells et al., 2012). Interestingly, previous studies (Porter et al., 2007; Zanton and Heinrichs, 2009) reported that high-fiber diets and high DMI (Zanton and Heinrichs, 2008) compromise digestibility. Porter et al. (2007) reported that DM digestibility in animals fed high-fiber diets (27% NDF) was lower than that of calves consuming low-fiber diets (20% NDF). It seems that particle size of forage may play an important role in nutrient digestibility; in the present study, despite the fact that COARSE calves consumed more NDF than FINE calves, digestibility of DM, CP, ADF and NDF was greater in COARSE than in FINE calves. It could be speculated that the presence of chopped hay in the diet was associated with an improvement of the rumen environment, leading to an improvement in digestibility.

Table 7.5. Total tract nutrient apparent digestibility during the week after weaning (week 8) of calves fed mixed rations containing chopped hay at different particle size.

	Treatment			
	COARSE	FINE	SE	P-value
DM, %	72.3	69.2	0.68	<0.01
CP, %	77.4	74.5	0.86	0.03
Soluble Protein, %	75.4	73.5	1.23	0.29
ADF, %	40.7	34.0	2.18	0.04
NDF, %	42.7	35.6	2.11	0.03

7.3.3. Animal Behavior

The total time devoted to performing each recorded behavior is reported in Table 7.6. No differences were found in time spent standing, lying, feeding, ruminating, drinking or head-butting between treatments. However, calves receiving the FINE treatment spent more time performing NNOB that COARSE calves. NNOB are often considered indicators of poor welfare, since they are thought to be related to frustrated feeding

motivation due to thwarted ability to forage (Redbo, 1990; Redbo and Nordblad, 1997). Previous studies (Redbo and Nordblad, 1997; Phillips, 2004) reported that restricted roughage allowance has a considerable effect on the duration and frequency of bouts of stereotypies in heifers. Furthermore, Castells et al. (2012) reported that calves fed a starter concentrate and rye-grass hay devoted less time to performing NNOB than forage-deprived calves. The present results suggest that forage particle size, in addition to forage allowance,plays an important role in reducing NNOB in young calves; thus, providing calves with longer forage particles may improve their welfare. COARSE calves tended to spend more time idling than FINE calves (Table 7.6), which could be attributed, in part, to the increased time devoted to performing non-nutritive activities in FINE compared to COARSE calves.

Table 7.6. Effect of feed exposure treatment on average time (in minutes) standing or lying, or devoted to perform different behaviors per day during the 28 h of observations (2 h/d) conducted between two weeks before weaning (wk 6) and one week after weaning (wk 8).

	Treatment			P-values[1]		
Activity	COARSE	FINE	SE	T	DAY	T × DAY
Standing, min	59.9	68.4	4.39	0.17	<0.01	0.67
Lying, min	60.1	51.6	4.39	0.17	<0.01	0.67
Idle, min	72.0	64.2	2.88	0.06	0.05	0.86
Feeding, min	19.6	21.8	1.28	0.22	<0.01	0.41
NNOB[2], min	19.7	28.3	0.25	0.01	<0.01	0.34
Ruminating, min	5.88	2.26	0.64	0.94	0.50	0.38
Drinking, min	2.13	2.22	0.16	0.53	0.52	0.52
Head-butting, min	0.69	1.15	0.13	0.98	0.32	0.59

[1]T = Effect of treatment, DAY = Effect of day of sampling. T x DAY = Interaction between treatment and day.
[2]Non-nutritive oral behavior.

7.4. Conclusions

The provision of chopped hay mixed with a starter concentrate improved total DMI and nutrient digestibility after weaning as compared with the addition of ground hay to a starter concentrate. Consequently, the provision of chopped hay tended to improve feed efficiency during the first weeks of age. Furthermore, provision of chopped hay reduced the performance of non-nutritive oral behaviors.

Chapter 8

STUDY 6:

BLOCKING OPIOID RECEPTORS ALTERS SHORT-TERM FEED INTAKE AND ORO-SENSORY PREFERENCES IN WEANED CALVES

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8.1. Introduction

The neuroendocrine control of feed intake and energy balance is a complex process controlled by many overlapping, integrated pathways (Sartin et al., 2011). In mammals, the hypothalamus plays a central role in this regard (Clarke, 2001; Wagner et al., 2004), and opioid receptors have been found in the hypothalamus (H.kfelt et al., 1977) as well as in limbic structures involved in the control of food reward and eating behavior (Berridge et al., 2009). Opioid peptides may thereby influence feed intake by mediating hedonism and incentive motivation upon feed consumption (LeMagnen, 1990; Berridge, 2009), and it has been suggested that blocking their activity may render palatable feeds less rewarding (Drewnowski et al., 1992; Giraudo et al., 1993). Naloxone is an opioid receptor antagonist and its peripheral administration reduces DMI in ruminants (Cheema et al., 1991; Obese et al., 2007).

Our hypothesis was that blocking opioid receptors might blunt oro-sensorial preferences of calves, thereby decreasing the rewarding response associated with feed intake. Furthermore, we expected that the effects of blocking opioid receptors would depend on circulating insulin and anorexigenic hormones as dictated by the metabolic status of the calves. Thus, the objectives of the current study were to determine if naloxone would (1) decrease feed intake by modifying oro-sensorial preferences for a sweet feed; and (2) affect plasma glucose, insulin, CCK, and GLP-1 concentrations in fasted or fed calves.

8.2. Materials and Methods

8.2.1. Animals, feeding, and management

Thirty-two male Holstein calves participated in this study. All calves were purchased from commercial farms and raised in the facilities of the Institute for Research and Technology in Agrifood (IRTA, Caldes de Montbui, Spain). Calves were housed in individual hutches (1 x 1.55 m) and managed under the guidelines and approval of the Animal Care Committee of IRTA. Hutches were bedded with sawdust every 3 d. All calves had access to 2 buckets, one containing solid feed and the other containing water. A commercial MR (25% CP and 19.2% fat, Sprayfo Excellent 60, Sloten BV, Deventer, the Netherlands) was offered in 2-L 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 of MR at 12.5% DM. Animals were weaned at 57 d of age. Starter feed (Table 8.1) and water were offered ad libitum throughout the study.

Table 8.1. Ingredient and chemical composition of the experimental starter feed.

Item	Starter feed
Ingredient, % DM	
Wheat	20
Corn	15
Barley	11.2
Sorghum	12
Soybean meal	23
Wheat middling	12
Soybean hulls	5
Premix[1]	0.2
Calcium carbonate	0.5
Dicalcium phosphate	0.3
Sodium chloride	0.8
Nutrient, % DM	
CP	19.5
NDF	17.7
ADF	8
Ash	5.6

[1]Contained 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; nicotinicacid: 656 mg/kg; pantothenicacid: 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.

