Universitat Autònoma de Barcelona

Influence of feeding and social behaviors and the use of sodium bicarbonate on ruminal pH of beef cattle fed high concentrate diets.

Influencia del comportamiento de alimentación y social, y del uso de bicarbonato sódico sobre el pH ruminal en el cebo intensivo de terneros.

TESIS DOCTORAL

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Certifican:

Que la memoria titulada "Influencia del comportamiento de alimentación y social, y del uso de bicarbonato sódico sobre el pH ruminal en el cebo intensivo de terneros" presentada por Luciano Adrián González para optar al grado de Doctor en Veterinaria, ha sido realizada bajo nuestra dirección y, considerándola concluida, autorizamos su presentación para que sea juzgada por la comisión correspondiente.

Y para que así conste, firman la presente en Bellaterra,

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LIST OF ABREVIATIONS

ADF	Acid detergent fiber
ADG	Average daily gain
ADL	Acid detergent lignin
ADV	Angular dominance value
ATP	Adenosin triphosphate
b	Linear reggression coefficient
BCVFA	Branched-chain volatile fatty acids
BICARB	Sodium bicarbonate
BW	Live body weight
CNS	Central nervous system
CP	Crude protein
d	Day
DCAD	Dietary cation anion difference
DM	Dry matter
DMI	Dary matter intake
EE	Ether extract
EU	European Union
g	Gram
G:F	Gain to feed ratio
kg	Kilogram
h	Hour
IU	International unit
L	Liter
LPS	Lypopolisaccharide
m	Meter
min	Minute
mg	Miligram
mL	Mililiter
mM	Milimolar
mmol	Milimol
NDF	Neutral detergent fiber

NEFA	Non-esterified fatty acids
NFC	Non-fiber carbohydrates
ng	Nanogram
N:L	Neutrophils to lymphocyte ratio
OM	Organic matter
SARA	Subacute ruminal acidosis
SCFA	Short chain fatty acids
SD	Standard deviation
s.e.	Standard error of the mean
SEM	Standard error of the mean
STFB	Short-term feeding behavior
TMR	Total mixed ration
USA	United States of America
VFA	Volatile fatty acid
WBC	White blood cells
WC	Water consumption
wk	Week
TCT	Total chewing time
T1, T2, T4	1, 2, and 4 feeding places per pen
T2, T4, T8	2, 4, and 8 heifers per feeding place
β-HBT	β-hydroxybutyrate

ABSTRACT

The objective of the present thesis was to study the effects of some behavioral factors, and the use of sodium bicarbonate, on the digestion processes affecting ruminal function of beef cattle fed concentrate and straw ad libitum. In the first experiment, four ruminally fistulated Holstein heifers were used in a 4 × 4 Latin square design to determine the effect of increasing levels of sodium bicarbonate (0, 1.25, 2.50 and 5 %, on concentrate DM basis) on intake, water consumption, ruminal fermentation, and chewing and feed intake behaviors. Concentrate and barley straw were fed once daily at 0830 and ad libitum. Concentrate decreased and straw DMI increased linearly with buffer level. Water consumption increased linearly when expressed in L/kg of DMI but not in L/d. Daily mean and lowest ruminal pH were not affected by treatments. However, ruminal fluid pH increased linearly at 2 and 4 h after feeding and the number of hours in which ruminal pH was below 5.8 was greatest when no buffer was added. Daily average molar proportion of propionate decreased linearly but acetate, butyrate, and branched-chain VFA increased linearly as the level of bicarbonate increased. Meal frequency and eating rate decreased linearly. The size of meals occurring within the 4 h post-feeding increased linearly but those occurring from 8 and 12 h tended to decrease. Feed intake tended to decrease from 6 to 10 and from 12 to 14 h post-feeding. Time spent eating per unit of DMI increased linearly with buffer level, which was the only chewing activity affected on a daily basis. Time spent ruminating decreased linearly from 0 to 2, from 12 to 14, and from 18 to 22 h post-feeding. In the second experiment, 72 Friesian calves were distributed in a factorial design with 3 treatments and 3 blocks of similar fasted BW to study the effect of increasing competition on performance, behavior, and welfare indicators throughout the 4 wk after arrival. Treatments consisted of increasing levels of social competition with the use of 1, 2 or 4 concentrate feeding places/pen (8 calves/pen). Concentrate and straw were fed ad libitum at 0830 and in individual feeders. Increasing competition resulted in a linear decrease of concentrate DMI and ADG during wk 1 after arrival but the response was quadratic in wk 3 and 4, being lowest at the greatest competition. Straw intake and the within-pen SD of ADG tended to increase linearly with competition during the 4-wk receiving period. Increasing competition at the concentrate feeders reduced lying time, increased standing time, and changed the diurnal feeding pattern (concentrate eating time decreased but straw eating time increased during peak feeding times). The number of displacements from the concentrate feeders showed a quadratic response, with the lowest levels of aggression at the lowest competition. Increasing social competition at the concentrate feeders accentuated the effects of dominance rank on ADG. However, the relationship between ADG and dominance rank were negative at wk 1 but positive at wk 3. Physiological indicators of welfare were not consistently affected by treatments. The same 72 heifers with the same experimental setup

were used to study the effect of increasing competition on performance, behavior, welfare, and ruminal fermentation of feedlot heifers. After the 4-wk adaptation period, DMI and ADG were measured, and blood and rumen samples were taken during 6, 28-d experimental periods. Fecal corticosterone and behavior were measured at periods 1, 3 and 6. Concentrate intake decreased linearly with competition but final BW, ADG, and G:F ratio were not affected by treatments. The proportion of abscessed livers responded quadratically with high proportion at the greatest competition. Concentrate eating time decreased, and eating rate, standing time, and aggressions increased linearly with competition for concentrate. The pen-average fecal corticosterone was not affected by treatments but that of dominants responded quadratically, being greatest with the greatest competition. Serum haptoglobin concentration increased linearly with competition, particularly within the most subordinate heifers. Increased competition reduced ruminal pH only in periods 1 and 2 but tended to increase the proportion of heifers with ruminal pH below 5.6 and increased ruminal lactate. The present thesis shows that feeding behavior in beef cattle allows better understand ruminal function and to assess welfare as well.

RESUMEN

El objetivo de la presente tesis fue estudiar el efecto de algunos factores relacionados con el comportamiento, y el uso de bicarbonato de sodio, sobre los procesos digestivos que afectan la función ruminal en terneros de cebo intensivo alimentados con pienso y paja ad libitum. En el primer experimento, 4 terneras Frisonas fistuladas en el rumen fueron asignadas a un cuadrado latino 4 × 4 para determinar el efecto del aumento en la concentración de bicarbonato sódico (0, 1,25, 2,50 y 5 % de la MS del concentrado) sobre la ingestión, el consumo de agua, la fermentación ruminal, y los comportamientos de masticación e ingestión. El pienso y la paja de cebada se suministraron a las 8 h 30 min y ad libitum. La ingestión de concentrado disminuyó mientras que la de paja aumentó linealmente con el aumento de bicarbonato. El consumo de agua aumentó linealmente sólo cuando se expresó en L/kg de MS. La media y el valor mínimo del pH diario del líquido ruminal no fueron afectados por el bicarbonato. Sin embargo, el pH ruminal aumentó linealmente a las 2 y 4 h post-alimentación, y el número de horas con pH ruminal por debajo de 5,8 mostró una tendencia cuadrática con altos valores en el tratamiento sin bicarbonato. La proporción molar de propionato disminuyó linealmente, mientras que las proporciones de acetato, butirato y ácidos grasos volátiles no-ramificados aumentaron linealmente con el nivel de bicarbonato. La frecuencia de las comidas y la velocidad de ingestión disminuyeron linealmente. Hubo un aumento lineal en el tamaño de aquellas comidas que ocurrieron dentro de las 4 h post-alimentación pero una tendencia a disminuir en aquellas que ocurrieron entre las 8 y las 12 h post-alimentación. El tiempo comiendo por unidad de ingestión (min/kg MS) aumentó linealmente con el nivel de tampón, siendo la única actividad de masticación afectada por el nivel de bicarbonato. El tiempo rumiando disminuyó linealmente entre las 0 y 2, 12 y 14, y 18 a 22 h post-alimentación. En el segundo experimento, se utilizaron 72 terneras Frisonas distribuídas en un diseño factorial con 3 tratamientos y 3 bloques con similar peso vivo para estudiar el efecto de la competencia social sobre la producción, el comportamiento y el bienestar durante las 4 semanas siguientes a la llegada al cebadero. Los tratamientos consistieron en aumentos en el nivel de competencia social a través del uso de 1, 2 y 4 espacios de comedero de pienso en cada corral (8 terneras/corral). El pienso y la paja se suministraron una vez al día, ad libitum y en diferentes comederos. Hubo una disminución lineal en la ingestión de pienso y en la ganancia media diaria durante la semana 1 después de la llegada al cebadero pero el efecto fue cuadrático durante las semana 3 y 4, con valores bajos cuando la competencia fue más alta. El consumo de paja y la variabilidad del crecimiento entre terneras dentro de cada corral tendieron a aumentar linealmente con el aumento en la competencia. El aumento de competencia también redujo el tiempo diario descansando, aumentó el tiempo de pie y cambió la distribución diaria del tiempo dedicado a comer (disminuyó el tiempo comiendo pienso y aumentó

el tiempo comiendo paja durante los picos diarios de alimentación). El número de desplazamientos entre animales en los comederos de pienso mostró una respuesta cuadrática con bajos valores en el tratamiento con competencia mas baja. El aumento de competencia por el pienso aumentó los efectos de la dominancia sobre el crecimiento. Sin embargo, la relación entre el rango de dominancia y el crecimiento fue negativa en la primera pero positiva en la tercera semana. Los indicadores fisiológicos de bienestar no mostraron un efecto consistente de la competencia durante el período de adaptación. Los mismos animales con el mismo diseño experimental se utilizaron para estudiar el aumento de la competencia sobre la producción, el comportamiento, el bienestar, y la fermentación ruminal a lo largo del cebo. Los parámetros fueron estudiados durante 6 períodos experimentales de 28 días cada uno, que comenzaron después de las 4 semanas de adaptación. La concentración de corticosterona en heces y el comportamiento se estudiaron en los períodos 1, 3 y 6. La ingestión de alimento disminuyó linealmente pero ésto no afectó a ningún parámetro productivo. El porcentaje de hígados con abscesos mostró un efecto cuadrático con altos valores en el nivel más alto de competencia. El tiempo diario comiendo pienso disminuyó, mientras que su velocidad de ingestión, el tiempo diario de pie y el número de agresiones aumentaron con la competencia por el pienso. No hubo efectos en la concentración media de corticosterona en heces pero los animales dominantes mostraron una respuesta cuadrática al aumento de la competencia, con alta concentración en el nivel más alto de competencia. La concentración de haptoglobina en sangre aumentó linealmente, particularmente en las terneras mas subordinadas. El aumento en la competencia redujo el pH ruminal sólo en los primeros 2 períodos experimentales pero hubo una tendencia por un aumento lineal en la proporción de terneras que mostraron un pH ruminal menor a 5,6 y un aumento en la concentración de ácido láctico. Esta tesis muestra que el comportamiento de la alimentación de los terneros en cebo intensivo permite entender mejor el funcionamiento ruminal y procurar así su bienestar.

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Chapter I

Literature review and objectives

1. Introduction

The beef industry in Europe is challenged by some problems that may be leading to a gradual decrease in the slaughter of cattle during the past 10 years (E.U. Agricultural Statistics, 2007) while consumption of beef coming from intensive farming might be more affected. The growing interest of the consumer for the welfare of our farm animals and the impact of some diseases such as ruminal acidosis are two examples of factors that may negatively impact to the beef industry, both of which are closely related to intensive beef production systems. Consumer concerns about animal welfare, good production practices (environmentally and socially), and food quality and safety are continuously increasing (Andersen et al., 2005; Thompson et al., 2007). However, these problems also lead to special markets where products have an added value, e.g. those under traceability and welfare programs, organic farming, or even functional beef.

Health problems in cattle are important for farmers and the industry because they result in production losses, lead to treatment costs, and are detrimental to animal welfare (FAWC, 1997). Bovine respiratory disease causes the greatest number of deaths (28% of all cattle deaths) while digestive deaths (20%) ranked second in USA in 1995 (USDA, 1997). In beef cattle, the total death loss in USA for the year 1999 was 1.3% of all beef cattle (USDA, 2000) and digestive disorders may account for 25 to 42% of all losses (Smith, 1998; Galyean and Rivera, 2003). Furthermore, the number of all deaths in USA increased by 40% while digestive deaths by 25% from 1991 to 1995 (USDA, 1997). The increase in mortality and disease rates in the past years suggest that cattle are currently under production stresses (Loneragan et al., 2001; Babcock et al., 2006). Digestive losses may be due to acute ruminal acidosis, bloat, and displaced abomasum although they may be closely related, as well as poliencephalomalacia and sudden death may have a digestive origin. Nevertheless, subacute ruminal acidosis (**SARA**) may be the economically most important type of this disorder because reduces intake and performance (Slyter, 1976; Britton and Stock, 1989; Owens et al., 1998). In a recent survey, 1.9% of all feedlot cattle developed digestive disorders and their cost of treatment was estimated to be around 6 U$S per treatment (USDA, 2000b). Other estimations indicate that SARA may reduce the income by 12 to 15 U$S/head in beef cattle (Schwartzkopf-Genswein et al., 2003a).

Galyean and Eng (1998) described several factors and interrelationships among those factors that may potentially lead to digestive disorders such as ruminal acidosis. The type of diet and the environment are among the 'better-known' factors whereas the effects of feeding and social behaviors are poorly understood in the etiology of digestive disorders (Galyean and Eng, 1998; Schwartzkopf-Genswein et al., 2003a).

2. Natural feeding behavior of cattle

The behavior of an animal is an essential reflection of its well being, its internal state (Appleby and Hughes, 1997). Therefore, the study of feeding behavior in cattle fits in the growing interest to improve animal welfare in modern farm production. The natural behavior of animals kept for production should be considered when assessing its welfare and designing housing systems (Lidfors et al., 2005) although this concept may be problematic (Ŝpinka, 2006). Lidfors et al. (2005) defined natural behavior as the repertoire of different behaviors that animals show when kept in environments where they can carry out behaviors created in the evolutionary process. However, because animals have evolved in an environment different from the one in which they are now living (Hartsock, 1982), then we should first carry out field observations on near-to-nature conditions such as on wild or feral animals, or after releasing the confined animals to natural conditions (Miller and Wood-Gush, 1991). Therefore, we should be able to construct the "normal" ethogram of our farm animals and then familiarize with the patterns of behaviors found in "artificial" environments. Finally, we must consider that natural selection has been replaced by artificial selection (Banks, 1982) because behavior is a product of a series of genotype-environment interactions to which the animal is exposed during its development (Klinghammer and Fox, 1971).

Despite the concerns about animal welfare with intensive production systems, supplying the right environment in order to allow performing the "natural behavior" in quantity and quality may be problematic and unviable nowadays. For instance, cattle under natural conditions have shown to spend between 7 and 12 h per day grazing (searching plus consuming) with around 5 feeding periods per day, although it will depend of forage quality and availability, stocking rate, weather, etc. (Phillips, 1993; Linnane et al. 2001; Vallentine, 2001). The long time spent grazing and ruminating each day in cattle is a natural and evolutionary behavior. Achieving the complete energy requirements of cattle with grazing is difficult because of the actual lack in land extension, lower productivity of grazing systems, and other financial and economical issues. Under intensive production systems, animals are fed in feeders, feed bunks or feeding troughs. Rations are generally prepared with mixtures of forages and grains, or by-products of the industry, at different ratios. The amount of time that beef cattle spend feeding per day decreases as the proportion of forage in the ration decreases (Beauchemin, 1991), up to minimum levels of 1.3 to 2 h/d (Shain et al., 1999; Rotger et al., 2006b; Faleiro et al., 2007). Nevertheless, cattle spend only around 2 h/d eating when forage and concentrate are offered both for ad libitum consumption (Robles et al., 2007). On the other hand, low levels of forage in ruminant's rations have been considered as one of the major causes of digestive disorders (Allen, 1997; Stone, 2004; Nagaraja and Titgemeyer, 2007). In

addition, other negative effects of intensive housing systems may be inevitable, e.g. increased competition for resources and aggression. Miller and Wood-Gush (1991) observed the same group of cows under "semi-natural" grazing conditions and indoors. They reported a much lesser synchrony of lying and feeding presumably due to the frustration of allelomimetic behaviors, and greater time spent socially "aware" or alert (34 to 56% of the active time) when the cows were indoors than outdoors.

Feeding behavior is likely one of the most important behaviors because it determines food intake and comes first than any other, animal without food do not reproduce. The study of feeding behavior has been used to assess the metabolic regulation mechanisms of feed intake (Allen et al., 2005), the coping mechanisms with the social and physical environment (Friend et al., 1977), health (immune system), and welfare (Stricklin and Kautz-Scanavy, 1984; Veissier et al., 1989). Veissier et al. (1989) carried out a factorial analysis of correspondence to determine the relative importance of different behaviors on the daily activity patterns of stressed calves. The contribution of each variable to the calculated activity level was: -0.67 for chin resting, -0.46 for lying, 0.41 for standing, 0.53 for moving, and 0.89 for feeding. This demonstrates the importance of feeding on the circadian rhythm of activity and places it as a putative behavior to measure adaptation to environmental or management factors. Finally, neural integration of numerous signals relating the environment, feed, and the physiological and psychological state of the animal will affect feeding behavior.

3. Short-term feeding behavior

The short-term control of intake involves those factors that determine the onset and termination of each meal (Forbes and Barrio, 1992). Meal size and meal frequency are the two parameters that determine the daily food intake and should result, at least in a pure formal sense, in the long-term intake (Tolkamp et al., 2000). The same daily intake level may be achieved through many different combinations of meals (or visits) size and frequency (Friggens et al., 1998; Nielsen, 1999; Tolkamp et al., 2002). In his review, Forbes (1988) presented "the cyclical nature of feeding" to describe and quantify the mechanisms or physiological changes that induce the start of a meal, its continuation and termination. However, the animal initiates and terminates feeding long before the various nutrients have been absorbed or cleared from blood and digestive system. Therefore, other mechanisms are required to balance feed intake with requirements in longer time frames. Learned associations between the organoleptic properties of a food or its gastrointestinal effects with the post-ingestive consequences may also be involved (Forbes, 1992) as well as signals arising from the stores of nutrients (Forbes, 1999). Finally, animals maintain well-being and balance within the

internal milieu through behavioral interactions with the external milieu (Provenza and Villalba, 2006). Thus, the consequences of behaviors are monitored in order to maintain a dynamic balance in the internal and external milieu through behavioral changes. Feeding behavior (satiety and hunger) is a consequence of the neural integration of all signals arising from the internal (tissues and organs) and external (social and physical) milieus, and acting during different time frames.

There are many different methods to collect the information and analyze feeding behavior. For instance, feeding behavior in cattle has been studied by measuring chewing activity (Beauchemin et al., 1989; Linnane et al., 2001; Kononoff et al., 2002), visits to the feeder (Putnam and Davis, 1963; Tolkamp et al., 1998a), animal's presence at the feed bunk (Schwartzkopf-Genswein et al., 1999; DeVries et al., 2003), feed disappearance from the feeder (Dado and Allen, 1993), or even visual observation of the animals (Vasilatos and Wangsness, 1980; Mitlöhner et al., 2001). Regardless the methodology used to collect the data, there is general agreement that the meal is the smallest unit in which feeding behavior is organized (Dado and Allen, 1994; Tolkamp and Kyriazakis, 1999a; Tolkamp et al., 2000). Therefore, the meal-based analysis may provide the common currency for comparison and estimation of the meal criterion is required to characterize feeding behavior in terms of meals. This meal criterion is the longest non-feeding interval between feeding events (e.g. chews, visits) that is considered part of the same meal. There are several methods to determine the meal criterion which lead, however, to different estimations and results (Tolkamp and Kyriazakis, 1999b) and, therefore, may affect the conclusions drawn (Forbes, 1988).

The log-survivorship methodology to determine the meal criterion fits a 'broken stick' to the log-transformed cumulative frequency distribution of the non-feeding interval length (Dado and Allen, 1993). However, it does not describe well the shortest non-feeding intervals and predicts that the probability of animals starting a meal is constant and independent from the time since last meal (Tolkamp and Kyriazakis, 1999b). A new methodology has been proposed that fits statistically and biologically better than previous models (Tolkamp et al., 1998a; Tolkamp and Kyriazakis, 1999b). This methodology is in agreement with the predictions from the satiety concept because it predicts that the probability to begin a meal increases with the time since the last meal (Figure 1b), and the probability of ending a meal also increases with the amount of food already consumed during the meal as shown in Figure 1a (Tolkamp et al., 2000). If feeding behavior is analyzed in terms of visits, then these observed probabilities do not follow such predictions (Figure 1a), but predictions from the model agree with observations and with the satiety concept if the analyses are based on meals (Figure 1b; Yeates et al., 2001; Tolkamp et al., 2000). The estimation of the meal criterion might differ depending on animal genotype, physiological status, type of food, method of data collection, age, and management (Tolkamp and Kyriazakis, 1999b; DeVries et al., 2003b). The way

in which the meal criterion is determined affects the number of meals per day and meal size. For example, the average meal frequency reported in beef cattle was 35 (Nkrumah et al., 2006), 23 (Gonyou and Stricklin, 1981), 16 (Schwartzkopf-Genswein et al., 2002), 9 (Schwartzkopf-Genswein et al., 2003b), and 4.5 meals per day (Erickson et al., 2003). Part of this huge variation among studies is likely due to a lack of a consistent and objective method to designate the meal criterion.

Figure 1. The probability of cows ending a visit and a meal in relation to the amount of food consumed during the visit or the meal (a). The observed (dots) and predicted (solid line) probability of animals to start a meal within the next 15 min as related to the length of the non-feeding interval length (b). From Tolkamp et al. (2002) and Yeates et al. (2001).

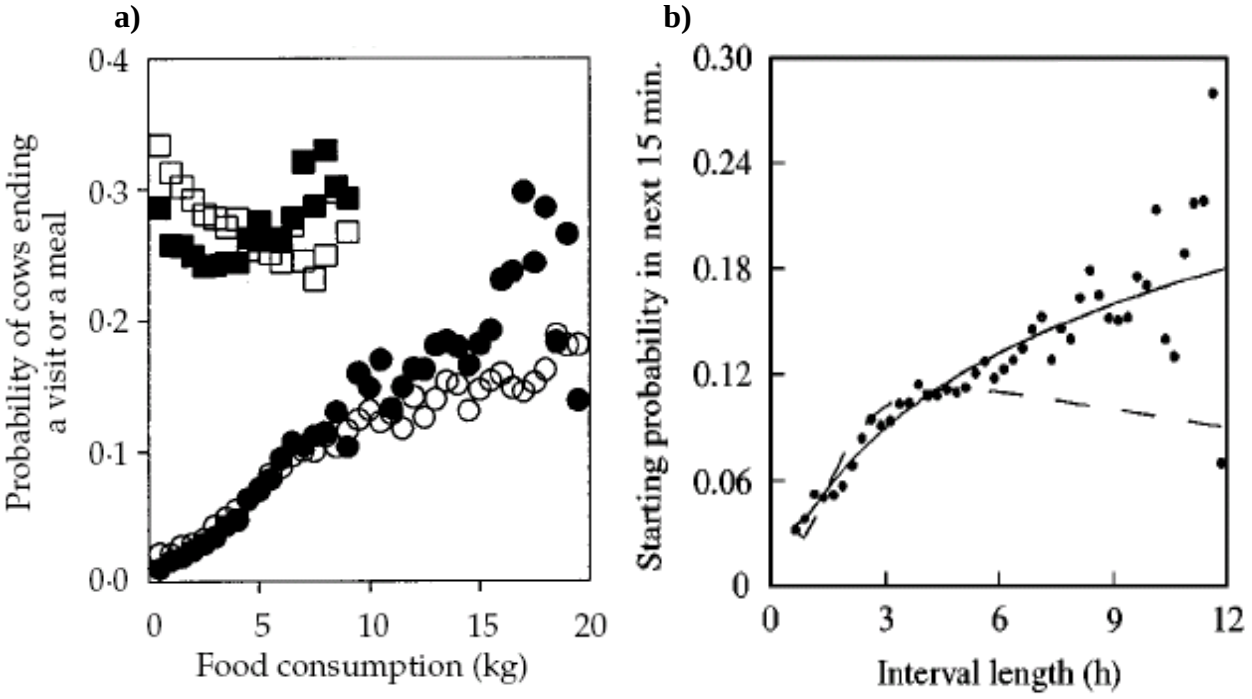
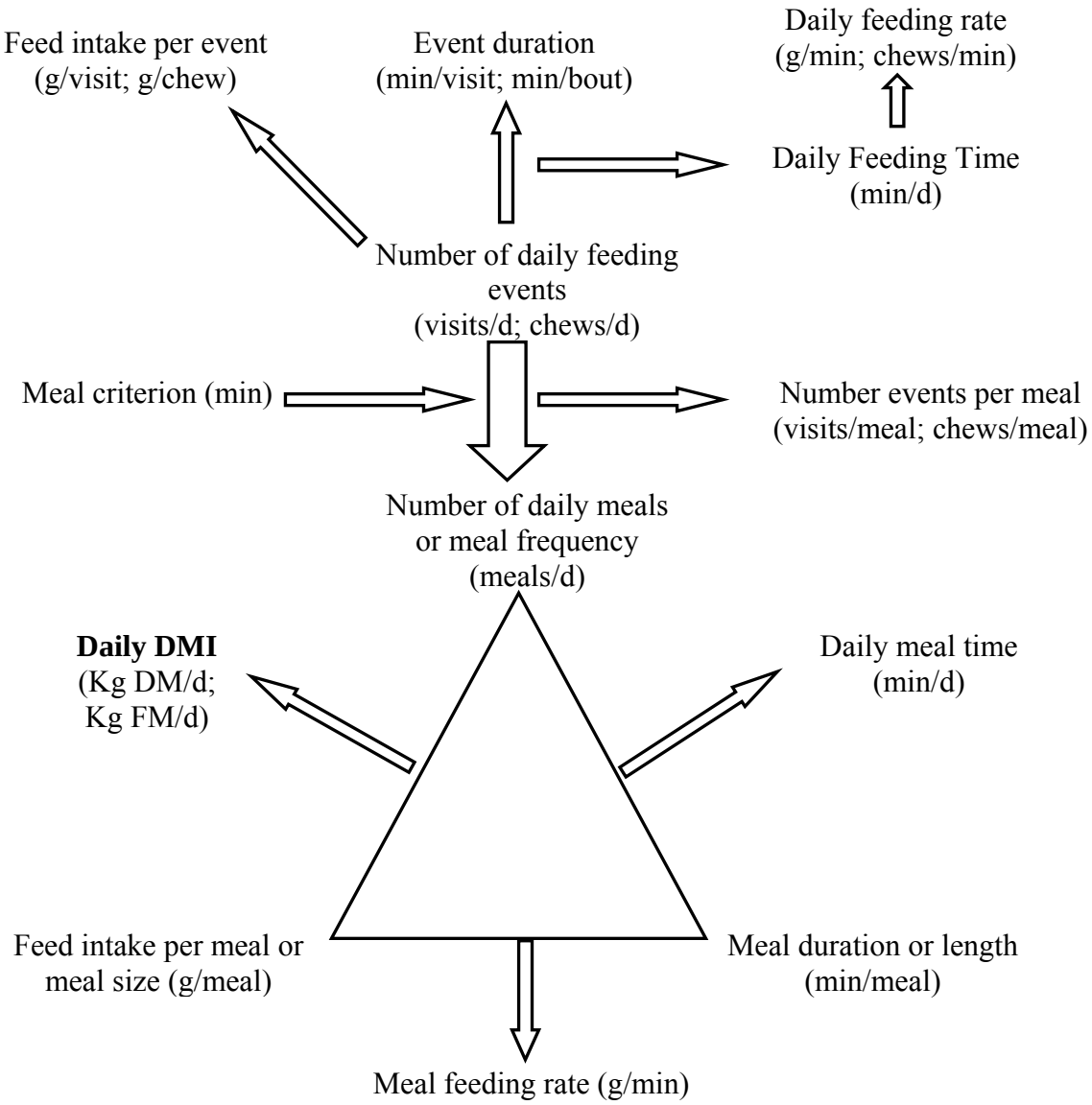


Figure 2 is a diagrammatical representation of the parameters that may be measured and the way they may be organized and calculated in order to study the short-term feeding behavior. The apices of the triangle indicate that if the length of each meal, the amount eaten per meal, and the number of meals per day are known then the other parameters are easily calculated. These parameters are the daily measures of feed intake, feeding time, and feeding rate. Daily DMI is determined by the number of meals or visits per day (meal frequency) and the average size of those meals (Figure 2). However, this is only true for a given animal within a day because the relationship between both measures is negative but does not follow a linear trend but curvilinear (Vasilatos and Wangsness, 1980; Nielsen, 1999; Tolkamp et al., 2000). Therefore, the product of these two is

consistently greater than the observed daily DMI. Similarly, the product of meal length and meal frequency is always larger than the actual daily meal time (Nielsen, 1999; Tolkamp et al., 2000).

