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Enhanced-growth feeding programs for dairy calves: nutrition, management, and long-term effects

MEMÒRIA PRESENTADA PER MARTA TERRÉ TRULLÀ

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

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RESUM

Es varen realitzar quatre estudis per avaluar l'efecte de donar molta llet a les vedelles lactants durant la fase de lactància, per tal de millorar el creixement de les vedelles de reposició durant aquest període. Els vedells que varen rebre una alimentació més rica en llet durant la lactància van créixer més, però van menjar menys pinso que els vedells que seguien una alimentació convencional. Tot i així, després del deslletament ambdos nivells d'alimentació van presentar el mateix ritme de creixement i consum de pinso. Per altra banda, el fet de criar vedells en grup i amb un nivell elevat de llet durant la lactància, no va estimular el consum de pinso en comparació als vedells criats individualment i alimentats amb un alt nivell de llet. A més a més, els index productius de creixement i l'aparició de problemes de salut van ser similar en vedells criats en grup o individualment. En general, els vedells criats en grups varen augmentar els comportaments orals amb finalitat no nutritiva, i disminuir els comportaments de succions creuades i succions dirigides a la zona pèlvica al llarg de l'estudi. Tant els vedells criats en grups com individualment van presentar un lleuger augment de la conducta de "self-grooming" al llarg de l'estudi. La concentració plasmàtica d'amino àcids va indicar que cap amino àcid limitava el creixement durant la fase de lactància quan les vedelles es varen criar seguint una alimentaió amb un alt nivell de llet. No obstant, en els vedells que es van criar convencionalment, les concentracions plasmàtiques de fenilalanina i triptòfan una hora després de menjar estaven positivament correlacionades amb el guany mig diari, i negativament correlacionades amb la concentració plasmàtica d'urea, suggerint que els creixements dels vedells criats convencionalment podria estar limitat per l'aportació d'aquests dos amino àcids quan s'utilitza una llet maternitzada i un pinso similar al d'aquest estudi. Per altra banda, la menor excreció urinària de derivats púrics observada en vedelles alimentades amb un alt nivell de llet en comparació a les vedelles criades convencionalment, va indicar un menor fluxe microbià a nivell duodenal que podria estar relacionat amb la menor ingestió de pinso en les vedelles alimentades amb un nivell elevat de llet durant el periode de lactància. A més a més, els vedells alimentats amb un nivell alt de llet durant la fase de lactància van tenir una menor digestibilitat aparent dels nutrients del pinso la setmana després del deslletament en comparació als vedells alimentats convencionalment. Les concentracions sèriques de glucosa i insulina varen ser majors en vedells alimentats amb un alt nivell de llet que en vedells alimentats convencionalment. Però, les concentracions sèriques d'urea no varen seguir un mateix patró en els diferents estudis. En dos dels tres estudis, les concentracions sèriques d'urea van ser superiors en vedells criats convencionalment que en aquells alimentats amb un alt

nivell de llet, però en l'altre estudi les concentracions sèriques d'urea varen ser similars en ambdos nivells d'alimentació. Finalment, l'avantatge de pes viu aconseguit durant la fase de lactància en vedelles seguint una alimentació amb un alt nivell de llet es manté numèricament superior fins els 385 d d'estudi, però aquest avantatge no redueix l'edat a la primera cubrició, i ni millora la fertilitat a la primera cubrició en vedelles criades amb un alt nivell de llet en comparació amb vedelles criades convencionalment.

SUMMARY

Four studies were conducted to evaluate the effects of feeding dairy calves on an enhanced-growth feeding program. Enhanced-fed calves showed greater average daily gain, but lower starter dry matter intake than calves fed conventionally during the preweaning period. However, after weaning both feeding programs resulted in similar rates of growth and starter consumption. On the other hand, rearing enhanced-fed calves in groups did not stimulate starter intake. Also, performance was not decreased nor the occurrence of health problems increased when calves were reared in groups. In general, calves raised in groups increased non-nutritive oral behavior, and decreased cross-sucking and inter-sucking behaviors throughout the study, and both individually- and grouped-reared calves slightly increased self-grooming behavior throughout the study. Plasma amino acid concentrations indicated that none amino acid was limiting growth during the preweaning period when calves were raised following an enhanced-growth feeding program. Nevertheless, with calves conventionally-fed, plasma phenylalanine and tryptophane concentrations one hour after feeding were positively correlated with average daily gain and negatively correlated with plasma urea concentrations, suggesting that growth of calves following conventional feeding programs could be limited by the supply of these two amino acids when using milk replacers and starters similar to those used in the present study. On the other hand, lower total purine derivatives urine excretions were observed in enhanced- compared with conventionally-fed calves, suggesting a lower microbial duodenal flow that was probably related to the low starter intake during the preweaning period of enhanced-fed calves. Furthermore, apparent nutrient starter digestibility was lower in enhanced- compared with conventionally-fed calves the week after weaning. Serum glucose and insulin concentrations were greater in enhanced- than in conventionally-fed calves, but serum urea concentrations did not follow a common pattern among studies. In two out of the three studies, serum urea concentrations were greater in conventionally- than in enhanced-fed calves, but in the other studies there were no differences between treatments. Body weight advantage obtained with enhanced-growth feeding program was numerically maintained later in life, but this advantage did neither reduce the age at breeding, and nor improve fertility at first breeding of enhanced-fed calves.

ABBREVIATIONS USED

AA: amino acid

ADF: acid detergent fibre

ADG: average daily gain

AFC: age at first calving

AOAC: Association of official analytical chemists

BW: body weight

CF: conventional feeding program

CP: crude protein

DE: digestible energy

DM: dry matter

DMI: dry matter intake

DNA: deoxy nucleic acid

EAA: essential amino acid

EF: enhanced-growth feeding program

EU: European Union

GE: gross energy

GP: grouped housed

HPLC: high performance liquid cromatography

I:G: ratio insulin to glucose

IGF-I: insulin-like growth factor I

IP: individually housed

ME: metabolize energy

MR: milk replacer

NE: net energy

NEAA: non essential amino acid

NEFA: non-esterified fatty acid

NDF: neutral detergent fibre

NRC: National Research Council

OM: organic matter

PBMC: peripheral blood mononuclear cells

PD: purine derivatives

PCR: polymerase chain reaction

SEM: standard error of the mean

TDMI: total dry matter intake

TMR: total mixed ration

T-RFLP: terminal-restriction fragment length polymorphism

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Chapter 1

LITERATURE REVIEW

Introduction

The most common practice for raising replacement dairy calves consists on feeding 4 l/d of milk replacer (MR) at a dilution rate of 12.5% DM from 3 to 60 d of age to attain ADG around 500 g/d. The aim of this conventional feeding program is to promote an early consumption of calf starter and to achieve a good rumen development while decreasing nutrition costs (Davis and Drackley, 1998). Nevertheless, calves allowed to suckle their dam (Bar-Peled et al., 1997) or receiving an *ad libitum* milk intake program (Jasper and Weary, 2002; von Keyserlingk et al., 2006) presented rates of growth around 0.7-1 kg/d. Therefore, the genetic potential growth of suckling calves is not fully expressed in conventional feeding programs for replacement dairy calves. Some strategies have been developed to raise calves at their potential growth rates, improving health and reducing the age at first calving without compromising the structural development of replacement heifers.

This review will cover different aspects that influence performance of dairy calves, and propose practices that may improve the growth of dairy calves during the nursing period.

1.1. Calves liquid feeding

Whole milk was the main dairy calves liquid feed before 1956 (Otterby and Linn, 1981). Studies evaluating MR date back to late 1960s and early 1970s (Colvin et al., 1969; Willett et al., 1969; Wood et al., 1971). Initially, milk replacers were manufactured with gruel ingredients (linseed meal, wheat middlings), but the use of spray-dried skim milk and dried whey, together with improvements on fat homogenization at manufacturing resulted in high quality products (Otterby and Linn, 1981). When comparing high quality MR with whole milk, performance parameters have been similar (Jaster et al., 1990). However, when using protein sources different to milk protein sources, MR have not support the same weight rates compared to whole milk or high quality MR (Gardner et al., 1990; Quigley 2002).

1.1.1. Colostrum, whole milk and discarded milk

Colostrum is the first meal offered to calves and has the important role of transfering passive immunity. It contains high amounts of immunoglobulins (Ig), mainly IgG1, leukocytes, cytokines, and greater amounts of nutrients and vitamins compared with cow's milk. Colostrum Ig absorption in calves depends on the amount of colostrum intake, the amount of Ig in the ingested colostrum, and the time elapsed between birth and first feeding (Besser et al., 1985; Tyler and Ramsey, 1991; Morin et al., 1997). The

failure of passive immunity transfer after birth has been associated with preweaning mortality in feedlot (Galyean et al., 1999), and dairy calves (Nocek et al., 1984a). The greater serum Ig concentration at 24 to 48 h after birth, the lower calf mortality up to 180 d of age (Robison et al., 1988), and it may enhance their future productivity (DeNise et al., 1989).

Although the main liquid feeding for dairy calves, once colostrum is fed, is MR, there are other available sources to nourish calves: whole milk, waste milk (mastitic or with antibiotics; fermented or row), and transition milk. All of these sources are good replacements to MR if they are used cautiously (Keys et al., 1980). Whole milk is the natural liquid feeding for calves, but it is the most expensive liquid feeding: approximately, feeding 4 l/d whole milk costs 1.32 €/d in contrast to the 500 g/d of MR powder that costs 0.72 €/d. Furthermore, when a spray (crude protein provided by milk proteins) MR was fed, there was no performance advantage compared to those calves offer whole milk (Jaster et al., 1990). Less expensive options to feed calves are waste or transition milk. Large farms use waste milk more often than small farms (Heinrichs et al., 1995), probably because they are likely to have mastitic or treated milk available because of the higher number of milking cows. When mastitic milk is offered, two management practices are recommended: not feeding mastitic milk to calves younger than 3 days of life, and not feeding mastitic milk to calves raised in groups, to avoid sucking and disease transmission (Kesler et al., 1981). However, feeding calves with mastitic or antibiotic milk has been associated with high mortality among preweaned calves, whereas feeding whole milk from the bulk tank has been associated with low deaths (Losinger and Heinrichs, 1997). Therefore, pasteurization of waste milk to reduce bacterial milk contamination may be envisaged. Pasteurization reduced morbidity and improved calves performance when compared with non-pasteurized waste milk (Jamaluddin et al., 1996b). A recent study (Godden et al., 2005) reported that feeding pasteurized non-saleable milk improved calf rate of growth, and decreased morbidity and mortality risk of disease compared with calves fed a conventional MR. It was estimated that pasteurization was economically feasible when feeding at least 315 calves daily (Jamaluddin et al., 1996a), this is possible in the recent appearance of commercial dairy calves contract operations. However, the comparison between pasteurized non-saleable milk with MR, yields that only 23 calves are needed to break even (Godden et al., 2005). This large difference between both studies may be attributed to the fact that the latter included the cost of MR, but the first one used waste milk. Moreover, the economic analysis to determine the feasibility of pastereuirzing waste milk in Jamaluddin et al. (1996a) and Godden et al.

(2005) studies included different fixed and variable costs, and performance parameters, which could also influence the differences between the studies.

Little information is available on the risk of antibiotics resistance when feeding discarded milk with antibiotics. Wray et al. (1990) did not find differences in the antibiotics resistance index when comparing calves fed milk with or without antibiotics. However, Langford et al. (2003) reported similar rates of growth and incidence of diarrhea in calves fed wasted milk with different levels of penicillin, but antibiotic resistance increased in relation to dosage, and it lasted at least 4 d after untreated milk was offered. Similarly, Selim and Cullor (1997) also observed antibiotic-resistance in calves fed treated milk.

1.1.2. Milk replacer

1.1.2.1. Ingredients

There are two main protein sources: milk (dried skim milk, whey protein concentrate, dried whey, sodium caseinate) or vegetable (soy protein, wheat protein, potato protein). In general, there are no differences among the use in either of the milk protein sources (Terosky et al., 1997), and vegetable sources are used to reduce MR costs. Although the replacement of milk proteins by plant protein leads to a decrease in MR apparent digestibility (Montagne et al., 2001), calf performance and digestible amino acid absorption can be improved by the inclusion of Thr, Met and Lys in MR containing soy protein (Kanjanapruthipong, 1998).

Other alternative sources of protein that replaced part of the milk protein supply, such as liquid egg (Touchette et al., 2003), spray-dried whole egg (Quigley, 2002), red blood cells (Quigley et al., 2000) or fish protein (Seegraber and Morrill, 1986), have been used to decrease the cost of MR with variable effects on calves performance.

However, in general, the more alternative protein sources replace milk protein in the MR the poorer the calf performance.

Although milk fat is highly digestible, milk fat is prohibitive in MR formulation because of its high cost. Then, manufacturers use animal fat (tallow, lard) as MR lipid sources. However, vegetable oils (coconut oil, palm oil) have been studied as a result of the consumer concern about the use of animal products in animal feeding. 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 fat MR source.

The only carbohydrate source in the MR is lactose from whey products. The intestine of calf mainly has lactase activity, and only lactose, glucose, and galactose can be effectively utilized in large amounts by calves (Davis and Drackley, 1998).

Vitamin A is necessary for normal growth development. The National Research Council (NRC, 2001) recommends 9,000 IU/kg of dry matter (DM) of MR, as suggested by Swanson et al. (2000) as the minimum amount necessary for proper development. The NRC (2001) recommendations are lower than the amounts found in whole milk (11,5000 IU/kg DM), and those used in the industry (between 25,000 and 40,000 IU/kg DM).

Vitamin E supports immune function and protects cellular membrane from oxidative damage. Whole milk contains 8 IU/kg DM compared with the greater values recommended by NRC (2001) (50 IU/kg DM of MR), and the cautious level in commercial MR (65 - 85 IU/kg DM). The main reasons for this difference are the stress conditions of neonatal calves, and the prevention to protect essential fatty acids from oxidation.

Vitamin D maintains calcium and phosphorus homeostasis. The NRC (2001) recommends 600 IU/kg DM of MR, which is two-fold the amount found in whole milk, and dramatically lower compared with commercial MR (4,000 – 10,000 IU/kg DM). Literature studies about vitamin D levels in MR are practically inexistent. Then, manufacturers formulate MR within a range between the minimum recommended by NRC (2001) and the maximum established by law (10,000 IU of Vitamin D_3, 2002/C 329/CE).

Macrominerals elements in MR recommended by NRC are similar to those found in whole milk. However, trace minerals are included in greater amounts than in whole milk to avoid deficiencies (NRC, 2001) without surpassing the legal threshold.

Several additives can be added to improve manufacture and preservation of MR: antioxidants (tocopherol), emulsifiers (lecithin, pectins), or preservative (citric acid). On the other hand, the association of the use of antibiotics in the MR with increasing levels of antimicrobial resistance (Berge et al., 2006), and the fact that the EU banned the use of antibiotics as growth promoters in animal nutrition (1831/2003 EEC) promoted the study of other additives to improve calf health and replace antibiotics. The replacement of antibiotics by oligosaccharides (Heinrichs et al., 2003) or a combination of several additives (allicin, fructooligosaccharides and bacteria, Donovan et al., 2002) was proved to sustain similar effect on preventing scours and improving performance, respectively, as MR supplemented with antibiotics. Moreover, the supplementation of lactoferrin in the

MR was proved to improve calves performance (Joslin et al., 2002), but it did not shown beneficial results on neutrophil function in neonatal calves (Dawes et al., 2004). On the other hand, some probiotics improved BW (Cruywagen et al., 1996) and incidence of scours (Abe et al., 1995; Timmerman et al., 2005), but sometimes its use has not shown any effect (Morrill et al., 1995). Furthermore, the use of clays has been used as diarrhea treatments or prophylaxis (Rateau et al., 1982), and their supplementation has resulted in improvements in nutrient digestibility in several species (Thielemans and Bodart, 1983). For instance, sepiolite tended to improve performance in chickens (Ouhida et al., 2000) and to improve protein and energy retention in pigs (Parisini et al., 1999), and bentonite was effective on preventing diarrhea of calves fed bentonite prophylactically for 6 days (Bartos and Habrda, 1974).

Overall, if the use of pasteurized discarded milk is economically profitable, it seems a good choice to raise calves. However, feeding milk with high antibiotics levels (> 25 µl/kg of penicillin) should be avoided to decrease calves antibiotic resistance. Moreover, vitamin and mineral contents of the MR formulation should be revised to adjust their contents to appropriate levels, which may decrease MR costs.

1.1.2.2. Nutrient composition

The nutrient composition of MR can influence body composition (Tikofsky et al., 2001, Blome et al., 2003), nutrient digestibility (Lodge and Lister, 1973), and starter intake (Kuehn et al., 1994).

Milk replacers usually contain from 18 to 28% crude protein (CP). The choice of MR protein content depends on the MR source, the desired calves growth, the energy density of the MR and the starter energy and protein supply.

Firstly, CP content 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.

Secondly, fast-growing calves would need greater CP supplies than calves growing more slowly. For instance, growth rates and N retention (g/d) increased linearly as the MR protein to energy ratio and the MR dilution rate increased, in calves fed isoenergetic MR with increasing CP levels (from 14% to 26%) (Blome et al., 2003), or fed a MR with 30% CP at a dilution rate of 15% or 18% (Diaz et al., 2001), respectively. However, plasma urea concentrations also raised as the protein to energy ratio increased, indicating that calves fed MR at high CP levels did not utilize dietary N as efficiently as calves fed MR

at low CP content (Blome et al., 2003). Even though, the decrease in protein efficiency should be contrasted against the reduced growth rate when CP content in the MR is low (Gerrits et al., 1996). In the study by Diaz et al. (2001), plasma urea N and efficiency of protein utilization suggested that protein was not limiting growth. Thus, the efficiency of utilization of absorbed amino acids (AA) could be improved by lowering the CP content of MR, and adjusting its amino AA profile.

Thirdly, restriction in protein to energy ratio in MR below those that would enable maximum lean tissue gain, will result in excessive body fat deposition (Donnelly, 1983).

Finally, calf starter composition should also be considered when deciding CP content in the MR. Following the NRC (2001) recommendations, when calves are offered 500 g/d of MR, if calf starter contains 16% CP and calves consume 500 g/d of starter, to support 500 g/d of growth rate, a 23% CP milk replacer would be needed. In contrast, when feeding a 20% CP calf starter to calves consuming 500 g/d of starter, a 19% CP milk replacer should be sufficient to maintain the same ADG.

Fat content in MR ranges between 10 to 22%, and carbohydrate content varies between 42 to 47%. In mild environmental conditions, higher fat contents (21.6% vs 15.6% fat) in MR decreased starter intake and ADG before and after weaning (Kuehn et al., 1994). However, the supplementation of MR with fat below calf critical temperature (< 8º-10ºC) has resulted in better growth rates (Jaster et al., 1990). This is mainly due to the greater metabolized energy requirements of calves reared at -4ºC (0.133 Mcal/$BW^{0.75}$) compared with calves raised at 10ºC (0.101 Mcal/$BW^{0.75}$) (Scibilia et al., 1987).

Calves consuming milk replacers containing 60.8% carbohydrate experimented moderate diarrhea, whereas calves consuming 67.3% and 72.3% carbohydrate in MR presented a high incidence of scours (Lodge and Lister, 1973). However, there were no differences in scours between calves consuming a 55% lactose MR and calves fed a 47% and 35% lactose MR (Tikofsky et al., 2001).

When protein is not limiting growth (dietary protein is adequate for the level of energy intake) carbohydrate is a more readily available form of energy than fat for lean tissue growth (Donnelly, 1983; Tikofsky et al., 2001). Moreover, considering isonitrogenous diets extra dietary fat will result in an increase of fat deposition, instead of retaining additional protein. Thus, changes in nutrient content of isonitrogenous and isocaloric diets can modify body composition maintaining similar growth rates (Tikofsky et al., 2001).

Data from several studies (Donnelly et al., 1983; Diaz et al., 2001; Tikofsky et al., 2001; Blome et al., 2003; Brown et al., 2005b; Bartlett et al., 2006) were analyzed to study the relationship between diet and carcass composition. Then, a stepwise analysis was performed to select the variables from the diet composition (fat, CP and GE of the MR and the ratio GE to CP, and fat to CP) that significantly influenced the percentage of CP and fat of the carcass gain and the daily amount of CP and fat deposited. The variables selected were checked to any possible correlations among them, and only variables with an R-square lower than 20 were selected for the final mixed-effects model. After that, a mixed-effects model, including the selected variables as fixed effects and adjusting the composition of the carcass gain to the random effect of the study, was performed to establish the relationship among the selected variables and the composition of carcass gain.

Data from Table 1.1 show that the percentage of the composition of the CP carcass gain was explained more accurately by the ratio fat to CP of the MR. The greater the fat to CP ratio of the MR the lower the percentage of CP in the carcass gain. Nevertheless, fat carcass gain was more influenced by CP and GE composition of the MR than the fat to CP ratio. The greater the CP in the MR, the lower the percentage of fat carcass gain, and the greater the GE content in the MR the greater the percentage of fat carcass gain. In contrast, the CP and fat content of MR directly influenced the daily amount of CP and fat carcass deposition, respectively. Furthermore, fat carcass gain (kg/d) was more difficult to predict using MR composition compared with CP carcass gain (kg/d) or CP and fat carcass content (%). Probably, because fat carcass gain depends more on nutrient intake rather than nutrient composition. Feeding a high-fat MR, but at a low rate, will not guarantee a high amount of fat carcass deposition (kg/d). However, if the same MR were fed at a high rate, calves would gain more fat in their carcass, but still this deposition would be limited by the CP deposition allowed by the CP supplied from the MR.

Table 1.1. Equations that predict the composition of carcass gain of calves resulting from the mixed-effects model performed with data from six studies (Donnelly et al., 1983; Diaz et al., 2001; Tikofsky et al., 2001; Blome et al., 2003; Brown et al., 2005; Bartlett et al., 2006).

	Predicting model	*P*-value	R^2	
CP carcass gain, %	22.87 - 3.32 fat:CP[a]	<0.001	0.54	[1]
Fat carcass gain, %	-1.48 – 0.97 CP % + 7.45 GE[a]	<0.001	0.94	[2]
CP carcass gain, kg/d	-0.044 + 0.0068 CP %[a]	<0.001	0.56	[3]
Fat carcass gain, kg/d	0.012 + 0.0029 fat %[a]	0.08	0.20	[4]

[a] fat:CP: ratio fat to CP of the MR; CP%: percentage of CP in the MR, GE: gross energy of the MR, kcal/g; fat%: percentage of fat of the MR.

The above articles studied body composition of calves fed MR only. But, body calf composition can also be influenced by the CP and GE sources of the total diet. The comparison of a high-energy diet composed of a MR (ratio GE to CP of 22.1) fed on a DM basis at 1.1% of BW and a calf starter (20.5% CP and 3.6 Mcal/kg) with a high-protein diet composed of a MR (ratio GE to CP of 14.5) fed on a DM basis at 2% of BW and a calf starter (25% CP and 3.7 Mcal/kg), shows that both resulted in similar fat and protein carcass composition (19.8 vs 19.9 % for protein and 4.4 vs 5.2 % for fat, respectively) at 8 weeks of age (Brown et al., 2005). Although the high-energy diet contained 19.94 Mcal GE/kg CP, and the high-protein diet contained 14.61 Mcal GE/kg CP, MR supplied 76% of GE consumed in high-protein diet compared to the 58% supplied by the high-energy diet (Table 1.2). Thus, the similar carcass composition might result from the different efficiency in fat and CP deposition when nutrients come from different sources.

Table 1.2. Percentage of energy and crude protein intake that comes from milk replacer or starter sources (Brown et al., 2005).

	High GE milk replacer	High CP milk replacer
GE intake from MR, %	58	76
GE intake from starter, %	42	24
CP intake from MR, %	52	76
CP intake from starter, %	48	24

Therefore, in very young calves (from 1 to 14 d of age) the use of all-milk protein MR should improve calf efficiency. On the other hand, if the aim is to improve growth rate during the nursing period it should be probably recommend to use a high CP milk replacer, even considering that protein efficiency will be low, and a low fat MR. Moreover, when raising dairy replacement heifers it would be recommended to promote lean tissue deposition by choosing a MR with a low fat to CP ratio.

1.2. Calf rearing systems

Dairy calves are usually fed 0.5 kg/d of MR and starter *ad libitum* to stimulate concentrate consumption. However, calves can be also fed high amounts of MR (1 kg/d of DM milk replacer) and starter *ad libitum* to increase calf growth during the nursing period. The liquid feeding program to follow in each situation depends on the desirable calves rate of growth, age at weaning, and the relative importance assigned to health or to economic returns. Furthermore, the European Directive (97/2/EC) encourages farmers to raise calves in groups, instead of in individual hutches, to stimulate calf contact and improve "welfare".

1.2.1. Conventional vs. Enhanced or Accelerated Growth Feeding Programs

Dairy calves are commonly fed at 10% of their BW twice a day to grow at the rate of 500 g/d. However, achieving greater growth rates at early stages in life (2-3 months) might be profitable because increases in relative BW and wither height are most rapid and cost-efficient during the first 6 months of life (Kertz et al., 1998). Recent studies have shown that greater ADG can be obtained when feeding milk *ad libitum* (Jasper and Weary, 2002), or feeding milk or MR at increasing rates (Diaz et al., 2001; Quigley et al., 2006).

However, high amounts of nutrient in the intestinal lumen may prompt intestinal disorders caused by undigested lactose that enters the colon and promotes water accumulation to the intestinal lumen caused by an increase of the osmotic pressure in the colon (Roy, 1980). Therefore, a major fear of feeding MR with high DM concentration is the risk of causing diarrhea by maldigestion.

Studies between the 50's and 80's evaluating several liquid feeding levels and MR concentrations, reported poorer fecal consistency or more days with moderate or severe diarrhea when MR feeding level increased (Hodgson 1971b; Stiles et al., 1974), and the dilution rate of MR was equal or greater than 20% DM (Pettyjohn et al., 1963; Jenny et al., 1978). However, similar feces fluidity was found when liquid offer was increased (Huber et al., 1984), and when MR dilution rate also increased (Burt and Bell, 1962; Hodgson, 1971b; Ternouth et al., 1985). In general, when feeding large amounts of MR, ADG is improved, but solid feed intake decreases (Hodgson 1971b; Huber et al., 1984). However, if a high incidence of scours occurs, it results in poor rate of growth and feed digestibility (Pettyjohn et al., 1963). Furthermore, Jenny et al. (1978) reported an increase of water consumption when the amount of MR intake increased, which probably attempts to compensate the loss of liquid by the loose feces obtained with high MR concentrated diets.