8.2.2. Experiment 1: *Preference for a sweetened starter feed.*

The aim of this experiment was to verify the establishment of a preference for a sweetener (Luctarom SFS-R; Lucta S.A., Montorn.s del Vall.s, Spain) in weaned calves at the age of 72, 73, and 74 d (i.e., 15 d after weaning). This experiment was performed in 2 periods, using 16 animals per period. During the experiment, all calves (n = 32) received a choice in 2 separated buckets of a control feed (CF; Table 8.1) that had previously been consumed by all calves, and the same starter feed with the addition of a noncaloric sweetener (sweet feed, SF; CF + 1 g/kg of Luctarom SFS-R). Calves were offered a known amount of fresh CF and SF in 2 separated buckets at 0800 h, and feed

consumption was recorded 2, 4, and 6 h after feeding. After 6 h, the remaining feed was removed and fresh CF was offered separately in 2 buckets to all calves.

8.2.3. Experiment 2: *Control of feed intake and the interaction between opioids and metabolic state of calves*

The aim of this experiment was to explore the potential interaction between opioid-mediated and metabolic (dictated by the fed state) control of feed preferences and intake. Similar to experiment 1, this experiment was conducted in 2 periods using half of the animals (n = 16) in each period. Calves were weighed and randomly distributed according to BW and age in 4 groups, following a 2 x 2 factorial design. One day before the onset of the experiment, calves (75 ± 0.66 d of age) were fitted with jugular catheters (Abbocath®- T 16G x 140 mm; Hospira Inc., Lake Forest, IL) to facilitate blood sampling and administration of treatments. Catheter patency was maintained by flushing with 5 mL of heparinized saline solution (1,000 USP units of heparin/mL). Half of the calves (n = 16; 8 calves per period) were either fed ad libitum (FED) until 10 min before the onset of the experimental period or fasted (FAS; n = 16; 8 calves per period) for 14 h until feeding at 0800. Within each of these groups, at feeding time (0800 h), half of the calves (n = 8; 4 per period) were injected i.v. with either 1 mg of naloxone (NAL; Sigma-Aldrich Quimica S.A., Madrid, Spain) per kilogram of BW diluted in 6 mL of saline solution (0.9% NaCl) or 6 mL of saline solution (SAL). Doses for NAL were based on previous studies (Alavi et al., 1991; Obese et al., 2007). Therefore, treatments were FAS-NAL (n = 8; 86.1 ± 4.74 kg of BW and 76.5 ± 1.18 d of age), FAS-SAL (n = 8; 87.9 ± 2.31 kg of BW and 76.5 ± 0.68 d of age), FED-NAL (n = 8; 86.1 ± 3.36 kg of BW and 77.1 ± 2.30 d of age) and FED-SAL (n = 8; 85.6 ± 4.16 kg of BW and 76.1 ± 0.77 d of age).

At 0800 h, known amounts of fresh CF and SF were offered in 2 separate buckets to all calves. At the same time, NAL or SAL was injected, and feed consumption was monitored at 2, 4, and 6 h thereafter. Blood samples were harvested at −10, 20, 180, and 240 min relative to NAL or SAL administration and feed offer. Between blood samples, catheter patency was maintained by flushing with 1 mL of heparinized saline solution (1,000 USP units of heparin/mL). All blood samples were harvested in 4-mL tubes containing EDTA as an anticoagulant (BD Vacutainer spray-coated K_2EDTA tubes) and 4-mL tubes containing a glycolysis inhibitor (BD Vacutainer fluoride tubes). Samples

were refrigerated at 4ºC until centrifuged at 1,500 × g at 4ºC for 15 min, and plasma and serum were decanted and stored at −20ºC until analyses of insulin, GLP-1, CCK, and glucose.

Concentrations of insulin, CCK-8, and GLP-1 were measured using RIA as described by Villalba et al. (2011). The intraassay CV averaged less than 3.1, 2.6, and 4.1% for insulin, CCK-8, and GLP-1 assays, respectively. The kit used for CCK determination binds to CCK-8 sulfate and cross-reacts with CCK-33 sulfate. For GLP-1 determination, the kit used was specific for total GLP-1 (1–37) and GLP-1 (7–37). Serum glucose was determined using a glucose RTU kit (bioMerieux S.A., Marcy l'Etoile, France) with an intra- and interassay of 1.4 and 1.7%, respectively.

8.2.4. Calculations and statistical analyses

In both experiments, oro-sensorial preference for SF was measured as the cumulative consumption of SF expressed as a percentage of total feed consumed: [consumption of SF/(consumption of SF + consumption of feed CF)] × 100. To assess whether the relative consumption of SF (as a percentage of total intake) differed from 50% (i.e., lack of preference), a one-sample comparison t-test using SAS (SAS Institute Inc., Cary, NC) was performed. Oro-sensorial preference values over 50% were considered to indicate a preference toward SF over CF.

In experiment 1, cumulative CF, cumulative SF, and total cumulative starter feed consumption, and orosensorial preference for SF at each recorded time (2, 4 and 6 h) were analyzed using the MIXED procedure (SAS Institute Inc.), treating time (h) as repeated measure. The 3 sampling days were analyzed separately. The model included time as a fixed effect, and animal and period as random effects. Data from cumulative CF, SF, and total starter feed consumption were rootsquare transformed to achieve a normal distribution. Least squares means presented herein for cumulative total starter feed consumption correspond to nontransformed data, and SEM and *P*-values correspond to the results from the mixed-effects model using root-square transformed data.

In experiment 2, cumulative CF, SF, and total starter feed consumption, oro-sensorial preference for SF, and blood metabolite data were analyzed using the MIXED

procedure (SAS Institute Inc.), treating time as a repeated measure. The statistical model included the effect of blocking opioid activity (NAL or SAL), the effect of metabolic state (fasted or fed), the effect of time relative to feeding, and their 2- and 3-way interactions as fixed effects, and period and animal as random effects. Due to the lack of normality, data from CF, SF, and total cumulative consumption were root-square transformed, and data from plasma metabolites were log-transformed. Least squares means presented herein for total cumulative consumption and metabolite concentrations correspond to nontransformed data, but SEM and *P*-values are results from the mixed-effects model using root-square or log-transformed data.

8.3. Results and discussion

8.3.1. Experiment 1: *Preference for a Sweetened Starter Feed*

Cumulative CF, SF, and total feed intake and oro-sensorial preferences for SF are presented in Table 8.2. The SF was preferred ($P < 0.01$) over the CF at all recorded times (2, 4, and 6 h postfeeding) on each sampling day. These data agree with the notion that calves select for sweet-tasting feeds (Goatcher and Church, 1970a; Hellekant et al., 1994; Nombekela and Murphy, 1995) and support the use of SF to evaluate the interaction between opioid-mediated and metabolic control of intake in experiment 2. As expected, cumulative feed consumption progressively increased ($P < 0.01$) over time. Because cumulative feed consumption increased with time, it seems that no negative postingestive feedback on intake (Forbes and Kyriazakis, 1995; Provenza, 1995) was triggered by the added sweetener.

Table 8.2. Daily cumulative consumption of control starter feed (CF), sweetened starter feed (SF), and total starter feed, and oro-sensorial preference for a SF [(cumulative consumption of SF / cumulative total feed consumption) x 100] during 3 consecutive days in Experiment 1.