Figure 2. Graphical representation of the components of feeding behavior and some interrelationships among them (adapted from Nielsen, 1999).



Meal frequency is the result of the intermeal interval or meal criterion that groups the feeding events into meals. Meal size may be the result of the length and the rate of eating during the meal, but these are usually considered as meal derived measures. Meal size and length varies greatly within the day (greatest after feed delivery and milking) and, therefore, daily average values may be of little significance (Dado and Allen, 1994; Tolkamp et al., 2000). Total daily meal time is the sum of the duration of all meals in a day and, therefore, it includes the non-feeding intervals within a meal, e.g. when drinking occurs or animals are momentanely displaced by a pen-mate from the

feeder. However, the total daily feeding time may also be calculated as time in which the animal was truly engaged in eating, e.g. the sum of the duration of all visits or feeding events in the day (Figure 2). Eating rate may be calculated as the total daily meal time or feeding time (including or not the pauses within a meal) divided the daily intake. These pauses within a meal have been reported to represent a high proportion of the total eating time under grazing conditions because the animal walks in search of better quality forage (selection). Therefore, time spent grazing per day is greater than when animals are fed the same feed from a trough (Lofgreen et al., 1957). Under intensive housing conditions the time spent non-eating within a meal is reduced and it has been estimated to be 16% of the total eating time in beef cattle fed high-concentrate diets (Schwartzkopf-Genswein et al., 1999).

4. Factors affecting feeding behavior

The feeding behavior of an animal may be seen as a result of the consequences of internal and external conditions integrated through the central nervous system (Provenza and Villalba, 2006). Learning from the consequences of behaviors and adaptation to those conditions play also an important roll. Genetic differences created through the evolutionary process and adaptation of feeding behavior patterns to environmental conditions of different seasons throughout the year also occurs. The social environment may also affect feeding behavior patterns as a consequence of, for instance, aggression among animals. Likewise, the characteristics of the feed ingested may be the result of physiological or metabolic consequences of the food ingested.

Among the different factors affecting feeding behavior in this review we want to focus the attention describing social, management, ration, and health-related factors, whereas differences due to genetic and physical environment are not considered because they are out of the objectives of this doctoral thesis.

4.1. Social environment

Social interactions among animals affect all aspects of behavior and feeding behavior is not the exception. The following section is a description of how the social behavior functions and which effects it has on feeding behavior. The description is basically focused on dominance behavior because it shows the greatest effects on feeding behavior. Although social behavior includes sexual activities, grooming, and parental relationships they do no markedly affect feeding behavior.

4.1.1. Social dominance

Social dominance is one component of social behavior and it has been defined as an attribute of the pattern of repeated, agonistic interactions between two individuals, characterized by a consistent outcome in favor of the same dyad member and a default yielding response of its opponent rather than escalation (Drews, 1993). Thus, dominance describes the pattern of an observable type or set of interactions without referring to their function. Social dominance is presumably mediated by aggression (Collis, 1976) and allows priority of access to resources for dominant animals (Syme, 1974; Wierenga, 1990). The outcome of social relationships within a group of animals can be expressed as a social order, rank order, peck order or social hierarchy. However, separate hierarchies may exist for access to different resources (Phillips, 1993).

Aggressiveness is the degree to which a particular animal shows aggressive behavior (Beilzharz and Zeeb, 1982). Butting, pushing and fighting are the main forms of aggression (Dickson et al., 1967). Other types of social relationships such as intimidation or threat, avoidance or fear, and the maintenance of individual space do not involve physical contact and are difficult to distinguish between each other. However, they also play an important roll in the social function and behavior in a group of cattle (Miller and Wood-Gush, 1991). Some authors stated that dominance and aggressiveness are separate characteristics (Arnold and Grassia, 1983; Beilzharz and Zeeb, 1982). Subordinate animals avoid dominants without the need for aggression and dominant animals are not necessarily aggressive (Beilzharz and Mylrea, 1963; Beilzharz and Zeeb, 1982). Nevertheless, the number of aggressions initiated by a cow is highly correlated with the social rank (Collis, 1976; Wierenga et al., 1990).

Individual space is the distance which the animal attempts to keep between itself and its conspecifics. Some authors stated that high-ranking individuals had greater personal space (Hinch et al., 1982) but others have reported that middle-ranking animals spaced themselves at greater distances from group-mates (Stricklin and Kautz-Scanavy, 1984). The housing of animals under intensive production systems usually violates this personal space and reduces the opportunity of subordinate animals to avoid dominants and, therefore, increases aggression (Miller and Woodgush, 1991). These authors have also concluded that 45 to 66% of the active time of cows under intensive housing was spent anticipating the movements of dominant and subordinate herd members, which was called “social awareness or tension”.

The social dominance and the competition for feed, water and space between animals living as a group produces profound changes in behavior. Resting, (Friend et al., 1977; Fisher et al., 1997; Galindo et al., 2000), drinking (Andersson et al., 1984), and feeding behavior are influenced by

social dominance and established through agonistic behavior. Under intensive housing, competition for space is the main reason of aggression. The behavioral changes observed will depend of factors related to space available and distribution such as space allowance, feeding management, and facilities design. Under extensive grazing systems, priority of access to the best grazing patch may form the basis of the herd hierarchy (Phillips and Rind, 2002). It was shown in calves (Kondo et al., 1984), steers (McPhee et al., 1964; Tennessen et al., 1985), and dairy cows (Kondo and Hurnick, 1990) that the proportion of aggressive interactions with physical contact decrease as time after grouping goes on. In contrast aggressions with non-physical contact increase because the development of psychological conditioning from the outcome of encounters (Kondo and Hurnick, 1990). The term of "socio-spatial pattern" has been previously used to describe the distribution of individual animals within a group since social behavior determines their spatial distribution, with a clear avoidance of dominants by the subordinates' counterparts (Beilzharz and Mylrea, 1963; Stricklin and Kautz-Scanavy, 1984; Manson and Appleby, 1990). The influence of social behavior on spatial organization might also be reflected in weighing and milking orders (Beilzharz and Mylrea, 1966; Phillips and Rind, 2002) but more importantly in all feeding characteristics.

Finally, it's good to pinpoint which factors may eventually determine the dominance rank of an individual although they are not exclusively related to it. Dominance is related to body size (height at wither and chest girth), BW, seniority, age (parity), presence of horns, and breed (Schein and Fohrman, 1955; Beilharz and Mylrea, 1963; McPhee et al., 1964; Wagnon et al., 1966; Dickson, 1970; Stricklin et al., 1980; Friend et al., 1977; Wierenga, 1990; Friend and Polan, 1974; Phillips and Rind, 2002). Previous social experience may also affect dominance because calves reared in isolation were dominated by those reared in groups (Warnick et al., 1977). However, the social organization and structure of the group is influenced by different factors and it might be measured through different ways. A detailed description of social structure and function, and dominance-aggressiveness in cattle was described by Beilharz and Zeeb (1982), Wierenga (1990), and Langbein and Puppe (2004).

4.1.2. The effects of social dominance on feeding behavior

The mere allowance of social interactions among animals (grouping) produces important changes in feeding characteristics compared to isolated animals. One of the greatest social pressures (competition) in a group of animals under intensive conditions is observed in the feeding area, where great amount of aggression among mates is usually observed (Friend and Polan, 1974; Miller and Wood-Gush, 1991; Grant and Albright, 1995). Furthermore, competition for feed is greatest

when fresh feed is delivered; dominant cows have priority of access over subordinates during the 15 to 90 min following feeding (Friend and Polan, 1974; DeVries et al., 2004; Huzzey et al., 2006).

The importance of social behavior on feeding characteristics was demonstrated by Harb et al. (1985). These researchers showed that group fed cows (1 cow/feeder) spent 27% less daily eating time compared to individually-fed cows while silage intake was not affected, which resulted from greater eating rate of the group-housed cows. Furthermore, even individually-fed cows in tie-stalls changed their drinking behavior due to dominance relationships in the shared water bowl between two adjacent cows (Andersson et al., 1984). Group-housing might also have positive effects on behavior. For instance, individually-fed gained less than group-fed beef cattle (Kidwell et al., 1954), either because social facilitation increases feeding activity in the group-fed or because of anxiety and restlessness due to isolation of individually-fed calves.

Under competitive situations, the changes in feeding behavior seem to be due to the effects of dominant animals over subordinates. Significant relationships were found between feeding characteristics and dominance rank, or between feeding characteristics and those variables directly related with dominance rank, such as BW and age (Collis, 1980; Katainen et al., 2005). Subordinate cattle are more often displaced or bunted from the feeding area by dominants (McPhee et al., 1964; Hasegawa et al., 1997). Therefore, the duration of subordinates' feeding visits is reduced because disturbed visits are shorter than undisturbed ones (McPhee et al., 1964; Katainen et al., 2005). As a result, total daily feeding time might be shorter, feeding 'bouts' more frequent, and eating rate higher in subordinates compared to dominants (McPhee et al., 1964; Stricklin and Gonyou, 1981; Harb et al., 1985; Olofsson, 1999). These characteristics of subordinates' feeding are likely a result of the psychological conditioning from the outcome of encounters. Aggressions received by subordinates may lead to fear and avoidance and, therefore, lead to 'behavior by consequence' of their social status. However, other studies have not shown such relationships (Ketelaar-de Lauwere et al., 1996) or were even opposite (Hasegawa et al., 1997). Moreover, dominance has also been negatively correlated with grazing time (Phillips and Rind, 2002). These discrepancies may be related to the extent of social pressure among experiments.

Psychological conditioning from social dominance also determines the spatial distribution of cattle when feeding. Subordinate cows prefer to eat apart from dominant pen-mates in the feeding trough (Manson and Appleby, 1990). Thus, cows with similar rank, and particularly those of medium social rank, feed significantly closer together than those with dissimilar rank (Friend et al., 1977; Manson and Appleby, 1990). However, cows choose positions and space themselves when there is enough feeding space or in times of the day when competition is low (outside major feeding periods). Conversely, the feeding position chosen is random when competition and feeder

occupancy is too high or too low (Manson and Appleby, 1990). In addition, the daily feeding pattern of subordinate cows is characterized by greater number of feeding visits during the night to avoid busy times and competition with dominant pen-mates (Collis, 1980; Ketelaar-de Lauwere et al., 1996; Wierenga and Hopster, 1991).

The development of adaptation mechanisms to the social environment, particularly the increase in the number of visits and in the eating rate, allow to subordinate animals to maintain a 'preferred' level of intake, unless competition is too high to allow compensation or other mechanisms are indirectly involved, e.g. stress. Leaver and Yarrow (1980) reported 10% lower OM intake, despite daily feeding time was longer, in subordinate Friesian steers compared to dominants in a 2-wk period trial, presumably because eating rate of subordinates was also 10% lower. Olofsson (1999) also reported that individual eating rate was correlated to DMI ($r > 0.68$) but not with dominance rank ($r < 0.38$). Stricklin and Gonyou (1981) reported that feed intake of dominants was equal to that of subordinates even at 15 bulls/feeder in a long term trial. Dominant cows produced more milk, spent less time grazing each day, and had faster pasture biting rate in the Phillips and Rind (2002) study. Animals seem to have a 'preferred' eating rate (Nielsen, 1999), which has genetic and phenotypic correlations with feed intake (Robinson and Oddy, 2004). In addition, the physical environment and management affects eating rate, and the methodology used affects the dominance ranks obtained (Langbein and Puppe, 2004). Therefore, the effects of social dominance on intake will depend on the characteristics of each animal and no strong relationships between daily feeding time, DMI and dominance rank are common in the literature. Although significant relationships between daily feeding time and DMI were found within studies under little environmental constrains ($r = 0.57$-0.38; Gibb et al., 1998; Schwartzkopf-Genswein et al., 2002). The relationship between daily feeding time and DMI may be stronger during certain stressing periods such as during adaptation to new environments or diets (Schwartzkopf-Genswein and Gibb, 2000).

Dominance hierarchies might be studied by measuring aggression in the feeding area where conflicts and aggressions are easily observed to displace a pen-mate from the feed containers (DeVries et al., 2004; Huzzey et al., 2006). In a displacement, one of the animals (the winner) completely displace physically to a pen-mate (looser) that was engaged in eating behavior to occupy that feeding space. However, visual observations are laborious and time-consuming. Computerized feeders may help to record when a cow left the feeder and which cow has replaced her feeding position. Rutter et al. (1987) found a high correlation between the dominance rank obtained with visual observation and that obtained with the computerized feeders during at least 3-wk (Spearman $\rho \geq 0.96$). Furthermore, a high consistency of ranks over time was observed when using the computerized system. However, they were not able to observe lower number of replacements when

the social pressure decreased from 3.6 to 2.4 cows per feeder because the registrations were done at the nearest minute, which seems a very long time, and the problems to differentiate between active (true) and passive replacements (a cow entering an unoccupied feeder).

4.2. Grouping

Grouping decisions refer to the management practice of grouping animals in pens, which is usually carried out in order to achieve more homogeneous animals within pens. This allows easier management of the groups or pens in order to adjust feeding or marketing programs. Thus, grouping should decrease the within-group and increase the between-group variability. These decisions should be done keeping in mind that grouping strategy and group feeding behavior influence cattle productivity and farm profitability (Grant and Albright, 1995).

4.2.1. Grouping decisions and group composition

The differences among individuals within a group will determine the dominance relationships as described previously and, therefore, determine their spatial distribution and feeding behavior. Those characteristics that are known to be related to dominance should be considered in order to attenuate the potential negative effects of dominance, e.g. age, BW or physical conditions. For instance, heifers housed isolated from older cows had 10-15% longer daily feeding times, 0.5 to 2 more visits to the feeder, and 18% greater feed intake compared to heifers housed with older cows (Konggard and Krohn, 1978). The daily feeding pattern of heifers also differs from that of older cows because they show greater eating activity during the night (González et al., 2003). Separating dominants from subordinates improved performance of both groups of grazing cows being offered hay as a supplement (Phillips and Rind, 2002). However, the time spent grazing and eating hay was greater when dominants and subordinates were kept apart than together.

Grouping animals of different BW also accentuate the effects of dominance, with lighter animals being affected at a greater extent than when they are housed in more homogeneous groups. Formation of homogeneous groups may be more important when competition for feed is high, e.g. restricted feeding or low feeding space per animal. Hindhede et al. (1999) studied three different groupings: small light homogeneous groups (6 heifers with 200 kg BW), small heavy homogeneous groups (6 heifers with 320 kg BW), and large heterogeneous groups (6 light and 6 heavy heifers). Heifers were fed restricted amounts of concentrates and ad libitum straw. Light heifers housed in the heterogeneous pens spent less time eating concentrate, had fewer eating periods and lower ADG

compared to those housed in homogenous pens. Contrarily, heavy heifers in the heterogeneous pens spend more time eating concentrate, had fewer eating periods but the same ADG than those in the heavy homogeneous pens. Nevertheless, there were no differences in ADG when heifers were fed ad libitum TMR, despite the fact that heavy heifers in heterogeneous groups had greater total eating time and were more aggressive than when housed in homogeneous groups. Conversely, the separate ingredients feeding reduced aggression of heavy heifers in heterogeneous pens but the reason for this result is not clear. Nevertheless, group size may be confounded with treatment in the Hindhede's experiments. For instance, increasing the number of animals per group increases aggression of housed beef cattle (Kondo et al., 1989) and increases the average length of grazing bouts (Takeda et al., 2000), likely as a result of less vigilance behavior.

Other factors known to affect dominance are likely to act in a similar way, altering the feeding behavior of subordinates mainly. For instance, BW was negatively correlated with the frequency of visits to a self-feeder and with the number of butts received (Katainen et al., 2005). Mixing different breeds may also accentuate the effects of dominance. Angus cows dominate Shorthorn and Hereford, and Shorthorn dominates to Hereford when grouped together in a competitive feeding situation (Wagnon et al., 1966). Therefore, the greatest percentage of all cows looking for a free feeding space in the feeding passage, and displaced from the feed trough, belonged to the Herefords. This may have important management considerations, especially in intensive production systems and under high levels of competition such as reduced space or other stressful situations.

Other situations may result in uneven motivation for feeding among cows sharing a pen and, therefore, alter feeding behavior apart from its relationship with dominance. Dry cows, high and low producing lactating cows might be all housed together in a pen but still receive different feeding. For instance, computerized self-feeders may be used to allow a small quantity of concentrates to low-producing lactating cows, a greater quantity to high-producing, and none to the dry cows. Such a management results in great alterations of the social and feeding behaviors of the group because the motivation to eat concentrate might be greater in dry compared to lactating cows. Therefore, aggressions are induced by the dry cows in order to displace lactating cows while eating concentrates and, therefore, gain access to some leftover concentrate belonging to the displaced lactating cow (Katainen et al., 2005). Half of the visits to the self-feeder were disturbed in that study and there was a positive correlation between received buttings and interrupted visits with leftover (lost) concentrate in the self-feeder of visits ended involuntarily.

4.2.2. Time after grouping

The first 1 or 2 weeks after grouping are very important for the establishment and stabilization of the social hierarchies in order to allow the function of animals as a social group. Social changes occur after marketing, parturition and introduction of cows to the milking herd, or as a management task in order to have more uniform groups. Grouping of unfamiliar animals may affect all aspects of behavior, decrease feed intake, body weight, growth rate, and milk yield (reviewed by Bøe and Færevik, 2003), change locomotor behavior, increase aggression, disturb the social hierarchy, change the dominance rank of individuals, and distress animals (Hasegawa et al., 1997; Gupta et al., 2005). Moved animals often exhibit aberrant feeding behavior and are more susceptible to metabolic disorders (Grant and Albright, 1995). However, there are no studies specifically dealing with feeding behavior changes after regrouping but they are expected to be of short-term (5 to 15 d) if the establishment of the new group is successful. Those animals lowered in their dominance rank after re-grouping, and subordinates, lowered their milk production and decreased their daily eating time (Hasegawa et al., 1997). The changes in feeding characteristics after regrouping will depend on the change in the competition level when animals are placed in the new group. For example, the increase in eating rate was of 67% when individually-fed cattle were grouped at 2 cows per feeder but it was of 27% when grouped at 1 cow per feeder (Harb et al., 1985).

4.2.3. Social facilitation

The social facilitation has been recognized through many years and refers to the stimulation to initiate or increase the frequency or intensity of a behavior, such as feeding, when shown in the presence of others engaged in the same behavior (Curtis and Houpt, 1983). Social facilitation may improve performance and welfare of cattle during stressful situations such as after weaning and marketing. The weaning process in calves is stressful because they are confronted with a diet change and the new feed has to be the only sustain of the energy needs at a time when the rumen might not be well developed. The adaptation process to new environment may constitute an extra challenge. Calves raised in groups during the pre-weaning period grew faster, started earlier to eat concentrate, and had more competitive feeding after weaning compared to those raised individually or in isolation (Warnick et al., 1977). This may be due to the fact that group-reared calves are dominant over individually-reared calves (Veissier et al., 1994) which may give them some advantages for competitive feeding under challenging situations such as after weaning, re-grouping or marketing, and under high competition for feed.

Newly arrived beef calves at a feedlot may require several days until they start to eat because the stressful condition reduces their desire to eat (Hutchetson and Cole, 1986). In addition, feed and water resources are foreign for the calves. Hutchetson and Cole (1986) reported that only 33, 64, and 95% of calves were eating by the d 1, 2, and 7 after arrival, respectively. Gibb et al. (2000) reported that 16% of newly received feedlot calves made no visits to the feed bunk for periods of 24 h during the first 10 d after arrival, with absences of up 4 consecutive days and, furthermore, one calf did not visit at all the feed bunk during the first 11 d. The use of trainer animals may improve the calves' adaptation to the feedlot through social facilitation, encouraging calves to attend the feeders quicker. However, the use of trainer cows did not influence daily feed bunk attendance or visit frequency of newly received calves (Gibb et al., 2000). Moreover, newly received calves attended the feed bunk during the morning whereas the trainer cows attended the feed bunk during the evening feeding period during the first 12 d after arrival, which is a clear avoidance behavior between calves and trainer cows. The ADG of calves received with trainers was also lesser during the first 3 d after arrival. Therefore, the use of trainer cows was detrimental for the calves' adaptation to the feedlot. Nevertheless, the use of trainer animals in the receiving pens increased the rate of gain, reduced stress-related behaviors, and improved health in some trials carried out by Loerch and Fluharty (2000), but not in all trials. The use trainer cows or steers resulted in greater percentage of calves eating during the 30 min after feeding. In contrast, trainer animals lost weight during the first wk after the arrival of the calves (Loerch and Fluharty, 2000). This indicates that the feeding behavior of trainers was altered negatively by the introduction of calves.

4.3. Design of the feeding facilities

There are several questions to consider when building new facilities for cattle and designing the feeding facilities. The space requirements in the feeding area is one of the most important but the spatial distribution of resources, the design of the feeders such as the feed barriers and orientation may also affect the feeding behavior of animals.

4.3.1. Feeding space requirements

There have always been industry interests to reduce building costs in beef and dairy farms by smaller housing designs and greater density of animals while maintaining welfare and production. The shape, length and spatial design of the feeding manger are the most important considerations when a reduction of feeding space is investigated (Collis et al., 1980; DeVries et al., 2004). In

addition, the reduction of manger space may make it practicable to introduce automatic systems for recording feeding patterns of individual animals. These could be an invaluable tool for farm management by detecting sick animals or to study the impact of management, feed or other changes.

The minimum feeding space required for cattle may be easily calculated considering the daily time that feed is available throughout 24-h determined by the feeding management, the average time spent eating per day determined by the forage proportion of the diet, the efficiency of feed bunk use which will be determined by the animals' hip width and social behavior (avoidance-intimidation). For the latter, the number (or proportion) of animals that are able to eat simultaneously is important (Gonyou and Stricklin, 1981). However, the desire to eat of the animals dictates that sufficient feeding space should be available at sunrise and sunset, or after milking, reducing the daily time to be considered for calculations. Gonyou (1986) reported that many behaviors are motivated at levels consistent with adequate comfort and well-being, and problems may arise if the facilities are not adequate for their timely expression reducing, therefore, the motivation for feeding. The degree of crowding and competition placed on the animals by the grouping strategy should also be considered (Grant and Albright, 2000). Cattle have a great ability to adapt feeding behavior so that intake is maintained but other problems may, however, arise, e.g. stress or digestive disorders in some individual animals within a group.

Some authors have reported that feeding space in dairy cows may be reduced up to 20 (Friend et al., 1977) or 15 cm/animal (Collis et al., 1980) without impacting animal production and welfare. However, feeding space in those studies was gradually reduced (weekly) in established groups of cows. The traditional rule of thumb is to allow 0.61 to 0.76 m/cow such that all cows could feed simultaneously (Albright, 1993; Grant and Albright, 2001). Nevertheless, the provision of 0.6 m/cow allowed only 70% of cows to eat simultaneously (Friend and Polan, 1974; DeVries et al., 2003c) even cows are able to fit in shorter feed bunk lengths than that provided by headlocks when competition increases (Huzzey et al., 2006). Aggressions among cows are even observed at one feeder per cow (Olofsson, 1999). Moreover, providing more than one feeder per cow or more than 0.6 m (DeVries et al., 2004; Huzzey et al., 2006) or more than 1 teat per calf (von Keyserlingk et al., 2004) still increase feeding time and reduce aggressions. This suggests, on one hand, that the 'appropriate' feeding space may neither be reached when all animals in a group are allowed enough space to eat together. On the other hand, it suggests that other mechanisms beyond the availability of a feeding space are involved in the behavior of animals when feeding, e.g. individual space requirement or the need of some animals to show dominance or aggressiveness.

The number (or proportion) of animals able to eat simultaneously can be expressed as social pressure or competition level at the feed bunk and is easily expressed in terms of number of animals per feeding place. However, data should be interpreted with caution as results obtained with small experimental groups of cattle may not extrapolate to large commercial herds. For example, some calculations were done from the data presented by Longenbach et al. (1999) who worked with 0.15, 0.31 and 0.47 linear m/heifer at the feed bunk (Table 1). The social pressure was calculated with the number of heifers able to feed simultaneously at the feed bunk divided the number of heifers in the group. Later, the linear space occupied by each animal was calculated dividing the total feed bunk length by the number of heifers able to feed simultaneously. As seen in Table 1, a 14-mo-old heifer has a hip width of 0.44 m while each heifer can fit in 0.27 m of feed bunk. How is this possible when 50 heifers are eating in line? In addition, the effects of social behavior may increase with group size since lower growth rates and lower feed conversion efficiency was reported at 30 than at 60 bulls/pen, even with the same floor and feed bunk space per bull (MacNeil et al., 1989).

Table 1. Relationships between linear feed bunk space available per heifer and body weight and size, social pressure, and space required per heifer (from Longenbach et al., 1999).

	6-mo-old		14-mo-old			19-mo-old		
Feeding space, m/heifer	0.15	0.31	0.15	0.31	0.47	0.15	0.31	0.47
Body weight, kg/heifer	171	174	380	375	374	516	513	516
Hip width, m	0.31	0.31	0.44	0.43	0.43	0.50	0.49	0.49
Nº heifers/group	9	9	9	9	9	9	9	9
Nº heifer at feed bunk	6	9	5	8	9	4	7	8
Social pressure, Nº/place	1.50	1.00	1.80	1.12	1.00	2.25	1.28	1.12
Space occupied, m/heifer	0.22	0.31	0.27	0.35	0.47	0.34	0.40	0.53

Feed bunk length requirements recommended in the literature for growing cattle is presented in Table 2. In growing beef cattle, either fed restrictively or ad libitum, research indicated that 0.15 or even 0.10 m/animal is sufficient to maintain adequate growth rate or even exceed it (Gunter et al., 1996; Zinn, 1989). Again, these results were obtained with small groups of up to 4 steers. So, it is likely that 4 steers in a small research group can feed simultaneously from a bunk of 0.60 m but, can 50 steers feed simultaneously from 7.5 m (0.15 m/steer × 50 steers)? More research is needed

with large groups of cattle to draw applicable conclusions. Increasing feeding space from 0.55 to 0.75 m/animal improved daily gain and feed conversion rate (Hanekamp et al., 1990, cited by EA-SCAHAW, 2001). Ingvartsen and Andersen (1993) stated that reducing feeding space to less than one per animal seems to reduce performance but Andersen et al. (1997) increased the number of bulls from 1 to 5 per feeding place and did not observed any effect on performance with ad libitum feeding. Feeding management may also affect the feed bunk length requirements. Faulkner and Berger (2003) have recommended that feed bunk length for beef cattle be increased to 0.60 m/animal when limit feeding is practiced or when the ration has high proportion of forages because this would allow all cattle to eat at one time. The scientific committee of the EU suggested that 0.3 m per adult animal fed silage or complete diets ensure adequate access to feed, and 0.6 m when concentrates are fed separately (EA-SCAHAW, 2001). Enough feeding space may be particularly important under stressful situations. A survey in USA reported that around 40% of the beef operations 'always or most of the time' provided new arrivals with additional pen space, waterer space, and bunk space compared to cattle that has been on feed for more than 30 d (USDA, 2000). This suggests that farmers perceive that the negative effects of competition are greatest during stressing situations.