In recent years, calves feeding program studies are in vogue again. When calves are fed whole milk *ad libitum*, they consume increasing amount of milk reaching up to 9-11 kg (1.3 kg of DM) of whole milk (Jasper and Weary, 2002; von Keyserlingk et al., 2006). Although increasing the liquid feeding rate of dairy calves increased fat deposition (Diaz, et al., 2001; Bartlett et al., 2006) and it may impair the mammary gland development (Silva et al., 2002), increasing protein intake at the same time as increasing energy intake during the preweaning period increased mammary parenchyma mass without increasing intraparenchymal fat content (Brown et al., 2005a). Thus, MR with a high content of CP (24-28%) are recommended when feeding high amounts of MR to dairy calves.

1.2.1.1. Advantages and Disadvantages

Advantages of an enhanced-growth feeding program are greater ADG during the preweaning period (0.56 vs 0.85 kg/d; 0.54 vs 0.81 kg/d for conventional and enhanced-growth feeding programs, Bar-Peled et al., 1997; Shamay et al., 2005, respectively), and a reduction of time needed to reach a target BW. For instance, calves following a conventional feeding program needed 28 more days to reach 85 kg of BW compared to calves following an enhanced-growth feeding program (Diaz et al., 2001). Furthermore, the BW and structural (wither height, heart girth and hip width) growth advantage reached during the preweaning period was maintained until 270 and 180 days of age, respectively, for calves fed *ad libitum* compared with calves fed 0.45 kg/d of MR (Shamay et al., 2005). Feeding calves high amounts of milk or MR increased efficiency in body growth gain (0.61 vs 0.68 or 0.54 vs 0.73 in calves fed conventionally or *ad libitum*, respectively, Jasper and Weary, 2002 and Shamay et al., 2005, respectively). However, feed cost per kilogram of BW gain was higher for calves fed additional MR compared with calves fed conventionally (1.92 vs 1.77 $/kg, respectively, Quigley et al., 2006), but no differences in feed cost per kg of BW gain were found when calves were fed a MR at 1.1% BW and starter restrictively to grow 0.4 kg/d, and calves were fed a MR at 2% BW, and starter *ad libitum* (2.65 vs 2.87 $/kg, respectively, Brown et al., 2005b).

On the other hand, a relatively premature age at first calving (AFC), between 23 and 25 mo of age, may reduce the feeding cost of replacement heifers (Tozer and Heinrichs, 2001), but it may also reduce BW at calving (Abeni et al., 2000), and milk yield at first lactation (Abeni et al., 2000; Ettema and Santos, 2004). Body weight at first lactation is more correlated to milk yield during first lactation than AFC (Hoffman and Funk, 1992). Thus, to reduce heifer replacement costs, AFC should be targeted about 22 mo of age ensuring a freshening BW at calving that maximizes milk yield at first lactation (544-567

kg, Keown and Everett, 1986). Although most of the studies based on reducing AFC without impairing BW at calving focus on the effect of nutrition on growth before and after puberty (Pirlo et al., 1997; Lammers and Heinrichs, 2000; Zanton and Heinrichs, 2005), AFC and milk yield at first lactation may be also affected by nutritional, health, and environmental factors during the first 4 mo of life (Losinger and Heinrichs, 1996; Heinrichs et al., 2005). Thus, diminishing the time spent rearing replacement calves, without impairing calves BW, may reduce the AFC, and consequently it may also reduce the cost of rearing dairy replacement heifers. Calves that were allowed to suckle their dam calved 31 d earlier and weighed 37 more kg of BW at calving than calves fed 3 l of MR by bottle-fed once daily (Bar-Peled et al., 1997). Moreover, calves fed MR at 2.1% of BW during the preweaning period calved 18 d earlier and with a similar BW to calves fed MR at 1.2% BW during the preweaning period (Davis Rincker et al., 2006). Nevertheless, no differences in the AFC were found in calves fed milk *ad libitum* or 0.45 kg/d of MR (Shamay et al., 2005). Overall, milk yield at first calving was improved by feeding high amounts of milk or MR during the nursing period (milk yield at 300 DIM, 9171 vs 9624 kg, $P = 0.08$, Bar-Peled et al., 1997; daily 3.5% fat-corrected milk 28.6 vs 29.8 kg/d, $P < 0.05$, Shamay et al., 2005), or no differences in milk yield at 60 DIM were found between two preweaning feeding programs (Davis Rincker et al., 2006). In general, reducing AFC when following an enhanced-growth feeding program did not seem to impair future milk production.

The main disadvantage when feeding high amounts of milk or MR was the reduction of starter or hay intake during the preweaning period (Jasper and Weary, 2002; Shamay et al., 2005; Quigley et al., 2006). Therefore, a low dry feed consumption early in life may negatively influence the metabolic activity of the rumen, and may delay rumen microbial development (Anderson et al., 1987b). Mature ruminal function of young calves has been reported to occur 2-3 weeks after dry feed was first offered (Lallès and Poncet, 1990), and the low starter intake during the preweaning period in calves following an enhanced-growth feeding program may delay rumen maturity. Consequently, a common pitfall reported in studies using enhanced-growth feeding programs is that ADG was reduced more than half the week before weaning (when MR was reduced to stimulate starter intake) (Jasper and Weary, 2002; Brown et al., 2005) or the week after weaning (Bar-Peled et al., 1997) compared with the ADG achieved during the preweaning period. However, similar starter intakes between calves fed conventionally or following an enhanced-growth feeding program were reported after weaning (Jasper and Weary, 2002;

Quigley et al., 2006). Although fecal consistency increased as MR offer was increased, it did not affect the incidence of diarrhea (Diaz et al., 2001; Jasper and Weary, 2002).

1.2.1.2. Effect on Immune System

The neonatal calf immune system is immature and more susceptible to infectious diseases the first days postpartum (Hauser et al., 1986). Calf immune system differs from the adult one in the peripheral blood mononuclear cells (PBMC) population, and neutrophils (Hauser et al., 1986). Furthermore, the classical and alternate pathways of complement activation (Mueller et al., 1983) activities are reduced in calf immune system. For instance, young calves present greater percentage of circulating γ∂T-cells, CD4 T-cells and CD8 T-cells with lower capacity to produce IFN-γ and lower percentages of B cells compared with the adult ones (Rajaraman et al., 1997; Tizard 2000). Moreover, neonatal immune system is characterized by a T-cell population with a high proportion of naïve T-cells that can suppress Ig production (Clement et al., 1990). Leukocytes from the young calves are hyporesponsive compared with those of the adult cow (Rajaraman et al., 1997) and phagocytic leukocytes produce less nitric oxide than those from the adult cows (Rajaraman et al., 1998). Furthermore, concentration of C3 component of complement in the serum of calves during the first month of life is 43% of that found in adult animals (Mueller et al., 1983).

Calves fed high amounts of MR presented greater plasma insulin-like growth factor I (IGF-I) concentrations compared with calves fed conventional amounts of MR (Smith et al., 2002). Because IGF-I plays an important role as a cell proliferation cofactor and differentiation factor on B cell development and it enhances the proliferative response of lymphocytes to mitogens (Clark, 1997), it was hypothesized that enhanced-growth feeding programs may benefit the immune system of the neonatal calf (Nonnecke et al., 2003).

High planes of nutrition to improve immune responses were initially studied by Pollock et al. (1994). Calves receiving a high feeding level resulted in a decrease of serum antibody responses to an immune stimulator (keyhole limpet haemocyanin). Recently, this hypothesis has been widely studied (Nonnecke et al., 2003; Foote et al., 2004; Foote et al., 2005a; Foote et al., 2005b; Nonnecke et al., 2005; Foote et al., 2007). A minimal effect was found when calves PBMC were stimulated with a mitogen (pokeweed mitogen, concanavalin A). PBMC from enhanced-fed calves produced less IFN-γ and more nitric oxide than PBMC from conventionally-fed calves (Foote et al., 2005b; Foote

et al., 2007) at the end of the preweaning period, which might influence cell-mediated immune responses pivotal in the destruction of intracellular pathogens.

On the other hand, the expression of the IL-2 receptor (CD25) occurs when T-cells, B-cells, and monocytes are activated (Waters et al., 2003), the leukocyte adhesion molecule (CD44) is considered essential for extravasation of T-cells at sites of inflammation (Dailey, 1998), and the lymph node homing receptor (CD62L) is required for entry of cells into the lymph nodes. The study of these receptors in calves following a conventional or an enhanced-growth feeding program may serve as an indicator of the functional capacity of the T-cell population of calves. A reduction in the expression of activation antigens CD25, CD44 and CD62L following an *in vitro* stimulation of CD4 T-cells, CD8 T-cells and γ∂-TCR cells was reported in calves fed intensively compared with calves fed conventionally (Foote et al., 2005a). The influence of an intensive diet on neonatal nutrition in the immune system might be induced by the effect of leptin, a hormone synthesized by adipocytes, and which plasma levels are correlated with total fat mass. The inhibitory effect of leptin on *in vitro* immune responses was suggested because leptin inhibited memory T-cells proliferation, and naïve T cells were markedly enhanced (Lord et al., 2002). Furthermore, leptin also increased the release of nitric oxide (Raso et al., 2002), as it was observed in enhanced-calves PBMC (Nonnecke et al., 2003). A recent study, indicated that high planes of nutrition in young calves decreased the viability of T cell populations that play pivotal roles in the development and regulation of antigen-specific immune responses, with unknown consequences on calf susceptibility to infectious disease (Foote et al., 2007).

1.2.2. Individual vs. Group Housing

The Directive (97/2/EC) regulates that no calf shall be confined in an individual pen after the age of eight weeks from 31 December 2006 on. This Directive dictates that the pen area for calves reared in groups should be of 1.5 m^2, and for calves in individual pens the width of the pen shall be at least equal to the height of the calves, and the length shall be at least the body length of the calves multiplied by 1.1. In this section we will discuss if there is a clear advantage on rearing calves in groups.

1.2.2.1. Performance

Literature comparing calves reared individually or in groups does not show dramatic performance differences between both rearing systems: Warnick et al. (1977) and Kung et al. (1997) reported an earlier starter consumption of calves reared in groups, but starter

intake was greater in individually-reared calves at 6 and 7 weeks of age (Kung et al., 1997). In contrast, Richard et al. (1988) found greater MR intake in calves raised in groups, but no differences in starter (Richard et al., 1988; Chua et al., 2002), and in MR intake (Chua et al., 2002) were observed in other studies. However, fresh grass intake was greater in calves housed in groups than in individually-housed calves, and it seemed that the sight of grass being taken into the mouth was the relevant stimulus to increase grass intake in calves reared in groups (Philips, 2004). Even though, starter intake did not seem to be stimulated when calves were raised in groups (Philips, 2004). On the other hand, lower final BW was found in calves reared in groups than in individually-housed calves (Maatje et al., 1993; Stull and McDonough, 1994), but no differences in ADG were observed in other studies (Warnick et al., 1977; Kung et al., 1997). Moreover, greater growth rate in pair-housed calves (Chua et al., 2002), but numerically lower ADG in calves housed in triplets were found at the beginning of weaning compared with calves reared individually. However, different results on calf health problems were reported when comparing calves raised in groups with calves reared individually such as more clinical respiratory problems and diseases (Warnick et al., 1977; Maatje et al., 1993), no differences in incidence of scours or diseases (Chua et al., 2002), and lower number of medicated days (Kung et al., 1997) in grouped compared with individually raised calves. Also, grouped-penned calves reared outdoors presented a greater mortality rate than calves grouped-penned indoors or individually (Peters, 1986). Overall, the US Department of Agriculture examined management practices indicators of dairy heifers health and productivity, and reported greater mortality in preweaned calves raised in groups of seven or more compared with preweaned calves in groups of two to six or reared individually (Willard et al., 1997). Similarly, Svensson and Lidberg (2006) concluded that calves fed from automatic milk-feeders were best kept in groups of less than 10 calves.

As a result of the move towards group housing in the EU, computer-controlled milk feeding seems a good option for large dairy facilities to raise calves in groups. Feeding calves with a computer-controlled milk system did not affect feed efficiency when compared with an individual bucket-fed system (Veissier et al., 2002). However, the risk of respiratory diseases and the presence of a more severe diarrhea increased in calves fed with the computer-controller system than those calves reared individually (Svensson et al., 2003). However, calves fed with automatic feeding *ad libitum* compared with calves fed in groups twice daily by buckets presented greater MR intake, grew faster, increased the number of meals (which may improve digestion) but decreased starter intake

(Appleby et al., 2001). Although automatic-feeding system is economically viable when raising more than 20 calves together, increasing the number of calves from 12 to 24 increased the number of disturbances without influencing negatively growth performance (Jensen, 2004). Closing the rear after calf entrance to the automatic feeder may help to palliate disturbances among calves. Moreover, closing the rear after calf entrance also increases non-nutritive sucking behavior (i.e. sucking the teat of the feeder without obtaining milk) after milk ingestion, and reduces cross-sucking (i.e. sucking a body part of a group member) during the first 15 min after ingestion (Weber and Wechsler, 2001). Alternatively, increasing the number of teats per feeder (von Keyserlingk et al., 2004), and offering milk in fewer and larger meals to calves (Jensen, 2004) also reduced competitive behavior, increased feeding time, and increased milk consumption.

1.2.2.2. Welfare

It seems that the opportunity for locomotion in grouped calves improves calf welfare. Calves housed in groups adopt more comfortable resting postures and display increased social behavior than calves reared individually (Andrighetto et al., 1999). Also, as the pen size increased the occurrence of locomotion play at 5 weeks of age increased (Jensen and Kyhn, 2000). Moreover, rearing calves in groups reduced the bucket licking and searching behaviors, and increased satisfaction behaviors (i.e. grooming and tail swishing) (Phillips, 2004). Furthermore, group-penned calves removed behavioral restrictions such as interaction with congeners, and explore the surroundings (de Wilt et al., 1986). Although early social contact facilitated the development of normal social responses after mixing with other calves (Veissier et al., 1994), calves housed in groups increased reactions at human handling because contacts with caretakers were less efficient than those for calves reared individually (Veissier et al., 1998), and this may explain an increase of serum cortisol concentrations during weeks 8 and 16 in group-penned calves compared with calves housed in stalls (Stull and McDonough, 1994). Some management factors such as sufficient number of feeding and resting places or early social experience may help to reduce the occurrence of aggressions when grouping calves. However, other factors such as introducing a group or a single animal in a resident group, grouping calves heterogeneous in weight, or space allowance present contradictory results or have not been yet studied (Bøe and Færevik, 2003).

Overall, feeding calves in groups with an automatic-feeder may facilitate the management of raising calves on an enhanced-growth feeding program, because milk consumption would be partitioned in several meals, and the calf adaptation to high levels of milk or

MR would be easier. Although, EU defends rearing calves in groups, performance and health results in favor of housing calves in groups are not consistent. Probably, management practices in each study may influence results. Generally, group-housing systems may reduce problems of behavioral and social deprivation but may increase aggression and disease transmission. Even though, there is as yet no simple way of elucidating which particular threats to welfare are the most serious (Rushen and de Passillé, 1992), and have the greatest repercussions on animal performance.

1.3. Rumen development

Digestive tract of newborn calf suffers important changes from birth to several weeks after weaning. For instance, reticulo-rumen represents 35% of stomach compartments size at birth, but 65% at 8 weeks of age (Lyford, 1988). In contrast, the abomasum represents 51% of total stomach compartments at birth, but 21% at 8 weeks of age, and 14% in adult cattle. Throughout this section, the influence of different feeding regimes and the effect of calf age on rumen development will be discussed.

1.3.1. Rumen metabolites

Main changes in rumen development occur at weaning when diet changes from liquid to solid. After weaning, dry matter intake increases, and rumen pH and volume also increase as a result of the production of volatile fatty acids (VFA) within the rumen. But rumen NH_3 concentration decreases with calf age, indicating N utilization by rumen microorganism (Anderson et al., 1987a; Vazquez-Añon et al., 1993; Beharka et al., 1998). The acetate to propionate ratio in the rumen decreases in a cubic fashion with age, as a result of the quadratic decrease of acetate molar proportions, and the linear increase of propionate molar proportions with age (Anderson et al., 1987a). Generally, diets high in roughage increase the proportion of acetate in the rumen at the expense of propionate, butyrate, and valerate molar proportions (Stobo et al., 1966; Leibholz, 1975). Furthermore, butyric acid is the short chain fatty acid that stimulates the most the rumen mucosa papillae growth, in part, because it has been shown to be a specific inhibitor of ruminal apoptosis *in vivo* (Mentschel et al., 2001).

1.3.2. Rumen microbiota

Rumen microbial development also occurred early in life. Total anaerobic bacterial counts increase during the first 3 weeks of life and remain fairly constant until 12 weeks of age (Anderson et al., 1987b; Beharka et al., 1998), but the types of microbial population change with calf age. 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). Similarly, ruminal microbial cellulolytic activity did not appear in *Bos indicus* x *Bos taurus* crossbred calves after 4 weeks of age, but afterwards it increased with age (Sahoo et al., 2005). In addition, the physical form of the diet can influence ruminal microbiota. For instance, feeding ground diets stimulated the presence of amylolytic and reduced the

amount of cellulolytic bacteria compared with feeding unground diets (Beharka et al., 1998).

1.3.3. Rumen epithelial metabolism

Rumen epithelial metabolic activity also increases with age. Although the first energy substrate for ruminants is glucose absorbed in the small intestine, during rumen epithelial growth the importance of glucose and lactate oxidation decrease, while that of butyrate increases (Giesecke et al., 1979). The rumen epithelium ketogenic capacity to produce D-3-hydroxybutyrate and acetoacetate from butyrate increases with age independently of solid feed intake and intraruminal VFA concentration in lambs (Lane et al., 2000). The rate of ruminal ketogenesis is probably controlled by the expression of the gene encoding the enzyme 3-hydroxy-3-methylglutaryl-CoA synthase, that controls the synthesis of 3-hydroxy-3-methyl-glutaryl-CoA from acetoacetyl-CoA and acetyl-CoA, and the expression of this gene increases with calf age, independently of their dietary treatment (Lane et al., 2002). Parallel to changes on diet, the liver must adapt to the patterns of nutrients absorbed. Thus, preruminant liver shifts from being glycolytic to becoming gluconeogenic in ruminant animals, increasing glucose 6-phosphatase activity as rumen develops (Baldwin et al., 2004). On the other hand, rumen mucosa ability to absorb VFA increases with solid feed intake, but not with age (Sutton et al., 1963). The rate of VFA absorption is also influenced by rumen pH (Danielli et al., 1945). As acids are absorbed in the free form (non-dissociated) and blood pH is ordinarily alkaline, lower rumen pH increases the proportion of free acid and, thus it increases the rate of VFA absorption towards the blood stream (van Soest, 1994).

1.3.4. Rumen physical structure

Rumen physical development consists on increasing rumen mass (rumen weight and musculature development) and growing rumen papillae. Recently, it has been suggested (Lesmeister et al., 2004) to use papillae length and width, and rumen wall thickness as indicators of rumen development for detecting differences across treatments. However, the ability of finding differences in papillae per square centimeter seems to be limited. Papillae denseness measurements varied across different rumen sections in contrast to papillae length and width that were similar among rumen sections (Hill et al., 2005). Rumen mucosa development especially depends on the stimulation of VFA, whose production requires the requisite of bacteria and substrate (van Soest, 1994). Type and physical form of the diet intake influence rumen physical development.

Feeding only milk to veal calves or lambs did not stimulate papillae development, but the consumption of solid feed, and the resulting increase in rumen VFA concentrations stimulated rumen morphological development (Assane and Dardillat, 1994; Lane et al., 2000). Similarly, reducing age of calf at weaning, early stimulate solid food consumption and increased papillae length, width and surface (Zitnan et al., 1999). Contrarily, veal calves supplemented with 250 g/d of dried beet pulp compared with only milk fed calves (Cozzi et al., 2002), and calves weaned at 17 days of age compared with calves weaned at 28-day (Klein et al., 1987) presented greater empty reticulo-rumen weight, but no changes in papillae length and rumen wall thickness were found. Hay diets stimulated rumen volume displacement and weight of rumen content, in contrast to concentrate diets that increased the weight of reticulo-rumen tissue (Stobo et al., 1966). Much of this increase in weight of reticulo-rumen tissue in calves fed a high-concentrate diet can be attributed to a greater papillae length rather than a growth of the muscular rumen wall (Stobo et al., 1966). Similarly, calves fed a concentrate diet compared with a concentrate plus alfalfa diet presented rumen papillae with protrusions, that increase surface for absorption, but the presence of protrusions was not related to rumen hyperkeratosis. In contrast, longer papillae without protrusion were observed with a concentrate-alfalfa hay diet compared with a concentrate diet (Zitnan et al., 1998). In general, high-concentrate diets stimulate rumen mucosa development by increasing papillae length and surface, in contrast to diets containing some forage that increased the proportion of muscle of rumen wall, and presented less vacuolation of stratum granulosum and thickening of the stratum corneum suggesting a favored metabolic uptake (Nocek et al., 1984b). Hence, butyrate and propionate are the main fermentation products from a concentrate diet, which are more responsible for stimulating papillae growth, but the "scratch factor" of roughages, whole plastic sponges or wood shavings have no impact per se on rumen mucosa development (Warner, 1991).

On the other hand, there were no differences in VFA concentration in calves fed ground or unground diets, and both physical form of diet presented similar rumen wall thickness (Beharka et al., 1998). However, calves fed an unground diet had longer rumen papillae in the dorsal sac in contrast to calves fed a ground diet that showed evidence of branched papillae, which were not accompanied by an increase of rumen mucosa parakeratosis (Beharka et al., 1998). Corn processing slightly influenced rumen physical development. Lesmeister and Heinrichs (2004a) suggested that roast-rolled corn is the type of processing that best converts ingested nutrient in growth and prepare rumen for weaning.

Overall, as mentioned above, one of the major disadvantages of raising calves on enhanced-growth feeding programs is a low starter intake. Thus, it probably decreases the amount of rumen metabolites, which may delay VFA absorption by rumen mucosa. Consequently, the liver shift from glycolytic to gluconeogenic metabolism might be also delayed. Finally, rumen mucosa development can also be affected when feeding high amounts of milk or MR, especially its papillae growth. Feeding calves on enhanced-growth feeding program and an unground diet and roast-rolled corn might help to stimulate rumen mucosa development.

1.4. Weaning

Weaning is the transition time from liquid to solid feeding. At this time, the rumen will take more importance than during the preweaning period at the expense of the abomasum. At weaning, starter intake, as an essential promoter of rumen development, will be a key factor to wean calves successfully.

1.4.1. Changes at weaning

Although young calves seem to possess mature ruminal function within 2-3 weeks after dry feeds are first offered (Lallès and Poncet, 1990), DM digestibility decreased immediately after weaning compared with calves of the same age that were still nursing (Funaba et al., 1994), probably because milk is more digestible than starter. However, starter DM digestibility linearly increased the weeks following weaning (Funaba et al., 1997). Similarly, the utilization of N absorbed decreased immediately after weaning, but increased 6 weeks after weaning. This might reflect compensatory growth, to compensate the nutrient restrictions and low DM digestibility immediately after weaning (Funaba et al, 1994). Furthermore, the nutrient restriction of early-weaned calves has also resulted on an increase in plasma NEFA concentration that may reflect body fat mobilization (Luchini et al., 1993). Thus, weaning is a stressful event that should be managed cautiously to avoid negative effects on performance.

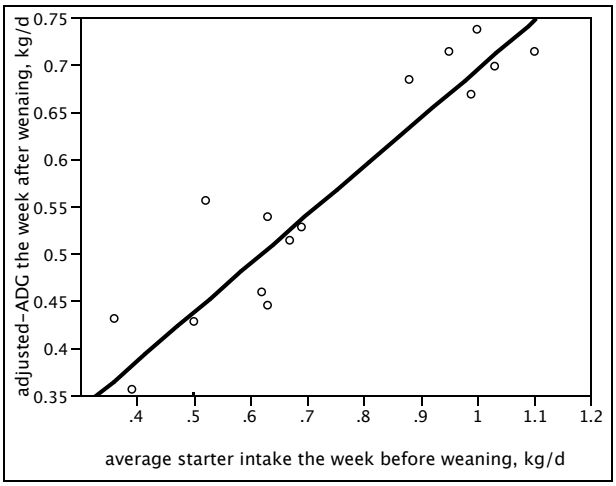
1.4.2. Weaning methods

In a questionnaire performed in Sweden, the median age at weaning was 8 weeks in dairy herds (Pettersson et al., 2001). Similarly, the average age at weaning was 7.9 weeks in the National Dairy Heifer Evaluation Project in US (Heinrichs et al., 1995). The Swedish study also reported that 46% of the herds used calf age as weaning criterion, and only 18% of the herd the concentrate consumption (averaging 1 kg of starter intake at weaning). The major management practices to wean were diluting the milk with successively increasing volumes of water for 7 days (32%), weaning calves abruptly (21%), or offer one MR meal per day for 5 days (21%) (Pettersson et al., 2001). Neither performance nor starter intake after weaning was affected by weaning Jersey calves abruptly at 35 d of age, or reducing MR offered one week before weaning (Quigley, 1996a). Greenwood et al. (1997) considered dry feed intake as a percentage of initial BW as a weaning criterion. When they considered dry feed intake of 1% of initial BW, calves reduced weaning time without impairing performance up to 20 weeks of age compared with calves weaned with a starter intake of 1.5% or 2% of initial BW (Greenwood et al., 1997). Moreover, comparing early (4 weeks of age), and conventional weaning (6 weeks

of age) resulted in similar growth rates up to 12 weeks of age, but early-weaned calves had higher feed intake than conventionally weaned calves (Anderson et al., 1987a). Similarly, early weaning at 17 days of age and feeding a prestarter diet until 4 weeks old resulted in greater dry feed intake and a more rapid rise in total VFA compared with calves weaned at 28 days of age without prestarter (Klein et al., 1987). 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). This observation has been evaluated in Figure 1.1. Data from 7 studies were adjusted for the random effect of each study and a mixed-effects linear regression was performed between the study-adjusted average daily gain the week after weaning and starter intake the week before weaning.