	Time after feed offer, h				
Day 1	2	4	6	SEM[1]	P-value
Cumulative CF intake, g	101.4[a]	144.2[b]	172.8[c]	2.01	<0.01
Cumulative SF intake, g	354.2[a]	508.6[b]	624.7[c]	0.86	<0.01
Total cumulative feed intake, g	455.6[a]	652.8[b]	797.5[c]	0.90	<0.01
Oro-sensorial preference for SF, %	76.6[*]	79.1[*]	79.7[*]	4.66	0.39
Day 2					
Cumulative CF intake, g	95.5[a]	144.8[b]	223.6[c]	1.10	<0.01
Cumulative SF intake, g	378.8[a]	507.2[b]	607.3[c]	0.91	<0.01
Total cumulative feed intake, g	474.2[a]	652.0[b]	830.9[c]	0.91	<0.01
Oro-sensorial preference for SF, %	80.2[*a]	79.5[*a]	74.7[*b]	2.19	<0.01
Day 3					
Cumulative CF intake, g	81.7[a]	124.2[b]	200.5[c]	3.57	<0.01
Cumulative SF intake, g	367.2[a]	546.1[b]	716.1[c]	0.90	<0.01
Total cumulative feed intake, g	448.9[a]	670.3[b]	916.6[c]	1.30	<0.01
Oro-sensorial preference for SF, %	84.3[*a]	83.5[*a]	79.2[*b]	9.25	0.04

[abc]Values with uncommon superscripts within row differ at $P < 0.01$.
[*]Values differ from 50% at $P < 0.05$.
[1]Standard error of the mean. SEM of the cumulative feed intake corresponds to root-transformed data, whereas mean values are non-transformed.

8.3.2. Experiment 2: *Control of feed intake and the interaction between opioids and metabolic state of calves*

Cumulative CF, SF, and total feed intake and orosensorial preferences for SF are presented in Table 8.3. As expected, FAS calves consumed more ($P < 0.05$) feed (684.1 ± 1.18 g) than FED calves (515.2 ± 1.18 g) on average throughout the study, but NAL treatment tended ($P = 0.08$) to reduce cumulative starter feed consumption only in FED calves (Figure 8.1). In contrast to these results, other authors (Baile et al., 1981; Alavi et al., 1991; Burgwald-Balstad et al., 1995) have reported an inhibitory effect of naloxone on feed intake when administered to fasted animals. Previous studies have been conducted in heifers (Burgwald-Balstad et al., 1995) and sheep (Baile et al., 1981; Alavi et al., 1991); therefore, differences in age and species coupled with different fasting times could explain the described discrepancy among studies. It would be reasonable to expect that as hunger increases, metabolic signals gradually become more relevant in

controlling feed intake, eventually overcoming the positive effects on intake elicited by oro-sensorial preferences. Indeed, in rodents, the sensitivity of the endocannabinoid system, which is involved in the generation of hedonic reactions along with the opioid system (Berridge et al., 2009), is modulated by appetite-controlling signals such as leptin, CCK, and ghrelin (Di Marzo and Matias, 2005). Assuming that similar mechanisms are operative in ruminants, fasting would also upregulate appetitestimulatory neurotransmitters, which could nullify the effects of NAL. On the other hand, SAL calves consumed more ($P < 0.05$) starter feed (442.2 ± 1.33 g) than NAL calves (319.4 ± 1.33 g) 2 h after feeding. Similar to other studies (Baile et al., 1981; Burgwald-Balstad et al., 1995), the inhibitory effect of naloxone on feed intake was only observed during the first 2 h after feeding. Naloxone is a short-acting opiate antagonist with a half-life of about 40 min in rats (Tepperman et al., 1983) and 1 h in humans (Ngai et al., 1976). As reported herein, therefore, any potential effect of naloxone on feed intake would be expected to last shortly after its administration.

As for the cumulative consumption of CF and SF, NAL calves consumed more ($P < 0.05$) CF (381.0 ± 1.73 g) than SAL calves (212.6 ± 1.73 g) by 6 h postfeeding. On the other hand, SAL calves consumed, on average, more ($P < 0.05$) SF (439.6 ± 1.37 g) than NAL calves (297.4 ± 1.37 g). Overall, calves receiving SAL had a greater ($P < 0.05$) preference for SF (70.3 ± 7.54%) than NAL calves (51.9 ± 7.54%), and FAS-SAL calves had a greater ($P < 0.05$) preference for SF (73.5 ± 8.32%) than FAS-NAL calves (52.0 ± 8.32%) at 6 h after feeding.

During the first 4 h, FAS-SAL calves did not discriminate between starter feeds, although this group had a numerically greater preference for SF (Table 8.3). Forbes (2007) suggested that metabolic and physiological states could modify eating behavior. Furthermore, recent evidence from murine models indicate that opioids, endocannabinoids, and γ-aminobutyric acid (GABA)-benzodiazapines mediate hedonic responses to feed consumption by stimulating well-defined regions in the nucleus accumbens and ventral pallidum that have been jointly termed “hedonic hotspots” (Berridge et al., 2009).

Table 8.3. Influence of naloxone (NAL) or saline (SAL) injection on cumulative feed consumption of control feed (CF), sweetened feed (SF), and total solid feed, and oro-sensorial preferences in fed or 14-h fasted calves

	2 h after feed offer				4 h after feed offer				6 h after feed offer											
	Fed		Fasted		Fed		Fasted		Fed		Fasted			P-Values						
Item	SAL	NAL	SAL	NAL	SAL	NAL	SAL	NAL	SAL	NAL	SAL	NAL	SEM	N	M	T	N x M	N x T	N x M x T
Cumulative CF intake, g	115.6	91.3	156.9	236.9	183.1	234.4	226.9	341.9	196.3	280.6	228.8	481.3	2.45	0.25	0.31	<0.01	0.48	<0.01	0.43
Cumulative SF intake, g	330.6	78.1	281.3	232.5	421.3	205.0	446.9	374.4	553.1	401.9	604.4	492.5	2.18	0.02	0.21	<0.01	0.27	0.23	0.23
Total cumulative feed intake, g	446.3	169.4	438.1	469.4	604.4	439.4	673.8	716.3	749.4	682.5	833.1	973.8	1.86	0.28	0.02	<0.01	0.08	0.02	0.39
Oro-sensorial preference for SF, %	71.8^{*abc}	49.9^{abc}	71.5^{abc}	50.1^{abc}	68.1$^{\dagger abc}$	44.7^{c}	66.6^{abc}	54.3^{abc}	70.2^{*ab}	60.3$^{\dagger ab}$	73.5^{*a}	52.0^{bc}	10.22	0.04	0.95	0.04	0.99	0.71	0.04