Despite there is a lack of scientific evidence, changes in feeding behavior as competition in the feeder increases were suggested as risk factors for ruminal acidosis (Shaver, 2002; Cook et al., 2004; Stone, 2004; Krause and Oetzel, 2006) or left displaced abomasum (Cameron et al., 1998; Shaver, 2002). Acidosis may be a consequence of variable feeding patterns that result in high eating rate or large meal size (Schwartzkopf-Genswein et al., 2003a). Left displaced abomasum seems more likely to be the result of reduced DMI at high levels of competition or because bacterial endotoxins release at low rumen pH decrease digestive tract motility (Andersen, 2003). Nagaraja and Chengappa (1998) suggested that changes in feeding patterns, allowing cattle to become hungry, may be a predisposing factor of ruminal acidosis and liver abscesses. Harman et al. (1989) found that the incidence of liver abscesses at slaughter was higher for cattle grown in confinement (17.2% abscessed; 0.3 m of feed bunk and 2 m^2 of floor space per steer) compared to outdoor pens (13.4%; 0.6 m in feed bunk and floor space of 20 m^2 per steer).

4.3.2. Feeding behavior with increased competition

Reduced feeding space increases competition, alters social behavior and dominance relationships and, therefore, changes feeding behavior. Increasing the stocking density at the feeders, either because of more animals per feeding position or because of shorter linear feed bunk

space per animal, increases competition for feed and aggression among animals (Olofsson, 1999; Olofsson and Wiktorsson, 2001; Huzzey et al., 2006). More aggressions lead to more interruptions or displacements while feeding, and subordinate animals are more likely to be displaced (Olofsson, 1999; DeVries et al., 2004; Huzzey et al., 2006).

Table 2. Some recommendations done by different authors for growing cattle under different conditions.

Description	Trough space (m/animal)	Feeding	Author
< 400 kg	0.50	General	Swedish recommendation
> 400 kg	0.60		Jordbruksinformation 2-1999
130 – 250 kg	0.3 – 0.45	General	Hardy and Meadowcroft (1986)
250 – 350 kg	0.45 – 0.55		
>350 kg	0.55 – 0.70		
Dairy heifers	0.27	High forage	Keys et al. (1978)
4-8 mo heifers	0.15	High forage	Longenbach et al. (1999)
11.5-15.5 mo	0.31		
17-21 mo	0.47		
200-350 kg	0.15	High concentrate	Zinn (1989)
Feedlot cattle	0.15 – 0.23	High concentrate	Elam (1971)
Adult cattle	0.3	Silage or TMR	EA-SCAHAW (2001)
	0.6	Separate concentrates	

In addition, the frequency of aberrant or unexpected interactions also increases with competition (Wierenga, 1990) because hunger can drive an animal to behave contrary to its social rank (Stricklin and Gonyou, 1981; Rutter et al., 1987). Contradictory displacements (an animal displacing to a higher ranking one), unsuccessful butting, and penetrations (forcing the way between 2 eating cows) may be considered as aberrant interactions (Wierenga, 1990). The number of visits to the feeder increases in a direct proportion to aggressions and displacements because of greater turnover of animals in the feeders as competition increases (Corkum et al., 1994; Longenbach et al., 1999; Olofsson, 1999; Olofsson and Wiktorsson, 2001). This may lead to shorter meals and with smaller meal size (Corkum et al., 1994; Elizalde and Maine, 1993). However, an increase in the number and a reduction in the size of visits does not mean the same apply to meals because the number of visits

per meal. Visits seem to terminate at random, e. g. because of interactions between animals, but meals are less affected by these random processes (Tolkamp et al., 2000). Therefore, even if an animal is displaced from the feeder it is expected that they will return to feed within a time interval shorter than the meal criterion until the animal is satiated. This should just lead to more visits per meals. Otherwise, if visits and meals are terminated involuntarily due to aggressions then animals may return to feed at less busy times after the meal criterion, resulting in more daily meals. Results are contradictory in the literature. For instance, von Keyserlingk et al. (2004) observed that increased competition resulted in greater number of visits to milk-teats by dairy calves but the number of meals were not affected (determined by the frequency distribution). However, Longenbach et al. (1999) reported that both the number of visits and meals per day decreased as feed bunk space increased, whereas Olofsson (1999) reported a decrease in the number of daily meals only (both authors determined the meal criteria through the log-survivorship method).

As competition for feed increases daily feeding time decrease sharply whereas eating rate increase as a compensating mechanism but daily feed intake is generally not affected at the group level (Keys et al., 1978; Gonyou and Stricklin, 1981; Harb et al., 1985; Corkum et al., 1994; Olofsson, 1999; Olofsson and Wiktorsson, 2001; DeVries et al., 2004). In table 3 some proportional changes in feeding time, feeding rate, and feed intake are presented. Nielsen (1999) suggested that eating rate is a relatively robust feature of an individuals and suggested animals have a 'preferred' eating rate when no constraints are present. Eating rate increases with competition because animals prefer to defend the synchronization of behavior and photoperiodicity rather than the 'preferred' eating rate. If social constraints continue to rise then eating rate will eventually reach a maximum and some animals may lower intake before the point where physical constraints reach the limit. Nielsen (1999) proposed that eating rate might be used as a tool to assess the degree to which an animal is constrained by its social environment. The increase in eating rate with competition may be the result of fewer and shorter pauses during a meal, larger mouthful, less chewing of food boluses or greater chewing speed.

The daily feeding pattern may also be altered towards greater feeding activity during the night (Metz, 1981; Olofsson, 1999). The daily feeding pattern was completely abolished at 15 beef cattle per feeder containing a 45% forage diet (Gonyou and Stricklin, 1981). Animals displaced from the feeders or not able to displace other pen-mates while feeding still hungry and thus, they are likely to wait inactively around for an unoccupied feeder (Gonyou and Stricklin, 1981; Olofsson, 1999; Huzzey et al., 2006).

Table 3. Proportional changes in feeding characteristics with increasing competition

Author	Change in competition	Type of diet	% variation on daily average		
			Feeding time	Feeding rate	Feed intake
Elizalde and Mayne, 1993	1 to 9 cows/feeder	Grass Silage	- 70.5	+ 232	- 2.0
Corkum et al., 1994	1 to 4 steers/feeder	Grass Silage	- 25	+ 35	+ 6.4
Olofsson, 1999	1 to 4 cows/feeder	50 silage:50 concentrate (ad lib)	- 18.6	+ 26.9	+ 3.8
Olofsson and Wiktorsson, 2001	1 to 4 cows/feeder	50silage:50 concentrate (+10% NE_m)	- 49.7	+ 75.4	+ 0.1

Increasing competition for feed also strengthens the relationship between dominance, daily feeding time, and DMI (Friend et al., 1977; Metz, 1981; Harb et al., 1985; Olofsson, 1999). As a result, the variation in performance between animals within a group might increase with competition level (Longenbach et al., 1999) because subordinates are affected to a greater extent than middle or high ranking animals (Friend et al., 1977; DeVries et al., 2004; Huzzey et al., 2006). Therefore, increasing competition is more likely to reduce intake of subordinates rather than at the group level. For example, dominant cows ate 14% more feed than submissives at 1 cow per feeder but 23% more at 4 cows per feeder (Olofsson and Wiktorsson, 2001). The most submissive cows fed at 2 cows/feeder of the Harb's study showed a two- to three-fold increase in eating rate compared to individual feeding. Low ranking cows are more likely to shift their daily feeding patterns towards more night feeding (Metz, 1981).

The characteristics of the group and feeding management are also important factors influencing the outcome of increased competition. In addition to reduced feeding space, competition increases further with reduced feed availability (restricted feeding). For instance, the variability of intake among cows and negative correlation between social rank and intake as a result of increased competition are greater with restricted compared to ad libitum feeding (Harb et al., 1985; Olofsson, 1999; Olofsson and Wiktorsson, 2001). The increase in the number of visits and eating rate due to greater competition were proportionately greater under restricted compared to ad libitum feeding

(Olofsson, 1999; Olofsson and Wiktorsson, 2001). If feeding space is reduced in socially established groups and in a gradual fashion then DMI and behavior are less likely to be affected (Albright, 1993), unless the feed bunk space available reaches very low values such as 0.10 or 0.15 m/cow (Friend et al., 1977; Collis et al., 1980). The reduction in feeding space in the feeders supplying supplements of grazing cattle increases the proportion of animals not consuming the supplement and the variability of intake (Bowman and Sowell, 1997).

Similar changes of milk drinking behavior were observed in dairy calves as a result of more bucket-teats per calf. Daily milk intake increased by 25%, number of visits increased by 33%, average visit length was not affected, and the number of displacements from the bucket-teats decreased 50% when the number of teats per calf increased from 0.33 to 1.33 (von Keyserlingk et al., 2004). However, increasing competition even from 12 to 24 calves per teat did not reduce milk intake when computer-controlled milk feeding was used (Jensen, 2004). In this latter study, aggressions among animals increased, duration of visits decreased, daily time ingesting milk decreased, and rate of milk ingestion increased. This suggests that the negative effects of increasing competition on behavior and, eventually, on social stress can not be avoided by the use of computer-controlled feeding systems. However, they may be effective to reduce the negative effects of competition on intake as observed in calves (Jensen, 2004) and dairy cows (Ketainen et al., 2005) because individual intake is programmed.

In conclusion, reductions in the feeding space available increases aggression and the frequency of visits but reduces the length and size of visits or bouts. Daily feeding time also decreases and eating rate increases in a pressure-dependent fashion but daily intake at the group level may be affected under some circumstances. Feeding behavior and intake of subordinates are more likely to be negatively affected as feeding space is reduced (Albright, 1993). Effects are particularly greater at popular or desired eating times such as after feed delivery or milking, and at sunrise and sunset. Therefore, the ability of some cows to access the feed might be limited during these times. As feed becomes restricted in space and time then social dominance becomes more important because dominants have priority of access. The characteristics of the group in relation to social stability and grouping strategy are also important but other factors may also interact. For instance, the reduction of feeding time was further affected when using headlocks compared to post-and-rail but the increase in the number of displacements was reduced (Huzzey et al., 2006). Grant and Albright (2000) stated that less than 0.2 m/cow reduce eating time and DMI, between 0.2 and 0.51 m/cow increase competition with variable effects on DMI, and more than 0.51 m/cow do not affect DMI. Therefore, it is suggested that feeding management (feed availability, amount offered, forage

proportion of the ration) and the design of facilities be considered for feeding space recommendations.

4.3.3. Feeding area design

The design and size of the resting and feeding areas play an important role in the maintenance of the individual's space and may result in aggressive feeding behavior (Grant and Albright, 2000). Grant and Albright (1997) reported that insufficient width of the feeding alley disrupted the normal movement of dairy cows behind the feed manger, precipitated fights and interfered with intense, focused feeding activity. Meanwhile, Grant and Albright (2000) recommended at least 4.3-m width in the feed alley, between the feed line and the first rows of free-stalls, to allow comfortable cow movement and avoid aggressive feeding behavior. Stone (2004) pinpointed that feed bunk and stall overcrowding and comfort, as well as heat stress, are environmental factors that may alter cow behavior, resulting in a shift in the ruminal balance which may decrease rumen pH. Interesting recommendations concerning to the formulation of rations and feeding management under these on-farm situations where made in order to maintain ruminal balance. When separate ingredients feeding is practiced and self feeders are used, it might be important to place the containers further away from each other in order to avoid aggressions among cows trying to reach different resources, as well as from the water resources.

4.3.4. Feeder design

The design of the feeders or feed bunks may have a great influence on feeding behavior, aggression and, perhaps, production. Feed barriers and mangers should allow: free access to feed without risk of injury or discomfort, undisturbed (comfortable) feeding activity, minimum feed wastage, facilitate normal cow behavior, and minimize undesirable feeding behavior such as excessive competition. Feed bunk partitions (Bouissou, 1970), geometrical distribution (Buskirk et al., 2003), and the elevation and slope of the bunk may affect feeding behavior (Albright, 1993). Cows eating in downward, natural grazing position secreted 17% more saliva than when eating with their heads in a horizontal position, which may result in better ruminal function (Albright, 1993). In addition, when cows were given a choice to eat from feed bunks elevated at different heights from the floor, they chose the feed bunk at the ground level and exhibited less feed tossing behavior (throwing the feed over their backs). Manger slopes greater than 3-5% results in cows shifting and moving in the direction of the slope (Grant and Albright, 2000).

Feed containers might be divided in two separate groups: those that allow a physical separation among two adjacent animals while eating and between the feed and the cow (headlocks or self-locking stations, vertical feed barriers); and those with no separations such as troughs, feed bunks or mangers. The post-and-rail system has horizontal feed barriers over the open trough (Endres et al., 2005). Headlocks or vertical partitions may protect cattle while eating since the number of aggressions was 20% lower compared to the post-and-rail (open trough) feeding system (Endres et al., 2005; Huzzey et al., 2006). Buskirk et al. (2003) reported that the number of agonistic interactions was three times greater when feeders did not provide separation between beef cows fed round bales in different feeder designs, although DMI was not affected. With post-and-rail or open troughs, dominant animals move alongside the manger, displacing other cows aside, until they reach the desired position, e.g. where best feed is or encounter a dominant (Wagnon et al., 1966). Contrarily, headlocks prevent this behavior but also the lateral swinging motion of a cow's head needed to displace a neighbor while eating. Cows with low daily feeding time relative to pen-mates increased their feeding time when moved from post-and-rail to headlocks, suggesting more equal access to feed among cows, particularly at peak feeding times and for subordinates (Endres et al., 2005). However, the fact that daily feeding time seems to be lower with headlocks compared to post-and-rail suggests that they are less comfortable when eating from headlocks (Huzzey et al., 2006). Headlocks may result in a developed aversion because they are commonly used for uncomfortable procedures, e.g. health examination, and because aggressions received from pen-mates may be also more traumatic. Wierenga et al. (1990) suggested that protective feed barriers, such as headlocks, may either protect cows from being butted while eating or she may feel unsafe because of inability to defend herself. In larger studies, DMI with the post-and-rail was 3 to 6% greater than with the headlocks (Batchelder, 2000) but not difference was observed by Brouk et al. (2003). Bouissou (1970) reported that subordinate cows showed longer feeding times when partitions extending into the feeder were used to separate the head or muzzle of two adjacent cows while eating, compared to a feeder with no partitions. The disposal or inclination of the feed barriers may also modify cattle behavior. Buskirk et al. (2003) fed round bales in different feeder designs and observed lower hay DM losses when using slanted bars designs because they provide some constraint to behaviors such as tossing and feeding transitions between openings in the feeders. This may be due to more difficulty for cows to get in and out of a feeder with slanted bars because they have to turn their heads in the direction of the inclination of the bars. The amount of waste was positively correlated to the number of agonistic interactions and feeder occupancy rate. The authors suggested that animals usually reallocated its pen-mates along the full length of a linear feeder but

this did not occur in round feeders. Furthermore, round geometry of a feeder may maintain a larger flight zone (individual space) among individuals.

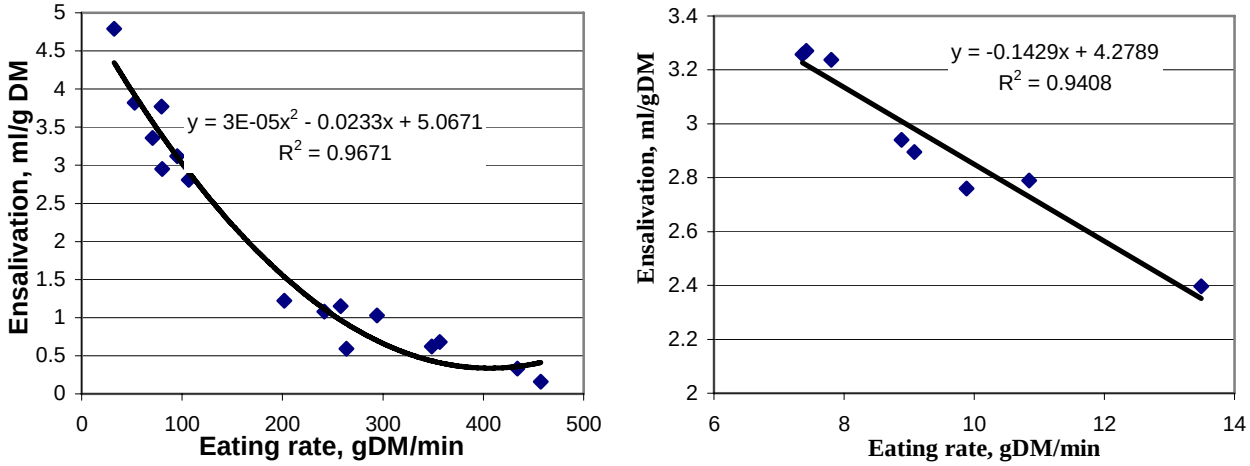
4.4. Feeding management

Feeding management is an important issue in animal production because it may affect feed intake, growth rate, milk yield, and feed conversion efficiency. Feeding behavior is particularly sensitive to feeding management because it may eventually alter the 'normal' or 'preferred' feeding behavior of cattle and become the cause of metabolic disorders, such as ruminal acidosis, due to disruption in the ruminal acid balance. For example, feeding managements that reduce meal size and eating rate, and increase meal frequency may result in more uniform feed intakes and, consequently, reduce the risk of SARA (Schwartzkopf-Genswein et al., 2003a). Greater meal size may increase the rate and extent of the ruminal fluid pH fall because a greater amount of rapidly fermentable carbohydrates results in rapid production of organic acids into the rumen without a synchronized increase of buffering agents such as saliva, ruminal absorption, metabolism or bypass of organic acids (Allen, 1997). Feeds that are eaten at a faster rate show lower ensalivation (g saliva/gDM; Bailey (1961) shown in Figure 3a; Carter et al. (1990) shown in Figure 3b), with concentrates showing at least 3 times lesser ensalivation than forages (Balch, 1958; Beauchemin, 1991). However, it is not known whether ensalivation is a result of eating rate per se or of differences among feed characteristics (DM content and chemical composition). Data suggests that not only eating rate is involved in the ensalivation of feed because feeds with low DM content and long particles have a lower eating rate (Bailey, 1961). Increasing the frequency of feeding and decreasing meal size, and the chop length of alfalfa hay, decreased the eating rate and increased ensalivation of feed in sheep (Carter et al., 1990; Carter and Grovum, 1990b). Therefore, it's suggested that ensalivation of feed is more related to eating rate (time spent eating) rather than to salivation rate, although intrinsic characteristic of feed is also a factor. Salivary secretion rate decreases by about six-fold from the start (first 15 min) until the end of a meal at 75 min in sheep (Carr and Titchen, 1978). However, eating rate also decreases throughout the meal (Tolkamp et al., 2002) and, therefore, ensalivation might not be affected.

Therefore, feeding regimens that act in harmony with feeding behavior and allow the maintenance of the ruminal acid balance are desirable whereas those that disrupt the feeding pattern should be avoided. It was suggested that avoiding large variations in daily feed intake would result in more homogeneous and constant feeding patterns and, therefore, reduce the incidence of clinical acidosis in beef cattle fed-high concentrate diets (Elam, 1976; Pritchard and Bruns, 2003). They

pointed out that bunk management has to be dynamic in response to type of diet, class of cattle, changes in climatic conditions, and bunk space allocations. Bunk management should also consider the capability to manufacture and deliver sufficient quantities of feed in a timely fashion.

Figure 3. Relationships between eating rate and ensalivation of different feeds by cattle (a) from Bailey (1961), and by sheep (b) from Carter et al. (1990).



4.4.1. Quantity of feed delivery

Feed can be delivered following different approaches in beef and dairy cattle, which is usually done by varying the amount of feed offered and/or the time it is available per day. In the "limit-fed" system a substantial restriction at 75 to 80% of ad libitum consumption is offered. In "programmed or restricted feeding" animals are fed 5 to 10% less than the expected DMI (Pritchard and Bruns, 2003). The advantages of restricted feeding programs are that they allow 'predicting' cattle performance, day-to-day DMI is supposed to be more consistent, facilitates feed delivery schedules, avoids feed wastage, reduce sorting behavior, and improves feed conversion efficiency of beef cattle (Loerch and Fluharty, 1998). The "clean-bunk management" attempts to achieve an empty feed bunk at certain time of the day and thus, there is no feed available for around 8 to 12 h/d (Erickson et al., 2003). This management allows cattle to achieve long-term average DMI that will meet or exceed that of cattle fed ad libitum but 'training' of cattle and feeding records play an important roll (Galyean, 1996; Pritchard and Bruns, 2003). The 'traditional or ad libitum' feed bunk management attempts to achieve minimal amounts of feed remaining right before the next feeding to avoid weighbacks and maintain bunk hygiene (Ericksson et al., 2003; Schwartzkopf-Genswein et al., 2003a). Another 'ad libitum' feeding may be achieved using self-feeders where animals are

allowed unrestricted access to feed at all times and little human intervention is required because hoppers are filled of concentrate at long time intervals (usually once each several days). Despite the fact that animals should have absolute control of daily feed consumption with ad libitum programs (Pritchard and Bruns, 2003) it is likely that feeding behavior differs between feed bunk and self-feeders because feed quality in the feed bunk may decrease along the day due to environmental effects and animal manipulation of feed. In addition, the routine management, e.g. personnel and machinery, may encourage animals to come to the feeders and consuming more feed in the first 1 or 2 h after feed delivery even when feed is available at all times.

The greatest critic to restricted feeding is that animals may become meal eaters (consuming a few large meals each day) and the variability in ruminal pH within the day increases Schwartzkopf-Genswein et al. (2003a). These conclusions are supported by Erickson et al. (2003), who reported that beef cattle consumed fewer meals (4.5 vs. 8.2 /h), with greater size (3.5 vs. 1.6 kg/meal) and length (130 vs. 70 min/meal), greater eating rate (32 vs. 18.5 %/h), and had greater daily ruminal pH variance when fed with "clean bunk management" compared to the "traditional" ad-libitum. Similar observations were made by Soto-Navarro (2000b) and Schwartzkopf-Genswein et al. (2002) in beef cattle fed high-concentrate diets and by Harb et al. (1985) in dairy cows. These alterations of feeding characteristics are likely due to hunger and anxiety resulting in greater motivation for feed at time of delivery as feed is not available for variable times each day. Finally, as the amount of feed offered (% of BW) increases then daily feeding time increases while time spent idling and licking (Schake and Riggs, 1969) and performing stereotypic behaviors (Redbo et al., 1996) are reduced.

4.4.2. Feed availability

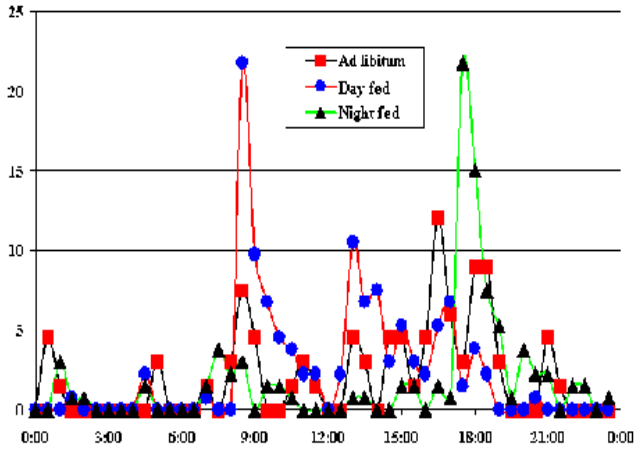
Restricting the daily time that feed is available may be a better way to train animals for a given feeding behavior without impacting daily DMI but will also conduce to greater intake and meal size after feed delivery (Grant and Albright, 1995; Nocek et al., 1997; Shaver et al., 2002). This is the goal in the clean bunk management, where animals are trained to eat as much as they can during around 12 h/d. Increasing feed access time from 8 to 20 h/d in dairy cows, increased DMI from 23.5 to 24.7 kg/d but had no effect on DMI in %BW (Erdman et al., 1989). Friesian steers allowed access to corn silage during 5 or 3 h/d reduced DMI by 8%, increased the within-group coefficient of variation in intake from 8 to 15%, and increased eating rate (Leaver and Yarrow, 1977). However, this trial was 2-wk long and is likely that adaptation time was not enough to learn and modify behavior.

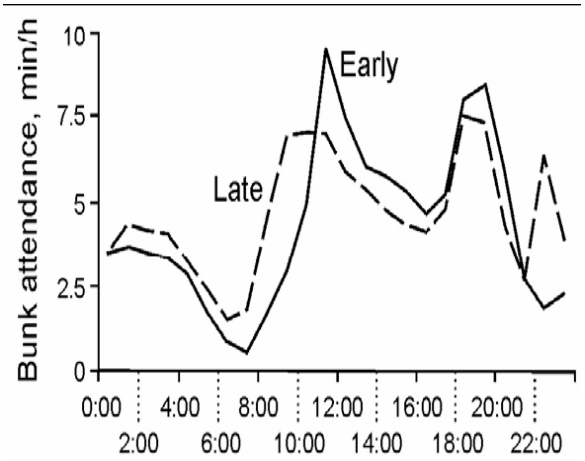
4.4.3. Time of feeding

Both the delivery of fresh feed and the routine management (e.g. milking, arrival of the feeding wagon to the pens, noises or personnel) affect feeding behavior. One of the greatest competition and desire for feed is usually observed when fresh feed is delivered in dairy cows (Grant and Albright, 1995; Grant and Albright, 2000). However, other authors stated that feeding activity of feedlot cattle was relatively independent of time of feed delivery (Gonyou and Stricklin, 1984). Sheep learned the routine management (time of feeding) and they voluntarily fasted before eating a large meal of fresh food (Driver and Forbes, 1981). If food is moist or dusty and deteriorates quickly, or the mixed ration is heterogeneous and allows sorting, then animals will be more motivated for the fresh feed. Cattle seem also to notice subtle changes in the environment (e.g. noises) that they associate with feeding activity. For example, cows learn the routine of the feed allotment program of automatic self-feeders of concentrates, and visit the self-feeders frequently when the feed becomes available (Wierenga and Hopster, 1991; Livshin et al., 1995; Katainen et al., 2005).

Changing the natural daily feeding patterns of cattle seems a very difficult task. Some authors have attempted to invert the 'natural or inherent' daily feeding patterns in feedlot cattle towards the nighttimes in order to improve feed efficiency and performance. The maximal ruminal fermentation rates of feed during the night may be thermodynamically interesting to decrease the heat load during the daylight in summer or to alleviate nighttime cold stress during the winter. Prawl and Owens (1998) fed steers once daily at the morning for ad libitum consumption (0800; 24 h/d), and either once at morning (0800; 9 h/d) or at night (1700; 9 h/d) but reducing the time feed was available to 9 h/d by closing the feed gates. Ad libitum-fed steers had more homogeneous eating patterns throughout the day, avoiding the typical high eating intensity after feed delivery while the other groups spent most of their daily feeding time during the first hour after feed delivery (Figure 4a). However, night-fed steers spent less time eating each day and had greater eating rate, than day-fed steers suggesting they were hungrier. Night-fed steers ate all of their feed during the first hour post-feeding, had the greatest F:G but DMI was not affected. Contrarily, Schwartzkopf-Genswein et al. (2000, 2004) were not successful when attempting to change the feeding patterns through early (0800) or late (2100) feeding even when the amount of feed offered was restricted at 85% of ad libitum (Figure 4b). Total time at the feed bunk and meal frequency were not altered despite the fact that DMI, ADG and G:F tended to be greater in the evening compared to the morning feeding.