Overall, weaning calves at 4 weeks of age has been feasible (Anderson et al., 1987a; Luchini et al., 1993; Hopkins, 1997), but DM starter intake after weaning did not increase sufficiently to compensate the lack of MR intake (Quigley, 1996a), and consequently growth was reduced immediately after weaning. According to the linear regression presented in Figure 1.1, to achieve an ADG of 0.5 kg/d after weaning, calves should be consuming a minimum of 0.62 kg/d of solid feed the week before weaning. Similarly, Davis and Drackley (1998) proposed that calves should be consuming 0.79-0.91 kg/d of starter after weaning to meet calf needs for maintenance plus a modest weight gain.

Figure 1.1. Linear relationship between daily starter intake the week before weaning and study-adjusted ADG the week after weaning of calves from different studies: adjusted-ADG postweaning = 0.183 + 0.514 daily starter intake before weaning [5]; R^2=0.86; P < 0.001; Anderson et al., 1987a; Jaster et al., 1992; Quigley et al., 1994; Quigley et al., 1996a; Chua et al., 2002; Jasper and Weary, 2002; Quigley et al., 2006.



1.5. Starter

Consumption of calf starter is important in early life, because it will be the main source of nutrients after weaning. Thus, it is important to stimulate calf starter intake the weeks before weaning to supply calf requirements after weaning. As calf digestive tract is not fully mature at birth, high quality ingredients should be chosen to formulate calf starters. This section will review calf starter composition and the factors that may stimulate intake, especially the weeks around weaning.

1.5.1. Ingredients

The most commonly used grain in calf starters is corn because of more ideal rate of breakdown in the rumen and its high digestibility (Davis and Drackley, 1998). In a recent study (Lesmeister and Heinrichs, 2004a), roast-rolled corn was reported as the processed-corn that showed the best ability to converted ingested nutrient into growth and prepare the calf's rumen for weaning. Oats are also used in calf starters because they add bulk, are very palatable, and a good source of fiber (Davis and Drackley, 1998).

Meal or ground alfalfa is a main fiber source in calf starter composition. However, alternative sources are wheat middlings (Coverdale et al., 2004; Lesmeister et al., 2004b), wheat bran (Bartley, 1973), soybean hulls (Coverdale et al., 2004), cottonseed hulls (Murdock and Wallenius, 1980), or beet pulp (Murdock and Wallenius, 1980; Vazquez-Añon et al., 1993; Mattiello et al., 2002).

The most common protein source used in calf starter is soybean meal. However, the replacement of soybean meal by lupines, maintaining the same CP level, resulted in similar performance of calves (Wright et al., 1989). Soybean and sorghum grains roasted have also been shown to promote similar performance when compared with soybean meal or raw sorghum, respectively (Abdelgadir and Morrill, 1995; Abdelgadir et al., 1996a). However, the synchrony of carbohydrate and protein ruminal availability (soybean roasted and raw corn or soybean meal and corn roasted) seemed to improve BW gain and calf starter efficiency (Abdelgadir et al., 1996b). Similarly, feeding less rumen degradable carbohydrates and protein (corn and extruded soybean meal) tended to improve DMI and ADG compared with barley or dried whey, as carbohydrate sources, and soy bean meal, as protein source (Maiga et al., 1994).

To improve energy intake, calf starter may be supplemented with fat sources. For instance, soybeans roasted and ground (Stewart and Schingoethe, 1984; Kuehn et al., 1994), hydrolyzed animal fat (Luchini et al., 1993), calcium soaps of fatty acids (Fallon et

al., 1986) have been used in high-energy calf diets. However, the inclusion of 7.3% fat in the starter decreased starter intake after weaning, and did not improve ADG (Kuehn et al., 1994). Similarly, calves fed a 6.3 (Stewart and Schingoethe, 1984) or a 5.8% (Luchini et al., 1993) fat starter did not benefit from the supplemental fat compared with calves fed a 2.2 or a 1.7% fat starter, respectively. Overall, starters above 5% fat generally result in a depression of DM intake, which negates the potential of increased energy density to improve energy intake (Davis and Drackley, 1998).

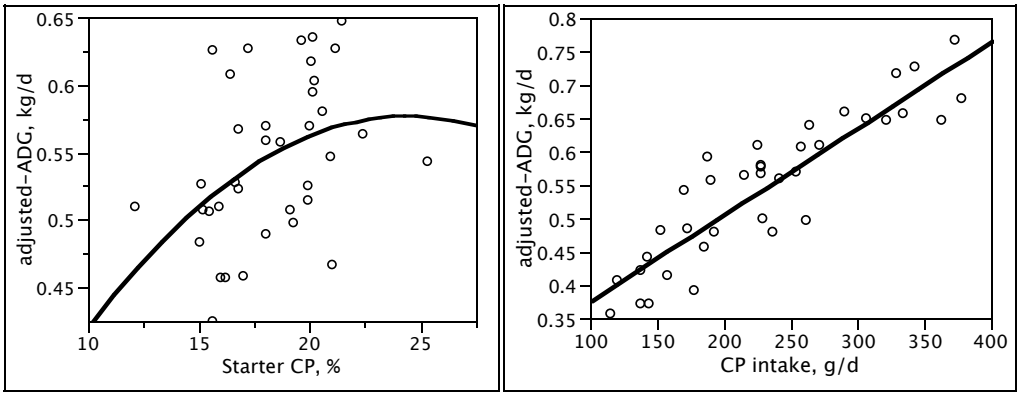
Calf starters commonly contain approximately 5 to 12% liquid molasses to increase palatability, minimize particle separation, and decrease dust (Morales et al., 1989). However, 12% molasses starter decrease DM intake and may have mixing, feeding, prehension and possible palatability problems (Lesmeister and Heinrichs, 2005).

Some additives have been studied in calf starter to improve performance, health or rumen development. Supplementation with 2% yeast in calf starter improved DMI and growth, and slightly enhanced rumen development (Lesmeister et al., 2004b). However, no performance and health benefit was observed in calves fed a mixed concentrate of microbial cultures (*Lactobacillus acidophilus*, *L. lactis*, *Bacillus subtilis*), or *B. subtilis* concentrate (Jenny et al., 1991). A fermentation extract of *Aspergillus oryzae* has also been studied in dairy calves, showing no significant effects on performance, but increasing the number of ruminal bacteria (Beharka et al., 1991).

1.5.2. Crude protein composition

Several authors (Whitelaw et al., 1961; Stobo et al., 1967; Morril and Dayton, 1978; Luchini et al., 1991; Akayezu et al., 1994) have studied the CP level in calf starter to maximize performance and CP efficiency. In summary, these studies agree that CP level in calf starter should be between 15 and 18 % CP. However, 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 most appropriate, because the amount of CP ingested, as well as DM starter intake, is the decisive value that determines ADG instead of CP %. Figure 1.2 shows data from thirteen different studies that were adjusted for the random effect of each study and a mixed-effects linear regression was performed between the study-adjusted average daily gain postweaning and CP % of the starter and CP intake at the same period. This Figure indicates that the CP content in the starter only explains 17% of the variation observed in ADG postweaning, whereas CP intake explains 87% of the variability of ADG postweaning.

Figure 1.2. Relationship between CP % in the starter (ADG-postweaning = 0.126 + 0.037 starter CP% - 0.00076 (starter CP%)^2) [6], R^2 = 0.17; P = 0.04) and CP DMI (ADG-postweaning = 0.037 + 0.00202 CP DMI, g/d [7], R^2 = 0.87; $P < 0.001$) with ADG of postweaned calves. n=37. Data adapted from: Stobo et al., 1967; Anderson et al., 1987a; Wright et al., 1989; Luchini et al., 1991; Jaster et al., 1992; Luchini et al., 1993; Jenny et al., 1991; Vazquez-Añon et al., 1993; Akayezu et al., 1994; Kuehn et al., 1994; Abdelgadir et al., 1996a; Lesmeister and Heinrichs, 2004a; Lesmeister and Heinrichs, 2005.



1.5.3. Factors modulating starter intake

The National Research Council for dairy cattle (NRC, 2001) uses the NRC equation for beef cattle to predict DMI in growing heifers. This equation was validated with growing heifers from 58 to 588 kg and it considered metabolic BW and NE required for maintenance to predict DMI. However, as mentioned above, calf intake is highly variable in early ages and it is difficult to predict (Kertz and Chester-Jones, 2004).

The stimulation of starter intake in early calf life is necessary to have a gradual transition from the liquid to solid diet. There exist a number of factors that affect starter DM intake. For instance, Quigley (1996b) proposed a DMI predicting equation that included calf age, BW, BW gain, milk DM intake, and calf gender in the model. However, other factors such as hay availability, incidence and severity of scours, amount and method of milk feeding, and environmental temperature may be also included in the DMI predicting equation (Quigley, 1996b).

Overall, the detection of other parameters that affect the high variability of calf starter DMI is necessary to improve accuracy in the prediction of calf starter DMI.

1.5.3.1. Milk replacer intake

Starter intake during the preweaning period is highly dependent on milk feeding rate (Hodgson, 1971b; Jenny et al., 1982; Jaster et al., 1992). This effect is well observed in calves fed *ad libitum* or calves fed conventionally, at 10% of BW (Jasper and Weary, 2002). Although solid food was negligible for both treatment groups until calves were 2 wk old, by 35 d of age starter consumption was of 6.11 kg for conventional-fed calves, and 2.99 kg for *ad libitum*-fed calves. Once gradual weaning began on d 37, starter intake increased rapidly for both groups. Similarly, a reduction in the amount of MR fed had a linear effect on forage organic matter (OM) intake, the lower the MR consumption the greater forage OM intake (Broesder et al., 1990).

1.5.3.2. Physical form

The physical form of starter also influences intake. In general, texturized or mash starters increase starter intake and calf performance when compared with a pelleted starter (Warner et al., 1991; Franklin et al., 2003). However, similar performance and starter intake were reported in the literature when calves were fed texturized, coarse or ground starters, and ground or pelleted starters (Franklin et al., 2003; Coverdale et al., 2004). On the other hand, the extrusion of a calf starter was not advantageous compare with a pelleted starter (Morrill et al., 1981). Moreover, lower DM intake have been observed in unground compared with ground diets that may reflect the increase of time ruminating and the decrease of rumen liquid flow rate in the unground diets (Hodgson, 1971a; Beharka et al., 1998, respectively).

1.5.3.3. Forage provision

As mentioned above, feeding forage to calves has an effect on rumen development. In general, feeding an all-concentrate diet increases the thickness of rumen mucosal layer, but some authors (Nocek and Kesler, 1980; Nocek et al., 1984b) have found that feeding a concentrate/hay diet instead of a concentrate diet increased the thickness of rumen muscular layer. Some researchers concluded positive effects on the inclusion of forage in the young calf diet. For instance, calves improved BW and gain to feed ratio during the postweaning period when grass hay was offered (Coverdale et al., 2004). Similarly, the inclusion of roughage incorporated in the calf pellet improved ADG and starter intake compared with no roughage pellet diet (Thomas and Hinks, 1982). Moreover, grass can be included in calf diets with no effect in ADG neither in starter intake during the preweaning period (Philips, 2004). However, other authors have observed a negative effect on the inclusion of forage in the diet. The BW, DM digestibility, and N retention

decreased in postweaned calves as ground roughage content increased in the diet (Leibholz, 1975). Similarly, reducing the amount of concentrate offered, increased forage intake, but decreased average daily gain, and increased the percentage of body weight due to gastrointestinal content (Stobo et al., 1966). On the other hand, calves were rapidly adapted (increase apparent nutrient digestibility and N balance) when a change from a forage to a concentrate diet was performed (Stobo et al., 1966). In a recent study, it was observed that the provision of barley straw, chopped at 10 cm, mixed with 15% cane molasses, 15% pot ale syrup, and 0.5% propionic acid stimulated growth and starter intake compared with the provision of grass hay (Philips, 2004), suggesting that the provision of palatable forage may be beneficial for young calves.

1.5.3.4. Management

Water supply is also an important factor that influences starter intake (Kertz et al., 1984). Not offering water to calves during the first weeks of life, decreased starter intake and BW gain (Kertz et al., 1984).

Finally, several management methods to improve early feed consumption have been studied, showing different successful results. For example, putting small amounts of dry feed into the milk feeding pails after finishing milk intake stimulated starter intake and weight gain especially after weaning (Morrill et al., 1981), but feeding calf starter in a nipple bottle did not affect starter intake when compared with calf fed with an open bucket (Quigley et al., 1994; Hopkins, 1997).

Overall, when raising young dairy calves on an enhanced-growth feeding program it could be recommended decreasing MR intake before weaning, offering water *ad libitum*, and feeding texturized-coarse starter to stimulate starter intake. Furthermore, providing a highly palatable forage may be envisaged, because the European Union regulation requires that all calves over 2 wk of age have access to a minimum of fibrous food (100-200 g daily).

Chapter 2

OBJECTIVES

This thesis was conceived from the appearance of recent research articles that observed great growth rates in dairy calves when feeding high amounts of milk or milk replacer. The main objective of this work was to evaluate positive and negative effects when calves were raised following an enhanced-growth feeding program during the preweaning period. The specific objectives were:

1. Comparing growth performance and N metabolism of calves fed two feeding programs: conventional vs enhanced-growth.
2. Evaluating the potential benefit of rearing calves following an enhanced-growth feeding program in groups on starter intake around weaning and performance.
3. Evaluating the effects of conventional and enhanced-growth feeding programs on performance during the preweaning period and concentrate apparent nutrient digestibility at weaning.
4. Comparing the performance during the preweaning period, and the reproductive function at breeding of calves fed conventionally or following an enhanced-growth feeding program.

To achieve these objectives, four studies were conducted:

- Study 1: Performance parameters, plasma amino acid profiles, and total purine derivatives urinary excretion were compared between calves raised following a conventional and an enhanced-growth feeding program.
- Study 2: The management practice of rearing calves in groups was evaluated to determine whether grouping improves calf starter intake when calves were raised following an enhanced-growth feeding program.
- Study 3: This study assessed the hypothesis that the low starter intake during the preweaning period of enhanced-growth feeding program may entail a decrease of apparent nutrient digestibility at weaning, and it may explain the decrease in gain to feed ratio the weeks around weaning of enhanced-fed calves observed in the previous studies.
- Study 4: Possible long-term benefits when calves were raised following an enhanced-growth feeding program, such as improvements on reproductive function at breeding and advantage in body weight later in life, were evaluated in this study.

Chapter 3

PERFORMANCE AND NITROGEN METABOLISM OF CALVES FED CONVENTIONALLY OR FOLLOWING AN ENHANCED-GROWTH FEEDING PROGRAM DURING THE PREWEANING PERIOD

A fraction of this research has been published in:

Livestock Science, 2006. 105: 109-119.

3.1. Introduction

Calves following an enhanced-growth feeding program are fed with high CP level milk replacers (MR). However, plasma urea N and efficiency of protein utilization suggested that protein was not limiting growth, when MR contained 30% CP (Diaz et al., 2001). The study of plasma amino acid profile may help to adjust MR amino acid profile, and use N more efficiently. On the other hand, the low starter described during the preweaning period in enhanced-growth fed calves (Jasper and Weary, 2002), may delay rumen microbial development.

The objectives of this study were to compare growth performance, N metabolism, and rumen microbiota development of calves fed two feeding programs (conventional vs enhanced-growth).

3.2. Materials and methods

3.2.1. Animals and treatments

Initially, forty-two Holstein and eight cross-bred calves were purchased (15.3 ± 4.45 d of age, and 36.1 ± 3.34 kg of BW) predicting that some will undergo health problems consequence of their journey from France to Spain. During the time elapsed (about 2 days) between calves left their origin farms and they arrived to facilities of IRTA, calves were only offered oral rehydratant. The animals were raised in the facilities of IRTA (El Prat, Spain) under the approval and supervision of the Animal Care Committee of IRTA. Calves were housed in individual wooden pens (1 x 1.55 m) in a barn with forced ventilation. Upon arrival, all calves received 2 l of oral rehydratant (Karihidra liquido, Karizoo S.A., Spain). They were weighed, and randomly distributed according to BW in 2 groups of 25 calves each following two different feeding programs: conventional (CF) or enhanced-growth (EF). The crossbred calves were evenly distributed, four in the CF and the other four in the EF treatment. Milk replacer was offered in buckets twice daily at 07.00 and 18.00 hours. The first week of study was considered an adaptation period, during which calves in CF and EF treatment received 4 l/d of MR at 12.5 and 18% DM dilution rate, respectively. Time throughout the article will be referred to the first day after the adaptation period when calves averaged 21.6 days of age. After the adaptation period, CF calves were fed 4 l/d of MR at 12.5% DM throughout the preweaning period, and EF calves were offered MR at 18% DM at the rate of 6 l/d from 1 to 6 d, 8 l/d from 7 to 26 d, and 4 l/d from 27 d to weaning day (38 d). All groups received the same MR (Specilait, Karizoo S.A., Spain), and the same calf starter (Table 3.1). Water and starter

were offered *ad libitum* until the end of the study (87 d of study). The weaning time was set at day 38 after beginning of the study at which point calves were on average 59.6 days old. All calves were offered 200 g/d of alfalfa hay after 66 d from the beginning of the study. Alfalfa hay was offered relatively late in the study to avoid inaccuracies on DMI measurements, and also because hay usually limits rumen development (Stobo et al., 1966). Also, all animals were vaccinated against PI3, BRSV, BVD, and IBRV with Bayovac combo IV (Bayer, USA) during the adaptation week and revaccinated 3 weeks later.

3.2.2. Measurements and sample collection

After the adaptation period, consumption of MR was recorded daily. Starter intake was recorded once weekly for the first 5 wk, and daily afterwards. Water consumption was measured daily. Body weight and withers height (WH) were measured once weekly throughout the study. Any use of medical treatments was also recorded. Blood samples were taken only during the preweaning period because it was expected that most of the differences in blood metabolite concentrations due to the treatments imposed in the study would occur while MR was consumed, and also because the aim of the study was to determine the differences in metabolite concentrations while feeding two levels of MR. One hour after the morning MR was consumed, a 10-ml blood sample was collected by venipuncture of the jugular vein into a heparinized tube once weekly during the entire preweaning period. Because AA concentrations in blood depend on both absorption and utilization, blood samples were obtained 1 h after feeding to maximize the proportion of absorbed AA reflected in blood, and thus better depict the differences in AA supplied by the dietary treatments. Blood was kept cold on ice and centrifuged at 1,500 x *g* at 4ºC for 10 min to obtain plasma. Plasma samples were stored at -20ºC until subsequent urea, and glucose determinations. Also, plasma AA concentrations were determined in 10 randomly-selected animals from each treatment for the entire preweaning period. In addition, with the aim of evaluating the evolution of rumen microbiota as the starter consumption increased, urine spot samples from 6 randomly-selected calves from each treatment corresponding to 29, 40, 49, 63, and 75 d of experiment were obtained by manual stimulation and collected into a 50-ml tube containing 2 ml 3*N* HCl, and stored at -20ºC until subsequent creatinine and purine derivatives (PD) analysis.

3.2.3. Nutrient composition

Grab samples of MR and starter were analyzed for DM (24 h at 103ºC), ash (4 h at 550ºC), N content (AOAC, 1990), ether extract (AOAC, 1990) with a previous acid hydrolysis, and NDF and ADF (Van Soest et al., 1991). Also, MR and starter gross energies were determined with an adiabatic IKA-calorimeter C 4000 (Heitersheim, Germany). Amino acid composition of MR and starter were determined following the procedure of Llames and Fontaine (1994).

3.2.4. Blood samples

Plasma concentrations of urea, and glucose were measured by an Auto Analyzer (COBAS MIRA, Roche, Switzerland) using different colorimetric techniques. Enzymatic determination of glucose, urea were performed following the HK/G-6-PDH method (Burrin et al., 1985), and the urease-GLDH method (Gutmann and Bergmeyer, 1974), respectively. Plasma AA concentrations were analyzed using the kit EZ:Phaast, number KGO-7165 (Phenomenex, Torrance, CA), and determined using a gas chromatograph (Hewlett-Packard, 5890 Series II, Palo Alto, CA).

3.2.5. Urine samples

Urine spot samples were analyzed by HPLC (Agilent 1100 Series, Waldbron, Germany) to determine creatinine and PD (Balcells et al., 1992). Because hypoxanthine and xanthine are usually traces in cattle urine (Chen et al., 1990), only allantoin and uric acid were considered as PD. To determine daily allantoin and uric acid excretion, total urine volume was estimated by the following relationship: (0.883 x $BW^{0.75}$)/urine creatinine concentration (mmol/l), assuming a daily creatinine excretion of 0.883 mmol/kg of $BW^{0.75}$/d (Chen et al., 1992). Also, the endogenous excretion of PD was considered in the calculations of total daily PD excretion, and it was estimated according to Funaba et al., (1997) as 705 µmol/kg $BW^{0.75}$ d. Total urine PD excretion was expressed as mmol/d/kg $BW^{0.75}$.

Table 3.1. Ingredient and nutrient composition of milk replacer and starter.

	Milk replacer	Starter
Ingredients*		
Dried skim milk	62.0	-
Palm-coconut oil	20.0	-
Milk whey	12.0	-
Wheat starch	5.5	-
Corn meal	-	31.0
Oat meal	-	22.0
Soybean meal 48	-	20.0
Barley meal	-	9.7
Soybean hulls	-	5.0
Molasses	-	4.0
Green peas	-	4.0
Sodium bicarbonate	-	1.5
Soybean	-	1.0
Soidum chloride	-	0.8
Calcium carbonate	-	0.5
Dicalcium phosphate	-	0.3
Microminerals	0.5	0.2
Nutrient composition†		
Crude protein	26.5	23.2
Ether extract	22.2	4.0
Neutral detergent fibre	0.6	20.3
Acid detergent fibre	0.5	8.0
Ash	7.1	6.3
Gross energy, MJ/kg of DM	23.9	20.9
Threonine	1.1	0.7
Glycine	0.5	0.9
Valine	1.5	1.0
Methionine	0.8	0.3
Isoleucine	1.2	0.9
Leucine	2.5	1.7
Phenylalanine	1.1	1.0
Histidine	0.7	0.5
Lysine	2.0	1.1
Arginine	0.9	1.5

*Percentage as fed.

†Percentage of DM unless otherwise indicated**.**

3.2.6. Statistical analyses

Data in the text are expressed as least squares means with the standard error of the means. Data were processed with a mixed-effects analysis of variance with repeated measures using the procedure PROC MIXED of SAS (1999). The statistical model included calf as a random effect, and feeding program, and its interaction with time as fixed effects. Initial BW and initial age were both included in the model as covariates. The model was subjected to three variance-covariance structures: compound symmetry, autoregressive

order one, and unstructured. The variance-covariance matrix that yielded the smallest Schwarz's Bayesian criterion for each of the parameters measured was considered to be the most desirable structure. Differences between treatment means across time were assessed by the PDIFF test of SAS (1999).

Pneumonia treatments within each week were analyzed with a mixed-effects logistic regression using calf as random effect and feeding program and time and their interaction as fixed effects. Due to the low incidence of pneumonia after the third week of the study, the logistic regression was only performed for the first two weeks of study.

Also, mixed-effects linear regression analyses, adjusted for the random effects of each individual calf and week of sampling, were conducted between individual plasma essential AA (EAA) profiles and ADG, and between individual plasma EAA profiles and plasma urea concentrations for 10 calves in each CF and EF treatments during the preweaning period. These mixed-effects linear regressions were performed to determine whether the supply of AA from the diet would contribute to growth or would deaminated and converted into urea, with the assumption that the most important or limiting EAA that were sustaining growth were those whose relative concentration (%) in plasma increased in conjunction with an increase of ADG and a concomitant decrease of plasma urea concentration (consequence of a lower amount of AA being deaminated).

3.3. Results

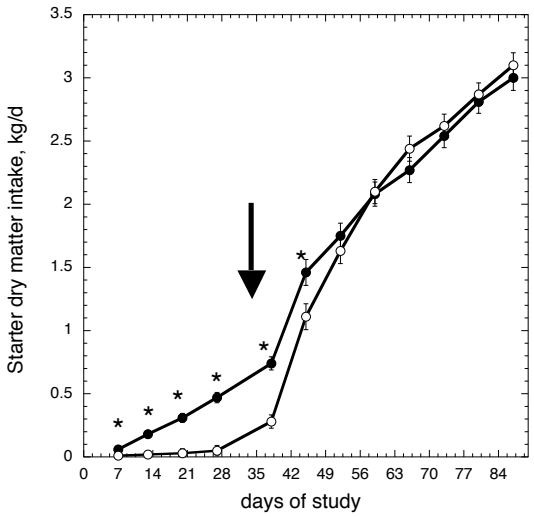
Three calves (two Holstein and one cross-bred) from the CF treatment, and three calves from the EF treatment were excluded from the study during the adaptation period due to severe health problems (with two in the CF and the three of the EF calves actually dying). Therefore, the study refers to data from thirty-seven Holstein and seven crossbred calves (22 animals per treatment), with an initial age of 16.1 ± 4.60 d, and an initial BW of 36.5 ± 3.19 kg of BW.

3.3.1. Intake and performance

Body weight was greater ($P < 0.001$) in EF than in CF calves throughout the 87 d of study, with 9.1 kg of final BW difference between both treatments in favor of EF calves. In contrast, WH was similar ($P = 0.15$) in both treatments throughout the study (Table 3.2). However, the ratio BW:WH evolved differently between treatments as indicated by the significant ($P < 0.001$) interaction between feeding program and time (Table 3.2). This ratio increased linearly in both treatments with the exception of EF calves from days 27 to 38 of study when this ratio was maintained constant (Figure 3.4). Furthermore, the

BW:WH ratio was greater ($P < 0.001$) in EF compared with CF calves throughout the study. There was a significant ($P < 0.001$) interaction between feeding program and time, indicating that ADG was greater during the first 27 d of study in EF than in CF calves, but from days 27 to 45 ADG was greater in CF than in EF calves. Beyond day 45 of study, there were no differences in ADG between feeding programs. Furthermore, calves in the EF treatment had greater ($P < 0.01$) ADG compared with CF calves (Table 3.2). Milk replacer intake was greater ($P < 0.001$) in EF than in CF calves (Table 3.2). But, a different evolution of starter intake throughout the study was indicated by the significant ($P < 0.001$) interaction between feeding program and time. The starter DM intake (DMI) was greater ($P < 0.001$) in CF compared with EF calves during the first 45 d of study (0.41 and 0.10 ± 0.035 kg/d, respectively) but similar ($P = 0.89$) afterwards (Figure 3.1). However, during the preweaning period total DMI was greater in EF compared with CF calves (1.16 and 0.87 ± 0.034 kg/d, respectively).