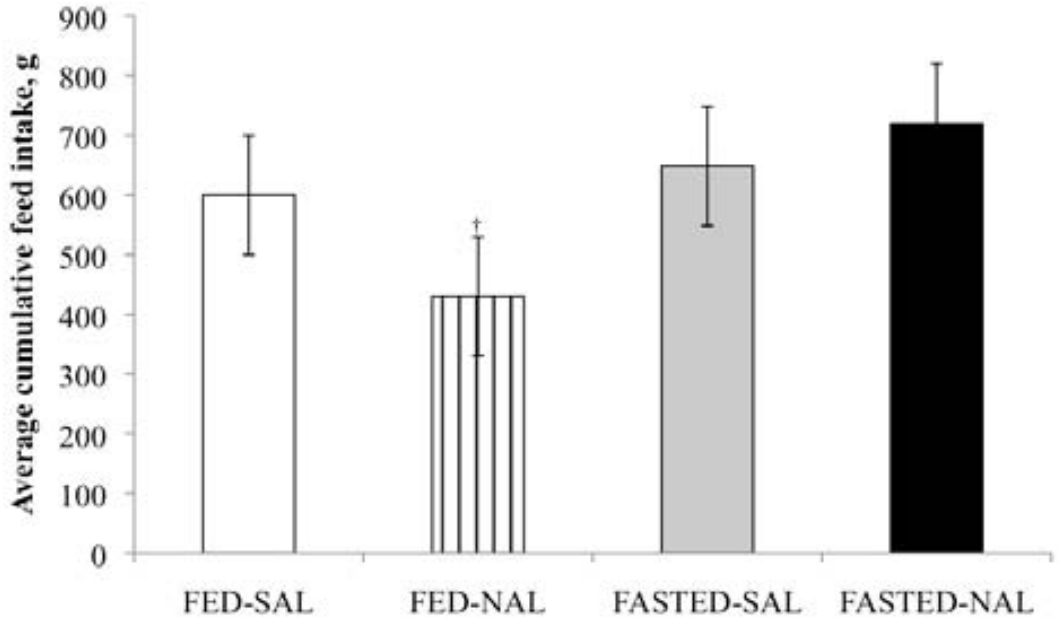
[1]N = Naloxone effect (NAL or SAL); M = Metabolic state effect (Fed or Fasted); T = time relative to feed offer (2, 4, or 6 h); N × T = interaction between naloxone and time; M × T: interaction between metabolic state and time; N × M × T = interaction between naloxone, metabolic state, and time; no M × T interaction was observed (P > 0.05).

[2]Mean values for cumulative feed intake are nontransformed, whereas SEM values correspond to root-square transformed data.

[abc]Values with uncommon superscripts within row differ at P < 0.01.

[*]Oro-sensorial preference differs from 50% (P < 0.05); [†]Oro-sensorial preference tends to differ from 50% (P < 0.10).

Figure 8.1. Average cumulative feed intake of well-fed calves that received 6 mL of saline solution (0.9% NaCl; FED-SAL), well-fed calves that received 6 mL of naloxone (1mg/kg of BW; FED-NAL), calves fasted for 14 h and that received 6 mL of saline solution (FAS-NAL), and calves fasted for 14 h that received 6 mL of naloxone (FAS-NAL).



† Average total cumulative feed intake in FED-NAL calves tend to differ ($P < 0.10$) from that of the other groups.

In rats, administration of an opioid-receptor agonist within those brain regions increases liking and eating behaviors triggered by sucrose, but the magnitude of such responses is influenced by several factors such as hunger, satiety, and taste preferences (Berridge, 2009; Berridge et al., 2009). Based on this notion, we hypothesize that in FAS calves, hunger diminished the sensitivity or activation of the opioid system and inhibited pleasure-dependent behaviors, ultimately minimizing the preference for SF during the first 4 h of the experiment. Interestingly, 6 h after feeding, FAS-SAL calves preferred ($P < 0.05$) SF over CF, whereas FASNAL continued to show no preference for SF (Table 8.3). On the other hand, FED-NAL calves did not discriminate between the 2 starter feeds during the first 4 h of experiment, and only tended ($P = 0.07$) to prefer SF 6 h after feeding (Table 8.3). These results indicate that the influence of opioids on oro-sensorial preferences was more pronounced and lasted longer than their effect on the control of total feed consumption (which disappeared 2 h posttreatment). In humans, it has been reported that blocking opioid receptors decreases the degree of pleasantness elicited by consumption of certain foods, including sweet solutions

(Fantino et al., 1986; Bertino et al., 1991; Yeomans and Wright, 1991; Drewnowski et al., 1992). In rats, opioid antagonists are most effective in reducing intake of palatable sweet liquids such as glucose, sucrose, or saccharin solutions (Holtzman, 1975; Lynch and Libby, 1983; Kirkham and Cooper, 1988; Morabia et al., 1989). Based on data in other species demonstrating that opioid receptors moderate effects on feeding, our results therefore suggest that naloxone inhibited the sensorial pleasure elicited by feed consumption, thereby contributing to control voluntary feed intake and voiding the preference for sweetened feeds.

Serum glucose and plasma insulin concentrations did not differ with sampling time (Table 8.4). As expected, FED calves had greater ($P < 0.05$) mean serum glucose (4.08 mM) and plasma insulin (121.5 pM) concentrations than FAS calves (3.80 mM and 84.0 pM, respectively). Similar to the present study, fasting reduced concentration of glucose and insulin in the plasma of calves and sheep (Trenkle, 1976; Cole and Hallford, 1994). In the current study, calves were fasted for 14 h, and FAS calves had greater starter feed consumption during the first 4 h after feeding compared with FED calves. However, serum glucose and plasma insulin concentrations continued to be greater for FED compared with FAS calves. Withholding feed has been shown to increase the hepatic removal of insulin (Brockman and Bergman, 1975), reduce secretion of insulin by isolated pancreatic tissues (Boden et al., 1981), diminish in vivo insulin secretion rate, and reduce postprandial insulin concentration (Trenkle, 1971). Serum glucose and plasma insulin concentrations did not differ between NAL and SAL calves. In rats, endorphins are capable of affecting circulating insulin and glucose directly via peripheral opiate receptors and (or) indirectly via opiate receptors located in the central nervous system (Appel et al., 1987; Curry et al., 1987). In agreement with our findings, however, no changes in serum glucose or plasma insulin concentration were observed when sheep were injected with naloxone (Alavi et al., 1991; Cheema et al., 1991).

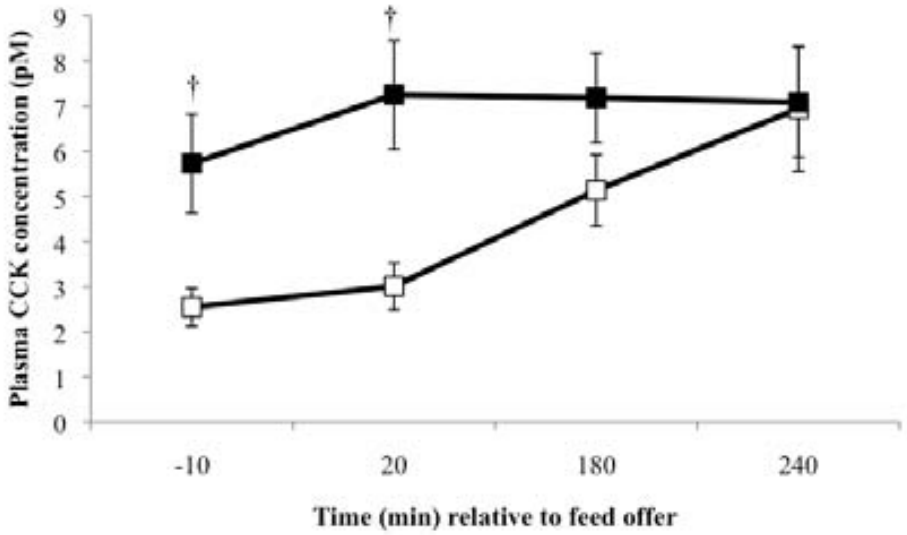
Table 8.4. Influence of naloxone (NAL) or saline (SAL) injection on circulating glucose, insulin, glucagon-like peptide-1 (GLP-1), and cholecystokinin (CCK) in fed or 14-h fasted calves relative to treatment administration