Figure 4. Feeding activity (scan sampling) for animals fed for ad libitum (red square) or feed availability restricted to 9 h/d and fed either at 0800 (blue circle) or 1700 (black triangle; from Prawl and Owens, 1998); and feed bunk attendance patterns for steers fed once daily at 85% of ad libitum either at 0800 (Early) or 2100 (Late) (from Schwartzkopf-Genswein et al., 2000).





Mader and Davis (2004) have also shown that restricting the time feed was available per day was more effective than restricting the amount of feed offered (85% ad libitum) with regard to more nigh feeding. However, no consistent effects on daily DMI and performance were observed (Mader and Davis, 2004). In conclusion, the natural and preferred daily feeding patterns of feedlot cattle (crepuscular) are more easily changed by restricting the time feed is available per day rather than the time and amount of feed delivery.

4.4.4. Consistency of feeding (time and amount)

Variable day-to-day feed intake by feedlot cattle has been seen as a risk factor for ruminal acidosis (Elam, 1976; Galyean and Rivera, 2003). Programmed or restricted feeding may favor more consistent day-to-day DMI, which is presumed to decrease the likelihood of overeating and ruminal acidosis. As a result, ADG and gain:feed ratio may be improved (Pritchard and Bruns, 2003; Schwartzkopf-Genswein et al., 2003a). Experimental designs to prove this hypothesis used imposed variations of the day-to-day DM offered. However, feeding characteristics (daily patterns, meal size, eating rate) may vary greatly from day to day even if daily DMI does not show day-to-day variations. Therefore, other designs may also be appropriate to test the hypothesis that variable and aberrant feeding behavior increases acidosis risk, e.g. variations in the time of feed delivery.

When feeding time is delayed in relation to the routine feeding time, animal anxiety may increase and, therefore, conduce to greater intake after feed delivery. This could happen accidentally in commercial situations but it was also used as models for the induction of acidosis

(Nagaraja and Titgemeyer, 2007). Erickson et al. (2003) failed when using a model of acidosis challenge by feeding 125% of the previous' day DMI at 4 h later than the routine feeding time. Although comparisons between the prechallenge against postchallenge were no made, no differences in feeding behavior characteristics (meal size, eating rate, etc.) are suggested by the data using this model. Erickson et al. (2003) concluded that their intake challenge may not have been severe enough to cause acute acidosis symptoms or effects on the animal. However, no differences in daily feed intake or feeding behavior were observed after re-feeding when the length of feed deprivation in steers increased from 12 to 48 h (Bond et al., 1976). Steers of this study were well-adapted to diets containing 0, 30, and 88% of forage but in no case they went 'off-feed' or suffered acidosis. Heifers fed an 88% forage diet, deprived of feed for 96 h, and then re-fed an all-concentrate diet did not engorged themselves although DMI was 36% lower after re-feeding (Bond et al., 1975). Therefore, feed deprivation does not seem to increase the risk of acidosis.

Galyean et al. (1992) produced a 10% daily or weekly fluctuation in the amount of feed delivered to beef cattle and observed a reduction of 6.5% in ADG and a 7% increase in feed:gain compared to groups fed a constant, programmed schedule based on BW. The reduced performance of that study was attributed to subclinical acidosis arising from variation in intake, but ruminal pH was not measured. Nevertheless, other studies testing this hypothesis under similar experimental designs did not support this theory (Soto-Navarro et al., 2000; Hickman et al., 2002; Schwartzkopf-Genswein et al., 2004) or obtained contradictory results among trials (Cooper et al., 1999). Cooper et al. (1999) observed that steers fed restricted amounts of a high-concentrate diet with a 10% variation of feed offered resulted in a 20 to 30% numerical increase in eating rate and increased the area with ruminal pH below 5.6. However, imposed feed intake variation of ad libitum fed steers did not affect meal size and eating rate, and seemed to alleviate ruminal acidosis (Cooper et al., 1999). Schwartzkopf-Genswein et al. (2004) reported that mean ruminal pH was numerically lower by 0.10 units whereas daily time with ruminal pH below 5.8 was 2.2 h/d greater in groups fed at 10% ad libitum fluctuations compared to a control. Therefore, the authors concluded that an inconsistent feed delivery may be a risk factor for ruminal acidosis. Nevertheless, figures presented by Cooper et al. (1999) and Schwartzkopf-Genswein et al. (2003) indicates that the 'normal' feeding behavior of feedlot cattle fed ad libitum is characterized by daily DMI variations even greater than 20% from the average intake. Hickman et al. (2002) concluded that the best-performing cattle had the most variable feeding patterns because steers with high ADG had daily variations in DMI of 0.36 kg greater, consumed 2.1 kg/d more feed, and had 3.7 min/d shorter feeding time than steers with low ADG. Moreover, Hicks et al. (1989) reported that 7.5 to 20% of beef cattle might

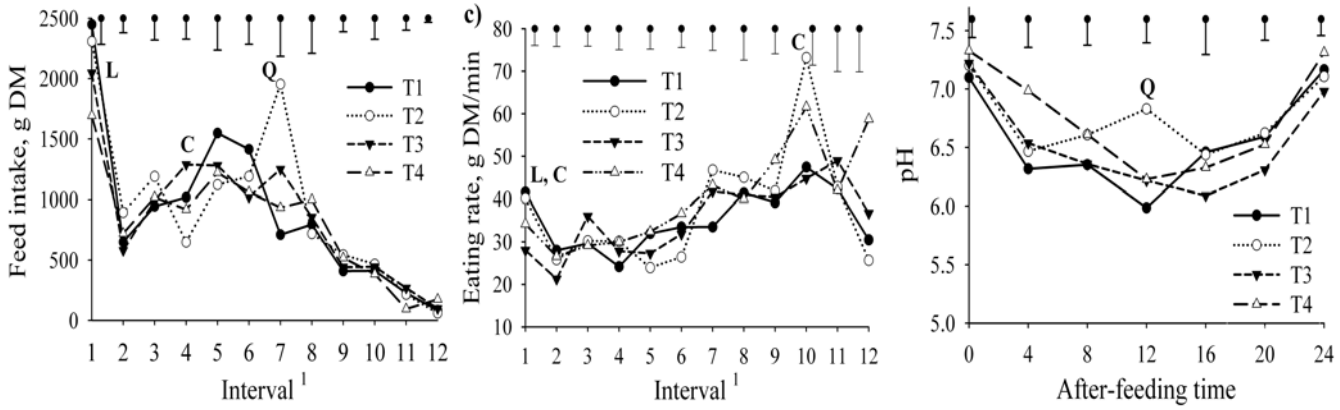
not be observed eating for a 24-h period but it is not known if this is due to repetitive cycles of subclinical acidosis, other environmental effects, e.g. temperature, or just the natural behavior.

4.4.5. Feeding frequency

Increasing the daily frequency of feeding might decrease acidosis by favoring more stable ruminal fermentation throughout the day if this leads to more meals per day and smaller meal size. More frequency of feeding attempts to avoid the very high intake typically occurring after the delivery of fresh feed with one daily feeding and distribute intake more uniformly throughout the day. This will lead to more stable ruminal pH with less daily fluctuations and reduce the risk for SARA (Soto-Navarro et al., 2000). Furthermore, more frequent feeding may reduce social competition for feed and aggressions after delivery (DeVries et al., 2005), reduce the magnitude of feeding errors and abnormal feeding patterns, or increase the accuracy of the animal short-term regulation of feed intake (Pritchard and Bruns, 2003).

In a study carried out by our group in Barcelona (Robles et al., 2007) the frequency of feeding was increased from 1 to 4 times per day in beef cattle fed concentrates and straw for ad libitum consumption. Increasing the frequency of feeding did not affect intake, meal frequency or size, ruminal pH or fermentation profile as daily averages. However, feed intake patterns were effectively changed by treatments.

Figure 5. Effect of feeding frequency on feed intake (a), eating rate (b) and ruminal pH (c) patterns of heifers fed high-concentrate diets at 1 (T1), 2 (T2), 3 (T3), and 4 (T4) times per day. L, Q, and C = linear, quadratic, or cubic effects of treatment at those time points ($P < 0.05$). Intervals of time were 1 (0800 to 1000) to 12 (0600 to 0800).



Eating rate and the amount of feed consumed (-31%) during the 2 h after the first morning feeding decreased linearly with feeding frequency, which may be an indication of lower appetite or motivation for feeding. In addition, the twice daily feeding showed 2 almost equal peaks of feed intake after each feed delivery (Figure 5) which led to a modification of ruminal pH pattern, being 0.85 units greater at 12 h post-feeding than the once feeding.

4.5. Ration

The characteristics of the short-term feeding behavior, such as meal size and frequency, determine the daily feed intake. Therefore, the study of factors affecting STFB has been used to address the mechanisms controlling feed intake. This approach allows the integration of feed characteristics, with the temporal patterns of feed intake and signals resulting from the ingestion of food such as nutrients absorption and utilization, and hormones patterns (Allen et al., 2005). The temporal patterns of those signals may potentially induce satiety and hunger changing the meal patterns. The satiety concept suggests that animals stop eating (terminate a meal) when satiated and, subsequently, begin another meal when the satiating effects of the previous meal disappear (Forbes, 1995; Yeates, 2003). For example, the temporal pattern of satiety signals may be related to the length and size of the meals, e.g. VFA and gut distention, or to the interval between meals, e.g. clearance of nutrients from the blood (Allen et al., 2005). Other measures, such as the rate of food consumption, may also indicate the degree of hunger (Forbes, 1988). For example, infusion of ammonium salts reduced intake through a reduction in meal frequency (presumably because the toxic effects of ammonia reduce hunger) whereas propionate reduces food intake through a reduction in meal size and thus, propionate may increase satiety (Oba and Allen, 2003d,e). Other indexes, such as the satiety ratio which is the intermeal interval length divided by the meal size, were also used to study the satiating effects of diets (Rossi et al., 1998; Tolkamp et al., 2002). The correlation between the size of a meal and the length of the non-feeding interval previous and after a meal (pre- and post-prandial, respectively) were also used to interpret hunger and satiety mechanisms (Forbes, 1995). Therefore, if the pre-prandial is higher than the post-prandial correlation then meal patterns are more determined by satiety than hunger mechanisms (Dado and Allen, 1994; Forbes, 1995). Finally, meal patterns may also be linked with the pattern of signals sensed in, or arising from, different tissues or organs, e.g. biopsies of the liver and adipose tissue, or sampling of rumen fluid or blood. The presence of stretch receptors, osmoreceptors and chemoreceptors along the gut were described by Forbes (1988) and Forbes and Barrio (1992). Hormones, nutrients absorbed, and products of their metabolism in the blood, as well as gastric

motility and digesta passage, might also be important signals involved in the regulation of feed intake (Allen et al., 2005).

Feeding behavior was used to answer questions regarding the two main groups of theories of intake regulation. One group of theories predicts that animals attempt to maximize food intake to meet their genetic potential, e.g. growth, subject to physical or metabolic constraints, e.g. rumen fill (Allen, 1996). Digestive capacity, ruminal fluid pH (Fulton et al., 1979) and osmolality (Carter and Grovum, 1990), starch digestion products (Allen et al., 2005), environmental temperature, fecal output, and palatability (Ingvartsen and Andersen, 2000) were some of the constraints previously considered to limit intake in ruminants. The other group of theories considers that animals optimize their intake (Emmans and Kyriazakis, 1995), rather than maximize, in order to achieve the lowest level of discomfort (Forbes, 2003) or to minimize any costs, relative to benefits, associated with food intake (Ketelaars and Tolkamp, 1996; Illius et al., 2002). Under this group of theories, food intake is a matter of benefit to cost ratios that can be expressed as minimum discomfort or maximum oxygen efficiency. For example, the costs of eating a high-concentrate diet may be the low ruminal pH, the damage to the ruminal tissue, the high levels of fermentation products that have to be metabolized or oxidized, and the toxic products of ruminal bacterial growth, e.g. LPS and histamine. Similarly, eating from a trough or feeder has the cost to compete against pen-mates, which requires energy expenditure and receiving aggressions. The benefits derived from food intake are the net energy for maintenance, growth, or milk production.

The ruminant's body seems to have a complete set of 'biosensors' distributed throughout the body that allows the animal to sense different signals in order to regulate food intake. Moreover, different signals may be involved in the regulation of feed intake within different time frames. Forbes (1999) described some of the signals and sensors that are involved in the transmition of a whole 'cascade' of information to the central nervous system to regulate, therefore, feeding behavior and intake. The food is identified first by sight and odor that will lead the animal to select and ingest the food based on innate and learned associations. Then the food is monitored by taste and texture through sensors in the mouth to transmit the information to the CNS based on palatability and innate and learned associations. Once the food is ingested, different receptors along the wall of the gastrointestinal tract transmit the information of the physical (e.g. stretch) and chemical (e.g. pH or osmolality) characteristics of the digesta to the CNS. The digestion products are then absorbed into the bloodstream, which are then sensed by different organs such as the liver and the CNS. Finally, absorbed nutrients will follow a wide range of metabolic pathways into the animal's body until they oxidized or stored in different tissues determining, therefore, the composition of the body. Signals arising at all levels (mouth, rumen fluid, liver) transmit the

information to the CNS to trigger behavioral responses that regulate intake. The information available to the CNS becomes more accurate in the regulation of intake the earlier or the sooner they are sensed. Therefore, learned associations between the sensory properties of the food and its nutritional value, potential toxicity or metabolic consequences seem to be the most accurate sensing mechanism (Forbes, 1999).

No single signal is though to be the only controller of intake and feeding behavior (Yearsley et al., 2001; Forbes, 2003). Additive, synergistic, and antagonistic effects among nutrients, hormones, and metabolic pathways regulate intake and behavior (Forbes, 1988; Forbes, 1992; Allen et al., 2005). For example, the effects of ruminal VFA and distension are additive for depressing food intake in ruminants (Anil et al., 1987; Mbanya et al., 1993). The hypophagic effect of ammonium salts is also greater when the accompanying anion was propionate instead of acetate (Oba and Allen, 2003d,e). However, these conclusions should be interpreted with caution because all these studies were of short duration (infusion of products during less than 24 h), do not allow learned associations, or used supra-physiological ranges.

4.5.1 Learned associations or conditioned food intake

When a variety of feeds are available, the metabolic consequences or previous experiences with the intake of a food may determine which food is going to be selected next (Forbes, 1988, 1999; Provenza and Villalba, 2006). Feeding behavior under such conditions is expected to result in a 'preferred diet selected' that is safe for the animal (Kyriazakis et al., 1999). Cattle react to potentially harmful feed with the appropriate behavior through instinct, learning as an individual by feedback mechanisms, and learning as part of a social group (social facilitation), or a combination of these behaviors (Albright, 1993).

In the continuous close-looped system used by Kyriazakis et al. (1999) to describe diet selection, feeding behavior determines the consumption of food, which results in changes in the animal internal state. These changes in the internal state are closely monitored and lead finally to a learning process that affects feeding behavior again. This repetitive cycle continues until a stable feeding behavior is reached, and the food is safe and in harmony with the animal metabolism. If food intake results in changes for the worse in its internal state then it will be followed by an appropriate modification of its feeding behavior. Then, new foods may be incorporated into the diet after learning the positive post-ingestive consequences that they have. The internal state include several 'signals' such as levels of several metabolites, hormones, protein and lipid stores, body temperature, etc. In ruminants, the pH and NH_3 concentration in the rumen fluid seem to be

important state variables. Therefore, short-term fluctuations of these variables may be the consequence of feeding but also the cause of the observed feeding behavior (Kyriazakis et al., 1999). Learned associations involve linking the organoleptic, e.g. taste, with nutritional properties of foods. Evolution has developed these abilities to identify foods based on these post-ingestive feedbacks (Forbes, 1999). 'Conditioned food preferences' occur when positive post-ingestive consequences are associated, or the opposite, 'conditioned food aversions' when negative. The speed of learning and the persistence of food aversions are likely to be related to the extent of the previous disturbance changing the internal state of the animal. Several examples can be cited about this topic in animal production. Gibb et al. (2000) suggested that the lack of eating activity during long periods of time in stressed calves being adapted to the feedlot environment may be due to apprehension or feed aversion.

4.5.2. Palatability

Forbes (1999) and Provenza and Villalba (2006) advised against the term palatability because it may be confounded with learned associations, physiological requirements of the animal, and the physical environment under many circumstances. Low eating rates are indicative of low palatability (Forbes, 1988) although it has usually been studied through choice preference tests. Nombekela et al. (1994) studied the taste preferences of dairy cows by giving the choice of a control diet paired with different additives. Taste preference ranking was sweet (1.5% sucrose) > control > bitter (1% urea) > salt (4% NaCl) > sour (1.25% HCl). The maximum intake of corn silage was obtained at a silage pH of 5.6 in dairy cows (Grant and Albright, 1995) suggesting a direct effect of feed pH on intake. However, Buchanan-Smith (1990) added acetate to different feeds fed to oesophageally fistulated sheep and observed a reduction of eating rate, concluding that the acceptability of feeds with acetate was reduced. On the other hand, butyrate increased eating rate and, therefore, enhanced the palatability of the feed. Condensed tannins decreased feed intake by 14% when added to the diet of heifers. During the 3 h after feed delivery, feed intake decreased by 40%, the number of eating bouts increased almost two-fold (20 s interbout criterion), and eating rate decreased by 25% compared to the control diet. In addition, the greatest effects were observed in the first post-feeding 'meal' but they were reversed by adding polyethylene glycol to 'neutralize' the tannins in the diet. These results suggest that palatability (astringency) of tannins is responsible for the reduction in feed intake although not the only one. The diets containing tannins had greater intake than controls during the rest of the day (Landau et al., 2000), which may suggest that post-ingestive consequences of eating tannins do not explain the reduction of intake.

4.5.3. Physical form

The selection of food based on post-ingestive feedbacks and palatability was previously described. However, sheep offered choices of rations with several physical properties (chop length) preferred those that they can eat more quickly and had, therefore, the easiest prehension (Kenney and Black, 1984). The optimal foraging theory predicts that animals select feeds in order to maximize the energy intake rate (Krebs and McCleery, 1984). Similarly, dairy cows fed TMR sort against coarse particles (Leonardi and Armentano, 2003).

Chop length and the proportion of forage in the diet determine the amount of time that cattle spend eating per day, which may be a result of the greater time required to chew and form the bolus as forage chop length and proportion increase (Forbes et al., 1972; Beauchemin, 1991). Typical feed bunk attendances of 100 min/d on average (85 to 120 min/d) were reported for feedlot cattle consuming concentrate diets with 15% barley silage (Schwartzkopf-Genswein et al., 2002, 2003b). Daily feeding time increases with forage proportion whereas eating rate decreases: diets with 89 vs. 25% of chopped forage resulted in a reduction of 30% in daily feeding time (188 vs. 127 min/d) and increase in eating rate (56.8 vs. 84.7 g/min) (Putnam and Davis, 1963; Putnam et al., 1968; Bond et al., 1976). Reducing the particle size of forages reduces daily feeding time and increases eating rate but these changes are proportionally greater as the forage content of the ration increase (Putnam and Davis, 1963; Putnam et al., 1964; Putnam et al., 1968).

4.5.4 Chemical composition

In the following section we describe the effect of some chemical characteristics of the diet, as expressed through the concentration of nutrients, and its digestion products on the short-term feeding behavior. However, it is good to pinpoint that many confounding effects are present when interpreting the results because when the concentration of one nutrient increase then the concentration of others decrease. In this review, not much emphasis is given to those short-term studies with gastric infusions or artificial fill because these protocols do not allow learned associations between post-ingestive feedbacks and taste or odor (Forbes, 1999), neither to compensating mechanisms usually carried out after animals adapt metabolism to maintain internal balance, among other drawbacks of these techniques.

4.5.4.2. Starch and non-structural carbohydrates

Highly digestible cereal grains might depress food intake in cattle (Allen, 2000). Increasing the fermentability of the diet depress daily feed intake because it triggers the 'metabolic' control of feed intake (Forbes and Barrio, 1992), e.g. when including more fermentable ingredients or increasing the energy concentration (Krehbiel et al., 2006) or the extent of grain processing (Owens et al., 1997). This results in lower ruminal pH, increases VFA production rate and the propionate molar proportion whereas acetate decreases. In principle, all SCFA may be involved in the regulation of feed intake but VFA are more important, and were more studied, because they predominate among the fermentation products (Forbes, 1980; Vandermeerschendoize and Paquay, 1984). It was hypothesized that the monitoring of the concentration of SCFA in the rumen may be involved in the metabolic control of feed intake in ruminants but their oxidation in the liver seems more likely to inhibit feeding (Forbes and Barrio, 1992). Therefore, propionate has been proposed to have greater hypophagic effects than other SCFA because it is more extensively metabolized in the liver of ruminants (Allen et al., 2005) and increases the blood concentration of glucose and insulin (Oba and Allen, 2003a,c). Intraruminal infusions of iso-osmotic mixtures of acetate and propionate at different molar proportions resulted in a linear decrease of meal size and length, and DMI, as the proportion of propionate in the infusate increased from 0 to 100% (Oba and Allen, 2003b). The frequency of meals decreased whereas the meal criterion increased (log-survivorship) in several infusion experiments and thus, Allen et al. (2005) suggested that propionate has satiating effects but it also may decrease hunger (see Allen et al. (2005) for his review). It was suggested that propionate reduces feed intake by stimulating the oxidative metabolism in the liver. The reason to propose propionate as the primary satiety signal in ruminants is that propionate flux to the liver increases during the meal and it is extensively metabolized here, whereas other VFA are not. However, propionate may either be oxidized in the liver to yield ATP or metabolized to produce glucose (consuming ATP). Therefore, the effects of propionate on feed intake will depend on other pathways affecting this partitioning of energy. Hypophagia is expected to be greater if the oxidation of propionate in the liver increases but gluconeogenesis may reduce its satiating effects. Therefore, it was hypothesized that feeding behavior is regulated through both satiety signals arising from the hepatic oxidation of fuels and hunger signals arising from the clearance of fuels from the blood (Allen et al., 2005). The oxidation step of metabolites in the liver seems to be involved in transmitting the information to the nervous system to terminate feeding rather than the presence of the products per se (Forbes, 1988; Allen et al., 2005).

4.5.4.3. Structural carbohydrates, protein, and fat concentration

A short description of the effects of the forage, protein, and fat of the diet is done because it is over the scope of this thesis. Daily DMI was reduced when the proportion of forage in dairy cows diets increased from 45 to 80% (Dado and Allen, 1995) or from 27 to 59% (Friggens et al., 1998; Tolkamp et al., 2002), whereas daily feeding time and meal length increased significantly, resulting in a reduction of eating rate. However, meal frequency and the meal criterion were not affected in either study. Smaller meals were observed in the high (2.52 kgDM/meal) than in the low forage ration (3.55 kgDM/meal) of the Tolkamp et al. (2002) study. DMI during the 3 h following feeding, as a proportion of the daily DMI, was greater for the high forage diet but tended to be lower thereafter (Dado and Allen, 1995). Similar observations were made by Thiago et al. (1992) and Gill and Romney (1994) in beef cattle with forages differing in their digestion and passage rates.

Tolkamp et al. (2000) observed greater intake in a diet containing 18.5% CP compared to a 13.1% CP diet in dairy cows. Meal size (8.0 vs. 6.4 kg/meal) and feeding rate (340 vs. 269 min/d) were lower in the low CP diet while other feeding characteristics remained unchanged. Cows eating the low protein feed had a greater probability of ending a meal with the same intake during the meal than cows consuming the high protein feed. These results suggest that consumption of low protein feed was more satiating than the high protein feed (Tolkamp et al., 2000) and that signals are sensed in the short term, e.g. during the course of a meal. However, cows given a choice between a high CP and a low CP feed were able to select a diet that differed from random (66% high CP) but achieved it in a medium term (3 to 6 d) rather than within a meal (Yeates et al., 2002). The addition of fat to ruminant's diets has the goal of increasing energy density of feed to increase energy intake. However, the response to supplemental fat depends on the fat source. The reduction in feed intake when feeding less saturated fat was due to decreased meal size without compensating with increased meal frequency (Harvatine and Allen, 2004).

4.5.5. Osmotic pressure

The chemical composition of the diet determines, in part, the osmolality of the gut contents and its post-prandial kinetics (Bennink et al., 1978; Carter and Grovum, 1990). The ingestion of feed, dissolution of minerals, and accumulation of fermentation products are the main 'contributors' to the post-prandial increase of osmolality of gut contents while drinking water, trasruminal water flux, saliva, and the disappearance of 'contributors' (absorption and passage) decrease its

osmolality. The gastrointestinal wall is provided with osmoreceptors to monitor the osmolality of its fluids (Carter and Grovum, 1990; Forbes and Barrio, 1992). High osmolality in different parts of the gastrointestinal gut of ruminants was considered as another factor regulating feed intake and, consequently, affecting feeding behavior (Forbes, 1988; Carter and Grovum, 1990; Langhans et al., 1995). It was hypothesized that the increase of gut osmolality after the onset of a meal may induce the termination of feeding because it acts as a satiety signal (Carter and Grovum, 1990). Then, satiety would be maintained until osmolality decreases again and a new meal is initiated. Rumen osmolality may cause satiety by inducing vasopressin release (Langhans et al., 1991).

Ruminal osmotic pressure levels at pre-feeding is usually lower than 250 mosmol/kg but can be over 500 mosmol/kg after eating a large meal (Maloiy and Clemens, 1980; Warner and Stacy, 1968) or in acidotic cattle (Huber, 1976; Owens et al., 1998). Ruminal osmolality ranges from 240 to 265 mOsm/L with roughage diets and 280 to 300 mOsm/L with concentrate diets (Garza et al., 1989, cited by Owens et al., 1998; Peters et al., 1990; Whetsell et al., 2004) but 370 mosmol/kg were observed at 2 h post-feeding rations with 2.5% of sodium bicarbonate (Okeke et al., 1983). It should be pointed out that many confounding effects may also be present when dealing with osmolality and the control of feed intake. For instance, the accumulation of fermentation products (VFA) increases osmolality but it may also increases the flux of fermentation products and liver oxidation metabolism in the liver. High ruminal osmolality can also affect intake by reducing rumination (Welch, 1982) and saliva production (Warner and Stacy, 1968).

The addition of osmotically active substances to the feed (minerals), such as salt, reduces the daily feed intake (Rossi et al., 1998). Short-term intraruminal infusions of NaCl reduced meal size by 27% but did not affect intake in dairy cows because the interval between meals was reduced by 31% (Choi and Allen, 1999 as cited by Allen et al., 2005). Ternouth and Beattie (1971) reported that when sheep were given a salty feed, they ate more if the rumen was loaded with water because it counterbalance the effect of minerals on osmolality. Goats fed a complete pelleted diet with 3% NaCl reduced DMI by 40% compared to 0.5% NaCl whereas water intake was not affected. The reduction in DMI was achieved through a reduction in both meal size and frequency (15 min meal criterion) whereas meal duration increased, leading to a decrease in the eating rate. The greatest effects were observed in those meals not associated with drinking (Rossi et al., 1998). This suggests that ruminal osmolality is a feedback signal controlling food intake since drinking antagonizes the meal-induce increase in ruminal osmolality, which increases during the course of a meal (Ternouth and Beattie, 1971; Carter and Grovum, 1990).