Figure 3.1. Starter dry matter intake throughout the study in calves on a conventional (●) or an enhanced-growth feeding program (○). On a time point, least squares means are significantly different:* ($P < 0.05$). The arrow indicates the weaning time.



Water consumption was greater from the beginning of the study to d 27 and from days 38 to 45 in EF compared with CF calves as indicated the significant ($P < 0.001$) interaction observed between feeding program and time. However, water consumption was similar ($P = 0.12$) between treatments (Table 3.2). Similarly, the ratio water consumption to total DMI evolved differently ($P < 0.001$) between treatments from days 28 to 45 as indicated

the interaction feeding program with time, being greater in EF than in CF calves (5.8 and 4.0 l water/kg total DMI, respectively) (Figure 3.5).

The significant ($P < 0.001$) interaction between feeding program and time indicated that the gain to feed ratio was greater from the beginning of the study to day 13 and from days 45 to 52, but lower from days 38 to 45 in EF than in CF calves. However, the gain to feed ratio was similar ($P = 0.18$) between treatments (Table 3.2).

Table 3.2. Least square means of performance parameters and intake of calves on a conventional or an enhanced-growth feeding program.

	Treatment*			*P*- value**	
Item	CF	EF	SEM†	FP	FPxtime
From 1 to 87 days of study					
Final body weight, kg	102.6	111.7	1.72		
Body weight, kg	68.3	78.1	1.61	0.001	<0.001
ADG, kg/d	0.74	0.86	0.027	0.005	<0.001
Water consumption, l/d	9.9	10.8	0.40	0.12	<0.001
Height, cm	82.9	84.0	0.57	0.16	0.15
Gain:feed	0.52	0.53	0.009	0.18	<0.001
Body weight:wither height	0.81	0.92	0.014	<0.001	<0.001
Preweaning period‡,					
DMI of milk replacer, kg/d	0.47	1.07	0.013	<0.001	<0.001
DMI of starter, kg/d	0.41	0.10	0.035	<0.001	<0.001
Total DMI, kg/d	0.87	1.16	0.034	<0.001	<0.001
Postweaning period§,					
DMI of starter, kg/d	2.27	2.25	0.085	0.89	<0.001

*CF: conventional feeding program; EF: enhanced-growth feeding program
**FP: effect of feeding program; FP x time: interaction between feeding program and time
†SEM = standard error of the mean
‡Preweaning period refers to days 1 to 38 of study
§Postweaning period refers to days 39 to 87 of study

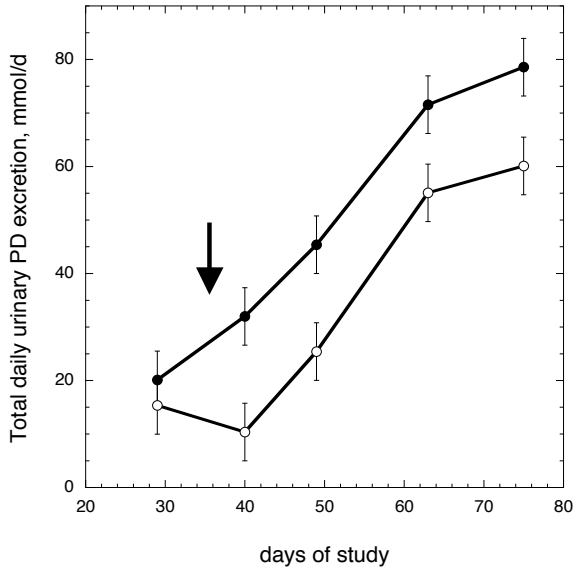
Several medical treatments were needed to address respiratory problems that mainly occurred during the first two weeks of study. Calves in the EF treatment tended to have a greater number of pneumonia treatments during week 2 than CF calves (1.41 vs 0.64 ± 0.294 treatments/calf, respectively) as indicated by the interaction ($P = 0.08$) between feeding program and time. However, there were no differences on the number of

pneumonia treatments during the first week of study (1.45 vs 1.46 ± 0.370 treatments/calf, in EF and CF calves, respectively).

3.3.2. Urine excretion of purine derivatives

Total PD urine excretion increased in a similar fashion ($P = 0.42$) throughout the study on both treatments (Figure 3.2) as indicated by the non-significant interaction of feeding program with time. However, it was greater ($P < 0.01$) in CF than in EF treatments (1.90 and 1.14 ± 0.119 mmol/d/$BW^{0.75}$, respectively). Furthermore, total PD urine excretion was quadratically correlated ($P < 0.001$) with starter intake, in both CF (r = 0.71) and EF treatments (r = 0.88).

Figure 3.2. Total daily urinary purine derivatives (PD) excretion (mmol/d) throughout the study in calves on a conventional (●) or an enhanced-growth feeding program (○). The arrow indicates the weaning time.



3.3.3. Plasma metabolites and amino acids

Plasma urea concentrations evolved differently ($P < 0.01$) during the preweaning period depending on the feeding program, being lower in CF than in EF calves during the first 27 d of study (2.94 vs 3.11 ± 0.206 mmol/l, respectively) and similar afterwards. But, there were no differences in plasma urea concentrations (Table 3.3) between feeding programs (2.83 and 2.92 ± 0.133 mmol/l in CF and EF treatments, respectively). Plasma glucose concentration evolved differently between treatments ($P < 0.001$), it increased from days 7 to 13 of the study in EF calves, but decreased in CF calves. Afterwards, it

tended to decrease in EF calves and to increase in CF calves. However, plasma glucose concentrations were greater ($P < 0.001$) in EF than in CF calves (7.08 and 5.60 ± 0.205 mmol/l, respectively) during the preweaning period (Table 3.3).

Table 3.3. Least squares means of weekly plasma metabolite concentrations (mmol/l) of calves following a conventional or an enhanced-growth feeding program during the preweaning period (from 1 to 38 days of study).

	Treatment*			*P*- value†	
Item	CF	EF	SEM‡	FP	FPxtime
Urea, mmol/l	2.82	2.92	0.133	0.63	<0.001
Glucose, mmol/l	5.60	7.08	0.206	< 0.001	<0.001

*CF: conventional feeding program; EF: enhanced-growth feeding program
†FP: effect of feeding program; FP x time: interaction between feeding program and time
‡SEM = standard error of the mean

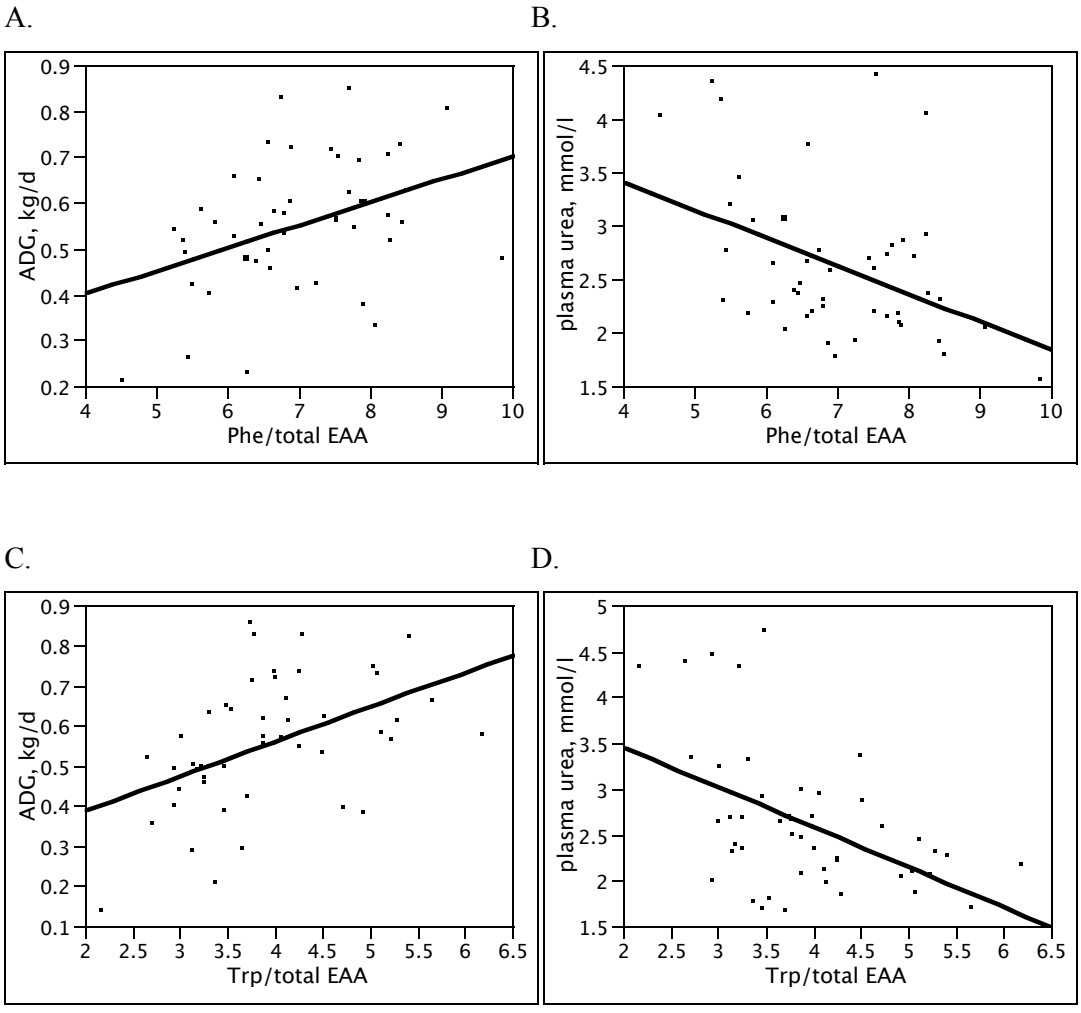
During the preweaning period, total EAA and non-essential AA (NEAA) consumptions were both greater ($P < 0.001$) in EF than in CF treatments (Table 3.4). Glycine was the only AA that was consumed in similar ($P = 0.11$) amounts by EF and CF calves (6.28 and 5.34 ± 0.375 g/d, respectively), due to the fact that MR contained less Gly compared with starter (0.50 and 0.93 % of DM, respectively). During the preweaning period, total plasma EAA concentrations 1 h after feeding were greater ($P < 0.05$) in EF than in CF calves (1109.0 and 915.1 ± 50.25 µmol/l, respectively). In contrast, total plasma NEAA concentrations did not differ ($P = 0.29$) between treatments (1369.8 and 1278.5 ± 55.88 µmol/l in EF and CF, respectively). As shown in Figure 3.3, Phe and Trp plasma concentrations in the CF calves presented a negative relationship with plasma urea concentration ($P < 0.01$, r = -0.42 and $P < 0.001$, r = -0.49, respectively) and a concomitant positive relationship with ADG ($P < 0.01$, r = 0.4 and $P < 0.001$, r = 0.47, respectively).

Table 3.4. Least squares means of weekly amino acid (AA) consumption (g/d) by calves fed conventionally or following an enhanced-growth feeding program during the preweaning period (from 1 to 38 days of study).

	Treatments[*]			*P*-value[†]	
Item	CF	EF	SEM[‡]	FP	FPxtime
Essential AA					
Phe	8.56	13.36	0.439	< 0.001	<0.001
His	4.61	7.55	0.221	< 0.001	<0.001
Ile	8.39	14.19	0.384	< 0.001	<0.001
Leu	17.01	28.81	0.776	< 0.001	<0.001
Lys	12.57	22.63	0.524	< 0.001	<0.001
Met	4.61	8.71	0.178	< 0.001	<0.001
Val	10.22	17.49	0.457	< 0.001	<0.001
Thr	7.27	12.24	0.332	< 0.001	<0.001
Arg	9.10	11.35	0.610	0.02	<0.001
Nonessential AA					
Glu	39.14	64.76	1.839	< 0.001	<0.001
Asp	16.23	23.90	0.902	< 0.001	<0.001
Ala	7.41	10.53	0.430	0.002	<0.001
Cys	2.49	3.12	0.166	0.02	<0.001
Pro	16.08	28.70	0.650	< 0.001	<0.001
Ser	9.25	15.14	0.443	< 0.001	<0.001
Gly	5.34	6.28	0.375	0.11	<0.001
Tyr	6.86	11.71	0.308	< 0.001	<0.001
Total EAA	82.29	136.44	3.857	< 0.001	<0.001
Total NEAA	102.59	164.43	5.062	< 0.001	<0.001
Total AA	184.88	300.87	8.915	< 0.001	<0.001

[*]CF: conventional feeding program; EF: enhanced-growth feeding program
[†]FP: effect of feeding program; FP x time: interaction between feeding program and time
[‡]SEM = standard error of the mean

Figure 3.3. Relationship, adjusted for the random effect of each individual calf and week of sampling, between plasma Phe and Trp profiles (% of EAA) and plasma urea concentration (Fig. A and C, respectively), or ADG (Fig. B and D, respectively) during the preweaning period of conventionally-fed calves. Data (n = 50) derived from 10 calves during the 5 wk of the preweaning period. Figure 2A: ADG, kg/d = 0.2043 + 0.05014 Phe/EAA; $P < 0.01$, r = 0.40. Figure 2B: Plasma urea concentration, mmol/l= 4.4654 – 02617 Phe/EAA; $P < 0.01$, r = -0.42. Figure 2C: ADG, kg/d=0.2196 + 0.08648 Trp/EAA; $P < 0.001$, r = 0.47. Figure 2D: Plasma urea concentration, mmol/l= 4.3368– 0.436 Trp/EAA; $P < 0.001$, r = 0.49.

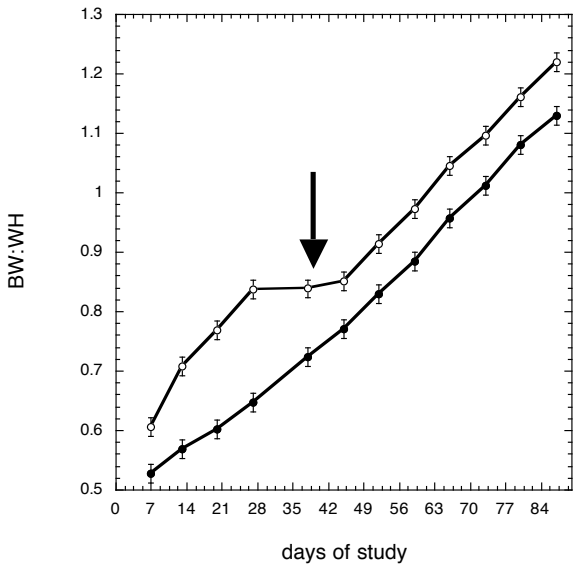


3.4. Discussion

The greater MR intake obtained in EF calves compared to CF calves resulted in greater ADG in EF calves during the first 27 d of study. In contrast, the lower ADG in EF compared with CF calves during the last 10 d before weaning was a consequence of the reduction of MR offered and the low starter intake of EF calves when MR was reduced. The reduction of the amount of MR offered during 10 d was not sufficient to stimulate starter intake of EF calves, and the slow rate of weight gain found at weaning in many calf-reared systems (Anderson et al., 1987b; Luchini et al., 1993; Greenwood et al., 1997) also occurred in EF calves, in contrast to CF calves that maintained their ADG at weaning. Despite the decrease in ADG during the days before and after weaning, EF calves were still heavier than CF calves at the end of the study. Similarly, female calves fed *ad libitum* slowed their rate of growth at weaning, but still maintained a significant weight advantage 20 d after weaning (Jasper and Weary, 2002).

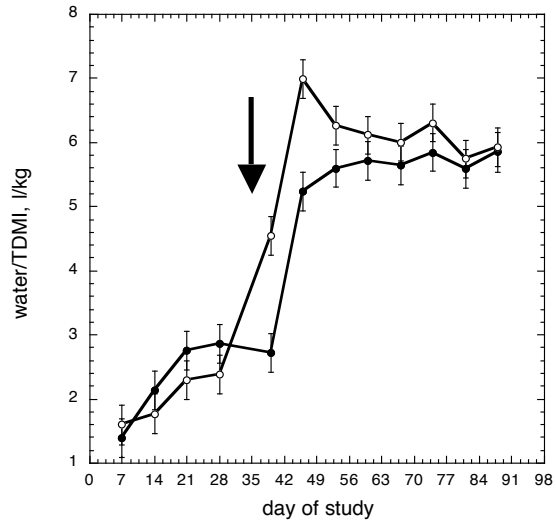
There were no differences in WH between treatments, but WH was numerically greater in EF compared with CF calves. Shamay et al. (2005) observed greater body measurements (BW, WH, hip width, and heart girth) in calves fed whole milk *ad libitum*, that consumed 9.8% more DM, than calves that were fed a restricted amount of MR. The ratio of BW to WH increases linearly with age (Kertz et al., 1998). However, calves in EF treatments lost this linearity (Figure 3.4) when MR was reduced by half, probably due to the decrease in ADG around weaning.

Figure 3.4. Ratio between body weight and withers height in calves on a conventional (●) or an enhanced-growth feeding program (○). The arrow indicates the weaning time.



Water consumption was greater in EF compared with CF calves for the first 27 d of study, as a consequence of the greater MR concentration used in the EF (18%) compared with that used in the CF calves (12.5%), as it was reported in the study by Jenny et al. (1978). However, coinciding with the days around weaning, EF calves further increased water consumption and the ratio between water consumption and total DMI (Figure 3.5), suggesting that EF calves tried to compensate for the reduction of MR offered with an increase consumption of water.

Figure 3.5. Ratio water consumption and total dry matter intake in calves on a conventional (●) or an enhanced-growth feeding program (○). The arrow indicates the weaning time.



The amount of antibiotic therapy tended to be greater in EF than CF calves during the second week of study. However, Diaz et al. (2001) did not report differences in antibiotic treatments, in contrast to Quigley et al. (2006) that observed greater health problems in calves fed additional MR (up to 900 g/d). Probably, differences in calves management may explain the variation among studies.

The greater gain to feed ratio observed in EF calves during the first 13 d of study could be explained by the greater amount of MR consumed by these calves. Similar gain to feed ratios, as seen for both treatments in the present study, have also been reported by other authors (Diaz et al., 2001; Blome et al., 2003) feeding preweaning calves conventionally or enhanced-growth feeding programs. Assuming that needs for digestible energy (DE) for maintenance (NRC, 2001) and that the energy digestibility of the starter was 63% (data not published), the lower gain to feed ratio from days 38 to 45 observed in EF compared with CF calves was a consequence of the lower ($P < 0.01$) proportion of the consumed digestible energy from starter that was above requirements of DE for

maintenance in EF compared with CF calves during this week (11.3% vs 50.4 % ± 0.09 in EF and CF calves, respectively).

On the other hand, the greater total PD urine excretion obtained in CF compared with EF calves estimated a greater duodenal microbial flow (Balcells et al., 1991; Funaba et al., 1997) in CF than EF calves, reflecting rumen development (Funaba et al., 1997) and probably a greater capacity to digest starter as it was observed in early weaned calves up to 6 wk after weaning (Funaba et al*.,* 1994), that also explained the better gain to feed ratio in CF than in EF calves after weaning. Average total PD urine excretion increased throughout the study, indicating that duodenal microbial flow progressively increased up to 6 weeks after weaning. Similar observations have been reported elsewhere (Funaba et al*.,* 1995). The increasing microbial duodenal flow is more likely, a reflection of the progressive increase in DMI with age. The difference in gain to feed ratio from days 45 to 52 between EF and CF calves might be attributed to compensatory growth of EF calves once DMI was recovered after weaning. A similar compensatory growth was also found in early-weaned calves (Funaba et al., 1997), and in weaned calves moved to a different pen after transport (Loerch and Fluharty, 1999).

Plasma glucose concentration was greater in EF than in CF calves, as a consequence of the greater supply of dietary lactose in EF than in CF calves (0.49 and 0.34 ± 0.012 kg/d, respectively). Plasma glucose concentrations were also greater in calves fed additional amounts of MR as a result of changes in nutrient source (lactose) as suggested by Quigley et al. (2006). Plasma urea concentrations observed in this study were lower than those reported by Diaz et al. (2001). These differences in level could be due to the energy to protein ratio in the MR, which was greater in the present study compared with that of Diaz et al. (2001). The greater plasma urea concentration of EF compared with CF calves observed during the first 27 d of study may indicate a greater AA deamination in the EF calves, suggesting that in EF either energy was limiting growth, or AA supply was in excess, or both. The results of the adjusted mixed-effects linear regressions suggested that growth of the EF calves did not seem to be limited by any of the measured EAA, because no positive relationship between plasma EAA and ADG, and no concomitant negative relationship between plasma EAA and plasma urea concentration were found for any of the EAA studied in the EF calves. Thus, it could be concluded that the energy to protein ratio in EF calves was too low to promote an adequate utilization of AA. The greater plasma EAA concentrations were found in EF than in CF calves may have resulted from the greater protein intake in EF than in CF calves. However, plasma NEAA concentrations did not differ between treatments, and this could be due to the fact that

despite Ala, Gly, and Ser are the NEAA absorbed in the greatest quantities, they are also the ones removed the most rapidly by the liver from the portal blood (Bergman and Heitmann, 1978). Thus, the similar concentration of plasma NEAA on both feeding programs could be explained, in part, by a greater NEAA deamination in EF calves as indicated also by the greater plasma urea concentrations observed in EF compared with CF calves during the first 27 d of study. Therefore, the amount of CP supplied by the MR used in this study could be lowered without impairing performance of EF calves, or additional energy should be included in the MR to enhance CP utilization. In addition, the results of the adjusted mixed-effects linear regressions indicated that, in CF calves, as more Phe and Trp were available, ADG increased linearly, and simultaneously plasma urea concentrations decreased, suggesting that lower amounts of AA were deaminated when more Phe and Trp were available (Figure 3.3) during the postprandial phase. These results indicate that Phe and Trp might potentially be limiting growth in CF calves with the starter and milk replacer used levels in this study. However, more research would be needed with more intensive blood samplings to further reinforce this theory.

3.5. Conclusion

Enhanced-growth feeding programs increase average daily gain and gain to feed ratio during the preweaning period despite the lower preweaning starter intake and the apparent delay in rumen development compared with conventional feeding programs. However, the improvement in body weight obtained in the preweaning period with enhanced-growth feeding programs is maintained at least up to 2 months after weaning. Moreover, with calves conventionally-fed, plasma phenylalanine and tryptophane concentrations one hour after feeding are positively correlated with average daily gain and negatively correlated with plasma urea concentrations, suggesting that growth of calves following conventional feeding programs could be limited by the supply of these two amino acids when using milk replacers and starters similar to those used in the present study.

Chapter 4

PERFORMANCE AND BEHAVIOR OF CALVES REARED IN GROUPS OR INDIVIDUALLY FOLLOWING AN ENHANCED-GROWTH FEEDING PROGRAM

A fraction of this research has been published in:

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4.1. Introduction

A common pitfall in enhanced-growth feeding program is the low starter intake during the preweaning period, and it affects calf performance when milk replacer is reduced to increase starter intake or the week after weaning (Bar-Peled et al., 1997; Jasper and Weary, 2002). The eating behavior can be learnt through the sight of an experienced animal (Phillips and Youssef, 2003). For instance, calves reared in groups increased fresh grass intake compared with calves reared individually (Phillips, 2004). The author suggested that the sight of feed being taken into the mouth was the relevant stimulus to learn the feeding behavior from each other.

The objective of this study was to evaluate the potential role of rearing calves in groups on starter intake and performance following an enhanced-growth feeding program.

4.2. Materials and Methods

4.2.1. Animals and treatments

Forty Holstein male calves (11.5 ± 1.92 d of age, and 40.6 ± 3.83 kg of BW) were purchased from commercial farms and raised in the facilities of IRTA (Prat, Spain) in spring under the guidelines of the IRTA Animal Care Committee. Upon arrival all calves received 2 liters of an oral electrolyte solution (Karihidra líquido, Karizoo S.A., Spain), were weighed, blocked by initial BW (36.5 ± 1.99; 40.4 ± 1.37; 43.1 ± 1.67; and 50.7 ± 6.15), and randomly distributed in 2 groups: 20 calves were allocated individually (IP) in wooden pens (1 x 1.55 m), and the other 20 in 4 pens (5 x 1.55 m) of 5 calves each (GP). Although pens were separated by wooden walls, all calves had visual contact with the calves that were in front of them. Pens were bedded daily with sawdust. All pens were in the same building under forced ventilation. All calves received the same milk replacer (Specilait, Karizoo S.A., Spain) and the same calf starter (Table 4.1). Milk replacer (MR) was offered in buckets twice daily at 7.00 and 17.30. The first 7 days were used to adapt calves to MR. Afterwards, both groups received the same feeding program: MR was offered at 15% DM, 4 l/d from 1 to 5 d, 5 l/d from 6 to 13 d, 6 l/d from 14 to 19 d, and MR offered was decreased to 4 l/d from 20 d to weaning day at 28 d of study to stimulate starter intake. A pelleted starter and water were offered *ad libitum* until the end of the study at 56 day. Water was offered in bowl drinkers at the ratio of one drinker per calf in both treatments. Starter was offered in an individual metal trough (0.35 x 0.16 x 0.22 m of length, width, and height, respectively) for IP calves, and in a common metal trough (1.40 x 0.30 x 0.16 m of length, width, and height, respectively) for GP calves. Starter

was removed and weighed each morning, and in the evening, troughs were checked to ensure that concentrate was available to all animals. Straw (200 g/d) was offered in hay racks the last week of study at the ratio of one hay rack per calf. All calves were treated with 700 mg tylosin, 875 mg sulfametroxazole, 175 mg trimethoprim, and 280 mg gentamicin on days 5, 6, and 8 of study to prevent respiratory problems. Also, calves were vaccinated against Infectious Bovine Rhinotracheitis (IBR), Infectious Pustular Vulvovaginitis virus, Parainfluenza-3, Bovine Virus Diarrhoea, and Bovine Respiratory Syncytial Virus (Hiprabovis-4, Hipra, Spain) on day thirteen. Vaccination was repeated on day 34 of the study.