	-10 min				20 min				180 min				240 min									
	Fed		Fasted		Fed		Fasted		Fed		Fasted		Fed		Fasted			P-value[1]				
	SAL	NAL	SAL	NAL	SAL	NAL	SAL	NAL	SAL	NAL	SAL	NAL	SAL	NAL	SAL	NAL	SEM[2]	N	M	T	N × T	M × T
Glucose (mM)	4.1	4.1	4.2	3.8	3.9	4.1	3.9	3.8	4.1	4.0	3.7	3.8	4.1	4.0	3.7	3.9	0.05	0.46	0.04	0.27	0.49	0.26
Insulin (pM)	118.2	95.7	87.7	61.2	108.9	112.1	63.6	71.0	152.7	88.2	99.3	92.8	95.8	153.3	85.6	166.2	0.20	0.31	0.01	0.09	0.62	0.14
GLP-1 (pM)	46.6	45.1	47.4	41.7	48.3	45.6	45.7	41.2	50.8	46.9	48.9	45.2	48.5	45.0	51.1	47.1	0.05	0.04	0.65	<0.01	0.03	0.02
CCK (pM)	6.4	5.3	2.5	2.6	5.5	6.3	2.9	3.3	6.2	5.3	6.0	7.0	7.7	6.1	8.2	8.2	0.26	0.58	0.02	<0.01	0.71	0.08

[1]N = naloxone effect (NAL or SAL); M = metabolic state effect (fed or fasted); T = time relative to naloxone (or saline) administration (−10, 20, 180, or 240 min); N × T: interaction between naloxone and time; M × T: interaction between metabolic state and time; no N × M and N × M ×T interactions were observed ($P > 0.05$).

Overall, FED calves had greater (P < 0.05) plasma CCK concentration than FAS calves (6.79 and 4.41 pM, respectively). The presence of fat and protein in the duodenum stimulates CCK secretion by endocrine cells of the upper small intestine (Liddle et al., 1985). CCK acts on the hypothalamus as a neuropeptide decreasing intake (Della Fera and Baile, 1979; Grovum, 1981). Plasma CCK concentration (Figure 8.2) tended to be greater ($P = 0.08$) in FED calves than in FAS calves at −10 and 20 min relative to feeding time (5.86 and 6.16 pM vs. 2.49 and 3.05 pM, respectively). Considering only plasma CCK concentration at −10 min relative to feeding time, FED calves had a greater ($P < 0.05$) concentration than FAS calves. However, plasma CCK concentration did not differ between NAL and SAL calves. As a result of the 14 h of fasting, the content of nutrients in the intestine of FED calves might have exceeded that of FAS calves (particularly during the first 2 sampling times), which could have led to a greater plasma CCK concentration in the former group of calves. In agreement with our results, Suominen et al. (1998) reported that plasma CCK concentration decreased during feed deprivation in heifers. However, in the current study, the lack of an effect of NAL on plasma CCK-8 concentration does not rule out an effect on other CCK forms secreted in ruminants that could affect the hypothalamus.

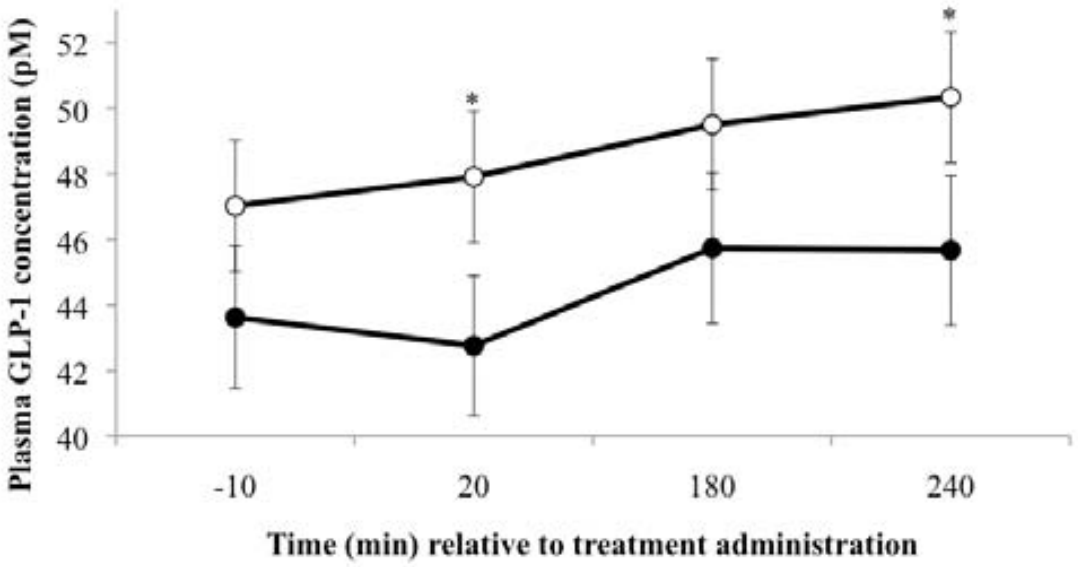
Figure 8.2. Plasma cholecystokinin (CCK) concentration (mean ± SEM) at −10, 20, 180 and 240 min relative to feed offer in fed (■) or 14-h fasted (□) calves.



† Plasma CCK concentration in fed and fasted calves tended to differ (P < 0.10).

The metabolic state of calves had no effect on plasma GLP-1 concentration. GLP-1 is secreted in response to feed consumption but it has an exceptionally short half-life of less than 2 min (Scheen, 2007). In the present study, blood was harvested 20 min after the feed was offered, and thus no differences in GLP-1 between FED and FASTED calves were expected. However, NAL calves had lower ($P < 0.05$) GLP-1 concentration (Figure 8.3) compared with SAL calves at 20 and 240 min after feeding (42.1 vs. 47.7 pM and 45.3 vs. 51.2 pM, respectively). Recent studies with rodents provide the first demonstration that the gastrointestinal mucosa expresses taste signaling elements, such as the sweet taste receptor T1R2/T1R3, that form part of a chemosensing system by which luminal nutrients (e.g., glucose) and other nonnutritive chemicals (e.g., sweeteners) trigger physiological responses, including secretion of incretin hormones such as GLP-1 (Jang et al., 2007). In the current study, SAL calves had similar starter feed intake but a greater ($P < 0.05$) preference for SF than NAL calves (70.3 vs. 51.9%, respectively). Therefore, it would seem reasonable to speculate that the intestinal mucosa of calves also has a chemosensing system that mediated changes in circulating GLP-1 in response to the larger intake of SF (sweetener) by SAL compared with NAL calves.