Drinking water is normally hypotonic to body fluids and, therefore, it decreases the osmolality of the digestive tract contents (Langhans et al., 1995). Therefore, water deprivation was also used to

assess the effects of ruminal fluid osmolality on food intake regulation. Daily intake of grass and corn pellets by dairy cows was reduced when drinking water was withheld during 48 h (Senn et al., 1996). This was accomplished through a reduction of meal size and length, despite meal frequency increased for the grass (8 min meal criterion). Burgos et al. (2000) observed that ruminal osmolality in dairy cows increased from 260 at the start of a baseline meal to 266 mosm/kg at the end of it. When drinking water was restricted at 65% of ad libitum ruminal osmolality increased from 267 to 282 mosm/kg from the start to the end of first meal, respectively. This subtle increase resulted in a significant reduction to half in the meal size while plasma osmolality was not affected. When water deprived cows were infused with tap water into the rumen then plasma osmolality and feeding was normalized (Burgos et al., 2000). In subsequent experiments, Burgos et al. (2001) restricted the water allowance at 25 and 50% of that consumed ad libitum during 8 d in dairy cows. The reduction of DMI was related to the extent of the restriction and was due to a reduction of meal size but the first meal in each day showed the greatest reduction (> 50%). Meal frequency increased for both levels of water restriction (8 min meal criterion). In addition, oxygen consumption, carbon dioxide and heat production decreased after water deprivation (Burgos et al., 2001). Based on these results seems likely that ruminal osmolality affects daily intake when animals are not able to maintain the electrolyte balance through other mechanisms (e.g. water consumption) and, therefore, decreasing intake is the mechanism used to maintain osmolality within certain limits.

4.6. Immune system and health

The immune system triggers many mechanisms to change behavior in order to help the sick animal to cope with the disease. Weingarten (1995) emphasized the importance of the immune system in the regulation of feeding behavior. However, there are not many studies dealing with changes in short-term feeding behavior in cattle as a result of disease. The immune system can cause hypophagia via cytokines, but mainly through those involved in the acute phase response (TNF-α, IL-1β and IL-6) which are thought to regulate feed intake in sick animals (Ingvartsen and Andersen, 2000). For example, Plata-Salaman (1994) reported that the hypophagic effect of IL-1β on rats is achieved through decreasing meal frequency and meal size. The immune system is a collection of mechanisms within an organism that protects against disease by identifying and killing pathogens and tumor cells. However, there are many ‘disorders’ that are not related with the immune system because pathogens are not the cause, e.g. metabolic disorders. Metabolic disorders include ketosis, hypocalcemia, ruminal acidosis, or left displaced abomasum. The behavioral response to a disease will depend of the physiological mechanisms affected by each desease and,

therefore, changes in feeding behavior will depend of the disease considered and its effects on apettite. In this section, a brief description of the changes in feeding behavior as a result of some diseases or disorders in beef cattle is reviewed.

4.6.1 Ruminal acidosis

In previous sections we have described some of the factors that may produce changes in feeding behavior and, consequently, precipitate, or increase the risk of appearance or the degree of ruminal acidosis. Some factors associated with increased risk for SARA in dairy or beef cattle are: high dry matter intake, intake of large meals at irregular intervals, high eating rate, and large variations in feeding patterns and feed intake, limited bunk space, rations inappropriately mixed (allowing sorting), limited feed access time, restricted feeding versus feeding for 5% to 10% refusal, inconsistent feeding schedule, infrequent TMR push-up, bunk competition, heat stress, excessive time spent in holding areas or in exercise lots without access to feed and water, unstable feed (silage particularly), poor ventilation, slippery floors, inadequate or poorly maintained free stalls, rough feeding mangers, and overcrowding (Grant and Albright, 1995; Shaver, 2002). The combination of two or more of these factors may usually occur on commercial farms, increasing the incidence risk of acute or SARA. However, if ruminal acidosis is addressed from the 'behavior by consequences' theory (Provenza and Villalba, 2006) then variable or erratic feed intake or feeding patterns are behaviors triggered as a consequence of acidosis. Nevertheless, the limit between cause and effect (consequence) is sometimes not clear. In this section, we will try to address feeding behavior as a consequence of ruminal acidosis. However, it is highlighted that isolating each factor occurred as a consequence, or in association, with low ruminal pH is difficult and their effects are often confounded.

Many causes were hypothesized to reduce DMI during SARA periods and, therefore, the consequence (behavior) may be different, or not, depending of the feedbacks or signals involved. These putative theories of DMI reduction include the following:

1) ***Ruminal pH.*** Ruminal pH is the most commonly used indicator of ruminal acidosis. However, rumen fluid pH does not always explain the typical signs and symptoms of ruminal acidosis (Huber, 1976), and low rumen pH do not always result in those signs and symptoms, e.g. variable and reduced intake (Koers et al., 1976). The accumulation of SCFA in the rumen result in low ruminal pH and their subsequent absorption into the bloodstream can potentially overwhelm the bicarbonate buffering system leading to systemic acidosis. The ruminoreticulum became static when the pH was below 5 in overfed sheep (Dougherty et al., 1975a) but H^+ receptors in the rumen

are difficult to demonstrate. Interestingly, steers ruminally infused with NaOH to maintain ruminal pH over 5.75 had greater intake than control steers whose ruminal pH was below 5.35 (Fulton et al., 1979b). Ruminal lactate and total VFA concentrations did not seem to play any important role and, therefore, a direct relationship between low rumen pH and DMI was suggested by Fulton et al. (1979b). Crichlow (1988) showed that intraruminal infusion of SCFA inhibited reticuloruminal contractions and raising the pH of ruminal fluid decreased their inhibitory potency.

2) ***Organic acids.*** The accumulation of SCFA may also be the cause of reduced DMI in acidotic animals (Britton and Stock, 1989). The concentration of fermentation products (VFA) in the rumen may be monitored by ruminal chemoreceptors (acetate mainly) to regulate intake (Forbes, 1992). However, it is more likely that the absorption of SCFA into the bloodstream is involved in the reduction of food intake of acidotic cattle, either because their hepatic oxidation (propionate mainly) increases (Allen et al., 2005), or the capacity of tissues to metabolize them or organs to excrete them is overwhelmed. In addition, high production rates of SCFA, rather than ruminal pH *per se*, may reduce rumen motility and, therefore, intake as suggested by Slyter (1976). More specifically, undissociated fatty acids (pH dependent) reduce rumen motility (Crichlow, 1988) because they are transported faster through the ruminal epithelium (Forbes and Barrio, 1992). Butyrate was among the VFA that activated the greatest amount of receptors in the ruminal wall and considered to be the most potent inhibitor of reticuloruminal motility. Reductions in gastrointestinal motility lead to decreases in the rate of passage of digesta, which may reduce food intake (Forbes and Barrio, 1992). Reduction of the digestive tract motility was considered as the primary cause of reduced DMI under SARA (Kleen et al., 2003).

3) ***Rumen osmolality.*** Owens et al. (1998) suggested that the accumulation of organic acids and glucose in the rumen of acidotic cattle result in high ruminal fluid osmolality, which may be responsible for the reduction of DMI. The absorption of organic acids and water pulled from the bloodstream lead to high blood osmolality. However, these suggestions are not supported by the results from the acidosis challenge experiments carried out by Brown et al. (2000). They found that ruminal osmolality was one of the variables selected to differentiate between steers suffering acute acidosis (ruminal pH < 5) and those not affected (ruminal pH > 5.6) at 3 and 7 d after induction, when acidotic steers went 'off-feed'. Ruminal osmolality between 500 to 700 mOsml was reported by Andersen et al. (1994) when ruminal pH decreased below 5. High ruminal osmolality (> 340 mosmhl) decreases absorption of VFA (López et al., 1994) which may conduce to a spiraling effect. Ruminal fluid osmolality has been linked to the control of feed intake by several authors (Carter and Grovum, 1990; Langhans et al., 1995).

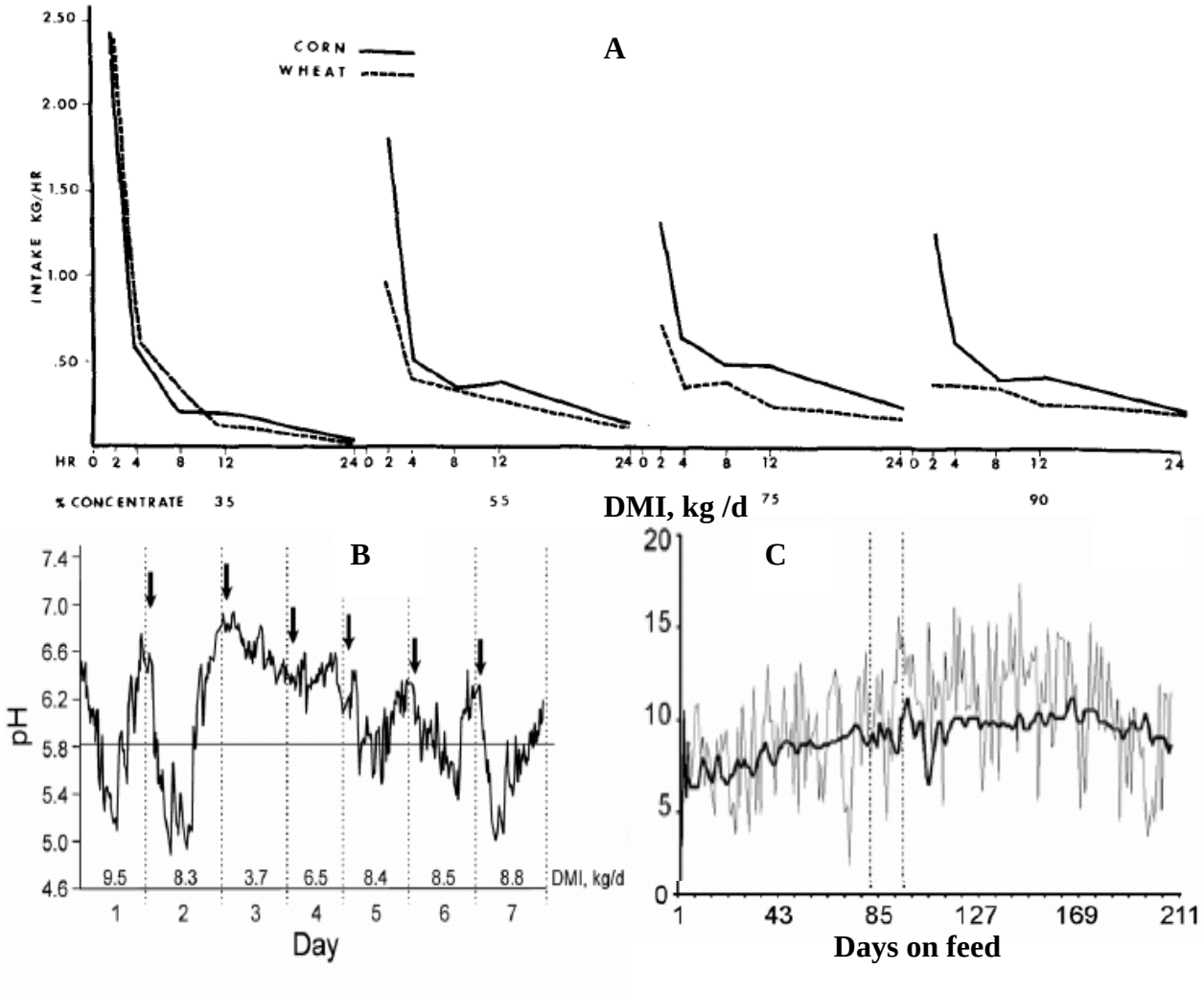
4) ***Bacterial endotoxins.*** The activation of the acute phase response has lately attracted the attention of researchers working with ruminal acidosis. On one hand, high starch diets favor growth of gram-negative bacteria (coliforms) and amino-acid decaroxylating bacteria. The latter may produce toxic amines which have been involved with laminitis (Nocek, 1997). Low rumen pH episodes occurring during SARA increase rumen gram-negative bacteria death and lyses (Nagaraja et al., 1978; Gozho et al., 2007). The cell walls of gram-negatives contain lipopolysaccharide (**LPS**) endotoxins (Andersen, 2003) that are released upon lyses, increasing ruminal LPS concentration (Gozho et al., 2006, 2007). Bacterial endotoxins (LPS) and histamine have been considered to inhibit rumen motility and, consequently, reduce or inhibit intake (Andersen, 2003). In addition, low rumen pH or high osmolality may produce ruminal epithelium damage or increase its permeability, or both, facilitating the translocation of LPS in to the bloodstream (Dougherty et al., 1975b; Nagaraja and Titgemeyer, 2007). This elevates the blood concentration of acute phase proteins such as serum amiloid A and haptoglobin (Gozho et al., 2005, 2006). Acidotic cattle showed increases in blood cytokines, such as interleukins and tumor necrosis factor (Aiumlamai et al., 1992). A systemic aseptic inflammation may be achieved when LPS enter into the bloodstream but individual differences in liver detoxification and tolerance to LPS would avoid it (Andersen, 2003). Alteration of the immune system by blood administration of LPS provokes the acute phase response, mediated by cytokines (Werling et al., 1996), and produces hypophagia in cattle (Elsasser et al., 1995; Bielefeldt et al., 1989; van-Miert et al., 1992; Steiger, 1999; Ingvartsen and Andersen, 2000). Therefore, the chronic inflammation caused by LPS or ruminal tissue damage under SARA, typically found in beef cattle on high-concentrate diets, and may antagonize intake and growth by releasing cytokines (Kleen et al., 2003). Other consequences of endotoxins in cattle are: increase in respiration rate, decrease in the number and strength of ruminal contractions (stasis), increase in body temperature, increase in plasma cortisol, respiratory vasoconstriction, decrease in plasma calcium concentration, alterations of cytokines concentrations, changes in energy turnover and fat and carbohydrate metabolism (Steiger et al., 1999; Andersen, 2003). Reticulo-ruminal stasis was also associated with displaced abomasum and reduced feed intake (Andersen, 2003). Steiger et al. (1999) infused LPS in the jugular of heifers during 100 min and reported reduced feed intake since 4 h after infusion.

The behavior of sick animals has been seen as an orchestrated strategy that facilitates recovery and survival of the animal and not a maladaptive response to the disease (Ingvartsen and Andersen, 2000). In addition, animals experience the consequences of their behaviors in order to survive in a world where the internal and external environments change constantly. Therefore, the positive and negative consequences of their behaviors are monitored in order to behave in accordance to the

animal's internal state (Provenza and Villalba, 2006). Likewise, acidotic cattle may respond to abnormal low fluid pH in the gut, e.g. rumen, with a change in feeding behavior in order to stop the accumulation of organic acids, allow the metabolization and excretion of acids, and regulate the acid load (Schwartzkopf-Genswein et al., 2003a). Behavioral responses may lead to a reduction of daily DMI, but smaller and more frequent meals may also help to regulate the acid load and synchronize the production with the neutralization or metabolization of acids. Therefore, the short-term regulation of intake may help to maintain the best physiological environment but has to be flexible enough to allow the variations required to achieve such a goal.

Brown et al. (2000) observed a high correlation between feed intake and the lowest daily ruminal pH on the previous day in cattle with induced ruminal acidosis. Several researchers observed that animals stop eating for several days after the induction of acute acidosis (Uhart and Carrol, 1967; Tremere et al., 1968; Brown et al., 2000). Cooper et al. (1998) and Bevans et al. (2005) presented some data of ruminal pH and feed intake suggesting that animals being adapted to high-concentrate diets have to go through a learning process likely departing from the post-ingestive consequences of eating more fermentable diets. When the proportion of concentrates increases then ruminal pH decreases. Feeding behavior is characterized by low meal frequency, high eating rate, and greater size of the meals occurring at the beginning of the feeding cycle (Figure 6A). This may lead to ruminal pH reaching values around or below 5.0 that may act as a feedback signal indicating to the CNS that homeostasis is in danger, which triggers a rapid behavioral response that lowers intake or stop eating (Figure 6B). Then, learning processes will modify feeding behavior in order to avoid these negative consequences. As adaptation progresses and learning occurs, feeding behavior should be characterized by lower DMI and more evenly distributed throughout the day, small and more frequent meals, and lower eating rates in order to maintain ruminal pH within physiological non-harmful limits. This is supported by Fulton et al. (1979a) who reported that steers being adapted to diets with greater grain content shifted their intake patterns from greater intake in the 0 to 12 h post-feeding towards the 12 to 24 h as concentrate level increased (Figure 6A). Moreover, the greatest reduction in intake was observed during the 2 h following feeding whereas intake later in the feeding cycle increased as adaptation to higher concentrate levels progressed. These authors suggested that behavioral adaptation required more time than ruminal microbial adaptations because intake patterns were not stabilized at the time lactic acid and VFA were. Fulton et al. (1979a) concluded that steers fed a corn diet attempted to maintain ruminal pH over 5.6 by adjusting intake patterns. Behavior should be stable and in balance with physiology (internal state) once the animal is adapted (Kyriazakis et al., 1999).

Figure 6. DMI of steers being adapted to higher concentrate diets (Figure **A**, from Fulton et al., 1979a). Ruminal pH and DMI of steers fed finishing diets throughout 7 consecutive days (Figure **B**, arrows indicate feed delivery times, from Schwartzkopf-Genswein et al., 2003a). Intake (DMI) and DM delivered (bold line) during 210 d in a steer fed a growing diet containing 80% silage and gradually switched (dashed vertical lines) to a finishing diet containing 20% silage (Figure **C**).



Nevertheless, large long-term variations in feed intake of beef cattle were reported by Schwartzkopf-Genswein et al. (2003a) and, therefore, it is not known if this is part of the normal feeding behavior in this type of cattle or to a very long-term learning process as shown in Figure 6C. In addition, different situations (social interactions, weather, management, stress) may cause aberrant feeding behavior, excessive production of organic acids occurs, and ruminal pH drops to uncomfortable levels. The animal lowers intake until ruminal pH is restored to resume high levels of feed intake until the cycle starts again (Schwartzkopf-Genswein et al., 2003a). The magnitude of the behavioral response may also be related to the strength of the negative feedback (ruminal pH).

In conclusion, adaptation to high-concentrate diets does not only requires ruminal microflora and epithelium adaptations but also behavioral adaptations. However, acidosis might still occur in well adapted animals as a consequence of environmental or social conditions that increase daily DMI and alter feeding patterns such as anxiety, weather changes, inconsistencies in feeding time and amount, and social interactions. Thus, measuring ruminal pH when cattle show low levels of feed intake will usually fail to find any relationship. This is a common observation either in the field or in research animals. Moreover, cattle showing the typical signs of ruminal acidosis such as locomotory problems, back-arched postures, and reduced feed intake did not show low ruminal pH during the development of the present thesis.

4.6.2. Other diseases

The behavior of sick cattle differs significantly from that of healthy cattle (Hart, 1987). Walking (O'Callaghan et al., 2003; Edwards and Tozer, 2004), standing, resting (Hassall et al., 1993), and feeding were the most important behaviors studied in sick animals (Urton, et al., 2005). There is increasing interest in last years for the automatic monitoring of these behaviors in order to identify sick animals without the need of personnel. These behavioral monitoring systems may be included in high-tech environments where several variables are integrated through computer programs in order to assist the farm staff to detect sick animals, e.g. fuzzy logic or neural networks (Mottram, 1997; DeMol et al., 1999; DeMol and Woldt, 2001). The use of radiofrequency technology to continuously monitor the feeding behavior of individual animals may offer great possibilities for the automatic detection of sick animals (Schwartzkopf-Genswein and McAllister, 2005). Changes in feeding behavior as a result of disease may depend of the mechanisms involved or affected during its development but the animal response to the disease will also depend of the mechanisms used to overcome the problem, the one that best fit.

Total time spent at the feed bunk has been a successful indicator to identify morbid beef cattle due to Bovine Respiratory Disease. For instance, morbid beef cattle spent 41 to 50% less time at the feed bunk and had 3.7 visits/d less than their healthy counterparts (Daniels et al. 1999; Sowell et al., 1998, 1999) and morbid beef cattle were identified by 4 d earlier than experienced personnel (Quimby et al., 2001). Cattle showing lung lesions at slaughter spent less time at the feed bunk and made fewer visits throughout the feeding period than those exhibiting no lesions (Schwartzkopf-Genswein and McAllister, 2005). Schwartzkopf-Genswein and McAllister (2005) stated that morbid beef cattle show longer non-feeding intervals and visit the feeders at the end of the major eating periods, when competition at the feed bunk is less intense. They suggested that daily feeding

patterns would be more effective for identifying morbid individuals within a pen. In fact, Sowell et al. (1998) reported that healthy animals had a greater response to feed delivery than sick cattle. Automatic milk feeders were also used to detect sick dairy calves, which showed lower drinking rate (Maatje et al., 1993) and number of unrewarded visits (Svensson and Jensen, 2007).

The previously described diseases are characterized by low daily feeding times, which probably is a result of reduced appetite triggered by the immune system although individual DMI was not measured in those studies. However, other diseases may not necessarily affect the appetite of the animal. During the development of this thesis, we analyzed the feeding behavior of dairy cows at the Scottish Agricultural College in Edimburgh. We have seen that chronic locomotory problems (lameness) does not significantly affect food intake. Lameness problems are a great welfare concern because it causes discomfort and pain (FAWC, 1997). Lame cows were seen to respond to this painful situation by abrupt and sustained decreases in the time spent eating per day and changing feeder positions less frequently during the day (less visits to the feeders and meals per day) while daily intake was maintained relatively constant.

The greatest advantage of using an automated detection of sick animals is that they can be detected before overt signs of the disease are observed. Therefore, the efficiency of the veterinary treatment should be improved, its cost and production losses reduced. However, one of the most important considerations in order to use an automated feeding behavior monitoring system is the repeatability of measurements over time within the same animal. In dairy cows, the greatest within-cow repeatability was obtained for daily feeding time, moderate for total daily meal time (including non-feeding intervals within a meal) and average meal duration, and lowest for meal frequency (DeVries et al., 2003b). In addition, a great variability was observed across cows and stages of lactation. Therefore, it is suggested that changes in daily feeding time within individual animals may be a good indicator of an animal's state. In addition, estimation of the meal criterion and its derived measures (meal length and frequency) would not be needed. Eating rate seems to be a good indicator of an animal's internal state and environmental constraints but it is difficult to measure on-farm.

4.7. Stress

Stress is an environmental effect on an individual which over-taxes its control systems and reduces its fitness or seems likely to do so (Broom and Kirkden, 2004). Stress may be seen, therefore, as the part of poor welfare which involves failure to cope, while welfare refers to a range from very good to very poor welfare. Less precisely, stress has been defined as any physiological

change from homeostasis (Lay and Wilson, 2001). There are many situations that may trigger a stress response in cattle under commercial and experimental conditions (e.g. thermal extremes, crowding, mixing of unfamiliar animals, transportation, weaning, branding, dehorning, vaccination, changes in the physical environment, etc.). Most production stresses may be classified within social, physical, psychological (behavioral), thermal, or metabolic stress (Lay and Wilson, 2001). Stress activates the hypothalamo-pituitary-adrenocortical and the sympatho-adrenal axis and could result in impaired growth, immunity, and reproduction (Minton, 1994; Lay and Wilson, 2001). However, the glucocorticoid response in the short-term is an adaptative mechanism that improves fitness in acute stress but seem to cause immunosupression in the long-term, chronic stress (Dhabhar and McEwen, 1997; Dhabhar, 2002; Avitsur et al., 2002). Some physiological or behavioral indicators may indicate that the animal is trying to cope with adversity and the extent of the attempt can be measured. However, some measures are only pathological when the animal is failing to cope.

There are not many studies dealing with feeding behavior changes as a result of stress in beef cattle. Some of those changes as a result of reduced welfare were described in previous sections, e.g. social constraints and disease. Conflict behavior (e.g. displacement and redirected behavior), destructive and injurious behavior, stereotypic behavior, and behavioral inactivity (lethargy) were considered as characteristic behaviors of stressed animals (von Borell, 1995). However, it is expected that the changes observed will depend on the type and duration of stress, whether or not the immune system is affected, and other physiological and psychological factors as well. For instance, the exposure of cows to novel stall and neighbors (acute stress measured during 15 min; Herskin et al., 2004) or deprived of lying for up to 8 wk (long-term stress; Munksgaard and Simonsen, 1996) increased the frequency but not the duration of feeding, which were seen as signs of motivational conflict. The anticipation of feeding in heifers also increases blood concentrations of cortisol and corticosterone (Willett and Erb, 1972) reaching maximum levels at around 15 min after feeding (Johansson et al., 1999) but decreasing at pre-feeding levels at 60 min (Samuelsson et al., 1996a). Both the oral manipulation of feed and rumen fill seem to be involved in the 'post-feeding' cortisol increase (Lindström et al., 2001). However, 36 h fasting increases cortisol of cows because the negative energy balance requires greater catabolism of stores and gluconeogenesis (Samuelsson et al., 1996b). Nevertheless, huger may also produce psychological stress because greater cortisol was also reported 2 h after re-feeding in that study. Certain feeding managements, such as those with low predictability of feeding, seem to cause stress, decrease growth, and increase aggressions in pigs (Carlstead, 1986). Another example of stress due to management was cited by Desirè et al. (2002) where preventing feeding reduced animal welfare because of increased hunger, which may be reduced even further if the individual sees neighboring animals feeding.

The most common stress situation of beef cattle is at the time of marketing, transport, and weaning. Stress depresses DMI and growth while the immunosupression increases the incidence of disease and death losses, e.g. during 2 to 4 weeks after cattle are placed in new environment and facilities (von Borell, 2001). Stress triggers a wide range of mechanisms aimed to face with the stressor (challenge) and several behavioral changes may be induced. Feeding behavior has been used to assess the adaptability of cattle under different managements when faced with the marketing stress. Sowell et al. (1999) reported that 94% of calves were detected at the feed bunk for an average of 30 min/d during the first day after arrival at the feedlot when the pre- and post-marketing management was good but only 13% when fair. All calves were detected at the feed bunk after the fourth day of arrival at the feedlot and spent 80 to 95 min/d feeding. Meanwhile, Hutcheson and Cole (1986) only observed 88% of cattle attending the feeder by the 7^{th} d after arrival. Sowell et al. (1999) stated that the length of stay in a holding facility, level of stress created by transportation, as well as initial health status of the animals were responsible for this delay in bunk attendance after arrival.

5. Objectives

5.1. General objective

The objective of the present thesis was to expand our knowledge about behavioral factors that may interact with the digestion processes and affect rumen function. Two different scenarios are used to study the changes in feeding behavior and its relationship with rumen function and welfare.

5.2. Specific objectives

Chapter II: "*Increasing sodium bicarbonate level in high-concentrate diets for heifers. I. Effects on intake, water consumption, and ruminal fermentation"*

1) To assess the ability of sodium bicarbonate to improve rumen function, intake, and water consumption in heifers fed ad libitum.

Chapter III: *"Increasing sodium bicarbonate level in high-concentrate diets for heifers. II. Effects on chewing and feeding behaviors"*

2) To assess the effects of sodium bicarbonate on feeding and chewing behaviors.

Chapter IV: "*Effect of the number of concentrate feeding places per pen on performance, behavior, and welfare indicators of Friesian calves during the first month after arrival at the feedlot"*

3) To study the effect of competition at the concentrate feeders on the performance and welfare of calves under a stressful situation that normally occurs on commercial farms.

Chapter V: "*Performance, behavior, and welfare of Friesian heifers housed in pens with 2, 4 and 8 individuals per concentrate feeding place"*

4) To study the long-term effects of competition at the concentrate feeders on production, welfare, and rumen function of calves fed concentrate and straw ad libitum.