Table 4.1. Chemical composition of milk replacer and starter.

	Milk replacer	Starter
Nutrient composition, % of DM		
Crude protein	24.2	20.8
Ether extract	20.3	3.7
Neutral detergent fibre	0.4	14.0
Acid detergent fibre	-	7.2
Ash	7.2	6.4

4.2.2. Measurements and Sample Collection

Consumption of MR was recorded daily for each calf at each offering. Individual starter intake was recorded for IP calves, and collective starter intake was recorded for each group of calves daily. Incidence of scours and veterinary treatments were recorded daily for each animal during the preweaning period, and throughout the study, respectively. Body weight was measured weekly. One hour after MR was consumed, a 10-ml blood sample was obtained by venipuncture of the jugular vein once weekly. Blood was centrifuged at 1500 x *g* at 4ºC for 10 min to obtain serum. Serum samples were stored at -20ºC until subsequent analyses.

4.2.3. Chemical Analyses

Samples of MR and starter were analyzed for DM (24 h at 103 ºC), ash (4 h at 550 ºC), N content (AOAC, 1990), ether extract (AOAC, 1990), and NDF and ADF (van Soest et al., 1991). Enzymatic determination of serum glucose, urea, and non-esterified fatty acid (NEFA) were conducted following the HK/G-6-PDH method (Burrin and Price, 1985),

the urease-GLDH method (Gutmann and Bergmeyer, 1974), and the ACS-ACOD method (Wako, Osaka, Japan), respectively. Serum insulin concentration was determined at 2, 3, 4 and 5 weeks of study using ELISA (Mercodia, Uppsala, Sweden). Serum cortisol concentrations were determined for all animals the week before and after weaning using an immunoassay (DRG-Cortisol ELISA EIA-1887, DRG Instruments, Germany).

To assess immune response to IBR vaccination, serum concentrations of antibodies against IBR were analyzed by a single-well blocking immunoenzymatic technique (Seralisa™ IBR Ab mono blocking kit, Synbiotics Corporation, Lyon, France) at 2, 5, and 8 week of study. To determine whether calves reacted positively to vaccination the competition percentage was calculated as the ratio of the difference between optical densities of a negative control and the sample, and the difference between the optical density of the negative and positive controls. When the competition percentage was greater than 60% the test was considered positive.

4.2.4. Behavior Measurements

Behavior was monitored for all animals twice weekly during the preweaning period. Calves were housed in a barn divided in two sides: there were two groups of GP calves, and 10 of IP calves in each side. One side was observed on Mondays and Wednesdays and the other on Tuesdays and Thursdays. Therefore, 20 animals were observed at any time by direct observations from an observing platform and continuous recordings of 20 min immediately following the morning and afternoon offers of MR. Once calves were weaned, the observations were conducted also twice weekly at the same time that in the preweaning period (7.30 and 17.30) for 20 min. The observer recorded oral behaviors, the animal or animals involved in the behavior, the time when the behavior started and finished, and the part of the body (head, ear, prepubertal zone, nose) or the object (walls, feeding trough, iron bars, bucket) that was touched by calves. Behavior patterns were grouped following Keil and Langhans (2001) in: 1) non-nutritive oral behavior: when calves were licking, sucking, or nibbling a part of the pen with their mouths, but no parts of the calf's body; 2) cross-sucking: when calves were licking, sucking or nibbling a body part of a group member with their mouths; 3) inter-sucking: when calves were licking, sucking, or nibbling the inguinal region of a group member with their mouths, and 4) self-grooming: when calves were leaking themselves.

4.2.5. Statistical Analyses

An analysis of variance with repeated measures was used to analyze performance data. The statistical model included calf within pen (to account for the dependence of animals within pen) as random effects and housing type and initial BW (block) as fixed effects. Repeated factors included time, and the interaction between type of housing and time. For each analyzed variable, calf nested within housing type and pen was subjected to 2 variance-covariance structures: compound symmetry and autoregressive order one. The variance-covariance structure that yielded the smallest Schwarz's Bayesian criterion was considered to be the most desirable matrix. Statistical significance was considered to exist for $P \leq 0.05$, and tendencies were declared for $P \leq 0.10$. Due to the lack of normality serum metabolites data were analyzed after a log-transformation. Results from this transformation are presented as arithmetic means with the SE and P values generated from the log-transformed distributions.

Medical treatments were analyzed as the number of days on medical treatment within week for each calf. Then, data were split in three categories: no medical treatment, from 1 to 3 d, and from 4 to 7 d on medical treatment within a week. After that, an ordinal logistic regression was performed including calf as random effect, and week, initial BW (block) and housing type as fixed effects. The occurrence of scours was analyzed as the number of days with scours within week for each calf. Data were split in 2 categories non scours and scours within each week and calf. Then, a binary logistic regression with calf as a random effect, and week, initial BW (block) and housing type as fixed effects was performed.

Immune response to vaccination was analyzed by contingency table analysis and a two-tailed Fisher's test. Serum cortisol concentrations of IP and GP calves were analyzed with an analysis of variance including calf within pen as random effect, BW as a block, and housing type, week of study, and their interaction as fixed effects.

Behavior data were summarized individually as the time (in seconds) devoted to each one of the monitored behaviors during the 80 min observed each week. After that, a Poisson regression model including calf as random effect, and housing type, week of study, MR intake, and the interactions between housing type and week, housing type and MR intake, and housing type, MR intake and week of study as fixed effects was performed to analyze behavior data.

4.3. Results

Five calves were removed from the study due to severe diarrhea during the adaptation period (3 in IP and 2 in GP), and were substituted by 5 new calves (21.5 ± 1.29 d of age, 55.8 ± 4.02 kg of BW). Later, one IP calf died after the adaptation period. Thus, data from the 4 groups of 5 calves, and 19 individually-housed calves are reported in this study.

4.3.1. Intake and Performance

There were no significant effects of housing type on final BW, starter (Figure 4.1) and MR intakes, and gain to feed ratio (Table 4.2). However, the evolution of ADG was different ($P < 0.05$) between treatments (Figure 4.2). The ADG of IP calves increased ($P < 0.05$) from 14 to 21 d of study (from 0.71 to 0.89 ± 0.071 kg/d, respectively). In contrast, the ADG of GP calves was maintained constant (0.85 ± 0.099 kg/d) during this period. Furthermore, the decrease in ADG from 28 to 35 d of study, the week after weaning (Figure 4.1), was greater ($P < 0.05$) in GP (from 0.60 to 0.17 ± 0.099 kg/d) than in IP calves (from 0.47 to 0.33 ± 0.071 kg/d). After that, ADG increased linearly in both treatments, being 1.09 ± 0.071 and 0.93 ± 0.099 kg/d in IP and GP calves, respectively at 56 d of study.

Table 4.2. Overall performance of calves housed individually (IP) or in groups (GP).

	Housing			*P*-value[a]		
Item	IP	GP	SE[b]	H	T	H x T
Final BW, kg	83.3	78.9	2.29	0.44	-	-
ADG, kg/d	0.69	0.67	0.085	0.84	< 0.01	0.03
DMI of MR, g/d	636.1	642.0	7.52	0.63	< 0.001	0.41
DMI of starter, g/d	975.2	954.1	0.119	0.91	< 0.01	1.00
Gain:feed ratio	0.63	0.60	0.024	0.91	< 0.001	0.99

[a] H = effect of housing type; T = time; H x T = interaction between housing type and time.
[b] SE = standard error of the mean

Figure 4.1. Starter dry matter intake of calves housed individually (●) or in groups (○). The arrow points the weaning day.

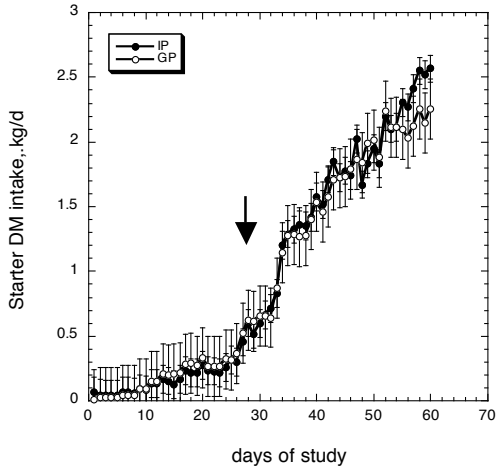
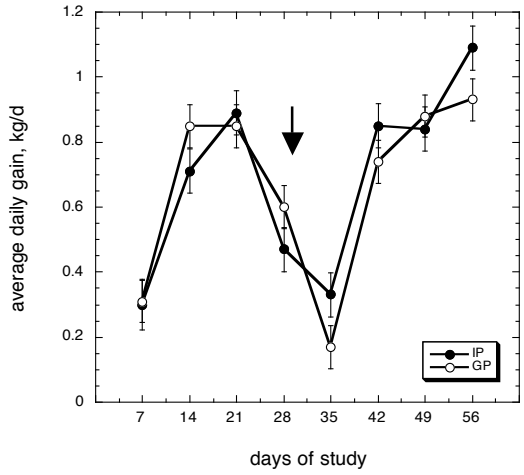


Figure 4.2. Average daily gain of calves housed individually (●) or in groups (○). The arrow points the weaning day.

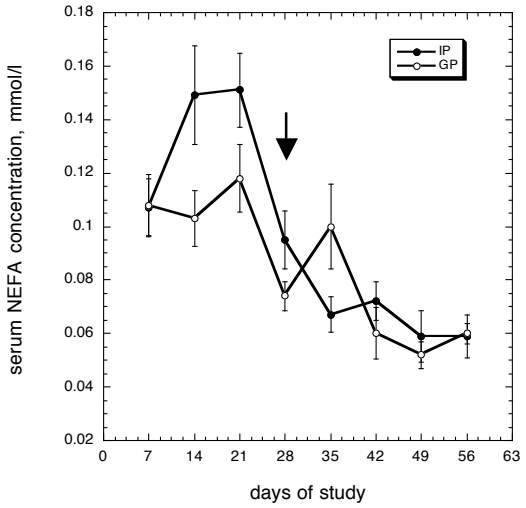


4.3.2. Serum Metabolites

Serum NEFA concentrations in GP calves were similar in both treatments (Table 4.3). However, a different ($P < 0.05$) evolution of serum NEFA concentrations was found between treatments (Figure 4.3). Serum NEFA concentrations in IP calves increased ($P < 0.05$) from 7 to 14 d of the study (from 0.11 to 0.15 ± 0.035 mmol/l), and then decreased from 21 to 35 d and from 42 to 49 d of study. Contrarily, serum NEFA concentrations in GP calves were similar (0.11 ± 0.035 mmol/l) from 7 to 14 d, decreased from 21 to 28 d,

were maintained steady from 28 to 35 d, and decreased again from 35 to 42 d of study (Figure 4.3).

Figure 4.3. Arithmetic means of serum NEFA concentrations of calves housed individually (●) or in groups (○). The arrow points the weaning day.



There were no differences between treatments in serum glucose concentrations (Table 4.3). Although serum glucose concentrations were similar between treatments, insulin serum concentrations were greater ($P < 0.01$) in GP than in IP calves (4.8 vs 2.7 ± 0.053 µg/l, respectively).

Table 4.3. Arithmetic mean of serum metabolite concentrations in calves housed individually (IP) or in groups (GP).

	Housing			*P*-value[a]		
Item	IP	GP	SE[b]	H	T	H x T
NEFA, mmol/l	0.09	0.08	0.035	0.48	< 0.01	0.02
Glucose, mmol/l	5.14	5.45	0.011	0.27	< 0.01	0.56
Urea, mmol/l	2.95	3.46	0.134	0.15	< 0.01	0.07
Insulin, µg/l	2.67	4.76	0.053	0.01	< 0.01	0.46
Cortisol, nmol/l	16.42	17.05	0.0811	0.70	0.02	0.89

[a] H = effect of housing type; T = time; H x T = interaction between housing type and time.
[b] SE = standard error of the mean

Serum urea concentrations were numerically greater ($P = 0.15$) in GP calves than in IP calves (Table 4.3) at 28 d (3.54 and 2.76 ± 0.134 mmol/l, respectively) and at 35 d (4.51 vs 2.67 ± 0.134 mmol/l, respectively) of study. The evolution of serum urea concentrations tended to be also different ($P = 0.07$) between treatments. Serum urea

concentrations in IP calves were stable throughout the study. In contrast, serum urea concentrations in GP calves increased from 28 to 35 d, and then decreased from 35 to 42 d of study. Serum cortisol concentrations were similar in both treatments (Table 4.3).

4.3.3. Scours and Immunological Response

There were no effects of housing type on the presence of scours within week, nor on the days of medical treatments within week.

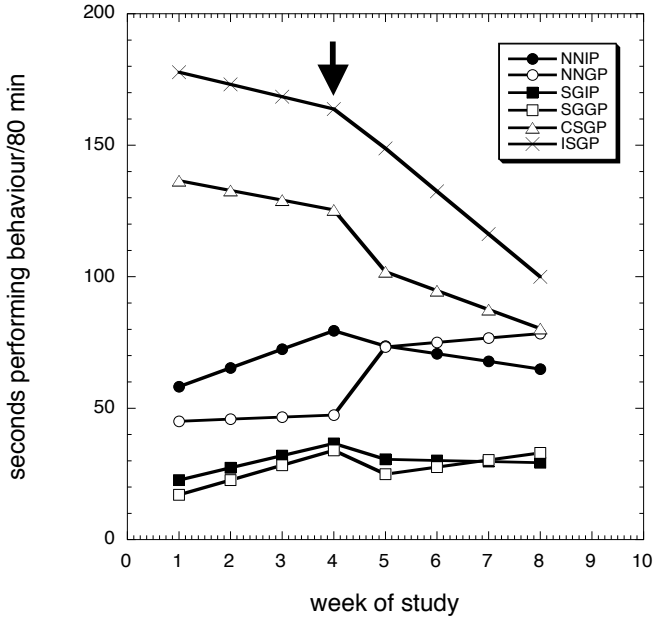
Immunity against IBR before vaccination was not different between the two housing types. However, IP calves tended (P = 0.08) to respond more effectively against IBR vaccination compared with GP calves 3 weeks after the first vaccination, as indicated by the percentage of animals that seroconverted positively in the IP (84%) and GP (55%) treatments. However, all calves seroconverted positively 3 weeks after the second vaccination.

4.3.4. Behavior

The time performing non-nutritive oral behaviors tended to be greater (P = 0.08) in IP than in GP calves, and it was numerically greater (P = 0.16) after weaning than before weaning. The availability of MR affected differently (P < 0.05) the time that calves devoted to perform non-nutritive oral behaviors (Figure 4.4). Calves reared individually increased non-nutritive oral behaviors with time before weaning, but their occurrence decreased after weaning. Contrarily, GP calves increased non-nutritive oral behaviors throughout the study. On the other hand, the occurrence of self-grooming behavior was numerically greater (P = 0.12) in IP compared with GP calves, but the increase of self-grooming behavior throughout the study tended (P = 0.10) to be greater in GP compared with IP calves. Although there were no differences in the time performing self-grooming between before and after weaning, the evolution of self-grooming behavior was different (P < 0.01) in IP calves, but similar in GP calves, before and after weaning. This behavior increased before weaning and decreased at weaning in both treatments, but it increased in GP calves and slightly decreased in IP calves after weaning.

In the GP calves, the time performing cross-sucking and inter-sucking behaviors decreased (P < 0.01) throughout the study. However, this decrease was numerically greater for cross-sucking (P = 0.19) and inter-sucking (P = 0.13) behaviors after weaning.

Figure 4.4. Time (in seconds) that calves devoted to perform non-nutritive oral behaviors (● for calves housed individually and ❍ for calves housed in groups), self-grooming (■ for calves housed individually and □ for calves housed in groups), cross-sucking (△) and inter-sucking (×) behaviors for calves housed in groups. The arrow points when no more MR was offered.



4.4. Discussion

The objective of this study was to test the hypothesis that rearing calves in groups would help to palliate the decrease in performance after weaning by increasing the consumption of dry feed. We hypothesized that when an animal would perform a given behavioral pattern, such as going to the feeder, the likelihood of another animal starting to perform that behavior would also increase (social facilitation). Although the degree of stimulation to attend the feeder when one calf visited it was not directly measured, the results obtained indicated that rearing calves in groups did not increase starter intake of calves receiving an enhanced-growth feeding program. In a recent study (Phillips, 2004), calves reared in groups only increased grass consumption, but not starter compared with calves housed individually. This author suggested that the sight of feed being taken into the mouth was the relevant stimulus for calves to learn the feeding behavior (Phillips, 2004). However, in the current study, starter consumption or the visit to the feeder by a calf might have not been a sufficient visual stimulus to improve starter consumption for other calves.

It was expected that the reduction of MR offer one week before weaning would have stimulated starter intake to avoid the weaning distress as occurred in calves fed increasing amounts of MR (Quigley et al., 2006). However, similar to calves weaned at 45 days (Greenwood et al., 1997), calves in both treatments decreased ADG the week after weaning indicating an insufficient availability of energy (due to low starter consumption) to maintain previous growth rates. Calves in GP treatment presented a more pronounced decrease in ADG the week after weaning compared with IP calves, which might be attributed to a greater energy deficit due to a higher behavioral activity. However, both treatments resulted in a similar increase in BW at 35 d of study (19.02 and 20.25 kg in IP and GP calves, respectively). It has been previously documented that rumen fermentation end-products (Anderson et al., 1987b) and digestive enzymes increased in activity and quantity as the age of calves increases (Le Huerou et al., 1992). Thus, the decrease in ADG after weaning in both treatments may also be attributed to the fact that calves were not metabolically mature.

The increase of serum NEFA concentrations during the first days of study in IP calves compared to GP calves might be attributed to a potential initial stress due to isolation of IP calves that may stimulate energy reserves mobilization, as occurred in transported calves that substantially increased serum NEFA concentrations during their journey (Knowles et al., 1999). In the current study, once IP calves adapted to the new situation serum NEFA concentrations decreased. On the other hand, calves in GP treatment maintained steady serum NEFA concentrations at the beginning of the study and at weaning, but decreased thereafter. The decrease on serum NEFA concentrations with calf age has also been reported by Knowles et al. (1999). In the current study, although calves decreased ADG at weaning, they did not mobilize energy reserves at that time as indicated by the maintenance or the decrease of serum NEFA concentrations in both GP and IP calves. The decrease in NEFA with age could have been related to an exhaustion of body energy reserves.

The tendency for GP calves to have greater serum urea concentrations the week after weaning compared with that of IP calves may suggest that GP calves diverted protein to obtain energy for maintenance.

The greater serum insulin concentrations 60 min after the meal in GP calves compared to IP calves may be explained by the greater sucking behaviors after the meal offer performed by GP compared with IP calves. Since, calves that are allowed to suck a teat after the meal have greater plasma insulin concentration (de Passillé et al*.,* 1993).

Calves reared individually presented a more rapid immune response than GP calves at 5 week of study. Immune response to a keyhole limpet hemocyanin was considered a good indicator of the well-being of water buffalo calves (Grasso et al*.,* 1999). Thus, the preceding results could suggest that, under the conditions of this study, immune responses indicate a greater welfare level of IP than in GP calves around weaning. However, immune response to vaccination in young calves may be interfered by maternal passive immunity acquired via colostrum (Meanteau-Horta et al., 1985).

Although the amount of time devoted to perform oral behaviors was greater in GP compared with IP calves, the behavioral pattern changed throughout the study. Calves reared in groups increased non-nutritive oral behavior, and decreased cross-sucking and inter-sucking behaviors throughout the study. On the other hand, both GP and IP calves slightly increased self-grooming behavior throughout the study. Although, non-nutritive oral behaviors decreased in IP calves after weaning, the amount of time performing non-nutritive oral behaviors was similar to GP calves after weaning. This could suggest a stimulated interest of calves towards their environment that could be tested by evaluating the reaction of the animals when new objects were introduced in the pen. Panivivat et al. (2004) found that, as calves grow older, they perform more self-grooming, investigate more their environment, and they also perform more behaviors involving pen contact. Thus, the interest for the environment as calves grow may explain the increase of non-nutritive oral and self-grooming behaviors throughout the current study. It was also observed (Kerr and Wood-Gush, 1987) that calves reared in confined groups presented higher frequency of investigation and self-grooming behaviors than calves reared in fields, suggesting that confined calves were motivated to perform investigation.

In the grouped-housed calves, cross-sucking behaviors decreased after weaning, as it was also reported in the study of Keil and Langhans (2001). A little occurrence of cross-sucking behavior after weaning has also been reported in the literature (Lidfors, 1993).

The incidence of inter-sucking behavior in group-reared calves is not consistent in the literature. De Wilt (1986) reported that calves reared in groups of 5 rarely performed inter-sucking from 8 to 20 weeks of age, but Chua et al. (2002) reported a prevalence of inter-sucking behavior of about 20% in calves housed in pairs. Furthermore, Keil and Langhans (2001) observed that calves that performed inter-sucking before weaning, also inter-sucked after weaning. The consumption of milk motivates sucking behaviors (de Passillé, 2001). Thus, cross-sucking and inter-sucking behaviors in GP calves probably decreased because MR was not offered and sucking was not stimulated by MR intake.

Furthermore, the increasing intake of solid feeds that occurs mainly after weaning also decreased cross-sucking and inter-sucking behaviors (Keil and Langhans, 2001). Although starter was offered *ad libitum* throughout the study, abnormal oral behaviors might have been reduced if hay had been offered earlier in life (Mattiello et al., 2002). Perhaps, if MR had been offered in a teat bottle rather than a bucket, GP calves would have spent less time performing cross-sucking behaviors, as reported Jung and Lidfors, (2001) in their study.

Overall, sucking behavior is partly a "behavioral need" developed as a consequence of feeding milk (de Passillé and Rushen, 1997). However, GP calves spent more time performing oral behaviors compared with IP calves, but it was not reflected in considerable metabolic or immunological differences.

4.5. Conclusion

In conclusion, rearing calves in groups following an enhanced-growth feeding program does not play a role in increasing starter intake. Calves reared in groups performed cross-sucking and inter-sucking behaviors, but there were no differences in days on medical treatments or with scours, and both calves performed similarly throughout the study.

Chapter 5

EFFECT OF LEVEL OF MILK REPLACER FED TO HOLSTEIN CALVES ON PERFORMANCE DURING THE PREWEANING PERIOD AND STARTER DIGESTIBILITY AT WEANING

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5.1. Introduction

Early feed consumption improves early rumen microbial development, resulting in a greater rumen metabolic activity (Anderson et al., 1987b). Then, the high level of milk replacer in calves following an enhanced-growth feeding program, may delay the start of dry feed consumption, and consequently it may delay rumen development (Davis and Drackley, 1998). The hypothesis of the current study was that the low starter intake during the preweaning period in calves following an enhanced-growth feeding program may delay the capacity to digest solid feed.

Therefore, the objective was to evaluate the effects of two different levels of milk replacer consumption on performance during the preweaning period and concentrate apparent nutrient digestibility at weaning.

5.2. Materials and methods

5.2.1. Animals and treatments

Nineteen Holstein male calves (12.4 ± 3.10 days of age, and 48.9 ± 5.98 kg of BW) were purchased from commercial farms in France and raised in the facilities of IRTA (El Prat, Spain) under the approval and supervision of the Animal Care Committee of IRTA. Calves were housed in individual wooden pens (1 x 1.55 m) in a barn with forced ventilation. Upon arrival, they were weighed, and randomly distributed according to BW in 2 groups: 9 calves being assigned to a conventional feeding program (CF) and 10 calves to an enhanced-growth feeding program (EF). Milk replacer (MR) was offered in buckets twice daily at 07.00 and 18.00 h. The first week of study was considered as an adaptation period, during which calves in CF treatment received 4 l/d of MR at 12.5% DM dilution rate, and EF calves received during the first 5 d of the adaptation week 4 l/d at 12.5% DM dilution rate, and during the last 2 d of the adaptation week 4 l/d at 15% DM dilution rate. After the adaptation period, CF calves were fed 4 l/d of MR at 12.5% DM dilution rate from d 1 to 28, while 2 l/d were offered in the afternoon feeding from d 29 to d 35 (54 d of age). The EF calves were offered MR at 18% DM dilution rate according to the following schedule: 4 l/d from d 1 to d 7, 6 l/d from d 8 to d 14, 7 l/d from d 15 to d 21, 6 l/d from d 22 to d 28, and then 3 l/d offered in afternoon meal from d 29 to d 35. All groups received the same MR (Sprayfo Excellent 60, Sloten BV, Holland) containing on a DM basis 25% CP, 19.2% ether extract, 0.15% NDF, 6.4% ash, and 21.7 MJ of gross energy/kg. The two groups received also the same calf starter (Table 5.1).

Water and starter were offered *ad libitum* throughout the study. The weaning time was set at d 35 after the beginning of the study, and the study finished at d 42 (61 d of age). Calves were vaccinated against *Clostridium perfringens* type A, B, C, D, *Clostridium septicum, Clostridium novyi,* and *Clostridium sauvoei with* SyvaBax (Syva, Spain) during the adaptation week and against PI3, BRSV, BVD, and IBRV with Cattle Master 4 (Pfizer, Exton) at d 5 of study, and revaccinated at d 19 and d 30, respectively.

Table 5.1. Ingredient and nutrient composition of the starter.