Figure 8.3. Plasma glucagon-like peptide-1 (GLP-1) concentration (mean ± SEM) at -10, 20, 180 and 240 min relative to treatment administration (saline or naloxone) in calves treated with a saline solution (○) or with naloxone (●).



*Plasma GLP-1 concentration in calves treated with saline solution or with naloxone differ at $P < 0.05$.

8.4. Conclusions

Injecting calves with naloxone alters short-term (2 h) feed intake. Furthermore, naloxone injection prevents calves from discerning between a sweetened and a plain starter feed, suggesting that in calves, as in other species, the opioid system controls short-term feed intake by modulating the oro-sensorial response to feed consumption. On the other hand, blocking the opioid receptors with naloxone had no effect on the concentration of glucose, insulin and CCK, but did reduce the plasma GLP-1 concentration. Whether the changes in GLP-1 are mediated directly by naloxone or indirectly by the reduced consumption of sweetened starter remains to be elucidated.

Chapter 9

GENERAL DISCUSSION

Time line and thesis development

All the studies in this thesis aimed at studying different factors influencing solid feed consumption around weaning. Although each study had concrete objectives, the results of each study offer information about oro-sensory preferences in short- and long-term, strategies that could improve solid feed consumption.

The first and the second studies were conducted with the objective of determining the oro-sensory preferences for common ingredients used to formulate starter concentrates. While the animal model was developed and oro-sensory preferences were defined, a third study was performed. In the third study, we realized that flavor characteristics of starter had an effect on animals with low appetence for solid feed, but no differences were observed among animals consuming high amounts of solid feed. Despite of the flavor effect, this result suggested that each calf perceived feed flavors in a different manner, and it could be due to the fact that each animal has individual feed preferences or even individual nutrient requirements. Using this hypothesis the fourth study was conducted, in which a group of calves was able to select a diet through voluntary consumption of starter ingredients offered separately. Furthermore, in the fourth study, several of the most palatable feeds (previously identified during the second study), were used to evaluate whether this preference was maintained long-term.

When developing the animal model, we observed that animals had a clear preference for sweet feeds. Furthermore, we observed that animals showed this preference for sweet feed using a non-caloric sweetener, indicating that there is a non-metabolic regulation of pleasure related to feed. With this hypothesis the fifth study was performed. Moreover, a sixth study was conducted according to the results of a parallel study from our department (Castells et al., 2012), where it was observed that forage provision in young calves could increase starter feed consumption, and consequently improve rumen development. Thus, we designed a sixth study to evaluate the relevance of particle size of forage on feed intake. The objective was to determine whether particle size of forage influences solid feed intake and digestibility around weaning.

9.1. Oro-sensory preferences in short- and long-term around weaning

Young calves showed preference for sweet feeds in the short-term assays performed in the first and sixth study. In the first study, sucrose was added to the starter concentrate to potentially increase its palatability. This result agrees with Nombekela and Murphy (1995) that reported an increase in sucrose-supplemented diet intake in cows. Recently, Broderick et al. (2008) reported that in cows, sucrose supplementation (from 2.5% to 7.5%) replacing starch in long term study increases DMI and milk fat content, and decreases ruminal concentrations of ammonia and branched-chain VFAs. However, in the results of our first study these young animals did not have previous experience with sucrose, and were only allowed to feed sucrose during 6 h, minimizing post-ingestive effects. These results suggest that calves have an oro-sensory preference for sucrose independently of its nutritional influence.

Furthermore, in the sixth study, when a non-caloric sweetener was added to the concentrate, the preference for sweet taste was maintained, reinforcing the concept of an innate preference for sweet taste. In agreement, several authors (Goatcher and Church, 1970a; Hellekant et al., 1994) have reported preferences for sweet taste in water solutions, even when no caloric supply was linked with the sweetener.

These results are consistent with the capability of cattle to perceive neurologically sweet stimuli (Hård af Segerstad and Hellekant, 1989a, 1989b). These stimuli could be explained because sweet taste could be related to carbohydrate content in feed (Ginane et al., 2011), which is the main energy source for ruminants, and it could explain the innate preference for sweet taste.

When the orange-sweetener flavor was analyzed in a long-term study, no differences were observed between flavored and unflavored treatment on solid feed consumption. But, although no significant, starter intake after MR reduction increased more in calves that received a flavored starter than calves that received an unflavored starter. It seems that flavor had a strongest effect on the animals that had a low level of consumption before MR reduction, because calves that received the flavor in the solid feed starter seemed to increase solid feed consumption and improve ADG compared to calves that received unflavored starter. Contrary, within calves that showed a high level of consumption before MR reduction, calves that received flavor showed a

numerically lower feed consumption than calves that received the unflavored starter. These results indicate that orange-sweetener flavor stimulates solid feed consumption in young animals, especially when the solid feed consumption is low. But, this preference seems to fade out over time, mainly when calves had a high level of consumption. Other authors suggested (Goatcher and Church, 1970c; Grovum and Chapman, 1988) that sweet taste could negatively influence solid feed consumption, and increasing doses of sweet compounds could even lead to rejection or intake depression.

These results suggested that sweet taste encourages solid feed consumption in short-term, and it could be useful to stimulate solid feed consumption in young calves. But in long-term or when calves consumed greater amounts of feed, it is necessary to consider other aspects, such as post-ingestive effects. For this reason, to maintain this stimulus to solid feed, balancing nutrient content could avoid negative post-ingestive effects and help to improve solid feed consumption. Determining the preferences for the most common ingredients used to formulate starter concentrate could help to increase solid feed intake minimizing negative post-ingestive effects. In the short-term assays conducted with the common ingredients used as energy source, wheat was the preferred ingredient and sorghum, corn, and barley were three of the most preferred ingredients. Whereas, wheat midlings and oats were two of the least desired, and rice and corn gluten feed were the least preferred ingredients. Therefore, it seems that wheat, sorghum, corn and barley could encourage solid feed consumption in short-term compared to the others ingredients. Surprisingly oats was one of the least desired ingredient, despite oats are commonly used in starter concentrates for its hypothetical palatability (Hill et al., 2008). In previous short-term studies (Klopfer, 1981; Spörndly and Åsberg, 2006), it was also reported that barley was one of the most preferred ingredients. However, the preference for wheat or barley in front of oats was not observed by Spörndly and Åsberg (2006) in a short-term study. The characteristics of the animals and the method used could explain these differences with our results. Spörndly and Åsberg (2006) only allowed heifers to feed one feed per day and used barley as a control feed between short-term assays.