Chapter II

Increasing sodium bicarbonate level in high-concentrate diets for heifers. I. Effects on intake, water consumption and ruminal fermentation

Chapter based on a paper sent to Animal

Abstract

Four ruminally fistulated Holstein heifers (BW = 264 ± 12 kg) were used in a 4 x 4 Latin square design experiment to determine the effect of increasing levels of sodium bicarbonate (**BICARB**; 0, 1.25, 2.50 and 5 %, on concentrate DM basis) on DM intake, water consumption and ruminal fermentation. Sampling was carried out in the last week of each four 21-d experimental periods. Heifers were offered concentrate (13.4 ± 0.04% CP, 13.3 ± 0.44% NDF, 51.7 ± 0.97% starch) and barley straw once daily at 0830 *ad libitum*. There was a linear decrease in concentrate DMI and a linear increase in straw DMI with increasing buffer level in the diet, resulting in a tendency towards a linear decrease in total DMI. Intake of concentrate was 6.89, 7.66, 6.72, and 5.72 ± 0.83 kg/d, whereas straw intakes were 0.73, 0.84, 0.94, and 1.06 ± 0.14 kg/d, for the 0, 1.25, 2.5 and 5% BICARB, respectively. Water consumption was not affected by treatments when expressed as L/d or percentage of BW, but increased linearly when expressed as L/kg of DMI. The percentage of total daily water drunk in the morning (from 0830 to 1230) increased linearly with the level of buffer. Mean ruminal pH and total area under the pH curve were not affected with increasing buffer level. The lowest daily pH (5.65 ± 0.09) was not affected by treatments. A quadratic tendency ($P \leq 0.10$) was observed in the number of hours and the area under the pH curve in which ruminal pH was below 5.8, with high values only at the 0% BICARB. Additionally, increasing bicarbonate level caused a linear increase in the ruminal pH at 2 and 4 h after feeding. Daily average NH_3 N (2.4 ± 0.9 mg N/100mL) and total VFA (143 ± 12 m*M*) concentrations were not affected by treatments. Daily average molar proportion of propionate decreased linearly, and acetate proportion and the acetate to propionate ratio were increased with increasing buffer level in the diet. Molar percentage of butyrate, isobutyrate and isovalerate, and branched-chain VFA concentration increased linearly as the level of bicarbonate increased in the diet. Results indicate that high levels of sodium bicarbonate to finishing heifers fed high-concentrate diets may result in a decreased DMI without significant effects on mean ruminal pH, which may affect animal performance. All individual volatile fatty acids proportions, except valerate, changed by bicarbonate addition.

Introduction

Bicarbonate is the dominant natural ruminal buffer and sodium bicarbonate (**BICARB**) the buffer traditionally added to diets in ruminant nutrition to moderate ruminal pH. In the literature, however, there are contradictory responses of variables measured to the addition of buffers, and confusion in the interpretation of results (Russell and Chow, 1993). For instance, the addition of up to 5% bicarbonate in high concentrate rations improved DMI in growing cattle (Nicholson et al., 1963;

Wise et al., 1965; Zinn, 1991) but 5% bicarbonate depressed DMI in dairy cows (Emery et al., 1964). Ruminal pH has also been ameliorated in some studies (Nicholson, 1963; Okeke et al., 1983; Zinn, 1991), but no effects have been reported in many others (e. g.: Thomas and Hall, 1984; Leventini et al., 1990). This fact could be the result of the different variables affected by buffer addition and interactions between them, such as intake level, ruminal fermentation and passage rates, water consumption, and blood biochemistry (Erdman, 1988). Therefore, different approaches to avoid confounding factors have been used: fixing the forage to concentrate ratio (Okeke et al., 1983; Hart and Polan, 1984); restricting the daily roughage intake (Emery et al., 1964) or the total ration daily intake (Nicholson, 1963; Okeke et al., 1983; Quigley et al., 1992); withholding feed on the day before sampling (Thomas and Hall, 1984); intra-ruminal infusions of BICARB (Rogers and Davis, 1982b); or even training the calves during the adaptation period to eat meals by removing feed shortly after feeding to facilitate pulse dosing of BICARB (Hart and Polan, 1984). However, there is little information about the effects of buffer addition on intake and ruminal fermentation when animals have *ad libitum* access to concentrate and roughage in intensive beef production systems. The objective of this experiment was to determine the effects of increasing BICARB level added to high-concentrate diets on feed intake, water consumption and ruminal fermentation on finishing beef heifers fed *ad libitum*.

Material and Methods

Animals, Experimental Design and Housing

Four Holstein heifers (average initial BW of 264 ± 12 kg) fitted with 1-cm i.d. permanent ruminal plastic trocars (Divasa Farmavic S. A., Vic, Spain) were used. Heifers were randomly assigned to one of four experimental diets in a 4 x 4 Latin Square design. The four 3-week periods consisted of 2 weeks of adaptation and 1 week of sampling and data collection. The experiment was conducted from March to June 2002. Animals were individually housed in tie-stalls on rubber comfort mats on the Experimental Farm of the Universitat Autònoma de Barcelona. Surgery was performed several months before the beginning of the experiment, following standard surgical procedures and conducted under local anaesthesia with full aseptic precautions. The research protocol was approved by the Institutional Animal Care and Use Committee of the Universitat Autònoma de Barcelona.

Feed, Water Supply and Data Collection

Heifers were offered concentrate and barley straw on an *ad libitum* basis. The concentrates were formulated according to the National Research Council (1996) for a 325-kg heifer with an ADG of 1.57 kg/d, and contained 0 (control diet), 1.25, 2.5 and 5 % of added BICARB, on a DM basis (Table 1). Soybean hulls were added to ensure the same level of highly fermentable non-structural carbohydrates and crude protein among the experimental diets. All ingredients of the concentrate were ground through a 3 mm screen, mixed and pelleted to 5 mm diameter. The particle size of barley straw was determined by dry sieving with the Penn State Particle Separator (Lammers et al., 1996). The percentage of DM retained in each sieve was 47.3, 27.0 and 25.7 %, for the 19-mm screen, 8-mm screen and bottom pan, respectively; with a geometric average particle size of 9.58 ± 4.31 mm. Feeders were cleaned and orts collected at 0800 each morning, and feed offered once daily at 0830. Concentrate and straw mixed orts were weighed before feeding, subsampled for later chemical analysis, and then both components were manually separated by the use of a screen to calculate the amount to be offered. Concentrate and straw were offered at115% of the previous day's intake. Intake of straw was recalculated through the NDF and ADF content of feed offered and refusals to check the accurancy of the measure and there was excellent agreement. Diet was changed gradually during the first three days of each period (33%, 66% and 100% of new treatment diet for d 1, 2 and 3, respectively). To register water consumption, individual water bowls with direct reading flow meters were used (B98.32.50, Invensys model 510 C, Tashia SL, Artesa de Segre, Spain), which allowed a minimum water measurement of 20 mL. Water was available at all times and consumption was read three times on each day of the sampling period at 0830, 1230 and 2030. The water consumption to DMI ratio for these intervals was calculated because continuous recording of feed intake was available (González et al., 2007).

Sample Collection and Analyses

Body weight was recorded before feeding and after withdrawal of refusals on 3 consecutive days at the start and the conclusion of the experiment. Intermediate weights were taken every 3 weeks.

Concentrate and barley straw refusals for each heifer were removed before feeding, weighed, sub-sampled and analyzed for DM content to record daily feed dry matter intake. Dry matter content of offered feed and refusals were determined by drying samples for 24 h at 103 ºC in a forced-air oven according to AOAC (1990; ID 950.01). Feed offered and refusal samples were collected daily for five consecutive days from d 15 to 19, composited for each heifer and period, mixed and dried in a forced air oven at 65 ºC for 48 h for later chemical analysis.

Table 1. *Ingredients and chemical composition of concentrates*

	Concentrates [a]				Straw
Item	0	1.25	2.5	5	
Ingredient, % DM					
Barley	34.1	34.0	34.0	37.45	
Corn	31.4	31.05	30.7	26.7	
Tapioca	16.4	16.4	16.4	16.3	
Soybean meal	10.0	10.3	10.5	10.8	
Soybean hulls	5.4	4.3	3.2	1.1	
Calcium carbonate	1.1	1.1	1.1	1.1	
White salt	0.2	0.2	0.2	0.2	
Sodium bicarbonate	0.0	1.25	2.50	4.95	
Tallow	1.1	1.1	1.1	1.1	
Vitamin-mineral premix [b]	0.3	0.3	0.3	0.3	
Chemical composition, % DM					
DM	88.7	88.9	89.0	89.3	91.9
OM [c]	95.3	94.8	94.1	92.8	93.9
Ash	4.7	5.2	5.9	7.2	6.1
CP	13.5	13.3	13.4	13.4	3.5
EE [d]	2.6	2.4	2.4	2.4	
NDF	14.4	13.6	12.9	12.4	75.8
ADF	7.6	6.9	6.3	5.5	44.0
NFC [e]	64.8	65.5	65.4	64.6	12.7
Starch	53.2	53.2	51.5	49.1	
K	0.53	0.57	0.56	0.54	
Na	0.09	0.34	0.80	1.25	
Cl	0.18	0.16	0.17	0.17	
S	0.35	0.33	0.35	0.35	
DCAD, mEq [Na + K] - [S + Cl] [f]	-9.69	4.16	22.29	41.64	

[a] Treatments were 0, 1.25, 2.5 and 5 % of sodium bicarbonate concentration in the concentrate

[b] Karimix® Terneros (Laboratorios Karizoo S.A., Barcelona, Spain): vitamin and mineral premix contained per kg DM premix: 3,333 kIU vitamin A, 666 kIU vitamin D_3, 2,166 IU vitamin E, 0.66 g vitamin B1, 0.66 g vitamin B2, 2 mg vitamin B12, 26 g coline chloride, 13.4 g Zn, 3.3 g Fe, 83.3 g S, 166.6 mg Co, 3.3 g Cu, 16.6 g Mn, 16.6 g Mg, 116.6 mg I, 66.6 mg Se, 100 mg Etoxiquine, 100 mg Butilhidroxitoluene.

[c] Organic matter: calculated as DM minus ash content

[d] EE: ether extract content

[e] NFC: non-fiber carbohydrates calculated as 100 – (CP + ash + NDF + EE)

[f] DCAD: dietary cation-anion difference

Feeds and refusals were ground in a hammer mill through a 1-mm screen (P. PRAT SA, Sabadell, Spain) and retained for analysis of DM and ash (AOAC, 1990; ID 950.05). Nitrogen content was determined by the Kjeldahl procedure (AOAC, 1990; ID 976.05). Organic matter was calculated as the difference between DM and ash content. Ether extract was performed according to AOAC (1990; ID 920.39). The NDF and ADF contents were determined sequentially by the procedure of Van Soest et al. (1991) using a thermostable α-amylase and sodium sulphite. Starch was analyzed by a modified method of Theander et al. (1995) for non-starch polysaccharides through enzymatic hydrolysis with α-amylase and amyloglucosydase, and later determination of glucose by spectrophotometry. Sodium (AOAC, 1990; ID 985.35) and K were determined by atomic emission spectrophotometry (model 410, Sherwood SCI, Cambridge, U.K.) previous digestion of the sample with HCl. Cloride was determined by flow photometry (Bran Luebbe, model AA3, Nordestedt, Germany) and sulfur through the $BaSO_4$ gravimetric method. Dry matter and nutrient daily intake were calculated as the difference between amounts offered and refused based on chemical analysis of the composited sample within heifer and period.

On d 18 of each period, ruminal samples (0.25 L) were taken with an electric vacuum pump connected to a 1-m iron tube that was introduced through the ruminal trocar to reach different locations within the rumen and obtain a representative sample. Times of sampling were: immediately before feeding and at 2, 4, 8, 12, 16 and 24 h after feeding. The ruminal fluid was squeezed through four layers of cheesecloth and pH was measured immediately with a glass electrode pHmeter (model 507, Crisson Instruments SA, Barcelona, Spain). Two sub-samples were taken for NH_3 N and volatile fatty acids (VFA) analysis as described elsewhere (Rotger et al., 2005). First, a 4-mL sample of filtered fluid was acidified with 4 mL of 0.2 *N* HCl and frozen at -20º C. Samples were later thawed, centrifuged at 25,000 x *g* for 20 min and the supernatant analyzed for NH_3 N by spectrophotometry (model Libra S21, Biochrom Ltd., Cambridge,

England). Second, 4 mL of filtered ruminal fluid were added to 1 mL of a solution made up of 1% (wt/wt) solution of mercuric chloride, to prevent microbial growth, 2% (vol/vol) orthophosphoric acid and 0.2% (wt/wt) 4-methylvaleric acid as an internal standard in distilled water and frozen at -20º C. Samples for volatile fatty acids (VFA) analyses were thawed and centrifuged at 15,000 g for 20 min, and diluted 1:1 in distilled water for subsequent analysis using gas chromatography (model 6890, Hewlett Packard, Palo Alto, CA). A capillary column treated with polyethylene glycol TPA (BP21, SGE, Europe Ltd., Buckinghamshire, UK) at 275 ºC in the injector and a 29.9 mL/min total gas flow rate were used in the chromatograph.

The daily average of ruminal fluid pH, NH_3 N and VFA concentrations were calculated with the area under the ruminal data vs. time curve and dividing by the total time (Pitt and Pell, 1997). The area under the pH curve and the number of hours during which ruminal pH remained below 5.8 were calculated assuming that the change in pH between two consecutive measures was linear.

Statistical Analyses

The individual animal fed a given treatment diet at each period was considered the experimental unit in all the analyses, which were conducted by mixed-effects regression model analysis of variance using the MIXED procedure of SAS (SAS Institute Inc., Cary, NC, v 8.2, 1999). All variables were averaged to generate period means on a daily basis for each heifer and treatment previous to the statistical analysis. Therefore, there was one daily mean value of feed intake, water consumption, ruminal pH and VFA for each experimental unit. The response to increasing BICARB levels on water comsuption and ruminal fermentation at any point in time within the day was also investigated. The average of 5-d water consumption during each interval of time (from 0 to 4, from 4 to 12, and from 12 to 24 h post-feeding) was calculated but it was not the case for ruminal pH (one sampling day per heifer period). The regression approach is appropriate for assessing relationships among BICARB levels and response. Thefore, the main focus of the present trial was to assess the trends in the response variables as BICARB increased. The model contained the fixed linear, quadratic and cubic effect of sodium bicarbonate level (continuous variable), the categorical effect of time of the day as repeated measure subjected to heifer by period nested within treatment, the interactions between both fixed effects (linear by time, quadratic by time, and cubic by time), and period and heifer as random effects. The linear, quadratic and cubic terms tested for the significance of overall or average regression coefficients of BICARB level regardless of sampling time. The linear quadratic and cubic terms by time interaction tested for the null hypothesis that regression coefficients were equal among all sampling times. Under a significant interaction, the

next step was to test the null hypothesis that all regression coefficients were equal among them and equal than zero at all sampling times. Then, linear, quadratic and cubic regression coefficients at each point in time were calculated and tested for their difference from zero using the SOLUTION statement (SAS/STAT, 2004). For those variables expressed on a daily basis, the same model was used but the main effect of time and its interactions were taken out from the model and, therefore, the main linear, quadratic and cubic effects were considered as fixed effects plus the random effects of heifer and period. The choice of the best covariance structure was based on biological meaning and fit statistics, where the model that minimized either Akaike Information Criteria Corrected or Schwarz's Bayesian Information Criteria was preferable (Littell et al., 1998). In most of the ruminal variables with repeated measures, Heterogeneous Toeplitz covariance structure provided the best fit because it yielded a cyclic (circadian) correlation matrix where the 0 h sampling time was more correlated to 24 h than to any other sampling time. It also allowed different variances among the repeated measures, and sampling time at unequal intervals. Significance was declared at $P < 0.05$ and tendencies are discussed at $0.05 < P \leq 0.10$. Multiple equations of regression were developed using the REG (STEPWISE) procedure of SAS taking all the variables available on the ruminal sampling day. Variables selected were tested for tolerance and collinearity (SAS/STAT, 2004).

Results

Average daily gain during the experiment and final BW of the heifers were 1.1 ± 0.23 kg/d and 361 ± 22.9 kg, respectively. The experiment was not designed to evaluate the treatment effect on animal performance. However, it should be noted that ADG for 0, 1.25, 2.5, and 5 % treatments were 1.46, 1.44, 0.98, and 0.52 kg/d, respectively.

Intake and Water Consumption

Concentrate DMI decreased linearly ($P = 0.03$; Table 2) with increasing BICARB level in the diet. In contrast, straw DMI increased linearly ($P < 0.01$), resulting in a tendency to a linear decrease in the total DMI ($P < 0.10$), and a linear increase ($P < 0.01$) in the roughage proportion of the total intake. The intake of OM and CP followed the same pattern as concentrate DMI, both decreasing linearly ($P \leq 0.05$) as the BICARB level increased. Total NDF intake was not affected by treatments, because it was counterbalanced by NDF intakes of both dietary components. However, the proportion of both NDF and ADF of the total intake increased linearly ($P < 0.05$; data not shown).

Table 2. *Effect of increasing sodium bicarbonate level in high-concentrate diets on intake*

	Treatment [a]					Effect [b]		
Item	0	1.25	2.5	5	s.e.	L	Q	C
Intake, kg/d								
Concentrate DM	6.89	7.66	6.72	5.72	0.83	*		
Straw DM	0.73	0.84	0.94	1.06	0.14	**		
Total DM	7.62	8.50	7.66	6.78	0.81			
OM	7.26	8.05	7.21	6.31	0.77	*		
CP	0.96	1.06	0.93	0.80	0.11	*		
NDF	1.54	1.68	1.58	1.56	0.13			
Straw, % total DMI	10.21	10.91	13.29	17.17	2.30	**		

[a] Treatments were 0, 1.25, 2.5 and 5 % of sodium bicarbonate concentration in the concentrate

[b] Effect of sodium bicarbonate level was significant at $P \leq 0.01$ (******), or at $P \leq 0.05$ (*****): L = linear, Q = quadratic and C = cubic

Daily water consumption, in L/d or in percentage of BW, was not affected by treatments (Table 3). However, the water consumption to dry matter intake ratio (L/kg DMI) increased linearly ($P = 0.03$) when buffer level increased. Moreover, heifers modified their drinking behavior pattern. There was a linear increase ($P = 0.01$) in the proportion of total daily water drunk in the morning period and a tendency ($P < 0.10$) to decrease the proportion of water drunk during the night period, when the BICARB level was increased. Nevertheless, linear and quadratic effects of treatment ($P < 0.05$) on the water consumption to dry matter intake ratio were observed during the intervals of time between 1230 to 2030 and 2030 to 0830 (Table 3).

Ruminal pH

Although the control diet resulted in 0.42 pH units lower daily average ruminal pH compared to the buffer treatments (5.91 vs. 6.33, respectively; Table 4), no effect was found (linear $P = 0.11$). No trends were found in the lowest (5.65 ± 0.09) or highest (7.11 ± 0.09) daily pH. The number of hours and area under the curve in which pH remained under 5.8 tended to a quadratic effect ($P \leq 0.10$).

Table 3. *Effect of increasing sodium bicarbonate level in high-concentrate diets on water consumption (WC), ratio WC to dry matter intake (DMI) and pattern of daily WC*

	Treatment [a]					Effect [b]		
Item	0	1.25	2.5	5	s.e.	L	Q	C
WC, L/d	28.04	32.12	27.00	30.13	3.86			
WC, % BW	8.14	9.76	8.44	9.41	0.74			
WC, % total daily								
0830-1230 [c]	18.35	21.95	21.42	30.09	2.62	**		
1230-2030	47.51	45.33	44.29	45.23	2.42			
2030-0830	34.14	32.72	34.29	24.68	3.05			
WC/DMI, L/kgDMI								
Daily average	3.60	4.04	3.65	4.48	0.50	*		
0830-1230 [c]	2.61	2.84	2.95	3.02	0.65			
1230-2030	4.81	4.38	4.21	6.12	0.73	**	**	
2030-0830	6.21	6.63	5.33	9.01	1.35	**	*	

[a] Treatments were 0, 1.25, 2.5 and 5 % of sodium bicarbonate concentration in the concentrate

[b] Effect of sodium bicarbonate level was significant at $P \leq 0.01$ (**), or at $P \leq 0.05$ (*): L = linear, Q = quadratic, and C = cubic

[c] Water consumption, as percent of total daily, and the water consumption to DMI ratio during the morning (0830-1230), afternoon (1230-2030) and night (2030-0830).

Analysing the pH patterns, the linear BICARB level by time interaction tended to be significant ($P = 0.06$). Increasing BICARB levels resulted in linear increases of ruminal pH at 2 and 4 h post-feeding ($P < 0.05$). The linear coefficient of regression indicated that the increase of one percentage unit of BICARB in the concentrate resulted in 0.13 pH units greater ruminal pH at at 2 ($b = 0.13 \pm 0.04$; $P = 0.004$), and 0.12 pH units greater at 4 h post-feeding ($b = 0.12 \pm 0.06$; $P = 0.04$). Whereas, quadratic ($P = 0.06$) and cubic ($P = 0.09$) tendencies were observed at 8 and 16 h post-feeding, respectively. When comparisons were made within treatment, the pH at 0% BICARB level fell ($P < 0.05$) at 4 h after feeding, whereas in the 1.25% diet no significant decrease was observed at any time during the after feeding cycle.

Table 4. *Effect of increasing sodium bicarbonate level in high-concentrate diets on ruminal pH*

Item	Treatment [a]					Effect [b]		
	0	1.25	2.5	5	s.e.	L	Q	C
Daily pH								
Average	5.91	6.36	6.26	6.38	0.15			
Lowest	5.43	5.74	5.74	5.71	0.19			
Highest	6.86	7.24	6.96	7.37	0.18			
pH < 5.8								
Hours	12.58	3.57	3.22	4.70	2.60			
Area	73.86	17.65	18.00	28.04	15.14			
Total area	142	153	150	153	3.51			
Time [c]								
0	6.86	7.12	6.92	7.37	0.22			
2	6.22	6.54	6.48	6.92	0.16	**		
4	5.93	6.20	6.03	6.59	0.21	*		
8	5.84	6.16	6.47	6.20	0.19			
12	5.66	6.04	5.95	5.92	0.25			
16	5.50	6.19	5.80	5.94	0.23			
24	6.42	6.96	6.94	7.00	0.25			

[a] Treatments were 0, 1.25, 2.5 and 5 % of sodium bicarbonate concentration in the concentrate

[b] Effect of sodium bicarbonate level was significant at $P \leq 0.01$ (******), or at $P \leq 0.05$ (*****): L = linear, Q = quadratic and C = cubic

[c] Time after feeding.

The average lowest pH was found at 16 h after feeding for 0 and 2.5 %, and at 12 h for the 1.25 and 5 % BICARB treatment. In all diets, ruminal pH decreased from 0 to 2 h and then from 2 to 4 h after feeding ($P < 0.05$). Thereafter, it remained low until 16 h after feeding. Nevertheless, ruminal

pH continued decreasing numerically to reach the lowest daily pH at 16 h, and then it increased at the 24 h sampling (24 h; $P < 0.05$).

Table 5. *Effect of increasing sodium bicarbonate level in high-concentrate diets on rumen volatile fatty acids (VFA) and ammonia nitrogen concentration (NH_3 N)*

	Treatment [a]					Effect [b]		
Item	0	1.25	2.5	5	s.e.	L	Q	C
Total VFA, m*M*	158	133	146	137	11.90			
BCVFA, m*M*	3.31	2.73	4.52	6.46	0.84	**		
VFA, mol/100 mol								
Acetate	53.64	50.72	56.73	62.12	4.80	*		
Propionate	35.76	36.79	30.46	21.33	5.62	*		
Butyrate	7.06	8.52	8.27	10.77	0.87	*		
Valerate	1.32	1.73	1.31	1.15	0.20			
Isobutyrate	0.62	0.76	0.83	1.08	0.13	*		
Isovalerate	1.58	1.48	2.39	3.55	0.67	*		
Acetate: Propionate ratio	1.85	1.74	2.27	3.25	0.61	*		
NH_3 N, mgN/100mL	1.84	2.93	2.10	2.90	0.87			

[a] Treatments were 0, 1.25, 2.5 and 5 % of sodium bicarbonate concentration in the concentrate

[b] Effect of sodium bicarbonate level was significant at P ≤ 0.01 (**), or at P ≤ 0.05 (*): L = linear, Q = quadratic and C = cubic

Ruminal Fermentation

Daily average total VFA concentration (m*M*) was not affected ($P > 0.10$) by treatments (Table 5). Daily average acetate molar proportion (mol/100mols) increased linearly ($P = 0.05$) with increasing BICARB concentration. Contrarily, the daily average propionate molar proportion decreased linearly ($P = 0.02$) with BICARB addition. As a result, the daily average ratio of acetate to propionate increased linearly ($P = 0.05$) as BICARB level increased. As buffer level increased, the daily average of n-butyrate increased linearly ($P = 0.02$). No effects of BICARB addition were observed on daily average valerate molar proportion. Daily average branched-chain VFA (BCVFA)

molar concentration (m*M*) increased linearly ($P = 0.01$) with increasing buffer level in the diet. The daily averages of iso-butyrate and iso-valerate molar proportions also increased linearly ($P < 0.05$) with BICARB level and were observed to be uniformly affected by treatments throughout the feeding cycle (data not shown). Daily average NH_3 N concentration was highly variable and not affected by treatments (Table 5).

Discussion

Intake and Water Consumption

Increasing buffer level resulted in a linear decrease of concentrate DMI. However, the 1.25% BICARB diet showed the highest concentrate and total DMI and the lowest was found with heifers fed the 5% BICARB diet. Hart and Polan (1984) and Thomas and Hall (1984) did not observe differences in DMI with levels of up to 4.5% BICARB in growing calves fed high concentrate diets. However, they reported an intake pattern very similar to that found in the present trial as level of buffer increased. Whereas Jackson et al. (1992) observed a quadratic trend with the highest intake when 1.88% of BICARB was added to calves' starter. Leventini et at. (1990) hypothesized that increases in liquid passage rate caused by BICARB addition lead to an increase in ruminal wash out of particles with the corresponding increase in feed intake. The reduction in DMI observed at the highest level of BICARB is in agreement with the results of Emery et al. (1964) in dairy cows fed high concentrate but contrary to those of Nicholson et al. (1963) and Wise (1965) in growing cattle fed all concentrate diets. The negative effects of high BICARB levels on intake could be attributed to reduced palatability, increased ruminal osmolality or dietary cation-anion difference (**DCAD**). Under the present experimental diets, heifers consumed an average of 286, 168 and 96 g/d of BICARB, for the 5, 2.5 and 1.25 % BICARB treatments, respectively. When dairy cows were given BICARB at free choice they did not eat more than 40 g/d, and the authors suggested that it would have adverse organoleptic properties (Keunen et al., 2003). On the other hand, osmolality of ruminal liquid and plasma is considered a triggering factor in feed intake regulation (Carter and Grovum, 1990), although the effect of DCAD *per se* on intake is difficult to isolate from that of osmolality or dietary Na with the literature available because BICARB has been usually used to increase the DCAD (Jackson et al., 1992). Hu and Murphy (2004) calculated that DMI peaked at a DCAD of 40 mEq/100 g of DM in a meta-analysis with dairy rations. The animal has to maintain an osmotic pressure balance of body fluids, which may be achieved by increasing water consumption and rumen influx of water from plasma, or decreasing feed intake (Langhans et al., 1995). In the

present experiment, we hypothesize that concentrate DMI was decreased by buffer addition in an attempt to avoid ruminal osmolality increases. Thus, heifers consumed more straw DMI in an attempt to maximize feed intake. Cooper et al. (1996) proposed that diet selection in ruminants is an attempt to promote high levels of feed intake while maintaining ruminal conditions within certain physiological limits. Those authors offered free choice of pelleted barley-based concentrates containing BICARB at 0, 1, 2 and 4% to sheep together with one of two forage sources, long-chopped or pelleted alfalfa. Total feed consumed was not affected and diet selection was not dependent of the concentration of BICARB in the pellets. However, the proportion of long-chopped alfalfa selected by sheep increased from 15.6 to 28.8% of the total intake, and the selection of pelleted alfalfa from 34 to 51%, when the proportion of BICARB in the concentrate increased from 0 to 4%, respectively. In addition, when Cooper et al. (1996) offered free choice of the 0% paired with the 4% BICARB sheep selected against the later. We especulate that animals under high BICARB diets of our study had a lower physiological limit in the level of feed intake, likely set by BICARB in order to avoid increases in ruminal osmolality.