	Starter
Ingredient composition[a]	
Milk whey	2
Cracked corn	27
Oats	10
Soybean meal	7.5
Wheat middling	11
Soybean hulls	8
Gluten meal	7
Distillers dried grains (wheat)	10
Beet pulp	5
Horse beans	5
Bakery waste	5
Calcium carbonate	1.5
Salt	0.5
Premix	0.2
Nutrient composition[b]	
Crude protein	19.7
Ether extract	3.8
Neutral detergent fibre	18.7
Acid detergent fibre	8.2
Ash	6.9
Gross energy, MJ/kg of DM	18.7

[a] Percentage as fed
[b] Percentage of DM, unless otherwise indicated

5.2.2. Measurements and sample collection

Consumption of MR and starter were recorded daily for each calf. Water consumption was recorded daily from 25 d to the end of the study. Incidence of veterinary treatments was recorded daily for each animal throughout the study. Body weight was measured at d 3, 17, 24, 31, 38 and 42.

Plastic bags were glued to each animal to collect feces in order to determine the apparent nutrient digestibility of the starter. A 5-d daily total fecal collection was conducted from d 38 to d 42. Bags were changed and weighed three times a day, and pooled by calf every day. Then, a 500-g sample was taken, dried at 103ºC for 48 h, ground in a Cyclotec 1093 mill, (Tecator, Sweden), pooled by each calf for the 5-d period and stored in plastic bags

until subsequent chemical analyses. Samples of starter and refusals for each calf were taken during the 5 d of fecal collection. These samples were ground, pooled for the 5-d period, and stored in plastic bags until analysis.

Furthermore, a 10-ml blood sample was obtained by venipuncture of the jugular vein 1 hour after the morning feeding at 23, 30, 37 d of study. Blood was centrifuged at 1500 x g at 4ºC for 10 min to obtain serum. Serum samples were stored at -20ºC until subsequent analyses.

At d 42 rumen samples were obtained from different locations with a stomach tube and a vacuum pump. The pH was immediately determined. Samples of rumen fluid were stored at -20ºC until subsequent total DNA extraction to determine rumen bacteria diversity by the terminal-restriction fragment length polymorphism (T-RFLP) technique.

5.2.3. Chemical analyses

Samples of MR and starter were analyzed for DM (24 h at 103 ºC), ash (4 h at 550 ºC), N content using the AOAC (1990) method (988.05) adapted for an automatic distiller Kjeldhal (Kjeltec Auto 1030 Analyzer, Tecator, Sweden) and using $CuSO_4$/Se as catalyst instead of $CuSO_4/TiO_2$, NDF, with sodium sulphite and heat-stable alpha-amylase, and ADF (Van Soest et al., 1991). Ether extract was also determined using the AOAC method (920.39) using petroleum ether for distillation instead of diethyl ether (AOAC, 1990), and hydrolyzing MR samples before distillation.

Enzymatic determination of serum urea, and NEFA were conducted following the urease-GLDH method (Gutmann and Bergmeyer, 1974), and the ACS-ACOD method (Wako, Osaka, Japan), respectively.

Feces, starter and refusals were analyzed for DM (24 h at 103 ºC), ash (4 h at 550 ºC), and N content (AOAC, 1990), gross energy (adiabatic IKA-calorimeter C 4000, Heitersheim, Germany), and NDF, with sodium sulphite and heat stable alpha-amylase, (Van Soest et al., 1991).

After thawing, rumen content was squeezed through 2-layer cheesecloth and the rumen fluid was used to extract rumen fluid DNA following a phenol-dependent bead-beating method described by Whitford et al. (1998). Then, a T-RFLP analysis of rumen bacteria community was performed following the procedure described by Höjberg et al. (2005), modified by Castillo (2006). This technique allowed to obtain small DNA fragments (50 to 700 base pairs) that corresponded to different rumen bacteria depending on the number of base pairs that resulted from a DNA enzymatic restriction (or sequence-specific

cleavage). Subsequently, an automatic sequence analyzer (ABI 3100 Genetic Analyzer, PE Biosystems, Warrington, UK) detected DNA fragments with the same number of base pairs and the sequence analyzer represented them by peaks. Then, the rumen bacteria diversity was defined as the number of peaks obtained by the sequence analyzer. Furthermore, a dendogram was constructed to determine the degree of similarity (based on the patters of peaks obtained from the sequence analyzer) among rumen bacterial populations using Fingerprinting II software (Informatix, Bio-Rad, Ca, USA) and unweighed pair group method with an averaging algorithm. The closer the lines represented in the dendogram the greater the degree of similarity between the rumen bacterial population of calves.

5.2.4. Statistical Analyses

Data originating from several measures within animal (BW, ADG, starter intake, transformation index, water consumption) were analyzed with a mixed-effects analysis of variance with repeated measures. The statistical model included calf as a random effect, while feeding program, and the interaction with time as fixed effects. Initial BW and initial age for preweaning data, and BW and age at weaning for the postweaning data, were both included in the model as covariates. The model was subjected to two variance-covariance structures: compound symmetry and autoregressive order one. The variance-covariance matrix that yielded the smallest Schwarz's Bayesian criterion was considered to be the most desirable structure. Comparisons with $P \leq 0.05$ were considered as significant, whereas comparisons with $P \leq 0.10$ were presented as tendencies. Differences between treatment means across time were assessed with a multiple test comparison using a Tukey's test.

Data from postweaning BW, ADG, transformation index, rumen pH, rumen bacteria biodiversity, and apparent nutrient digestibility were analyzed with a mixed-effects analysis of variance. The statistical model included calf as a random effect, and feeding program as fixed effect. Body weight and age at weaning were both included in the model as covariates, and starter intake the last day of the study was included in the model as covariate for the statistical analysis of rumen pH.

Medical treatments within each week were analyzed with a mixed-effects logistic regression model using calf as a random effect and feeding program and time as fixed effects. Due to the low incidence of medical treatments after the fifth week of the study, the logistic regression model was only performed for the first 4 weeks of study.

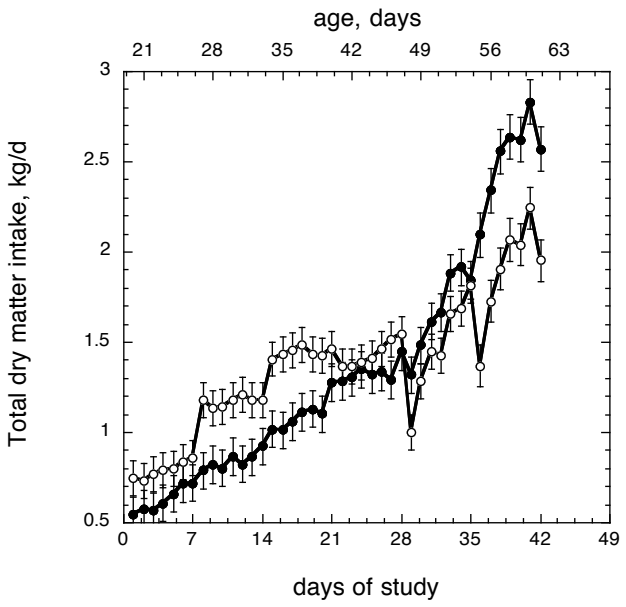
5.3. Results

There were no differences between treatments in days on medical treatments within any week of the study, with an average of 0.25 ± 0.140 treatments/calf/week.

5.3.1. Intake and performance

Calves in the CF treatment were characterized by a greater ($P < 0.05$) starter dry matter intake (DMI) compared with EF calves during the preweaning (0.68 vs 0.36 ± 0.078 kg/d, respectively) and postweaning (2.52 vs 1.90 ± 0.102 kg/d, respectively) periods, but total DMI was similar in both treatments (Table 5.2). However the evolution of total DMI was different ($P < 0.01$) between treatments (Figure 5.1). Calves in the EF treatment presented greater total DMI compared with CF calves from d 8 to d 20. Then, from d 21 to weaning, calves in both treatments had similar total DMI, and during the postweaning period, CF calves had greater ($P < 0.01$) total DMI compared with EF calves. Calves in both treatments presented similar water consumption from 25 d to the end of the study (Table 5.2). However, the ratio between water consumption and total DMI tended (P = 0.07) to be greater in EF compared with CF calves during the preweaning period, and it was greater ($P < 0.05$) the week after weaning in EF compared with CF calves.

Figure 5.1. Total dry matter intake of calves following a conventional (CF, ●) or an enhanced-growth (EF, ○) feeding program.



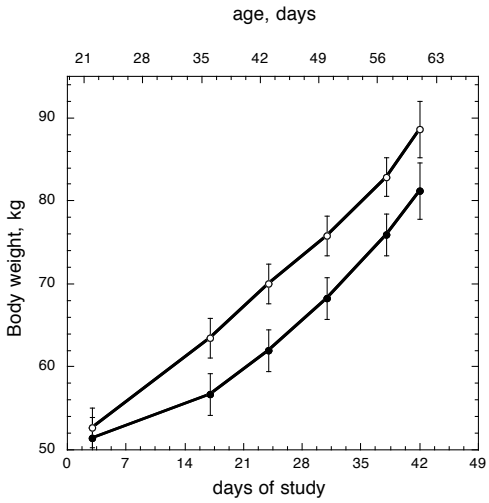
Calves in the EF treatment were numerically heavier than CF calves at the end of the study (Table 5.2), with a 7.4-kg difference in final BW (Figure 5.2). However, ADG of calves was similar in both treatments throughout the study, but the pattern of the ADG according to time was different ($P < 0.01$) during the preweaning period. Calves in the EF treatment maintained a constant ADG during the first 31 d of study (0.84 kg/d ± 0.092), whereas CF calves steadily increased their ADG (from 0.38 to 0.91 kg/d ± 0.098) during that same period. Then, ADG increased linearly in both treatments. Transformation indexes were similar between treatments during the pre and postweaning periods (Table 5.2), and they also evolved similarly throughout the study.

Table 5.2. Least squares means of performance in calves following a conventional (CF) or an enhanced-growth (EF) feeding program.

	Feeding Program			*P*-value[a]		
Item	CF	EF	SE[b]	FP	T	FP x T
Preweaning, 1-35 d						
Initial BW, kg	51.4	52.6	2.45	-	-	-
ADG, kg/d	0.79	0.88	0.076	0.38	< 0.001	0.001
DMI of MR, kg/d[c]	0.43	0.90	0.010	-	-	-
DMI of starter, kg/d	0.68	0.36	0.078	0.01	< 0.001	0.005
TDMI, kg/day	1.12	1.26	0.078	0.21	< 0.001	< 0.001
Water consumption, l/d	3.52	4.55	0.658	0.29	< 0.001	0.62
Ratio water to TDMI, l/kg	2.33	3.17	0.302	0.07	< 0.001	0.17
Transformation index[d]	1.88	1.65	0.117	0.19	0.65	0.11
Postweaning, 36-42 d						
Final BW, kg	81.2	88.6	3.36	0.14	-	-
ADG, kg/day	1.38	1.37	0.153	0.97	-	-
DMI of starter, kg/d	2.52	1.90	0.102	< 0.001	< 0.001	0.94
Water consumption, l/d	6.70	6.36	0.419	0.58	< 0.001	0.60
Ratio water to TDMI, l/kg	2.71	3.45	0.189	0.02	< 0.001	0.51
Transformation index[d]	1.98	1.99	0.452	0.99	-	-

[a] FP = effect of feeding program; T = time; FP x T = interaction between feeding program and time.
[b] SE = standard error of the mean
[c] arithmetic mean of the entire preweaning period
[d] calculated as the ratio total dry matter intake:average daily gain

Figure 5.2. Body weight of calves following a conventional (CF, ●) or an enhanced-growth (EF, ○) feeding program.



5.3.2. Nutrient apparent digestibility

Apparent DM, OM, NDF, CP, and GE digestibility coefficients were greater ($P < 0.05$) in CF compared with EF calves the week after weaning (Table 5.3).

Table 5.3. Least squares means of nutrient apparent digestibility in calves following a conventional (CF) or an enhanced-growth (EF) feeding program measured the week after weaning.

	Feeding Program			*P*-value[a]
Item	CF	EF	SE[b]	FP
Apparent digestibility, %				
DM	77.4	71.8	1.23	0.009
OM	78.7	73.2	1.18	0.007
CP	77.1	71.6	1.29	0.01
NDF	34.7	20.3	3.79	0.02
GE	75.6	69.8	1.25	0.007

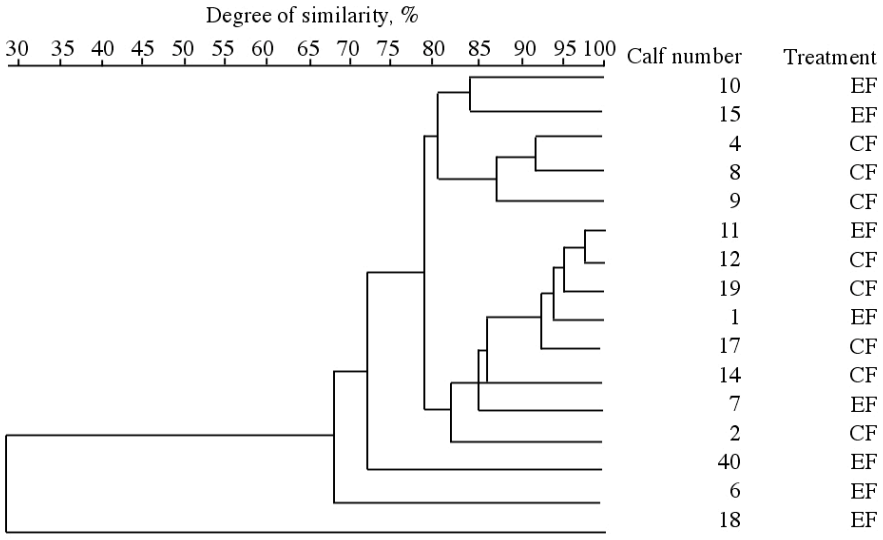
[a] FP = effect of feeding program
[b] SE = standard error of the mean

5.3.3. Ruminal pH and ruminal bacteria diversity

Rumen pH was lower ($P < 0.05$) in CF compared with EF one week after weaning (5.73 vs 5.99 ± 0.078).

The T-RFLP analysis was performed on 8 animals per treatment, since no liquid rumen sample was obtained from one EF calf, and 2 DNA samples (one for CF and another for EF treatments) did not amplify by the PCR analysis. Dendogram of T-RFLP analysis of rumen bacteria from ruminal liquid at the end of the study (Figure 5.3) indicated a 67% degree of similarity in ruminal bacteria population in any calf independently of treatment. There were no differences ($P = 0.56$) in bacteria biodiversity (number of bands from the T-RFLP analysis) between treatments (58.4 vs 53.6 ± 5.61, in CF and EF calves, respectively).

Figure 5.3. Dendogram of T-RFLP analysis of rumen bacteria from ruminal liquid at d 42. Each line corresponds to one rumen sample from one calf in one of the two treatments (CF: conventionally-fed calves; EF: enhanced-fed calves), as indicated by the treatment column. The closer the vertical lines, the greater the rumen bacteria similarity between calves.



5.3.4. Blood parameters

Serum urea concentrations were similar between treatments (Table 5.2), but they evolved differently depending on the feeding program as indicated by the interaction between feeding program and time ($P < 0.05$). Calves in EF treatment increased serum urea

concentration from 24 to 37 d of study, and CF calves presented similar serum urea concentrations from 24 to 31 d, and later increased until 37 d of study.

Serum NEFA concentrations were greater ($P < 0.05$) at 31 d of study, and they were numerically greater at 24 and 37 d of study in EF compared with CF calves (Table 5.4). Furthermore, serum NEFA concentration tended ($P = 0.10$) to evolve differently between treatments. Calves in CF treatment maintained unchanged serum NEFA concentrations throughout the study, in contrast to EF calves that increased serum NEFA concentrations from 24 to 31 d of study and, then decreased until 37 d of study.

Table 5.4. Least squares means of serum urea and NEFA concentrations in calves following a conventional (CF) or an enhanced-growth (EF) feeding program throughout the study.

	Feeding Program			*P*-value[a]		
Item	CF	EF	SE[b]	FP	T	FP x T
Urea, mmol/l	3.49	3.29	0.116	0.25	< 0.001	0.04
NEFA, mmol/l	0.07	0.11	0.010	0.02	0.03	0.10

[a] FP = effect of feeding program; T = time; FP x T = interaction between feeding program and time.
[b] SE = standard error of the mean

5.4. Discussion

The lower starter DMI during the preweaning period in EF compared with CF calves has been previously reported in several studies (Jasper and Weary, 2002; Shamay et al., 2005; Quigley et al., 2006; Terré et al., 2006). However, the lower starter intake maintained the week after weaning in the current study, have only been previously reported by Bar-Peled et al. (1997), Cowles et al. (2006) and Terré et al. (2006), but it has not been observed by Jasper and Weary (2002) and Quigley et al. (2006). This discrepancy among studies could probably be attributed to the weaning method applied in each study, and to the amount of milk or MR consumed the week before weaning. In the Bar-Peled et al. (1997) and in Cowles et al. (2006) and Terré et al. (2006) studies, milk or MR reduction before weaning was nil and lower, respectively, than in Jasper and Weary (2002) and Quigley et al. (2006) study. The decrease in ADG around weaning reported by Bar-Peled et al. (1997), Jasper and Weary (2002), Cowles et al. (2006) and Terré et al. (2006) was not found in the current study, but results of the current study agree with those reported by Quigley et al. (2006). One reason for this discrepancy can be found in the gender of calves. The studies by Bar-Peled et al. (1997), Jasper and Weary (2002), Cowles et al. (2006) and

Terré et al. (2006) were conducted with female Holstein calves, whereas the study by Quigley et al., (2006) was conducted with male Holstein calves in their study. Male calves following an enhanced-growth feeding program and a 25.5% CP calf starter decreased ADG after weaning, but they presented greater total DMI and ADG two weeks after weaning when compared with female calves with the same feeding program (Stamey et al., 2005; Stamey personal communication).

The immediate increase of water intake after weaning observed in the current study is consistent with previous reports (Quigley et al., 2006). The tendency of EF calves to drink more water per kg of DMI during the preweaning period than CF calves may be the result of the greater MR dilution rate in EF calves (Jenny et al., 1978). However, after weaning, it suggested that EF calves attempted to compensate the lack of MR offered with an increase consumption of water (Terré et al., 2006).

Although there were differences in nutrient apparent digestibility coefficients, no statistical differences for performance (ADG and transformation index) at weaning were found. This might be attributed to the high coefficients of variation in ADG (34%) and transformation index (46%) compared with the coefficients of variation of nutrient digestibility (6% for DM or CP digestibility), in conjunction with the relative low number of calves (Kertz and Chester-Jones, 2004). However, the purpose of this study was to evaluate differences in digestibility, and not to detect statistical differences in performance.

The apparent starter digestibility observed in the current study was greater than that found in calves weaned at 42 d of age (Spanski et al., 1997), but similar to that reported by Funaba et al. (1994). The improvement in nutrient digestibility coefficients in CF compared with EF calves may be attributed to an early starter consumption by CF calves, and consequently to an improvement on ruminal microbiota and fermentation activities as suggested by Anderson et al. (1987b). According to Abdelsamei et al. (2005), the feeding of increasing amounts of milk, decreased alfalfa hay intake, while there were only numerical differences in alfalfa hay DM digestibility with the greatest value for the calves fed the lowest milk level. Similarly, early-weaned calves, which consumed 1.79 kg/d DM of concentrate the week after weaning, had lower DM digestibility (71.4%) than late-weaned calves that consumed 3.44 kg/d DM of concentrate (77.0% of DM digestibility) 1 week after weaning (Funaba et al., 1994). High starter DMI has been associated with low nutrient digestibility due to a high passage rate (Seo et al., 2006). However, Broesder et

al. (1990) did not find any effect of high forage intake compared with low forage intake on passage rate, suggesting an increase of gut capacity as DMI increased.

The lower rumen pH in CF compared with EF calves suggested a greater ruminal activity in CF calves (Anderson et al., 1987). This may be a consequence of the greater starter intake in CF than in EF calves the day that rumen samples were obtained, as denoted by the significant effect of the covariate (starter intake of that day) in the analysis of variance performed. The absolute values for rumen pH reported in the present study may be overestimated since rumen samples were obtained using a stomach tube. The present values were higher than those obtained from cannulated-weaned calves as reported in the literature, 5.25 (Anderson et al., 1987), 5.69 (Vazquez-Añon et al., 1993), or 5.60 (Lesmeister and Heinrichs, 2004a). This may indicate some salivary contamination of rumen samples in the current study when using a stomach tube (Duffield et al., 2004).

Although changes the quantity and quality of the diet may affect ruminal species diversity, size and activity of the microbial population in the rumen (Theodorou and France, 2005), there were no differences in rumen bacteria similarity or biodiversity between treatments. Neither the quantity nor the activity of rumen bacteria were quantified by T-RFLP analysis. The improvement of apparent nutrient digestibility in calves raised conventionally should be attributed to differences in activity or number of rumen bacteria, but not in changes in population, as suggested by the increase of microbial duodenal flow in calves raised conventionally compared with calves raised following an enhanced-growth feeding program in a previous study (Terré et al., 2006).

However, an EF calf (number 18) showed only a 30% degree of similarity to other calves, and had 44 T-RFLP bands compared with the 56.8 bands that averaged the other calves of the study. Curiously, calf number 18 presented the lowest starter intake (0.4 kg DM/d) the week before weaning, and consumed the lowest starter DM (1.47 kg DM/d) the week after weaning. This low starter DMI the week before and after weaning, might have influenced rumen microbiota, and consequently EF calf number 18 showed a different rumen bacteria population when compared with the other calves. Thus, a low starter intake at weaning might change rumen bacteria population as suggested by Warner (1962) and Theodorou and France (2005).

Serum urea concentration was similar between treatments, and both treatments increased serum urea concentrations 2 days after weaning. This increase in serum urea concentration after weaning has been also observed in the literature (Quigley et al., 2006).

The increased of serum NEFA concentration on EF calves at 31 d, may be related to the fact of reducing MR to one feeding per day. An increase in serum NEFA concentration has also been reported in early-weaned calves in response to a low energy intake at weaning that may indicate body fat mobilization (Luchini et al., 1993). However, serum NEFA concentration returned to the same levels as CF calves 2 days after weaning.

5.5. Conclusion

Calves on an enhanced-growth feeding program had lower dry matter intake during the preweaning and postweaning periods, and lower apparent nutrient digestibility coefficients than calves reared conventionally the week after weaning.

Chapter 6

LONG-TERM EFFECTS OF AN ENHANCED-GROWTH FEEDING PROGRAM DURING THE PREWEANING PERIOD ON HEIFER PERFORMANCE

6.1. Introduction

Feeding costs per kg of BW gain with enhanced-growth feeding programs have been reported to be greater than these associated with conventional feeding programs (Quigley et al., 2006). However, the advantage in BW during the preweaning period may result in a decrease of age at the puberty onset, an increase of fat-corrected milk yield at first lactation (Shamay et al., 2005), or a reduction of age at first calving (Bar-Peled et al., 1997), that may improve the profit of enhanced-growth feeding programs.

The objective of this study was to compare growth of calves fed conventionally or following an enhanced-growth feeding program on performance during the preweaning period and reproductive performance at breeding.

6.2. Materials and Methods

6.2.1. Animals and Treatments

Sixty female Holstein calves (weighing 43.2 ± 0.58 kg and aging 9.8 ± 0.61 d) arrived from different farms to a commercial contract-heifer operation (Rancho Las Nieves, Mallén, Spain) under the approval and supervision of the Animal Care Committee of IRTA. Upon arrival, they were weighed and housed in individual hutches (1.07 x 1.60 m). Then, calves were randomly distributed according to BW in 2 groups: 31 calves were assigned to a conventional feeding program (CF) and 29 calves to an enhanced-growth feeding program (EF). The first week of study was considered as an adaptation period, during which calves in CF treatment received 4 l/d of MR at 12% DM dilution rate, and EF calves received during the first 3 d of the adaptation week 4 l/d at 12% DM dilution rate, and during the last 4 d of the adaptation week 4 l/d at 15% DM dilution rate. After the adaptation period, CF calves were fed 4 l/d of MR at 12% DM dilution rate from 1 to 27 d, and 2 l/d offered in the afternoon feeding from 28 to weaning day at 34 d (50 d of age). The EF calves were offered MR at 18% DM dilution rate according to the following schedule: 4 l/d from 1 to 6 d, 6 l/d from 7 to 13 d, 7 l/d from 14 to 20 d, 6 l/d from 21 to 27 d, and 3 l/d offered in afternoon feed from 28 to 34 d of study. All groups received the same MR (Sprayfo Excellent 60, Sloten BV, Holland) that was offered in feeding bottles twice daily at 0700 and 1700, and the same calf starter until 1 week after weaning (Table 6.1). Water was offered *ad libitum* throughout the study. Calves were housed individually until 1 wk after weaning. Then, they were moved to groups of 6 calves and were fed a TMR (Table 6.1) until 56 d of study (72 d of age). Heifers were kept within the same groups until they reached the target BW of 116 kg. Then, these pens were combined in

groups of 4 and transferred to other pens forming groups of 24 heifers and were fed a TMR containing 18.1% CP and 11.21 kJ of ME/kg (DM basis) until they reached 160 d of age and a target BW of 164 kg. These pens were later grouped again to form single groups of approximately 70 heifers and fed a TMR containing 17.4% CP and 9.67 kJ of ME/kg (DM basis) until they were 215 d old and reached a target BW of 213 kg. Later, these pens were further combined into single groups of approximately 130 heifers and were fed a TMR containing 16.8% CP and 9.41 kJ of ME/Kg (DM basis), and the animals did no leave this pen until they reached a target BW of 261 kg at an age of 270 d. In these pens, heifers were fed a TMR containing 16.2% CP and 9.20 kJ of ME/kg (DM basis), then at the age of 330 d all the animals weighing more than 310 kg were moved into other pens forming groups of approximately 120 heifers and fed a TMR containing 15.3% CP and 9.00 kJ of ME/kg (DM basis). Finally, at the age of 400 d heifers weighing more than 380 kg were moved to a breeding pen and were fed a TMR containing 14.1%CP and 8.83 kJ of ME/kg (DM basis). Once heifers were in the breeding pen, heats were checked three times a day, and heifers were inseminated 12 h after heat was detected. When a heifer did not reach the target BW at each specific age it was kept in the same pen, but its cohorts moved up to the following pen, and a new group of younger heifers moved in. Thus, the animals that were delayed (did not reach the target BW at a specific age) were regrouped with a new set of younger heifers. Animals that were delayed were weighed every 15 d until they reached the target BW and were, then, moved up one group.