In terms of protein ingredients, soybean was the most preferred ingredient, followed by dried distillers grains and sunflower meal. On contrary, peas meal, rapeseed meal

and corn gluten meal were the least desired. In previous short-term assays in bovine (Spörndly and Åsberg, 2006) it has been reported that 550-kg pregnant heifers preferred rapeseed meal (heat-treated) over soybean meal and ground peas. These different results could be related to the glucosinolates content of rapeseed, because the variety and product of rapeseed could vary on glucosinolates content, and decreasing glucosinolates content increases feed acceptance (Spörndly and Åsberg, 2006). Despite these differences in short-term studies, it has been observed that there are no differences on DMI between soybean meal and sunflower meal (Stake et al. 1973), and between soybean meal and distillers dried grains (Owen and Larson, 1991), in long-term studies, suggesting that these three ingredients are the most preferred ingredients among protein sources in long-term. However, rapeseed meal palatability is not clear in long-term assays. Lardy and Kerley (1994) described that rapeseed products usually result in low DMI compared to soybean meal, but, on the other hand, Bertilsson et al. (1994) reported that rapeseed meal (heat-treated) was preferred in front of soybean meal or rapeseed expeller.

Corn and barley were the preferred ingredients among the carbohydrate sources, whereas oats was the least preferred. Full fat soybean was also one of the three most preferred ingredients, whereas soyhulls was the second least preferred. Long-term preferences involve the interrelationship between the senses and post-ingestive feedbacks, as influenced by an animal's physiological condition and food's chemical characteristics (Provenza, 1996). Young calves have high protein requirements, and probably soybean products produced hedonic sensations from taste and smell as a function of a food's homeostatic utility (Provenza and Villalba, 2006) without being harmful due to the excess of nitrogen consumption. On contrary, oats meal was not preferred in short- and long-term studies, suggesting that feed characteristics (short-term) and post-ingestive effects (long-term) are not the most desired for young calves.

When protein and energy ingredients were combined and offered together, young calves showed short-term preferences mainly for wheat and soybean meal. Sorghum, barley, corn, dried distillers grains and sunflower meal were also among preferred ingredients in young calves. These ingredients have food characteristics that encourage solid feed consumption. Nowadays, most studies have investigated the importance of physical and chemical characteristics of the foods, such as density,

biomass or nutrient content. Less attention has been paid to their organoleptic characteristics, despite the potential role of these in feeding behavior. To consider the organoleptic characteristics of ingredients when a concentrate starter is formulate could improve calves' appetence for solid feed encouraging their intake.

Physical food characteristics also influence solid feed consumption. The particle size of roughage played an important role on solid feed consumption in the sixth study. Calves that received the mixture of starter with chopped forage (3-4 cm) sorted for forage, while calves that received the mixture of starter with ground forage (2 mm) sorted for starter or against forage. Perhaps, these observations were potentially due to increased keratinization of ruminal papillae of animals receiving small forage particles (McGavin and Morrill, 1976). This fact could have induced post-ingestive negative effects reducing consumption in a long-term. Chopped forage seems to be preferred in front of ground forage. Accordingly, Woodford and Murphy (1988) reported that reducing forage particle size in diets for early lactation cows decreased DMI. However, when feeding poor-quality forages or high-forage diets, reducing forage particle size significantly increased DMI (Moore et al., 1964; Osuji et al., 1975). In the sixth study, this preference for chopped forage seemed to be related to a positive post-ingestive effect, because calves that received chopped forage displayed greater digestibilities compared to calves that received ground calves. Previous studies reported that providing chopped diets or forage increase chewing activity, and consequently increases ruminal pH, and help to maintain the integrity and healthiness of the rumen wall (Krause et al., 2002; Yansari et al., 2004). It could be speculated that that young calves have preference for chopped hay because it improves digestibility and rumen development.

In summary, feed characteristics (physical and chemical) modulate solid feed consumption. Taste, related to chemical compounds, and particle size have a clear influence on feeding behavior, indicating that organoleptic characteristics have to be considered to improve solid feed consumption. But, the hedonic value of ingredients must be associated with a positive post-ingestive effect to encourage solid feed consumption in long-term.

9.2. Feeding strategies that could increase solid feed consumption around weaning

The potential effect of previous exposure to a familiar flavor early in life could increase solid feed intake. In humans, it has been shown that consumption of a flavor during breastfeeding facilitates acceptance for novel foods (Hausner et al., 2010). In the third study of this thesis, flavor effect around weaning influenced in different manner young calves. As described above, calves with high level of consumption at weaning did not increase solid feed intake. On contrary calves with low level of consumption increased solid feed intake. This fact could be explained because all calves were weaned at the same day, but calves differed on BW and solid feed consumption, suggesting that calves' characteristics were different. These differences could influence feed preferences (Provenza et al., 1996; Villalba and Provenza, 1996). Furthermore, animals had 22 d of age when the study began, and probably these animals experienced with solid feed before the study. Experiences early in life have been shown to affect the learning of feeding behavior in ruminants, with differences in feed preferences greatest when experiences occur earlier rather than later in life (Arnold and Maller, 1977; Provenza and Balph, 1987; Nolte et al., 1990). Miller-Cushon and DeVries (2011) suggested that feed familiarity affects initial diet selection post-weaning. This could explain why calves with high level of consumption consumed less starter when the orange-sweetener flavor was added, because the change of starter could cause neophobia or fear to novel feed in cattle (Herskin and Munksgaard, 2000). Moreover, a typical recommendation was to wean calves when intake of starter reached 700 to 1000 g/d for three consecutive days (Morril, 1992), and calves with low feed consumption consumed 615 g/d, whereas high feed consumption described 1330 g/d. Furthermore, in the third study calves received starter concentrate with flavor at 7 wk of age, whereas Morril and Dayton (1978) who reported an increase in solid feed intake when adding a commercial flavor in young calves during the first 5 wk of age. These results suggest that to achieve an increase on feed consumption adding a flavor on solid feed and MR, it has to be performed on young calves with low or null experience with solid feed, as early in life as possible, then this flavor could encourage solid feed consumption. Applying this flavor later in life could have negative effect as a consequence of previous experiences that could modify the intrinsic characteristics of each animal.

To determine the intrinsic characteristics of each calf, other proposed strategy to increase solid feed consumption around weaning, was to offer different ingredients separately. It has been reported that each animal may have different individual feed preferences, nutrient tolerances (Provenza et al., 1996; Villalba and Provenza, 1996; Scott and Provenza, 1999) and nutrient needs (Provenza et al., 2003). In the fourth study in this thesis, it has been observed that calves that received different ingredients separately described a similar DMI compared to calves that received concentrate intake. However, nutrient and ingredient consumption was different among these groups. Atwood et al. (2001) compared a mixed ration of rolled barley (31.3 %), rolled corn (31.3 %), corn silage (15.5 %) and alfalfa hay (18.9 %) to a choice among those feeds offered individually. In this study, no differences were observed in BW gain, ratios of protein to energy consumed. However, animals that received the mixed ration tended to eat more than animals that received a choice. In contrast, in the fourth study of this thesis, calves that were able to select their diet described similar growth than calves that received mixed ration, but a greater ratio of protein to energy was observed in calves offered a choice compared to calves offered a mixed ration. Consequently, protein efficiency was lower in choice group than in the mixed ration group. In contrast, Manteca et al. (2008) suggested that without the ability to choose, individual animals may be forced either to over- or under-consume particular nutrients, consequently animals could use nutrients inefficiently. Previous studies conducted in lambs or sheep have demonstrated the capability to select a diet balanced for macronutrients in response to their changing needs (Kyriazakis and Oldham, 1997; Villalba and Provenza, 1999; Scott and Provenza, 2000), and in some animals reaching their nutritional needs more accurately than feeding animals in a non-selecting feeding system (Provenza, 1996; Early and Provenza, 1998). The difference between results found herein and those reported in previous studies are probably due to the species used and the quantity and characteristics of feeds offered. In the fourth study of this thesis, calves had access to six different ingredients, whereas in previous studies lambs or sheep had less than six options of feeds available, and some of these studies were conducted offering imbalanced protein-energy diets and a choice of protein or energy supplement to correct the imbalanced diet offered (Kyriazakis and Oldham, 1997; Scott and Provenza, 2000). Offering six different ingredients made more difficult to achieve a balanced diet compared to animals that received protein-deficient diet with a choice of energy or protein