The BICARB level had no effect on water consumption though the reason is unclear. Warner and Stacy (1968) observed an increase in rumen volume and in outflow and dilution rates during and shortly after eating and drinking in sheep, and concluded that there is a physiological limit on those variables beyond a certain point. However, these limiting variables were not identified. Daily water consumption was positively correlated ($P \leq 0.01$; data not shown) with concentrate, total DM, NDF and ADF intakes, and with BW but not with straw DMI or BICARB level. This shows the close and positive relationship between DMI and water consumption. Surprisingly, water consumption was not correlated with buffer level. Water consumption decreases by 4.4 L/d for each one percentage unit increase of dietary salt in feedlot cattle (NRC, 1996), and increases by 0.05 kg water per each g Na ingested in dairy cattle (Murphy, 1992). Hoffman and Self (1972) observed similar values than the present study for water consumption of feedlot cattle. In agreement with our results, Rogers et al. (1982) found no effect of BICARB in high-concentrate diets on daily water consumption. Water consumption increased linearly during the morning but decreased during the night as BICARB level increased, whether expressed as percentage of daily water consumption or as total amount (L). When water consumption was expressed as L/kg DMI, a linear increase with buffer level was observed. Wheeler et al. (1980) also observed an increase in the water consumption to DMI ratio when adding 5 % BICARB, without affecting total water consumption. Because the water consumption to DMI ratio is a response to the need to maintain body water and electrolyte balances, under high mineral addition an increase of osmolality was prevented by decreasing electrolyte intake, rather than increasing water consumption (Carter and Grovum, 1990; Langhans et al., 1995).

Treatment effects on this ratio were observed during the afternoon (1230 to 2030) and night (2030 to 0830), and explained by a linear decrease in DMI during the afternoon period ($P < 0.10$) and a quadratic decrease at night ($P < 0.05$; data not shown). This ratio was lowest between 0830 and 1230 (2.85 ± 0.32 L/KgDMI), in the medium range from 1230 to 2030 (4.88 ± 0.36) and highest from 2030 to 0830 (6.80 ± 0.67). Ruminal osmolality kinetics follow a pattern related to the contribution of dietary minerals (rapidly dissolved) and the accumulation of fermentation products, which will depend on diet type (Bennink et al., 1978) and intake patterns. This may explain the patterns of water consumption but, unfortunately, ruminal osmolality was not measured in the present trial.

Ruminal pH

The increase observed in roughage intake and decrease in concentrate intake may be confounding the interpretation of results on ruminal fermentation. We decided *ad libitum* straw allowance because it is the common feeding management in many commercial facilities around the world, as it is in Spain. The proportion of straw consumed increased from 10.2 to 17.2 % as the level of sodium bicarbonate increased from 0 to 5 % of concentrate DM. However, this range of roughage intake variation is thought to have little effect on the measured parameters. Even higher increases in the forage-to-concentrate ratio of beef cattle did not show consistent effects on intake, ruminal pH or VFA (Rotger et al., 2005; White et al., 1971).

An increase of rumen fluid and solid passage rates, which results from increased water consumption (Rogers and Davis, 1982a and b), is thought to be the main factor increasing ruminal pH when using mineral buffers, because of reduced substrate availability for fermentation (Russell and Chow, 1993). More recently, however, there has been renewed support for the BICARB effect on ruminal pH through hydrogens neutralization (Kohn and Dunlap, 1998). In the present experiment, water consumption was not consistently affected by BICARB addition, this being a possible reason for the lack of buffer effect on daily average ruminal pH. Indeed, even a decrease in ruminal acid load could be expected as level of BICARB increased, due to decreased concentrate intake and increased forage intake, this did not affect ruminal pH. Erdman (1988) reported a mean increase of 0.26 pH units when BICARB was added to dairy cow diets containing less than 30 % forage, at a mean rate of 2.5 % of BICARB. Nicholson et al. (1963) observed an increase of 0.46 pH units in the mean 8-h post-prandial pH when adding 3% BICARB to all concentrate rations. The regression coefficients of the buffer concentration on ruminal pH at 2 and 4 h post-feeding were similar (0.13 and 0.12, respectively). These results indicate that BICARB alleviated the after feeding pH depression. In

fact, pH fell by 0.64, 0.58, 0.44 and 0.45 pH units at 2 h after feeding, for the 0, 1.25, 2.5 and 5 % BICARB treatments, respectively. Higher ruminal pH in single rumen samples taken at 4 h after feeding high-concentrate rations were reported by Zinn (1991) when feeding 0.75% BICARB diets to finishing steers and Quigley et al. (1992) 3% BICARB to calves. The effect of BICARB on post-feeding ruminal pH plus the large differences observed for the number of hours and area under the pH curve in which pH remained under 5.8, not previously reported, could be the most beneficial effect of the buffer on rumen environment.

Ruminal Fermentation

Values found for total VFA concentrations are typically high for concentrate finishing rations (Rumsey et al., 1970; Rotger et al., 2005). Although daily average total VFA concentration of the control diet was 12% higher than the rest of treatments, no effect was observed. This is in agreement with other reports (Nicholson et al., 1963; Rogers et al., 1982). However, decreases in total VFA concentration caused by increased water comsumption and passage rate were suggested as the main mechanism for this result when using mineral buffers (Rogers and Davis, 1982a and b; Russell and Chow, 1993). We expected an effect of BICARB level on total VFA concentrations at 2 and 4 h after feeding because of previously observed effects on ruminal pH (Table 3) and water consumption (Table 4). Although total VFA concentration at 2 h was 21% higher in the control diet compared to the 5% BICARB treatment, the differences were not significant. Regardless of diet, VFA concentration increased from 0 to 2 h and further to 4 h after feeding ($P < 0.05$; data not shown), as opposed to ruminal pH. Thereafter, it remained high until 16 h after feeding, and decreased again until 24 h ($P < 0.05$).

Whereas the average molar proportion of propionate decreased linearly with BICARB addition, molar proportion of acetate increased linearly, resulting in a linear increase in the acetate to propionate ratio (Table 5). When multiple regression of acetate and propionate molar proportion and their ratio were calculated against all the preceding variables, all three were mostly explained by the level of concentrate intake, in g DM / kg $BW^{0.75}$, which yielded an $r^2 \geq 0.75$. The number of hours in which ruminal pH remained under 5.8 explained a smaller proportion of the variation, with an $r^2 \leq 0.08$. These results are consistent with Rumsey et al. (1970), who demonstrated the necessity of recognizing the effect of feed intake level when interpreting ruminal data. Moreover, they pointed out that changes in ruminal acids due to feed intake level were greater when an all-concentrate diet was fed compared to a roughage diet, probably due to the inherently low liquid and solid ruminal passage rates. In the present experiment, however, confounding factors may be hidden

by the level of concentrate intake because linear effects were observed for many variables. The effect of the number of hours at suboptimal pH on the VFA molar proportions was previously demonstrated in vitro in our laboratory (Cerrato et al., 2007). The treatment effect on daily average propionate and acetate molar proportion is in agreement with Thomas and Hall (1984), Zinn (1991) and Quigley et al. (1992) with BICARB levels of 0.75, 3 and 5 %, respectively, in growing cattle fed high-concentrate diets. However, Hart and Polan (1984) and Nicholson et al. (1963) did not observe any difference in propionate at BICARB levels between 0.75 and 4.5 %.

The increase in daily average n-butyrate is in agreement with Nicholson et al. (1963) and Rogers et al. (1982). In contrast, Thomas and Hall (1984) did not observe any effect of adding 1 and 2.5% of BICARB on n-butyrate. Ruminal BCVFA originate primarily from dietary true protein degradation, although microbial protein recycling within the rumen also increases BCVFA (Miura et al., 1980). In the present experiment, there is no evidence of different dietary protein degradation, because there were no differences in ruminal NH_3 N concentration among diets. However, the linear increase of BCVFA as the BICARB level increased could be due to greater protein recycling or degradability, or both, caused by the decrease in CP intake as the BICARB level increased. In fact, when stepwise regression was performed, the selected variables affecting daily average BCVFA concentration were CP intake, BICARB level, and the water consumption to DMI ratio (adjusted R^2 = 0.66; $P < 0.01$). The CP intake contributed to the model with a negative coefficient of regression (b = -0.62), explaining 47% of the total variation in the BCVFA concentration. In contrast, Hart and Polan (1984) did not find any effect of linear increases of BICARB on isobutyric or isovaleric acids at 3 h post-feeding.

In conclusion, the addition of sodium bicarbonate to high-concentrate diets for growing heifers reduced the intake of concentrate that included bicarbonate whereas straw intake decreased. Therefore, animal performance may be affected. Total daily water consumption was not affected but the amount drunk per unit of feed intake increased as sodium bicarbonate increased. No consistent effects on daily ruminal pH were observed, perhaps because the buffer did not affect total daily water consumption. However, alleviation in the post-prandial ruminal pH depression was observed shortly after feeding. All the ruminal VFA proportions were affected by bicarbonate level, except n-valerate.

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Chapter III

Increasing sodium bicarbonate level in high-concentrate diets for heifers.

II. Effects on chewing and feeding behaviors

Chapter based on a paper sent to Animal

Abstract

Four Holstein heifers (264 ± 12 kg initial BW) were used in a 4 x 4 Latin square design with 21-d experimental periods to determine the effect of increasing levels of sodium bicarbonate (0, 1.25, 2.5 and 5 %, of concentrate dry matter (**DM**) basis) on chewing and feed intake behavior when fed high-concentrate diets. Concentrate (13.41% CP, 13.35% NDF) and barley straw were fed once a day at 0830 *ad libitum*. Feed bunks placed on scales and video recording were used to measure 24-h feed intake and chewing behavior, respectively. The patterns of feeding behavior (feed intake, meal size and length) and chewing behavior (eating, ruminating, and total chewing) were studied by dividing the day in 12 intervals of 2-h each, beginning at feeding (interval 1 through 12). Number of meals per day and eating rate decreased linearly with increasing buffer level, but meal length increased linearly. No treatment effects were observed in sum of daily meal lengths or average meal size. The treatment by interval interaction was significant on meal size, length and feed intake. The size and length of those meals occurring during the 4 h post-feeding increased linearly. However, meal size tended to decrease at the evening between 8 and 12 h whereas feed intake decreased linearly from 6 to 10 and 12 to 14 h post-feeding. Buffer concentration did not affect percentage of time spent ruminating, eating or drinking per day but the buffer level by interval interaction was significant. Time spent eating expressed as min per kg of DM or organic matter intake increased linearly with buffer levels. Proportion of time spent eating increased linearly during the intervals between 0 and 4 h post-feeding. Time spent ruminating decreased linearly during the 2 h post-feeding, and also at the evening from 12 to 14 h, and at nighttimes from 18 to 22 h post-feeding, but the effect was quadratic between 8 and 10 h when intermediate buffer levels showed the greatest ruminating time. Time spent drinking decreased linearly from 6 to 8 h but increased during the 2 h following feeding and from 10 to 12 h post-feeding. Daily eating rate and meal frequency decreased linearly as buffer level increased but average meal size and daily chewing times were not affected. However, significant time of the day by buffer level interactions were observed for feed intake, meal size and length, and chewing behavior.

Introduction

High proportion of concentrates in ruminant's diets may result in physiological disorders such as ruminal acidosis, which was related to anorexia, liver abscesses, poor performance, diarrhea, and lethargy (Owens et al., 1998). Ruminal buffers such as sodium bicarbonate (**BICARB**) have traditionally been used to improve performance and rumen health of beef cattle on these diets. However, saliva production is the main source of bicarbonate in the rumen environment (Erdman,

1988). Thus, eating and ruminating activities are important components of the digestive function because they are associated with increased saliva output (Bailey and Balch, 1961; Allen, 1997). The amount of daily sodium bicarbonate secretion through saliva is greater than that entering the rumen through dietary buffers (Erdman, 1988). However, in beef cattle fed high-concentrate diets mastication is reduced (Sudweeks et al., 1975; Carter and Grovum, 1990) and dietary buffers could, therefore, become more important for rumen function and health. Fulton et al. (1979) observed lower intake in steers being adapted to wheat compared to corn diets, concurrent with a lower ruminal pH. The times of the day when the lower DMI occur were also related to lower ruminal pH in the wheat diets. It was suggested that animals regulate ruminal pH through changes in feeding behavior (stop eating) to restore pH conditions to comfortable levels and, therefore, low ruminal pH results in erratic eating patterns (Fulton et al., 1979; Schwartzkopf-Genswein et al., 2003). However, other management factors may alter the eating patterns and become the cause of low ruminal pH, e. g. factors that increase meal size and eating rate may result in lower ruminal pH and greater risk of acidosis (Allen, 1997; Owens et al., 1998; Schwartzkopf-Genswein et al., 2003). Feeding BICARB increased ruminal pH and osmolality (Rogers and Davis, 1982). However, low pH and high osmolality in the rumen may reduce ruminating time (Welch, 1982). Sodium bicarbonate may improve ruminal pH and, consequently, change the chewing and feeding behaviors. However, bicarbonate may affect these behaviors without mediation of ruminal pH but in such a way that affects rumen function and health. Studying these factors together could provide a deeper insight to explain the mechanisms triggered when BICARB is added to high concentrate diets and to assess the relationships between behavior and rumen function.

The objective of this experiment was to investigate the changes in chewing and feed intake behaviors as BICARB level increases in the concentrate of beef heifers fed high-concentrate diets.

Material and methods

Animals, Experimental Design and Housing

Animals, diets and the experimental design were described in Chapter II. Briefly, four Holstein heifers (average initial BW of 264 ± 12 kg, final BW of 361 ± 23) were used in a 4 x 4 Latin Square design with four 21-d periods. Animals were individually housed in tie-stalls on rubber comfort mats on the Experimental Farm of the Universitat Autònoma de Barcelona. Light was continuously on during the sampling week to allow video recordings at night. The barn had small open windows occupying 6.5 % of the front and back wall surfaces.

Feed, Feeders and Feed Intake Behavior

Heifers were offered concentrate and barley straw on *ad libitum* basis at 115% of the previous day intake. Diets contained 0 (control diet), 1.25, 2.5 and 5 % of added BICARB to the concentrate, dry matter (DM) basis. Additional information on ingredients, chemical composition and processing can be obtained in Chapter II. To record feed intake from d 14 to d 18 of each experimental period, an automated system was used. Feed bunks (120 L capacity) were mounted on waterproof digital platform scales for each stall (model HW-60KV-WP, A & D Company Ltd., Tokyo, Japan). Iron bars were set between the heifers and the scale to avoid entrance, foot step or head resting on the scales. Each scale was programmed to transmit the feed weight at 1-min intervals. This interval was chosen because it was considered to be a reliable indicator of short-term feeding behavior. The information was downloaded onto a personal computer with a software application (WinCT, A & D Company Ltd., Tokyo, Japan). Feeding events were registered as minute-by-minute feeder disturbance. Each feed weight observation was classified as an "eating" observation when the as-fed feed intake (actual feed weight minus the prior one) was greater than 10 grams or when the measurement was recorded as "unstable" due to the animal's head pushing on the scale while eating. Otherwise, the observation was considered "no eating". The length of all inactive intervals, in min, in which feeding did not occur were registered during five days per heifer and period, log-transformed, and used to calculate the meal criterion (which is the minimum time required to consider two periods of eating activity as separate events) through a modification of the mixed distribution methodology described by Yeates et al. (2001). The Mixed Distributions Package on the R software (R Development Core Team, 2004) was applied. Gamma distributions resulted in the best fit of the data because the first population of non-feeding intervals was much skewed. Data was fitted to the non-feeding intervals length within and across heifers, periods, and also to the experimental unit (heifer in a period within a treatment). The latter resulted in the best goodness-of-fit and was, therefore, chosen for the determination of the meal criterion. The meal criterion was defined as the point where both first and second, or second and third populations of non-feeding intervals intersected. All minute-by-minute "eating" observations, separated by "no eating" observations shorter than or equal to the meal criterion, were grouped into meals. Meals smaller than 0.050 Kg of feed consumed were not considered an individual meal. Meal frequency (meals/d) was the number of intervals where eating activity was registered and that exceeded the meal criterion. Meal length (min/meal) was calculated as the time from the first eating observation until the time of the last eating observation (within a meal) before to an inactive interval that exceeded the meal criterion. Total daily meal time was the sum of each meal length (min/d). Meals were further characterized by DM ingested (meal size; g DM/meal), and rate of DM ingested per meal

(eating rate; g DM/min) calculated as the ratio of amount of feed ingested and the corresponding meal length. Calculations were required to account for changes in feed moisture during the day because of heifers drooling. Therefore, estimates of DM content in feed remaining were made assuming linear changes between DM content of feed offered and that of the refusals. Regardless of meal time, daily patterns of DM intake (**DMI**) were analyzed based on the amount of DM eaten between each 2-h after feeding interval throughout the day. To analyse daily meal size and length patterns, meals were assigned to a given after feeding interval depending on its starting time.

Behavior

To register animal behavior throughout the day a video-camera recording device was set in the barn. It consisted of a digital black and white camera (model LTC 0500/50, Philips, Eindhoven, The Netherlands), with iris vari-focal lenses (model LTC 3274/40, Philips), which was connected to a time lapse recorder (model RT 24ª/00T, Philips). Animal behavior was video-recorded for 24-h during d 15, 17 and 18 of each experimental period. Data processing was carried-out by scan sampling at 5 min intervals for posture and behavior of each heifer. The behavioral categories used were mutually exclusive and as defined later. Posture was recorded as standing when the body was supported by all four legs. Otherwise, posture was defined as lying on the right or left side of the body. Posture was recorded independently of the activity the animals were performing. Activities recorded included eating, ruminating, drinking, resting, self-grooming, social behavior and oral behavior. Data for each activity is presented as the percentage of total daily observations obtained by summing the number of times the activity was observed and divided by the total number of observations during the day, 288 observations per day or 864 observations per heifer and period. To analyse behavior patterns, the day was sub-divided in 12 intervals of 2-h each starting at time of feeding (intervals 1 through 12). Percentage of observations made for each activity was calculated by summing the number of times the activity was observed divided by the total number of observations during the interval, 24 observations per interval or 72 observations per interval and period.

Chewing Behavior. Chewing behavior was divided in three main categories: eating, ruminating and total chewing. An observation was defined as eating when the animal was eating, including manipulation and apprehension, from the feed bunk with muzzle in the feed bunk or chewing or swallowing food with head over it. Ruminating included the regurgitation, mastication and swallowing of the bolus. Total chewing was the sum of eating plus ruminating activities. To estimate time spent eating, ruminating or total chewing per kg of DM, organic matter (OM) and

NDF intake, each activity was assumed to persist for the entire 5-min period between each observation.

Drinking Behavior. Individual drinking cups and in-line water flow meters were placed in each stall. An activity was recorded as drinking when the heifer was with her muzzle in the water bowl or swallowing the water.

Other Behaviors. Non-chewing behavior categories were: resting, self-grooming, social behavior and oral behaviors. Resting was recorded when no chewing behavior and no apparent activity were being performed. Self-grooming was defined as non-stereotyped licking of the body or scratching with a hind limb or against the fixtures. Social behavior was registered when a heifer was licking or nosing a neighbouring heifer with the muzzle or butting. Oral behaviors included the act of licking or biting the fixtures, and tongue-rolling, considered a stereotypyed behavior.

Statistical Analyses

The experimental unit and statistical models used are described in Chapter II. All variables were averaged to generate period means for each heifer period that represented a mean daily value. In addition, a mean value was calculated for each 2-h interval of time within day to study the patterns of behaviors throughout the day. Behavioral activities expressed as percentage were statistically analyzed after square root-arcsine transformation (Mitlöhner et al., 2001). Data were analyzed with a mixed-effects regression model using the MIXED procedure of SAS for repeated measures (SAS Institute Inc., Cary, NC, v 8.2, 1999). Because equally spaced intervals within the day were taken, the heterogeneous autoregressive covariance structure generally resulted in the best fit of the data.

Results

Feed Intake Behavior

No significant effect of BICARB addition was observed on meal criteria (29.77 ± 2.81 min; Table 1). The frequency of meals decreased linearly ($P = 0.01$) with increasing buffer level, but it also showed a tendency for a quadratic decrease ($P = 0.10$), indicating that there could be a threshold response to dietary BICARB observed mainly at the 1.25% BICARB level that showed the greatest frequency but declined at higher BICARB levels. Meal length increased ($P = 0.02$) and eating rate decreased ($P = 0.02$) linearly with increasing buffer level. No treatment effects were observed for meal size (linear $P = 0.12$) or total daily meal time (cubic $P = 0.14$).

Daily patterns of DMI are shown in Figure 1a. The linear, quadratic and cubic treatment effect by interval interaction were all significant ($P < 0.05$), as was the interval main effect ($P < 0.001$). Those interactions indicate that linear, quadratic and cubic regression coefficients differed among intervals of time within the day. These interactions are explained by a linear decrease ($P \leq 0.05$) in the amount of feed consumed during the evening at intervals 5 (8-10 h post-feeding) and 7 (12-14 h), and a tendency at interval 4 (6-8 h; $P = 0.06$) when the BICARB level increased. However, DMI was affected cubically at interval 12 (22-24 h; $P < 0.001$). Additionally, eating rate decreased linearly during intervals 5 and 6 which were from 8 to 12 h post-feeding (data not shown; $P < 0.05$). In all diets, feed intake was highest ($P < 0.05$) during the first 2 h after feeding compared to any other interval of the day ($P < 0.05$). Thereafter, heifers maintained an average intake until the night, from interval 2 to 7 corresponding to 4 to 16 h after feeding. Feed intake decreased ($P < 0.05$) thereafter and remained very low during the night and increased ($P < 0.05$) again 2 h before the next feeding, from interval 11 to 12.

Table 1. *Feed intake behavior of beef heifers as affected by increasing levels of sodium bicarbonate in high-concentrate diets*

	Treatment [a]					Effect [b]		
Item	0	1.25	2.5	5	s.e.	L	Q	C
Meal criterion, min	26.99	25.08	34.37	32.64	5.88			
Meal								
Frequency, meals/d	10.62	11.07	10.62	7.66	1.12	**		
Length, min/meal	25.27	23.81	29.48	42.69	4.44	*		
Size, g DM/meal	646.02	706.09	677.21	846.46	80.19			
Eating rate, g DM/min	32.29	34.08	29.79	24.25	2.83	*		
Daily meal time, min/d	264.66	258.98	289.95	272.16	26.52			

[a] Treatments were 0, 1.25, 2.5 and 5 % sodium bicarbonate level in the concentrate

[b] Effect of sodium bicarbonate level was significant at P ≤ 0.01 (**), or at P ≤ 0.05 (*): L = linear, Q = quadratic and C = cubic

For the daily meal size patterns (Figure 1b), the after feeding interval ($P < 0.001$) and the linear treatment effect by interval of the day interaction was significant ($P < 0.05$). The interaction was the

result of a linear increase in meal size during the 4 h following the morning feeding, at intervals 1 and 2 ($P < 0.05$) as the level of buffer increased. Contrarily, meal size tended to decrease linearly at interval 5 (8-10 h) and quadratically at interval 6 (10-12 h; $P = 0.08$) when intermediate BICARB levels showed the greatest meal size. In all treatments, meal size (g DM/meal) during the 2 h following feeding was greater ($P < 0.05$) than at any other interval of the day. However, those meals did not differ from meals occurring from 10 to 12 h post-feeding (interval 6) because a slight increase in meal size was observed at this time. Thereafter, meal size decreased until the interval between 14-16 h ($P < 0.05$) to remain low until 22 h post-feeding.

Daily meal length patterns (min/meal) are shown in Figure 1c. Treatment, after feeding interval and their interaction were significant ($P \leq 0.05$). The length of the meals occurring during the 4 h post-feeding increased linearly (intervals 1 and 2), and also those from 16 to 18 h post-feeding (interval 9), with increasing level of BICARB, but they were affected cubically at interval 11 (20-22 h; $P < 0.05$). In all treatments, meals occurring from 0 to 2 h post-feeding (interval 1) were significantly longer than those from 4 to 8 (interval 3 and 4; $P < 0.05$) but not different from those occurred between 8 and 12 (interval 5 and 6). Thereafter, meal length decreased and remained shorter during the night intervals (8 to 11).

Chewing Behaviour

No effects of BICARB level were observed on the total proportion of time spent eating (Table 2), which is in agreement with the lack of treatment effect on total daily meal time (Table 1). The time spent eating per unit of DM or OM intake increased linearly ($P < 0.05$) when increasing the buffer level. Nevertheless, treatment effect on eating time per kg NDF intake was not significant ($P = 0.19$). When data from daily video recordings were analyzed in 2-h intervals (Figure 2a) a tendency for an overall linear treatment effect by interval interaction was observed ($P = 0.10$) on the proportion of time spent eating. It increased linearly with buffer level in interval 2 (2-4 h; $P = 0.05$) but also showed a tendency for a quadratic effect ($P = 0.08$). Additionally, a tendency for a linear increase was also noted during the 2 h post-feeding, interval 1 ($P = 0.08$). Mean daily pattern of time spent eating was similar to daily DMI patterns (Figure 1a). As in DMI patterns, the time heifer spent eating was greatest in interval 1 (0-2 h; $P < 0.05$), and decreased in interval 2 (2-4 h) and again in intervals 3, 4 and 5 which corresponded from 4 to 10 h post-feeding ($P < 0.05$). However, time spent eating from 2 to 4 h (interval 2) was not different ($P > 0.10$) from that of interval 6 (10-12 h). Heifers maintained a relatively constant eating time between 4 to 14 h post-feeding (intervals 3 to 7), and this time decreased thereafter ($P < 0.05$) remaining low at night, from interval 8 to 11.

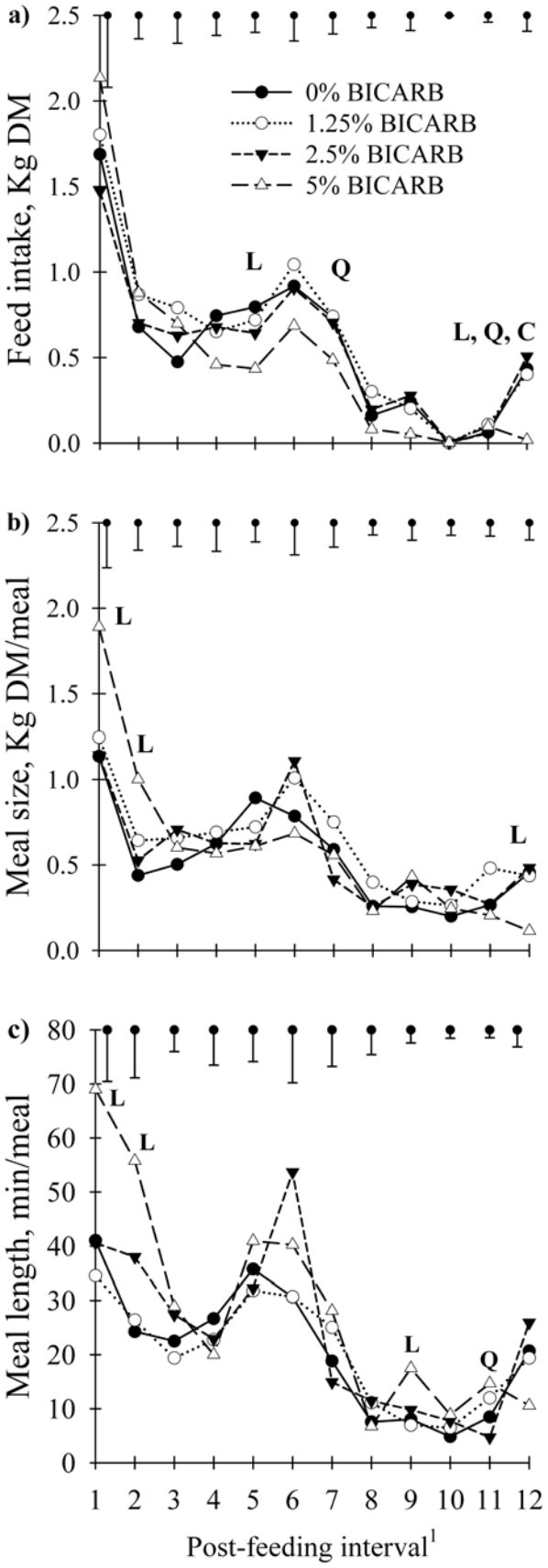

Figure 1. Daily dry matter intake (a), meal size (b) and meal length (c) patterns of finishing heifers fed high-concentrate diets with increasing sodium bicarbonate (BICARB) percent of concentrate dry matter. **L, Q, C** linear, quadratic and

cubic treatment effects within any given post-feeding interval are significant ($P < 0.05$). [1] After feeding intervals were: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12, corresponding to 0 to 2, 2 to 4, 4 to 6, 6 to 8, 8 to 10, 10 to 12, 12 to 14, 14 to 16, 16 to 18, 18 to 20, 20 to 22, and 22 to 24 h after feeding, respectively. Bars in the top of the graph represent one standard error.