Table 6.1. Chemical composition of milk replacer, starter, and first total mixed ration (TMR).

	Milk replacer	Starter	TMR
Nutrient composition, % of DM			
Crude protein	25.0	20.7	18.5
Ether extract	19.2	3.9	3.6
Neutral detergent fiber	0.2	20.5	27.5
Acid detergent fiber	-	9.7	14.5
Ash	6.5	5.9	6.9
Gross energy, MJ/kg of DM	20.78	18.73	18.81

During the preweaning period, animals were vaccinated against *Clostridium* spp. Miloxan (Merial, Lyon, France), and bovine respiratory syncytial virus (BRSV), *Parainfluenza-3*

virus, *Pasteurella haemolytica* with Bovipast RSP (Intervet, Boxmeer, Holland) at 23 d of age and they were revaccinated 3 weeks later.

6.2.2. Measurements

Milk replacer and starter intakes were measured daily from the beginning of the study to 42 d of study. Afterwards, from 42 to 56 d of the study, pen TMR intake was recorded daily. Body weight was measured once weekly from the beginning to 56 d of the study, and before every change from pen to pen (94, 149, 200, 387 d of study). Scour scores were recorded daily during the preweaning period following the scale (1=firm; not hard; 2=soft; 3=runny; and 4=watery) proposed by Larson et al. (1977). Treatments, if needed, were recorded throughout the study. During the preweaning period, 10 ml of blood were collected from each calves by venipuncture of the jugular vein into a collection tube under vaccum at 1, 3, 5, 6 and 8 wk of study between 2 and 4 h after the morning offer of MR. Blood was kept cold in ice and centrifuged at 1500 x *g* 15 min to obtain serum. Serum samples were stored at -20ºC until subsequent determination of serum urea, NEFA, and insulin concentrations. Additionally, a 5-ml blood sample harvested with sodium fluoride and potassium oxalate was also obtained to determine plasma glucose concentration.

6.2.3. Chemical Analyses

Samples of MR and starter were analyzed for DM (24 h at 103 ºC), ash (4 h at 550 ºC), N content using the AOAC (1990) method (988.05) adapted for an automatic distiller Kjeldhal (Kjeltec Auto 1030 Analyser, Tecator, Sweden) and using $CuSO_4$/Se as a catalyst instead of $CuSO_4/TiO_2$, ether extract using the AOAC method (920.39) using petroleum ether for distillation instead of diethyl ether (AOAC, 1990), NDF, with sodium sulphite and heat-stable alpha-amylase, ADF (van Soest et al. 1991), and gross energy with an adiabatic calorimeter (IKA-calorimeter C 4000, Heitersheim, Germany).

Enzymatic determination of plasma glucose, and serum urea, and NEFA were conducted following the HK/G-6-PDH method (Burrin and Price, 1985), the urease-GLDH method (Gutmann and Bergmeyer, 1974), and the ACS-ACOD method (Wako, Osaka, Japan), respectively. Serum insulin concentration was determined using an ELISA kit (Mercodia, Uppsala, Sweden).

6.2.4. Statistical Analyses

An analysis of variance with repeated measures was used to study the changes in body weight, gain to feed ratio, MR and starter intakes, and blood parameters during the preweaning period. The statistical model included calf as a random effect, feeding program, sampling time, and the interactions between the two main factors as fixed effects. Time entered the model as a repeated measure.

Performance from 42 to 56 d of study was analyzed considering calf within pen as random effect, and feeding program, sampling time, and the interaction between these two fixed factors as fixed effects. Total intake of TMR was analyzed considering pen as experimental unit. Time entered the model as a repeated measure.

For each analyzed variable, calf nested within feeding program was subjected to 3 variance-covariance structures: compound symmetry, autoregressive order one, and spatial power. The variance-covariance structure that yielded the smallest Schwarz's Bayesian criterion was considered to be the most desirable matrix. Comparisons with $P \leq 0.05$ were considered as significant, whereas comparisons with $P \leq 0.10$ were presented as tendencies. Differences between treatment means across time were assessed with a multiple test comparison using a Tukey's test.

Performance from 56 d to the end of the study was analyzed with the same model as data from the preweaning period, but time entered the model as a repeated measure only using the spatial power variance-covariance structure.

Due to the lack of normality, serum NEFA and insulin concentrations, and the ratio insulin to glucose were analyzed after a ln-transformation. Least square means for these parameters presented herein correspond to non-transformed data, and SE and *P*-values correspond to the ANOVA analysis using ln-transformed data, respectively.

Due to the low incidence of medical, oral rehydratant and presence of loose feces in the study, only data from the first two weeks of the study (where more incidences occurred) were used to perform the statistical analysis. Fecal scores were grouped in two categories: score of 1 and 2 were considered a single category illustrating absence of loose feces, and scores of 3 and 4 were grouped into a second category representing presence of loose feces. Then, data were split in 2 categories for every calf and week: absence of loose feces within a week (0), or presence of loose feces at least once within a week (1). Then, a binary logistic regression with calf as a random effect, and week and feeding program as fixed effects was performed. Medical and oral rehydratant treatments were analyzed

similarly to the presence of loose feces within a week. Therefore, data were split in two categories: no medical treatments or any medical treatment within a week. After that, a binary logistic regression was performed including calf as random effect, and week, feeding program, and their interaction as fixed effects.

Delays due to insufficient BW were analyzed as the number of delays throughout the study within the total number of weights per heifer. Then, data were analyzed as a Poisson regression model including feeding program as a fixed effect.

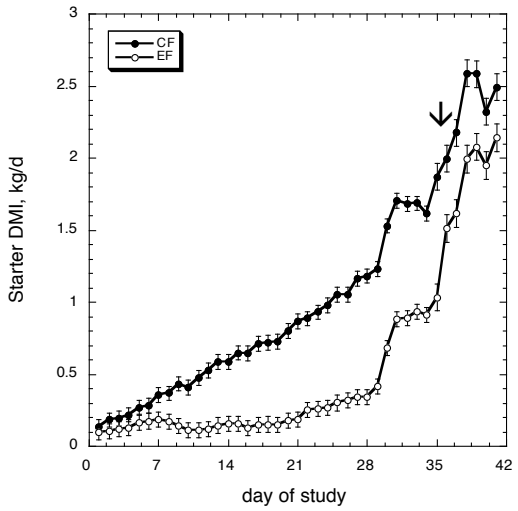
The reproductive performance parameters analyzed included: age of heifers at the entrance to the breeding pen, age at first breeding, age at pregnancy, and fertility at first breeding. These parameters were analyzed with an analysis of variance including feeding program as a fixed effect. The pregnancy rate at first breeding was analyzed by a binary logistic regression including feeding program as a fixed effect.

6.3. Results and Discussion

6.3.1. Performance

As a result of the high amounts of MR offered during the preweaning period, starter DMI was lower ($P < 0.001$) in EF compared with CF calves during the preweaning period and the week after weaning (Table 6.2). Furthermore, starter DMI evolved differently ($P < 0.001$) during the preweaning period. Starter DMI of CF calves increased linearly from the 7th d of the study and throughout the preweaning period, whereas EF calves presented a slightly linear increase of starter DMI from the 22th d of the study (Figure 6.1). Although total DMI was similar in both treatments during the preweaning period, it evolved differently throughout the preweaning period as indicated by the interaction between feeding program and time (Table 6.2). During the first 20 d of the study EF calves had greater total DMI than CF calves, as a result of the greater MR intake. Thereafter, as CF calves increased starter DMI, total DMI was similar between treatments, and from 25 to 34 d of the preweaning period, CF calves had greater total DMI than EF calves. After mixing calves from the same feeding program in groups of 6, similar DMI were observed in both treatments (Table 6.2).

Figure 6.1. Starter dry matter intake of calves following a conventiona (CF, ●) or an enhanced-growth (EF, ○) feeding program. The arrow points the weaning day.



Calves in the EF treatment had greater ($P < 0.05$) ADG compared with CF calves during the preweaning period. The rate of growth of EF calves during this period was similar to that observed by Shamay et al. (2005) when feeding high amounts of milk, but greater than the rates reported in other studies (Brown et al., 2005b; Quigley et al., 2006). Probably, calves age, gender and origin, and the presence of an adaptation week before the start of the study may explain these differences in ADG. However, similar ADG were observed in both treatments the week after weaning, and the first 2 wk after grouping calves (Table 6.2). Moreover, the interaction between feeding program and time indicated that during the first 3 wk of study ADG was greater ($P < 0.001$) in EF compared with CF treatment, but it was similar in both treatments during the 4th wk of study, and it was lower in EF than in CF treatment the week before weaning when MR was reduced to one meal per day. This decrease in ADG the week before weaning in EF calves was also observed in other studies (Bar-Peled et al., 1997; Jasper and Weary, 2002). It may be explained by the decrease of energy and protein intake of EF calves (6.4% less GE and 9.2% less CP intake than the week before) when MR was reduced to one feeding per day. Contrarily to CF calves, that continued to increase GE and CP intake the week before weaning (19.4% more GE and 17.3% more CP intake than the week before). A common pitfall encountered in EF programs is the low starter intake at weaning (Bar-Peled et al., 1997; Brown et al., 2005b; Cowles et al., 2006). To avoid the reduction in ADG and encourage starter intake around weaning, a more gradual weaning process should be used (Brown et al., 2005b). A recent study (Khan et al., 2007), showed that a step-down program successfully weaned calves. This program consisted on feeding milk at 20% BW

during the first 25 d of age, milk at 10% BW from 26 to 45 d, and finally calves were weaned gradually by diluting milk with water from 46 to 50 d. Alternatively, starter manipulations to increase its palatability and consumption should be envisaged.

Table 6.2. Least squares means of performance and intake of calves following a conventional (CF) or an enhanced-growth (EF) feeding program.

	Feeding Program			*P*-value[a]		
Item	CF	EF	SE[b]	FP	T	FP x T
Preweaning, 1-34 d						
Initial BW, kg	43.6	44.8	1.10	-	-	-
BW, kg	55.9	60.7	1.05	0.002	< 0.001	< 0.001
ADG, kg/d	0.80	0.90	0.031	0.02	< 0.001	< 0.001
DMI of MR, kg/d	0.41	0.90	0.174	< 0.001	< 0.001	< 0.001
DMI of starter, kg/d	0.79	0.29	0.043	< 0.001	< 0.001	< 0.001
TDMI, kg/d	1.20	1.19	0.043	0.84	< 0.001	< 0.001
Gain:feed ratio	0.70	0.77	0.040	0.11	< 0.001	0.04
Postweaning, 35-41d						
BW at 41 d, kg	79.9	84.5	1.71	0.06	-	-
ADG, kg/d	1.28	1.23	0.071	0.65	-	-
DMI of starter, kg/d	2.29	1.76	0.084	< 0.001	< 0.001	< 0.001
Gain:feed ratio	0.57	0.72	0.035	0.003	-	-
Postweaning, 42-56 d						
BW at 56 d, kg	95.7	100.7	3.52	0.37	-	-
ADG, kg/d	1.13	1.20	0.065	0.47	< 0.001	0.89
TMR intake, kg/d	2.73	2.58	0.179	0.57	< 0.001	0.98
Heifers, 57-387 d						
BW at 387 d, kg	401.3	406.3	4.05	0.22	-	-
ADG, kg/d	0.96	0.95	0.019	0.77	0.009	0.69

[a] FP = effect of feeding program; T = time; FP x T = interaction between feeding program and time.
[b] SE = standard error of the mean

Nevertheless, after mixing calves in groups, ADG evolved similarly in both treatments. All calves decreased ADG the first week after mixing, but calves recovered the ADG observed the week after weaning during the 2nd wk following mixing of groups. After that, heifers presented similar ADG throughout their growing period (Table 6.2). The

decrease of the rate of growth after grouping calves may be the result of mixing calves and diet change from a cracked starter to a TMR, since the fact of mixing calves (O'Driscoll et al., 2006) or change of regime (Girard et al., 1993) can affect feeding behavior. Overall, differences in BW tended to be greater ($P = 0.06$) in EF compared with CF calves until 41 d. But BW was only numerically greater in EF than in CF calves with a 5.0 kg of BW difference at 387 d of study (Table 6.2). Although 36% more delays to insufficient BW were observed in CF compared with EF heifers, this difference was not significant ($P = 0.22$). The gain to feed ratio tended ($P = 0.11$) to be greater in EF than in CF calves during the preweaning period. Furthermore, the interaction of feeding program with time ($P < 0.05$) showed that calves in EF treatment presented greater gain to feed ratio up to 13 d of the study compared with CF calves, but it was similar afterwards in both treatments throughout the preweaning period. Surprisingly, the gain to feed ratio was improved ($P < 0.01$) in EF calves the week after weaning (Table 6.2), because EF calves presented lower starter DMI the week after weaning than CF calves, and ADG was similar in both treatments that week. The improvement of feed efficiency during the first 2 wk of study in EF compared with CF calves were probably a result of the high degree of digestion in milk-based diets (Davis and Drackley, 1998). However, the decrease of gain to feed ratio in EF calves from 14 to weaning day may be attributed to the low capacity of EF to digest starter, as it was observed in enhanced-fed calves the week after weaning when compared with conventionally-fed calves (Terré et al., in press). However, no explanation was found to determine why EF calves consuming less starter than CF calves, performed similar to CF calves during the postweaning period.

The incidence of health problems was very low in both treatments and there were no differences in the incidence of loose feces between CF and EF treatments during the first 2 wk of the study. Similarly, there were no differences in the incidence of medical and oral rehydratant treatments in CF and EF calves throughout the first 2 wk of the study. However, other studies feeding additional MR have reported increased health problems (Quigley et al., 2006), higher fecal scores (Diaz et al., 2001), and higher medicated days (Cowles et al., 2006), although Jasper and Weary (2002) did not find any differences in the incidence of diarrhea when calves were fed milk conventionally or *ad libitum*. A recent study concluded that high growth rates in neonatal calves affected minimally the adaptive immune response (Foote et al., 2006). Thus, differences in incidence of health problems might be probably related to management and environmental practices rather than differences in calf immune response induced by the nutritional plan (Chua et al., 2002).

6.3.2. Blood Parameters

Plasma glucose and serum insulin concentrations were greater ($P < 0.001$) in EF compared with CF calves during the preweaning period (Table 6.3). Quigley et al. (2006) also observed greater plasma glucose concentrations when calves were fed additional amounts of MR. This can be explained by the greater amounts of lactose fed to EF compared with CF calves, since serum glucose and insulin concentrations follow lactose and total sugars intake (Hugi et al., 1997). Furthermore, both plasma glucose and serum insulin concentration showed the same evolution throughout the preweaning period. Calves in EF treatment showed greater ($P < 0.001$) plasma glucose and serum insulin concentration from 7 to 21 d of study than CF calves, and similar concentrations were observed in both treatments on 34 d. The increase of serum insulin concentrations from 7 to 21 d of the study in EF calves was probably the response of the high amount of ingested energy during the first 3 weeks of study (Hammon et al., 2002). However, after weaning, there were no differences between treatments neither in serum insulin concentration nor in plasma glucose concentration. Regarding the ratio insulin to glucose concentration during the preweaning period, it was greater ($P < 0.05$) in EF than CF calves, and similar in both treatments 2 wk after weaning. This indicated that more insulin was needed to be released to avoid an increase of plasma glucose levels during the preweaning period, but similar ratios insulin to glucose were observed in both treatments after weaning.

During the preweaning period, serum NEFA concentrations were greater ($P < 0.01$) in EF compared with CF calves, and their evolution tended ($P = 0.09$) to be different between treatments during this period (Table 6.3). Calves receiving the EF treatment showed an increase of serum NEFA concentration from 21 to 35 d of the study that was not observed in CF calves (Figure 6.2). This change occurred when MR was reduced to one daily feeding the week before weaning, and it may suggest the mobilization of energy reserves due to the decrease of energy intake that week, as it occurred in transported calves that substantially increased NEFA levels during their journey (Knowles et al., 1999). However, during the 2 wk after weaning, there were no differences in serum NEFA concentrations between treatments.

Figure 6.2. Serum NEFA concentration of calves following a conventional (CF, ●) or an enhanced-growth (EF, ○) feeding program. The arrow points the weaning day.

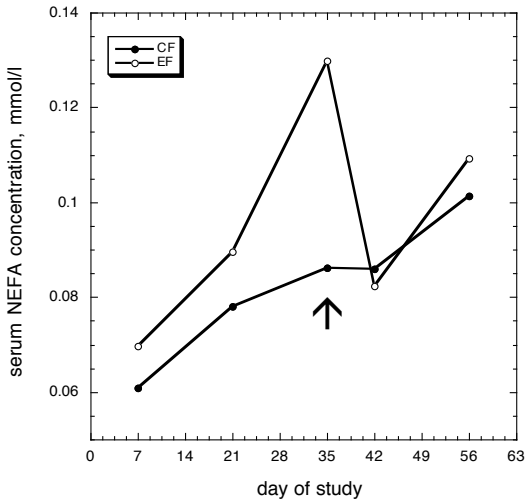


Table 6.3. Least squares means of blood parameters of calves following a conventional (CF) or an enhanced-growth (EF) feeding program.

	Feeding Program			*P*-value[a]		
Item	CF	EF	SE[b]	FP	T	FP x T
Preweaning, 1-34 d						
Plasma glucose, mmol/l	4.96	5.97	0.094	< 0.001	< 0.001	< 0.001
Serum insulin, µg/l[c]	0.57	1.12	0.108	< 0.001	< 0.001	< 0.001
Ratio insulin to glucose[cd]	0.117	0.190	0.106	0.002	< 0.001	< 0.001
Serum NEFA, mmol/l[c]	0.074	0.093	0.0402	0.002	< 0.001	0.09
Serum urea, mmol/l	3.50	3.22	0.096	0.04	< 0.001	0.014
Postweaning, 35-56 d						
Plasma glucose, mmol/l	4.66	4.81	0.079	0.19	0.46	0.36
Serum insulin, µg/l[c]	0.54	0.53	0.090	0.91	0.81	0.90
Ratio insulin to glucose[cd]	0.116	0.111	0.085	0.71	0.72	0.99
Serum NEFA, mmol/l[c]	0.093	0.095	0.0440	0.79	< 0.001	0.31
Serum urea, mmol/l	3.53	3.71	0.088	0.16	< 0.001	0.14

[a] FP = effect of feeding program; T = time; FP x T = interaction between feeding program and time.
[b] SE = standard error of the mean
[c] The least squares means for NEFA and insulin concentrations and the ratio glucose to insulin blood concentrations presented herein correspond to non-transformed data, and SE and *P*-values correspond to the ANOVA analysis using ln-transformed data, respectively.
d ratio insulin to glucose expressed as pg/l to mmol/l

Serum urea concentrations were greater ($P < 0.05$) in CF than in EF calves during the preweaning period (Table 6.3), and they also evolved differently ($P < 0.001$) during this period. Serum urea concentration decreased from 1 to 21 d of study in calves on the CF treatment, but serum urea concentration increased from 21 to 34 d of study in both treatments. However, during the 2 wk after weaning there were no differences between treatments. The lower serum urea concentrations observed in EF compared with CF calves during the preweaning period may indicate that although EF calves tended to consume more CP during this period compared with CF calves (data not shown), they utilized dietary N more efficiently than CF calves. Similarly, a recent study (Cowles et al., 2006) reported lower blood urea N when feeding calves on an enhanced-growth feeding program compared with conventionally fed calves. These results are surprisingly as literature usually describes a positive relationship between dietary CP and serum urea concentrations (Blome et al., 2003; Quigley et al., 2006). However, other studies (Smith et al., 2002; Terré et al., 2006) have not found differences in plasma urea concentration when comparing high and low levels MR or milk intake.

6.3.3. Reproductive Performance

There were no differences between treatments in age of entrance to the breeding pen, age at first artificial insemination, and age at pregnancy (Table 6.4). Although fertility at first breeding was similar in both feeding programs, EF heifers had 1.58 greater odds of becoming pregnant at first AI than CF calves ($P = 0.40$).

Overall, the long-term effects of calves fed high amounts of milk or MR are varied and they are not consistently repeated in the literature. Probably, different reproductive management practices after the preweaning period among the studies might reflect the different results. Shamay et al. (2005) synchronized heifer artificial insemination at 13 mo of age independently of their BW, but Bar-Peled et al. (1997) and Davis Rincker et al. (2006) based first artificial insemination on heifers BW above 330 and 390 kg, respectively. In contrast, the present study targeted first breeding when heifers were more than 400 d of age and weighed more than 380 kg.

On the other hand, the numerical improvement of fertility at first insemination in EF compared with CF heifers might envisage some positive effects on reproductive performance when feeding calves following an EF program.

Table 6.4. Reproductive performance of calves following a conventional (CF) or an enhanced-growth (EF) feeding program.

	Feeding program			*P*-value[a]
	CF	EF	SE[b]	FP
Age at entrance to breeding pen, d	421	418	3.2	0.60
Age at first breeding, d	429	430	4.4	0.81
Age at pregnancy, d	448	440	6.2	0.39

[a] FP = effect of feeding program
[b] SE = standard error of the mean

6.4. Conclusions

Enhanced-growth feeding programs improve calf average daily gain but impair starter intake during the preweaning period. However, the same dry matter intake level is achieved one week after weaning. Although a numerical body weight advantage is maintained in calves fed following an enhanced-growth feeding program, neither age at first breeding nor age at first pregnancy seem to be improved when calves are raised following an enhanced-growth feeding program during the preweaning period.

Chapter 7

GENERAL DISCUSSION

The first study was performed with the objective to compare the effects of a conventional and an enhanced-growth feeding program on N metabolism of dairy calves, since high amounts of milk replacer (MR) with high levels of CP had been recommended to improve performance of dairy calves (Diaz et al., 2001). During this study, we realized that weaning was especially stressful for calves on enhanced-growth feeding programs. Therefore, we designed a second study to evaluate the potentially positive effects of social facilitation when raising calves in groups with the aim of improving starter intake at weaning and address the weaning problem. Following the grouping study, we observed a decrease in feed efficiency when calves were weaned, and we decided to study the digestibility of a calf starter after weaning. Finally, we observed that the feeding cost (cost/kg ADG) of calves on enhanced-growth feeding programs during the preweaning period was greater than that of conventional feeding programs. Thus, we studied the long-term effects of enhanced-growth feeding programs to explore whether high amounts of MR during the preweaning period would contribute to improve productive parameters later in life.

Although all these studies were performed to analyze the effects of feeding high amounts of MR to dairy calves, not all studies followed the same feeding program; neither the same MR was used, nor the same calf starter was fed. Furthermore, calf origin was also different, and the studies were performed at different seasons and locations. This offers the possibility of discussing and relating the results with the factors that changed among the studies.

7.1. Performance

Feeding high amounts of MR to dairy calves improved ADG during the preweaning period in 2 out of the 3 studies that compared conventional and enhanced-growth feeding programs, consistent with most of the studies reported in the literature (Bar-Peled et al., 1997; Diaz et al., 2001; Jasper and Weary, 2002). However, in the digestibility study these differences were not observed, probably due, in part, to the low number of calves used. Performance parameters have a high variability that may preclude finding statistical differences between treatments when a low number of animals is used (Kertz and Chester-Jones, 2004). The comparison of the performance parameters of the long-term effect and digestibility studies showed that the difference in ADG between treatments was similar in both studies (0.10 kg/d vs 0.09 kg/d, respectively). However, in the long-term effects study, ADG was significantly greater in enhanced- than in conventionally-fed calves. This was probably due to the low number of calves used in the digestibility

study was not sufficient to find statistical differences in performance parameters (40 vs 10 in long-term effects and digestibility studies, respectively).

Obviously, MR intake was always greater in enhanced- compared to conventionally-fed calves, contrarily to starter DMI during the preweaning period that was always lower in enhanced- compared with conventionally-fed calves. The grouping study did not reach the high levels of MR offerings, because the dilution rate was 15%, in contrast to 18% dilution rate offered in the other studies, and because the preweaning period was one week shorter than in the other studies. Lower dilution rate in the grouping study was mainly used to avoid digestive disorders when MR increased.

However, total DMI during the preweaning period was not always greater in enhanced- compared with conventionally-fed calves. In the amino acid study, calves on an enhanced-growth feeding program presented greater total DMI compared with conventionally-fed calves. But, a similar total DMI in both treatments during the preweaning period was reported in the long-term effects and digestibility studies. This occurred because in the long-term effects and digestibility studies, starter intake in both treatments was greater than in the amino acid study. The main differences among the amino acid and the long-term effects and digestibility studies were the calf origin (purchased from commercial market vs directly harvested from the farm, respectively), and the calf starter presentation (pellet vs multiparticles, respectively). In relation to the first difference, calf origin, literature studies performed with purchased calves reported high rates of mortality, for instance, Blome et al. (2003) reported 14% of mortality, Bartlett et al. (2006) 21%, and Quigley et al. (2006) 12.3%. In contrast, literature studies conducted with newborn calves from a research facility did not observed death losses (Jasper and Weary, 2002; Heinrichs et al., 2003). Therefore, the fact of being shipped may affect performance parameters, especially those close to calf arrival to the research facilities. A greater lost of BW occurred in newly weaned beef cattle after transportation compared with newly weaned calves that remained in the preweaning period farm (Arthington et al., 2003). Furthermore, a reduction of DMI is normally associated with newly arrived feedlot cattle (Loerch and Fluharty, 2000). Regarding the second difference, calf starter presentation, calves fed a texturized starter consumed 43% more grain at 6 wk of age compared with calves fed a pelleted diet (Franklin et al., 2003). Similarly, a more recent study that used the same calf starter composition in a pelleted and a multiparticle form reproted a 15.4% increase in DMI when comparing a multiparticle with a pelleted starter the week before and after weaning (Bach et al., 2007).

Thus, either the calf origin or physical form of the diet may explain part of the differences in starter intake among the studies.

In the three studies where conventional and enhanced feeding programs were compared, enhanced-fed calves tended to have a better gain to fed ratio during the preweaning period, probably due to the greater MR intake, which is more digestible than starter. Some apparent dry matter digestibilities of MR reported in the literature are: 93.9% (Petit et al., 1988), 96.4% (Xu et al., 1998), 95.2% (Yuangklang et al., 2004), in contrast to lower values observed in apparent dry matter digestibilities of starter one week after weaning 74.8% (Funaba et al., 1994) or 73.1% (Funaba et al., 1997).