supplement. Furthermore, offering a choice makes it more difficult for the animal to associate a post-ingestive effect or a consequence with the food responsible (Duncan and Young 2002; Favreau et al., 2010). Despite these differences, in the fourth study of this thesis, calves were able to meet their nutrient requirements and did not reduce solid feed consumption and ADG compared to mixed ration group, although they were less efficient utilizing dietary protein. However, offering different ingredients in young calves could present some problems to increase solid feed consumption, because calves are not efficient and calves could excess some nutrient consumption.

Roughage could play an important role on rumen development and consequently on rumen health, minimizing negative post-ingestive effects. For this reason, to increase solid feed consumption around weaning an alternative option may be providing forage before weaning. As mentioned in the introduction, forage inclusion before around weaning is a controversial issue. But recent studies reported that forage inclusion could improve ADG, total DMI (Castells et al., 2012) and feed efficiency (Coverdale et al., 2004). Castells et al. (2012) reported that chopped grass hays or grass silages increased solid feed consumption, whereas chopped alfalfa hay reduced starter concentrate consumption (and as a result impaired performance). This study claimed that forage supplementation could improve solid feed, but the source of forage is crucial. In the sixth study of this thesis, offered chopped grass hay to pre-weaned calves increased solid feed consumption compared to ground grass hay after weaning. Furthermore, chopped grass hay improved feed, CP, NDF and ADF digestibilities. This result determined that the particle size of forage provision is also important to achieve an improvement in solid feed consumption.

The physical characteristics of roughages, such as coarseness, bulkiness, and abrasiveness, have been reported as necessary to maintain the integrity and healthiness of the rumen wall (Haskins et al., 1969). Recently, it has been observed that providing chopped diets or forage increases chewing activity, and consequently increases ruminal pH, improving rumen status (Krause et al., 2002; Yansari et al., 2004). In agreement with these beneficial effects on rumen development, chopped hay seems to improve rumen environment and, consequently, may elicit positive post-ingestive effects leading to increasing solid feed consumption around weaning. Furthermore the increase in nutrient digestibilities suggests that young calves that

received chopped forage during the first weeks of age are more efficient and more adapted to consume solid feed later on.

In summary, considering the use of preferred flavors early in life related to MR, offering solid feed as a mixed ration, voiding the possibility of ingredient choices, and mixing this diet with long particle size roughage represent interesting alternatives to increase solid feed consumption in young calves.

9.3. Intake regulation through hedonism in young calves

In humans, opiate peptides have been shown to play an important role in control of food intake (De Zwaan and Mitchell, 1992), basically influencing oro-sensory reward mechanisms in feeding (Berridge et al., 2009).

Blocking opiate receptors had an effect in regulating short-term (2 h) feed intake in well fed calves, but not in fasted calves. Accordingly, Barbano and Cador (2006) showed that the effects of opiates are only visible in well-fed animals because under feed-deprivation states the effect of other metabolic hormones overruns the feed control by opiates. Furthermore, it has been described that naloxone has a half-life of 1 h in humans (Nagi et al., 1976). These results support the hypothesis that opiates are implicated in short-term regulation of intake. Accordingly, previous studies in sheep reported that intravenous injection of an opiate receptor antagonist decreased feed intake for 2 h (Baile et al., 1981) or 4 h after administration (Alavi et al., 1991; Obese et al., 2007). Furthermore, Burgwald-Balstad et al. (1995) described that naloxone-injected heifers decreased intake compared to those receiving saline one hour and two hours after feeding.

The majority research conducted with opiates antagonist in ruminants has been performed to improve the knowledge about opiod systems role on intake regulation, i.e., the influence of naloxone on intake, ruminal fermentation, nutrient digestibilities, digesta kinetics, and plasma hormone and metabolite concentration (Burgwald-Balstad et al., 1995). But, to our knowledge until our study, the influence of the opioid systems on pleasantness related to feed consumption had not been studied in ruminants. As described above, calves have a preference for sweet feed. However, naloxone injection did not allow calves to discern between a sweet and control

concentrates, suggesting that the opiate peptide system may regulate short-term feed intake by mediating the pleasure response to feed. Accordingly, it has been reported that blocking opioid receptors decreases pleasantness of certain foods, including sweet solutions, sugar/fat mixtures, and salted soup in humans (Fantino et al., 1986; Yeomans and Wright, 1991; Bertino et al., 1991; Drewnowski et al., 1992) and reducing intake of palatable sweet liquids such as glucose, sucrose or saccharin solutions in rodents (Holtzman, 1975; Lynch and Libby, 1983; Kirkham and Cooper, 1988; Morabia et al., 1989).

Our results suggest that naloxone might regulate voluntary intake by inhibiting the pleasure response to feed, and consequently decreasing the energy intake in weaned calves. However, no clear interaction was observed with other metabolites related to energy homeostasis. It seems that opiates influence feed intake regulation through oro-sensory reward mechanisms in young calves, indicating that feed characteristics (odor, taste, etc.) could influence solid feed consumption apart from post-ingestive effects or nutritive characteristics.

Chapter 10

CONCLUSIONS

The results obtained in this thesis allow concluding that:

1. Young calves have oro-sensory preferences for sweet taste, through sucrose or sweetener supplementation around weaning in short-term assays. Orange-flavored sweeteners improve solid feed consumption and growth in animals with low appetence for solid feed.
2. Wheat and soybean meal are highly preferred by young calves in short-term assays. Young calves also have preference for soybean products (meal and full fat) in long-term assays. On the other hand, oats were the least desired in long-term studies.
3. Flavoring milk replacer and starter concentrate in the same manner increases consumption in young calves with low appetence for solid feed.
4. Young calves sort in favor of coarse particles of forage when it is offered in a mixed ration with starter concentrate. Contrary, young calves reject fine particles of forage when it is offered in a mixed ration with starter concentrate.
5. Calves offered a voluntary selection of ingredients separately have similar solid feed consumption and growth than calves offered a conventional starter. However, calves that receive different ingredients separately consume different amount of ingredients and nutrients, using crude protein more inefficiently.
6. Providing young calves a forage with a coarse particle size (3-4 cm) mixed with a starter improves dry matter intake and feed efficiency after weaning, as a consequence of an improvement of digestibility. Furthermore, it reduces undesirable behaviors, such as non-nutritive oral behaviors.
7. Opiate peptides are implicated in the control of feed regulation, and their participation in the modulation of intake is more important in well-fed than in undernourished animals.

Chapter 11

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