In any case, neither the negative effects (a decrease in the starter DMI), nor the positive effects (an increase of ADG) obtained from the enhanced-growth feeding program during the preweaning period were carried over beyond the first week after weaning. However, the differences in BW achieved due to the high levels of MR intake were significantly or numerically maintained during the postweaning (amino acid study) and growing (long-term effects study) periods, respectively.

In the three studies where a conventional and an enhanced-growth feeding program were compared, it was found that the feed cost per kg of BW gain during the preweaning period was greater in enhanced- compared with conventionally-fed calves (Table 7.1). The amino acid study presented the greatest difference of feed cost per kg of BW between the conventional and the enhanced-growth feeding program. It was mainly due to the lower cost of the starter in the amino acid study compared with the starter cost of the other two studies. This fact decreased the cost of the conventional-feeding treatment in the amino acid study. Furthermore, the greater the amount of MR offered to calves in the amino acid study raised the cost of kg of BW gain in the enhanced-growth feeding program. Although raising calves on an enhanced-growth feeding program is economically inefficient during the preweaning period, some long-term advantages are reported in the literature when feeding high amounts of milk or MR: a reduction of age at puberty (Shamay et al., 2005), a decrease of age at calving (Bar-Peled et al., 1997; Davis-Rincker et al., 2006), and an improvement of daily 3.5%-fat corrected milk at first lactation (Shamay et al., 2005). However, in the long-term effects study no differences were found in fertility at first breeding, age at first breeding, and age at first pregnancy

Overall, as mentioned in the introduction, there seems to be no benefit on the immune system function when feeding high levels of MR during the preweaning period. However, some benefits on milk yield or on reducing AFC may be envisaged when feeding high

levels of milk during the preweaning period. However, the same benefits are not consistently found in the literature. Thus, an accurate economic analysis should be conducted to either of the studies that evaluate enhanced-growth feeding program to determine whether the economic investment during the nursing period is recovered when reducing AFC or increasing milk yield at first lactation.

Table 7.1. Feed cost (€) per kg of body weight gain of calves following a conventional (CF) or an enhanced-growth (EF) feeding program during the preweaning period of amino acid, long-term effects, and digestibility studies.

	Feeding program			*P*-value
Study	CF	EF	SEM	FP effect
Amino acid[a]	1.77	2.82	0.117	< 0.001
Long-term[b]	1.19	1.68	0.040	< 0.001
Digestibility[c]	1.44	1.97	0.115	0.005

[a] cost of MR 1.58 €/kg, cost of starter 0.21 €/kg
[b] cost of MR 1.59 €/kg, cost of starter 0.31 €/kg
[c] cost of MR 1.63 €/kg, cost of starter 0.31 €/kg

7.2. Evaluation of average daily gain prediction by the NRC

The chapter on nutrient requirements of the young calf from the National Research Council (NRC, 2001) reports several equations to predict nutrient requirements of young calves. The NRC (2001) reported that these equations were in good agreement to 16 previous research studies. Mainly, these studies raised calves following a conventional feeding program, feeding MR at the rate of 8 to 12 % BW, or 0.54 kg/d. Therefore, it would be interesting to check whether data from the four studies presented in the current report fit well to the NRC (2001) predicting equations. Our data were used to compare the observed ADG during the preweaning period of calves with the predicted ADG based on the ME requirement equations from the NRC (2001). The NRC (2001) equations are:

$$ME_i\ (ingested) = (MRintake\ x\ ME_{MR}) + (starter\ intake\ x\ ME_s), \quad [8]$$

where ME_{MR} and ME_s were considered 5.12 Mcal/kg DM of MR, 3.28 Mcal/kg DM of starter for the amino acid and grouping study, and 4.72 Mcal/kg DM of MR, 3.38 Mcal/kg DM of starter in the long-term effects and digestibility study

$$NE_m\ (maintenance) = 0.086\ BW^{0.75}, \quad [9]$$

where BW is body weight in kg

$$ME_m = NE_m/efficiency\ of\ use\ of\ ME_m, \quad [10]$$

where efficiency was computed as the weighed average of efficiencies of MR (0.86), and starter (0.75)

$$NE_g\ (gain) = 0.84\ x\ BW^{0.355}\ x\ ADG^{1.2}\ x\ 0.69, \quad [11]$$

where ADG is the average daily gain in kg/d

$$ME_g = NE_g/efficiency\ of\ use\ of\ ME_g, \quad [12]$$

where efficiency was computed as weighed average of efficiencies of MR (0.69), and starter (0.57)

Therefore,

$$ADG = \exp((\ln[((ME_i - ME_m)\ x\ (efficiency\ of\ ME_g)) / (0.84\ x\ BW^{0.355}\ x\ 0.69)]/1.2) \quad [13]$$

After calculating the predicted individual ADG values using the NRC (2001) equations, a linear mixed-effects model similar to the model reported in each study was performed. The least square means for the predicted ADG were nearly equal for the conventional

treatment in the amino acid study, and for the enhanced-growth treatment in the long-term effects and digestibility studies (Table 7.2). Nevertheless, these equations overpredicted ADG of calves following an enhanced-growth treatment in the amino acid and grouping studies, and underpredicted the ones following a conventional treatment in the long-term and digestibility studies.

Table 7.2. Comparison of observed average daily gain of calves on a conventional (CF) or an enhanced-growth (EF) feeding program during the preweaning period in four studies with the predicted average daily gain values from the NRC (2001) metabolizable energy (ME) equation.

	Treatment[a]			
	CF		EF	
Study	observed	ME pred[b]	Observed	ME pred[b]
Amino acid	0.53	0.54	0.84	1.05
Long-term	0.80	0.71	0.90	0.91
Digestibility	0.79	0.62	0.88	0.87
Grouping	-	-	0.58	0.66

[a] CF: conventional feeding program; EF: enhanced-growth feeding program
[b] ADG = exp((ln[((ME$_I$ – ME$_M$) x (efficiency of ME$_g$)) / (0.84 x BW$^{0.355}$ x 0.69)]/1.2) [14]; ME$_i$ metabolizable energy intake, ME$_m$ metabolizable energy for maintenance, BW body weight

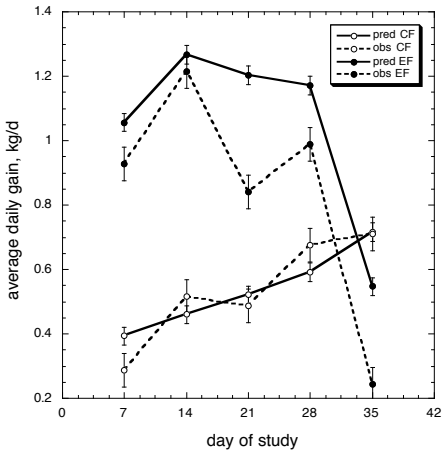
The NRC (2001) overprediction of ADG for the calves in the enhanced treatment in the amino acid study is mainly due to the lower ADG observed at 21 d of study compared with the predicted ADG estimate (Figure 7.1, A) which may suggest that EF calves at that week used part of their consumed ME to cope with outside factors that were restricting growth such as disease or stress. Lesmeister and Heinrichs (2005) reported a slightly overprediction of ADG during the preweaning period in conventionally-fed calves (370 g/d vs 357 g/d, predicted and observed ADG values). But, the predicted ADG values during the postweaning period in the Lesmeister and Heinrichs (2005) study disagree with the observed values, probably because few studies were conducted with weaned calves below 100 kg of BW to correctly determine efficiencies of utilization of protein and energy for growth.

On the other hand, the ADG underprediction in conventionally-fed calves is mainly due to the underprediction of ADG at 14, 21, and 35 d of study in the long-term effects study, and at 28 and 35 d of study in the digestibility study (Figure 7.1, B, C).

Several hypotheses may be envisaged to explain the ADG underprediction of conventionally-fed calves in the long-term effects and digestibility studies. Firstly, an oversupply of protein (relative to energy) in the conventional diet of calves in the long-term effects and digestibility studies that would allow calves to obtain energy to grow might explain the ADG underprediction obtained when using the ME requirement equation. This hypothesis is supported by the greater serum urea concentration obtained in conventionally-fed compared with calves following an enhanced-growth feeding program during the preweaning period in the long-term effects and digestibility studies (Table 7.4).

Figure 7.1. Comparison of observed and predicted average daily gain, using the equation based on ME requirement (NRC, 2001), values during the preweaning period of calves on a conventional and an enhanced-growth feeding program in different studies. A=Amino acid study. B=Long-term effects study. C=Digestibility study. D=Grouping study.

A.



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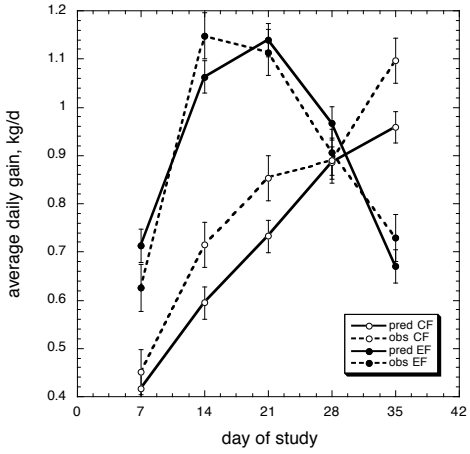

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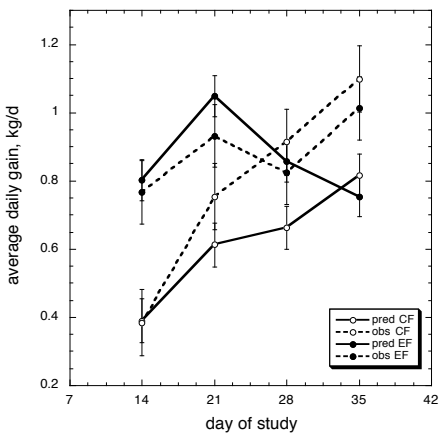
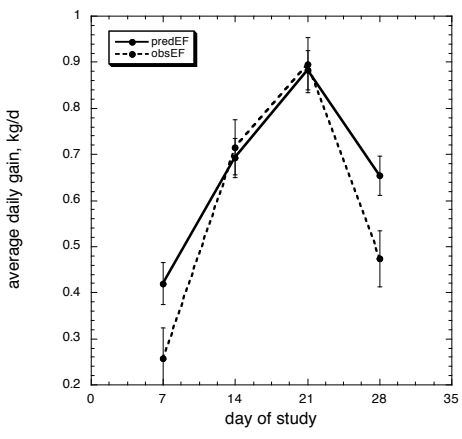


D.



A second hypothesis to explain the NRC (2001) underprediction of ADG for the conventionally-fed calves in the long-term effects and digestibility studies may be related to the greater starter intake of conventional treatment of these studies compared with the conventional treatment of the amino acid study. Calves from the long-term effects study had 47% more starter intake than calves from the amino acid study, and calves from the digestibility study consumed 40% more starter than calves from the amino acid study. Therefore, calves from the long-term effects and digestibility studies were more adapted to dry feed intake, and consequently they might have had a better starter digestibility and absorption of energy and protein, since rumen function seems to be independent of age at weaning, but mainly determined by dry feed intake (Lallès and Ponchet, 1990).

A third hypothesis may question the fixed estimated factors proposed in the NRC (2001). For instance, the NRC (2001) estimated the biological value of milk protein according to the literature (Donnelly and Hutton, 1976). A recent study suggested that 0.80 was a reasonable estimated for biological value of milk protein (Blome et al., 2003), and apparent biological values were similar when feeding different amounts of MR (Diaz et al., 2001; Bartlett et al., 2006). Therefore, 0.80 seems to be an adequate estimator of milk protein biological value for conventional and enhanced-growth treatments. However, data from calf starter biological values were based on NRC (1978), which values were obtained from studies of 7 wk-weaned calves weighing more than 100 kg (Stobo and Roy, 1973), or 75-kg Holstein heifers (Lofgreen et al., 1951). Moreover, Lofgreen et al. (1951) also reported that biological values for proteins in ruminants might vary considerably. Furthermore, these values are based on early studies, and current feed manufacturing process may have improved biological protein values of calf starter. Similarly, the efficiency of use of ME of MR for gain was considered 0.69, whereas Davis and Drackley (1998) reported a range of 0.72 to 0.75.

Overall, the last hypothesis may be the most reasonable. As we did not known accurately the “fixed values” for our experiments, they might have been over or under predicted by the average value used by the NRC (2001) prediction equations. For example, an efficiency of use of ME for gain of calf starter greater than 0.57 in the long-term effects study would have increased the predicted ADG for conventionally-fed calves.

7.3. Blood parameters

7.3.1. Glucose and Insulin

Serum insulin and glucose concentrations will be discussed as the ratio between both of them, to establish how much insulin was necessary to maintain serum glucose concentration steadily. Glucose concentrations in plasma were measured in the amino acid, grouping and long-term effects studies, but serum insulin concentrations were only measured in the grouping and long-term effects studies. Serum insulin to glucose ratio of the grouping and long-term effects study is presented in Table 7.3. Calves fed conventionally in the long-term effects study showed the lowest insulin to glucose ratio during the preweaning period, suggesting that less insulin was needed to maintain serum glucose concentration. This was probably related to the lower amount of MR fed to conventionally-fed calves compared with the other two studies. On the other hand, calves from the grouping study were fed only 0.64 kg/d of MR and they showed the greatest insulin to glucose ratio during the preweaning period, indicating that more insulin was needed to maintain plasma glucose concentrations constant. The greater insulin to glucose ratio in the enhanced-fed calves of the grouping study compared with the enhanced-fed calves from the long-term study may be explained by different sampling hours between the two studies (1 hour vs 2-4 hour after the morning feeding, in the grouping study, and in the long-term effects study, respectively). Plasma glucose concentration peaks at 60 or 75 min after feeding, and decreases thereafter (Herrli-Gygi et al., 2006). Nevertheless, some studies (Todd et al., 2000; Stanley et al., 2002) have reported that plasma glucose concentration appeared to be maintained several hours after feeding. Regarding the effect of feeding time between feeding and sampling moments on plasma insulin concentration, several studies (Kaufhold et al., 2000; Stanley et al., 2002; Herrli-Gygi et al., 2006) have reported a decrease 2 h after feeding. Thus, the ratio insulin to glucose concentrations may be lower when blood samples are obtained beyond 2 h after feeding than 1 h after feeding as occurred in the long-term effects and the grouping study, respectively.

Table 7.3. Serum insulin (µg/l) to glucose (mmol/l) ratio of calves on a conventional (CF) or an enhanced-growth (EF) feeding program from the studies reported above.

	Feeding Program			P-value[a]		
Item	CF	EF	SE[b]	FP	T	FP x T
I:G ratio preweaning[c]						
Grouping	-	0.472	0.188	-	-	-
Long-term	0.117	0.190	0.106	0.002	< 0.001	< 0.001
I:G ratio postweaning[c]						
Grouping	-	0.169	0.187	-	-	-
Long-term	0.116	0.111	0.085	0.71	0.72	0.99

[a] FP: effect of feeding program; T: effect of time; FPxT: effect of the interaction of feeding program with time.
[b] SE = standard error of the mean
[c] The least square means presented herein correspond to non-transformed data, and SE and *P*-values correspond to the ANOVA analysis using ln-transformed data, respectively.

Overall, the sampling time seems to be an important factor when comparing metabolic results among studies. Moreover, each study was conducted at different seasons, which may also influence some blood hormonal levels (Yokus et al., 2006). Furthermore, other hormones, which were not measured in these studies, such as leptin (Brown et al., 2005b), insulin-like growth factor I (Smith et al., 2002; Brown et al., 2005b), or cortisol (Todd et al., 2000) may be influenced by feeding practices or calves management.

7.3.2. Urea

Urea is a metabolite from the protein metabolism. In the long-term effects and digestibility studies it increased after weaning, as it was also reported by Knowles et al. (2000). Although in the grouping study, serum urea concentration was similar before and after weaning, it numerically increased every week throughout the postweaning period. Generally, the amino acid and grouping studies showed lower blood urea levels compared with the long-term effects and digestibility studies (Table 7.4). These differences between the two groups of studies may be associated to two factors: diet and season. The amino acid and grouping studies were performed with the same MR and a similar calf starter, and the long-term effects and digestibility studies were conducted with a different MR and starter from the amino acid and grouping studies, but the same MR and starter in both of them. Consequently, the diets were similarly balanced in the amino acid and grouping studies on the one hand, and in the long-term effects and digestibility studies on the other.

Table 7.4. Serum urea concentration (mmol/l) of calves on a conventional (CF) or an enhanced-growth (EF) feeding program from the studies reported above.

Item	Feeding Program		SE[b]	*P*-value[a]		
	CF	EF		FP	T	FP x T
Urea preweaning[c]						
Amino acid	0.97	1.03	0.045	0.30	< 0.001	0.005
Grouping	-	2.96	0.059	-	-	-
Long-term	3.46	3.16	0.028	0.03	< 0.001	0.009
Digestibility	3.25	2.92	0.043	0.07	0.022	0.33
Urea postweaning[c]						
Grouping	-	2.74	0.074	-	-	-
Long-term	3.46	3.63	0.028	0.21	< 0.001	0.009
Digestibility	3.82	4.02	0.024	0.32	-	-

[a] FP: effect of feeding program; T: effect of time; FPxT: effect of the interaction of feeding program with time.
[b] SE = standard error of the mean
[c] The least square means presented herein correspond to non-transformed data, and SE and *P*-values correspond to the ANOVA analysis using ln-transformed data, respectively.

Therefore, the metabolizable energy to apparent digestible protein ratio weighed by the proportion of MR or starter of the diet in each one of the studies was compared against the metabolizable energy to apparent digestible protein ratio predicted by the NRC (2001) according to the BW, ADG, MR, and starter intake of each study (Table 7.5). It was observed that calves of the amino acid and grouping studies were more in agreement to the NRC (2001) ratios than the long-term effects and digestibility studies. This suggested that the diets of long-term effects and digestibility studies diets were slightly more unbalanced than the diets used in the amino acid and grouping studies (Table 7.5), and it might also explain the differences of serum urea concentrations among studies.

On the other hand, the studies were conducted at different seasons, and this may have affected nutrient utilization, and consequently serum urea concentration. The amino acid and grouping studies were conducted in summer and spring, respectively. Thus, calves were in their thermo neutral zone, 15-25ºC (NRC, 2001). However, the long-term effects and digestibility studies were conducted in winter and fall, respectively. Thus, the environmental temperature dropped below 15ºC, and calves probably diverted some additional energy to maintain body temperature, and part of this energy might have come

from protein, which would explain the greater serum urea concentration in the long-term effects and digestibility studies compared with the amino acid and grouping studies.

Table 7.5. Comparison of the ratio between metabolizable energy and apparent digestible protein weighed by the relative contribution of milk replacer and starter intakes during the preweaning period of calves on a conventional and an enhanced-growth feeding program in different studies using the required metabolizable energy and apparent digestible protein ratio predicted from the NRC (2001) equations to sustain the observed performance.

	Feeding program[a]					
	CF			EF		
Study	Observed	Predicted	Obs/Pred	Observed	Predicted	Obs/Pred
Amino acid	21.46	22.31	0.96	21.96	21.54	1.02
Grouping	21.78	22.41	0.97	21.76	21.52	1.01
Long-term	21.27	21.33	1.00	20.63	21.66	0.95
Digestibility	21.20	22.48	0.94	20.70	22.84	0.91

[a]CF: conventional feeding program; EF: enhanced-growth feeding program

7.4. Postweaning starter intake

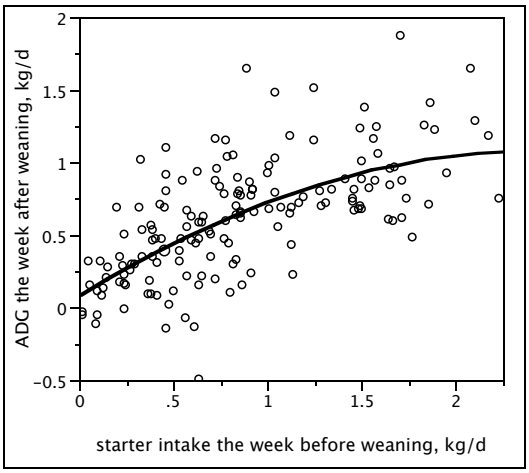
Weaning is the transition time from liquid to solid feed. As mentioned in the introduction, starter intake is essential to ensure proper rumen development and weaning calves successfully. Data from our studies were used to determine the minimum starter intake to wean calves avoiding an impaired calf growth during the weaning process.

After adjusting data of individual calves from the four studies for the random effect of each study and adding the age at weaning as a covariate in the model, a mixed-effects linear regression was performed between the study-adjusted ADG of the week after weaning and the average starter intake the week before weaning. The resulting equation was:

$$ADG\ (kg/d) = 0.095 + 0.812\ starter\ intake\ the\ week\ before\ weaning - 0.165\ (starter\ intake\ the\ week\ before\ weaning)^2 \quad [15]$$

$$R^2=0.43,\ P < 0.001$$

Figure 7.2. Quadratic relationship between starter intake the week before weaning and average daily gain (ADG) the week after weaning (data have been adjusted for the study effect).



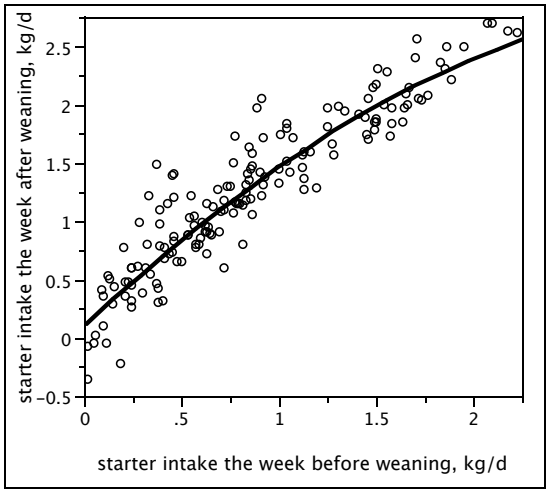
Considering 0.5 kg/d the threshold ADG that will allow weaning calves adequately, calves need to consume an average of 0.56 kg/d the week before weaning (Figure 7.2). This preweaning starter consumption will predict a postweaning starter intake of 0.95 kg/d as suggested by the mixed-effects linear regression [16] between study-adjusted average starter intake the week after weaning and the average starter intake the week before weaning (Figure 7.3). These results agree to the linear regression [5] proposed in

the introduction using data from the literature, where 0.62 kg/d of starter DM intake was recommended to wean calves properly.

Average starter intake the week after weaning (*kg/d*) = 0.126 + 1.584 *starter intake the week before weaning* – 0.221 (*starter intake the week before weaning*)^2 [16]

R^2=0.87, $P < 0.001$

Figure 7.3. Quadratic relationship between starter intake the week before weaning and starter intake the week after weaning (data have been adjusted for the study effect).

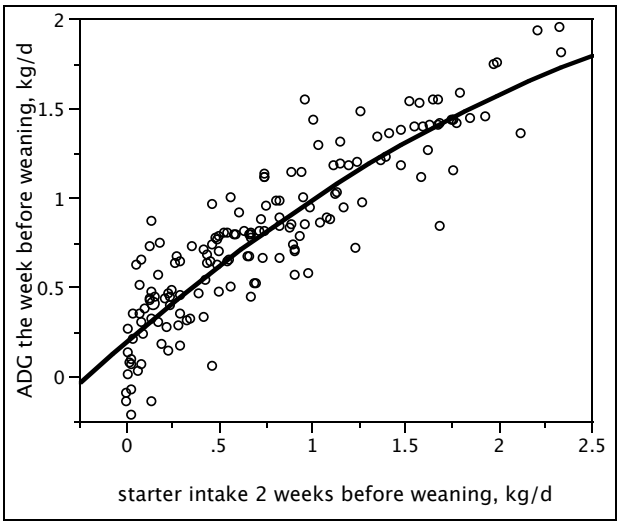


However, the major drop in performance observed in calves in the present studies was found when MR was reduced to one daily feeding. Hence, to ensure a rate of growth of 0.5 kg/d when MR was reduced to one feeding per day, calves should consume an average of 0.340 kg/d two weeks before weaning as indicated by the mixed-effects linear regression [17] performed between the average starter intake two weeks before weaning and the ADG the week before weaning, when MR was reduced to one daily feeding (Figure 7.4).

Average daily gain the week before weaning (*kg/d*) = 0.207 + 0.898 *starter intake two weeks before weaning* – 0.104 (*starter intake two weeks before weaning*)^2 [17]

R^2=0.81, $P < 0.001$

Figure 7.4. Quadratic relationship between starter intake two weeks before weaning and average daily gain (ADG) the week before weaning (data have been adjusted for the study effect).



Chapter 8

CONCLUSIONS

The results obtained in this thesis allow to conclude that:

1. Feeding calves following an enhanced-growth feeding program improves rates of growth during the preweaning period. Although this type of programs improve the gain to feed ratio of calves, the cost per kg of body weight gain during the preweaning phase increases.
2. The enhanced-growth feeding programs in dairy calves decrease starter consumption during the preweaning period and negatively influence microbial duodenal flow and starter digestibility at weaning.
3. The main negative aspects of enhanced-growth feeding programs include a decrease in average daily gain when milk replacer is reduced to one feeding per day, and a low starter intake the week following weaning.
4. The amino acid profile of milk replacers used in enhanced-growth feeding programs is less important than that of milk replacers used in conventionally-fed calves. In conventionally-fed calves, attention should be paid to the supply of phenilalanine and tryptophane as amino acids potentially limiting growth when feeding calves a diet similar to that used in the amino acid study.
5. Raising calves in groups of five does not improve starter intake neither affects performance during the pre- and post-weaning periods.
6. Enhanced-growth feeding programs increase glucose supply to the animal and consequently stimulate insulin secretion.
7. The advantage in body weight acquired with enhanced-growth feeding programs during the preweaning period is only numerically maintained along the development of heifers. Furthermore, this numerical advantage does not seem to improve age at first breeding when breeding policy is based on both a target age and a target body weight.

Chapter 7

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