A slight increase was noted at interval 12 ($P < 0.05$) indicating that heifers had an early morning eating period.

When the time spent ruminating was analyzed either as proportion of total observations or in minutes per kg of nutrient intake, treatments had no effects (Table 2). The daily rumination patterns resulted in linear treatment effect ($P = 0.04$), interval of the day effect ($P < 0.0001$) and their interaction ($P = 0.06$; Figure 2b). Thus, as BICARB level increased overall rumination time decreased linearly, which may indicate that the curves were generally lower throughout the feeding cycle as the buffer level increased. However, the linear interaction showed that increasing the level of buffer resulted in a linear decrease in the time spent ruminating at intervals 1 (0-2 h), 7 (12-14 h), 10 (18-20 h) and 11 (20-22 h) to different extents ($P < 0.05$).

In addition to the linear decrease observed during the 2 h post-feeding a quadratic tendency was observed ($P < 0.10$), with intermediate BICARB levels showing the lowest ruminating time. Additionally, BICARB level had a quadratic effect ($P \leq 0.05$) on the time spent ruminating during interval 5 (8-10 h) of the day because the two intermediate BICARB levels spent the greatest time ruminating. Regardless of BICARB level, ruminating time increased from interval 1 (0-2 h) to 3 (4-6 h; $P < 0.05$) and further to 4 (6-8 h; $P < 0.05$), contrarily to the decrease in time spent eating. Thereafter, ruminating time decreased from interval 4 (6-8 h) to 7 (12-14 h; $P < 0.05$), and increased again from interval 7 (12-14 h) to 11 (20-22 h; $P < 0.05$) where the peak ruminating time was observed.

The increased buffer level tended to increase linearly ($P = 0.09$) the total chewing time only when expressed in min per kg of OM intake (Table 2). Total time spent chewing was equivalent to 401.9, 444.4, 401.6 and 363.0 ± 40.8 min/d (for 0, 1.25, 2.5, and 5 % BICARB, respectively), with no treatment effect. Total chewing time patterns are shown in Figure 2c. Interval of the day was highly significant with regard to total chewing time and there was a linear treatment by interval interaction ($P = 0.01$). Total chewing time decreased linearly at intervals 7 (12-14 h) and 11 (20-22 h) but the effect was quadratic at interval 5 (8-10 h) with 1.25 and 2.5% BICARB showing the greatest values ($P < 0.05$). The mean daily total chewing time pattern was lowest at interval 7 (12-14 h) and highest at the interval 11 (20-22 h).

Table 2. *Chewing and drinking behavior of beef heifers affected by increasing sodium bicarbonate proportion in the diet*

Item	Treatment [a]					Effect [b]		
	0	1.25	2.5	5	s.e.	L	Q	C
Eating								
Daily time, % [c]	9.65	10.41	10.49	10.37	0.92			
min/KgDM	16.42	15.40	16.05	23.55	3.32	*		
min/KgOM	17.25	16.25	17.02	25.34	3.56	*		
min/KgNDF	82.90	74.59	77.79	96.76	11.84			
Ruminating								
Daily time, % [c]	19.63	22.43	19.60	16.08	2.47			
min/KgDM	35.64	39.28	36.09	36.41	4.20			
min/KgOM	37.43	41.44	38.28	39.19	4.51			
min/KgNDF	178.21	192.77	175.71	161.87	19.38			
Total chewing								
Daily time, % [c]	28.38	31.16	28.28	25.41	2.53			
min/KgDM	52.06	54.68	52.14	59.96	6.33			
min/KgOM	54.68	57.69	55.30	64.53	6.82			
min/KgNDF	261.11	267.35	253.50	258.63	25.45			
Drinking								
Daily time, % [c]	2.27	2.64	2.41	2.57	0.51			

[a] Treatments were 0, 1.25, 2.5 and 5 % of sodium bicarbonate in the concentrate, DM basis

[b] Effect of sodium bicarbonate level was significant at $P \leq 0.01$ (******), or at $P \leq 0.05$ (*****): L = linear, Q = quadratic and C = cubic

[c] Data were analyzed as square root-arcsine transformed (No. of counted daily behavioral activities/ No. of total daily observations)

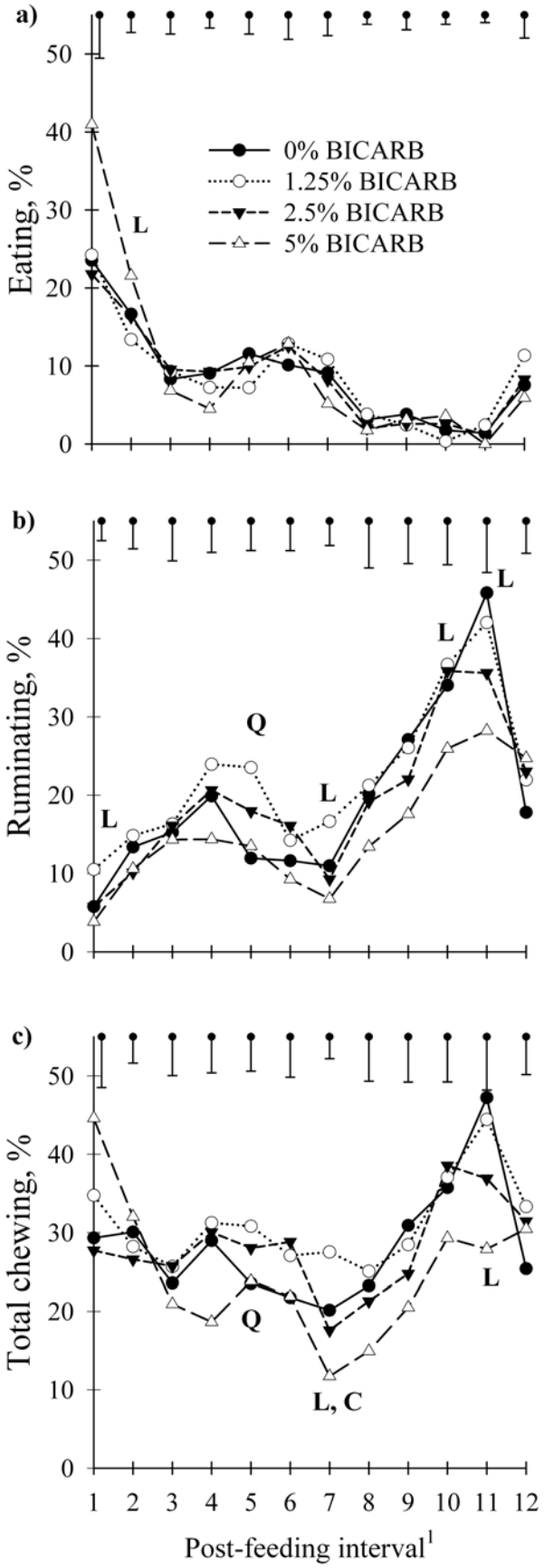

Figure 2. Eating (a), ruminating (b) and total chewing (c) time patterns obtained from video recordings of finishing heifers fed high-concentrate diets with increasing sodium bicarbonate (BICARB) percent of concentrate dry matter.

Data are presented as proportion of total observations but were analyzed as square root-arcsine transformed (No. of counted interval activity/ No. of total interval observations). **L, Q, C** linear, quadratic and cubic treatment effects within any given post-feeding interval are significant ($P < 0.05$). [1] After feeding intervals were: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12, corresponding to 0 to 2, 2 to 4, 4 to 6, 6 to 8, 8 to 10, 10 to 12, 12 to 14, 14 to 16, 16 to 18, 18 to 20, 20 to 22, and 22 to 24 h after feeding, respectively. Bars in the top of the graph represent one standard error.

Drinking Behavior

The proportion of time spent drinking was not affected by treatments (Table 2), which is in agreement with the lack of effect on total daily water consumption (Chapter II). When daily drinking patterns were analyzed, treatment had no effect, interval of the day was significant ($P = 0.001$) and the treatment by interval interaction was linear ($P = 0.04$). The interaction can partly be explained by the fact that within the 0% BICARB diet the time spent drinking was not affected by interval of the day ($P = 0.14$) whereas this proportion was highly affected by interval ($P \leq 0.002$) for the rest of treatments (Figure 3). Moreover, a linear decrease ($P = 0.05$) in time spent drinking was observed from 6 to 8 h post-feeding (interval 4) as BICARB level increased, but increased linearly from 10 to 12 h (interval 6; $P < 0.01$). A tendency for a linear increase was also observed during the 2 h post-feeding ($P = 0.10$). At interval 9 (16-18 h), a cubic effect of treatments was observed ($P < 0.05$). The pooled mean drinking pattern showed two peaks of drinking activity at intervals 2 (2-4 h) and 6 (10-12 h), which were higher ($P < 0.05$) than night intervals (8 to 12).

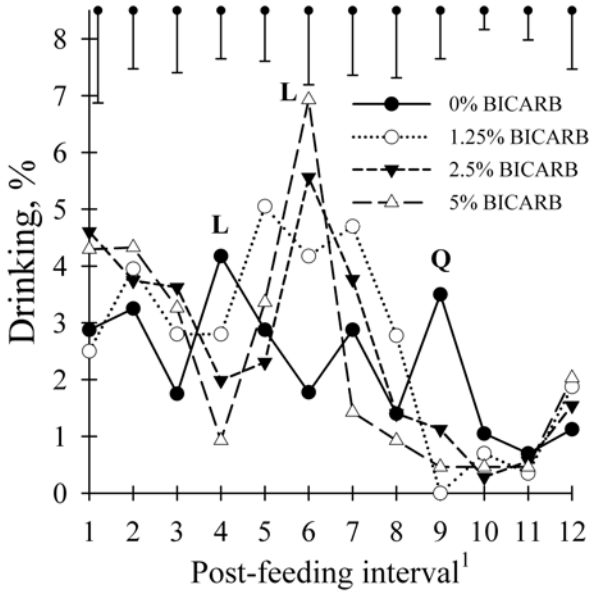

Figure 3. Drinking time patterns obtained from video recordings of finishing heifers fed high-concentrate diets with increasing sodium bicarbonate (BICARB) percent of concentrate DM. Data are presented as the proportion of observations within each interval but were statistically analyzed as square root-arcsine transformed (No. of counted interval activity/ No. of total interval observations). **L, Q, C** linear, quadratic and cubic treatment effects within any given post-feeding interval are significant ($P < 0.05$). [1] After feeding intervals were: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and

12, corresponding to 0 to 2, 2 to 4, 4 to 6, 6 to 8, 8 to 10, 10 to 12, 12 to 14, 14 to 16, 16 to 18, 18 to 20, 20 to 22, and 22 to 24 h after feeding, respectively. Bars in the top of the graph represent one standard error.

Other behaviors

Treatments did not affect time spent resting (Table 3) and heifers preferred to rest lying on their right side ($P < 0.001$), means being 46.8 ± 1.6%, 34.9 ± 2.6% and 18.3 ± 1.9% for right lying, left lying and standing, respectively. Moreover, BICARB level tended to decrease linearly right-lying time ($P = 0.10$) and left-lying time increased linearly ($P = 0.04$). Daily time spent on self grooming and oral stereotypies were not affected by treatments. Time spent on social behaviors showed a tendency for a cubic effect ($P = 0.06$).

Table 3. *Proportion of time spent performing different behaviors as affected by increasing sodium bicarbonate proportion in the concentrate of beef heifers*

	Treatment [a]					Effect [b]		
Item	0	1.25	2.5	5	s.e.	L	Q	C
Resting								
Daily time [c]	59.80	58.2	59.85	62.75	3.93			
Right side [d]	50.40	45.75	47.73	43.2	3.23			
Left side [d]	30.23	32.50	35.60	41.40	5.35	*		
Standing [d]	19.35	21.70	16.65	15.38	3.98			
Self-grooming [c]	4.15	4.07	3.75	4.02	0.75			
Social behaviors [c]	2.77	1.90	3.17	2.50	0.80			
Oral stereotypies [c]	2.62	2.05	2.75	2.65	0.85			

[a] Treatments were 0, 1.25, 2.5 and 5 % of sodium bicarbonate in the concentrate, DM basis

[b] Effect of sodium bicarbonate level was significant at P ≤ 0.01 (**), or at P ≤ 0.05 (*): L = linear, Q = quadratic and C = cubic

[c] Data were statistically analyzed as square root-arcsine transformed data (No. of counted daily behavioral activities/ No. of total daily observations)

[d] Data were statistically analyzed as square root-arcsine transformed data (No. of counted postures of activity/ No. of total observations of the activity)

Discussion

Feed intake behavior

Linear increases of BICARB level decreased linearly the amount of concentrate intake and increased that of barley straw, resulting in a tendency to decrease the total DMI (Chapter II). This was achieved through linear decreases in the number of meals per day. Although there was a numerical trend to increase meal size, it wasn't enough to compensate DMI because of the meal size by time of the day interaction. Daily average ruminal pH did not seem to play any roll on daily DMI. Meal size and length increased linearly during the 4 h following the morning feeding, while ruminal pH and water consumption also increased as buffer level increased (Chapter II). The linear increase observed in ruminal pH during the 4 h post-feeding may play a roll in these effects because animals may adjust their intake patterns to regulate ruminal pH (Schwartzkopf-Genswein et al., 2003). Fulton et al. (1979) observed lower intake in steers being adapted to wheat compared to corn diets, concurrent with a lower ruminal pH. Moreover, lower intakes were observed during the first 2 h, and from 4 to 8 h post-feeding, in relation to lower ruminal pH. The authors concluded that steers fed the corn diet were able to maintain ruminal pH over 5.5 by adjusting intake patterns. Nevertheless, ruminal fermentation products are more likely to be associated with meal size and length because they trigger satiety signals and, consequently, they may affect feed intake (Forbes et al., 1980; Allen et al., 2005). Ruminal propionate molar proportion and concentration decreased linearly at all after feeding times ($P < 0.05$), except at 0 and 24 h (data not shown). However, total volatile fatty acid (**VFA**) concentration just decreased numerically by 14%, on average, during the 4 h post-feeding as buffer level increased (data not shown). Dietary sodium bicarbonate has shown to increase pH and osmolality, and to reduce the molar proportion and production rate of propionate in the rumen (Rogers and Davis, 1982). Total VFA have been related to satiety signals (Forbes et al., 1980) but propionate has been proposed to cause satiety at a larger extent than other VFA decreasing both the size and frequency of meals (Allen et al., 2005). Thus, the increase in the size and length of meals observed during the 4 h post-feeding could be explained by a reduction in the accumulation of fermentation products, mainly propionate, which would cause a delay of satiety. Nevertheless, the opposite findings than those observed during the 4 h post-feeding were observed later in the day, from 6 to 12 h post-feeding. Accordingly, DMI decreased linearly from 6 to 10 and from 12 to 14 h post-feeding, whereas meal size and eating rate tended to decrease from 8 to 12 h, as dietary BICARB increased. Neither ruminal pH nor fermentation products can explain these effects during these intervals of time. Osmolality was not measured in the present study but it has

been related to the short-term control of feed intake, reducing meal size and increasing meal frequency, because it contributes to early satiation (Carter and Grovum, 1990; Langhans et al., 1995). Ruminants can avoid increases in ruminal osmolality through an increase in the water consumption to DMI ratio (Langhans et al., 1995). Water consumption per kg of DMI increased linearly from 4 to 24 h but was not affected from 0 to 4 h post-feeding, which was caused by decreased DMI because water consumption was not significantly affected (Chapter II). The production of volatile fatty acids and dissolution of minerals from ingested feed are the major determinants of the post-prandial increase in ruminal osmolality (Bennink et al., 1978). In the present study, concentrations of total VFA were highest 12 h post-feeding (160 mM) with no treatments effects (data not shown). Just a numerical trend was observed in total VFA concentration at 8 h post-feeding, values being 165, 137, 135, and 155 ± 15 mM (quadratic P = 0.11). Therefore, it is speculated that ruminal osmolality could be triggering the mechanisms of feed intake control from around 6 to 12 h post-feeding, similar to those described by Carter and Grovum (1990) and Langhans et al. (1995), but more noticeable when feed contained BICARB because its rapid contribution to osmolality when dissolved in the rumen. In conclusion, both proposed mechanisms of feed intake control could be acting at different moments within the day, as reflected by a meal size by time of the day interaction, likely as a result of variations in ruminal and other metabolic conditions throughout the day.

Reduced eating rate has been associated with reduced feeding motivation (Nielsen, 1999) suggesting reduced palatability, as was the case when the buffer was fed alone free-choice (Keunen et al., 2003). However, if low palatability was responsible for the reduction of feed intake, then eating rate, meal size and DMI should have been reduced from the beginning of the feeding cycle, which did not occur. Eating rate decreased linearly only from 8 to 12 h post-feeding (data not shown). Moreover, eating rate of heifers under 0 and 1.25% BICARB only showed a tendency to differ ($P \geq 0.06$) among intervals of the day whereas in heifers under 2.5 and 5% BICARB it differed among intervals ($P < 0.001$). Thus, feeding behavior of the present study was altered by high BICARB levels resulting in greater variation of eating rate throughout the day, whereas it was more homogeneous in the control and 1.25% BICARB. Results of mean meal size and length patterns emphasize the importance of the first after-feeding meals for cattle fed once daily. The size of the first meal as a proportion of the total daily DMI increased linearly ($P = 0.01$) as buffer level increased (data not shown). This could be conditioning the evolution of all subsequent feeding and ruminal fermentation patterns.

No studies on feeding behavior with high mineral levels were found for this type of cattle. Rossi et al. (1998) fed a complete pelleted diet containing 3% NaCl diet to pigmy goats and observed a

reduction in feed intake with no effect on water consumption, as in the present trial (Chapter II), and reported a reduction in the size and the frequency of meals, and in eating rate, whereas meal length increased. Our results for total daily meal time, meal frequency and eating rate are similar to those reported in a series of experiments working with steers by Putnam and Davis (1963) who fed 24% ground forage (15 min meal criterion), and Chase et al. (1976) who fed 28% chopped hay (20 min meal criterion). However, other researchers have reported a much higher daily meal time, size and length than the present trial when working with steers fed around 10% forage in diets and using a 20-min meal criterion (Cooper et al., 1999; Erickson et al., 2003). Results are difficult to compare because of different experimental conditions and the selection of the meal criterion may affect the estimation of all these variables (Tolkamp and Kyriazakis, 1999). The great DMI during the 2 h following feeding is similar to those reported by Fulton et al. (1979) while adapting steers to diets higher in concentrates. Although meal size and length were smaller at night, short and small meals were recorded in the present trial. On average, heifers spent 78% of their total eating time during the 12 post-feeding hours which agrees with results from Putnam and Davis (1963) and Krause et al. (1998) with beef cattle on high concentrate diets. We used continuous lighting because video recording at night was required. However, eating patterns were not significantly affected by complete 24-h controlled lighting in steers or dairy cows (Chase et al., 1971; Tanida et al., 1984).

Chewing behavior

Proportion of time spent eating in the present experiment agrees with Shain et al. (1999) and Rotger et al. (2006) but is slightly lower than that observed by Krause et al. (1998) in beef cattle fed high concentrate rations with about the same levels of straw. Although BICARB addition reduced DMI (Chapter II) no effects were observed in the proportion of time spent eating or total daily meal time, which led to a linear increase in the time spent eating per kg of DM and OM intakes, as reflected by a decrease in eating rate. Eating time per kg of OM and DM intakes were primarily due to an increased proportion of forage eaten ($R^2 = 0.41$; $P < 0.001$), likely because more time would be needed to form the bolus and swallow (Sudweeks et al., 1975). However, extra time might have been required to sort the concentrate and choose for straw

As in the present study, large variations among animals in ruminating time were also observed by Campbell et al. (1992), which reduce the likelihood of detecting differences. Daily ruminating time was probably the variable most affected by the low number of animals used in the present experiment, which reduces the statistical power. Ruminating time among treatments followed the same pattern as DMI (Chapter II) but was best explained by the total organic matter (OM) intake in relation to metabolic BW (g OM / $kg^{0.75}$; Adjusted $R^2 = 0.49$; $P = 0.001$), correcting for the high

minerals content of DM (Chapter II), which are not conducive to rumination at all. The 5% BICARB diet reduced total DMI by 20%, whereas straw intake increased 26%, and rumination time decreased by 28% compared to the 1.25% BICARB. Therefore, ruminating time seem to be more related to total DMI than to straw DMI. Decreasing intake level of steers fed high forage diets also decreased ruminating time in the study of Sudweeks et al. (1980) but not in that of Deswysen et al. (1987). Ruminating times of the present experiment are higher than those observed by Shain et al. (1999) who demonstrated that even all-concentrate rations resulted in 89 min/d of rumination time. Contrarily to our results, Rotger et al. (2006) fed heifers with 8 or 12% of straw and observed greater ruminating time, as well as Shain et al (1999) when adding 5% straw to an all-concentrate diet. Campbell et al. (1992) observed the lowest eating and ruminating times during the first after-feeding interval in a diet containing corn cobs and a higher ruminal osmolality during that interval (Marshall et al., 1992), and Welch et al. (1982) observed a delay in the onset of rumination after feeding (increased latency) by increasing ruminal osmolality with hypertonic solutions. The linear decrease in ruminating time observed during the 2 h post-feeding was primarily due to an increase in mastication time allocated to eating rather than ruminating, as reflected by higher meal length and time spent eating as BICARB level increased. However, the decrease in ruminating time observed from 6 to 10 and 12 to 14 h post-feeding could be due to expected high ruminal osmolality as discussed before. Because high ruminal osmolality was not expected, due to the very low DMI, and no competitive activities (eating) were observed, the linear decreases observed for ruminating time from 18 to 22 h post-feeding could be related to the linear decrease in the level of daily intake.

Drinking behavior

The lack of an effect of treatments on percentage of time spent drinking is in agreement with that of total amount of water drunk (Chapter II). Langhans et al. (1995) stated that in ruminants a relatively high proportion of drinking bouts are temporarily dissociated from eating because the mechanism that links those activities is ruminal osmolality. Nonetheless, from Figure 1a (eating pattern) and Figure 3 (drinking pattern) it may be concluded that the higher the BICARB level of the ration, the higher the association between the eating and drinking patterns, or higher the meal-associated drinking. Additionally, time spent drinking was not different among intervals of the day within the control diet but a significant variation between intervals was evident for the other treatments. This conclusion agrees with findings by Rossi et al. (1998).

Results of the present study and the companion paper (Chapter II) demonstrate that the addition of sodium bicarbonate leads to a wide rage of changes in intake, ruminal fermentation, and feeding and chewing behaviors. Moreover, significant treatments by time of the day interactions were

observed for most variables, some of which were of opposite directions during different times such as meal size and length. Feed intake and chewing behaviors were not previously studied when assessing the effects of sodium bicarbonate in high concentrate rations. Those behaviors may explain part of the variability in response among studies when sodium bicarbonate is added. Some of the changes observed when BICARB increased in the present study might have a positive effect on ruminal pH whereas others may negatively affect it. For example, ruminating time decreased linearly in a period of time when ruminal pH reached the nadir, from 12 to 14 h. Rumination is necessary during this time to increase saliva production which buffers the rumen (Bailey and Balch, 1961). Meal size increased linearly during the 4 h following feeding, which may negatively affect ruminal pH and its post-prandial drop, as suggested by Allen (1997), Owens et al. (1998), and Cooper et al. (1999). However, there is little doubt about the buffering effect of bicarbonate in the rumen (Kohn and Dunlap, 1998) which was supported in the present study by the finding that despite increasing buffer level increased meal size during the 4 h post-feeding the drop in pH was attenuated (Chapter II). In addition, the linear increase in the proportion of straw eaten and in the time spent chewing per kg of organic or dry matter intake were related to greater ruminal pH by Allen (1997) because the proportion of starch is diluted and ensalivation of feed increases. We observed a large animal effect on meal size and, for heifers that had a large meal size, ruminal pH fell significantly at 2 h even with 5% BICARB. This highlights the importance of the first after feeding meals on subsequent ruminal function under the current feeding management, which could be conditioning the evolution of the whole feeding cycle. Relating the present data with that in the companion paper (Chapter II), total chewing time per kg of OM and DM were the single variables that explained the greatest proportion of the variation in daily average ruminal pH in the present study (Adjusted $R^2 = 0.33$; $P = 0.01$; data not shown). In contrast, Krause et al. (1998) did not find any relationship with chewing activity in high concentrate diets. The objective of the present experiment was not to choose the best buffer level but to assess trends in, and relationships among, the changes of variables as BICARB levels increase. However, if multiple comparisons of means are considered then most significant differences were observed between the 1.25 and the 5% BICARB levels. Therefore, the 1.25% BICARB may be considered the best buffer level and was characterized by more homogeneous patterns of feed intake and chewing behaviors throughout the day. For instance, the 1.25% BICARB did not show differences among time intervals of the day on total chewing time ($P = 0.60$) whereas the rest of treatments did so ($P < 0.05$). Nevertheless, the variables affecting daily pH patterns or its variation may change in type and importance throughout the feeding cycle.

In conclusion, the addition of sodium bicarbonate as a ruminal buffer to high-concentrate rations decreased meal frequency and eating rate, increased meal length but did not affect meal size, meal time, and chewing time on a daily average basis. However, all these variables showed a treatment by time of the day interaction. As bicarbonate level of the diet increased, meals occurring within 4-h after-feeding were greater and longer, as well as eating time. Nevertheless, meals tended to be smaller and with a slower eating rate from 8 to 12 h post-feeding. Feed intake was also reduced from 6 to 10 and 12 to 14 h as well as ruminating and total chewing times. These behavioural variables may help to explain the mechanisms triggered by bicarbonate that may potentially influence intake and digestive function. However, those mechanisms might change throughout the day due to differences in ruminal conditions and physiology.

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Chapter IV

Effect of the number of concentrate feeding places per pen on performance, behavior, and welfare indicators of Friesian calves during the first month after arrival at the feedlot

Chapter based on a paper accepted in Journal of Animal Science

ABSTRACT

Seventy-two Friesian calves (BW = 102.0 ± 1.8 kg) were bought from a commercial calf farm and distributed to a factorial arrangement of treatments in a complete block design with 3 treatments and 3 blocks of similar fasted BW to study the effect of increasing the number of feeding places per pen on performance, behavior, and welfare indicators during the 4 wk after arrival. Treatments consisted of 1 (**T1**), 2 (**T2**) or 4 (**T4**) concentrate feeding places/pen (8 calves/pen). Concentrate and straw were fed at 0830 in individual feeders and animals were allowed to consume on an ad libitum basis. Dry matter intake and ADG were recorded weekly, and blood samples were taken on d 0 (before transport), 7, 14, 21, and 28. Time spent in maintenance activities, number of displacements between calves, and the angular dominance value (**ADV**) were registered at wk 1 and 3 after arrival. Increasing the number of feeding places per pen resulted in a quadratic response of concentrate and total DMI, ADG, and BW during the 28-d period, with T1 showing the lowest values. Straw intake and the within-pen SD of ADG tended to decrease linearly ($P = 0.10$) as the number of feeding places per pen increased. During the 4-wk receiving period, and particularly on d 7 after arrival, serum NEFA responded quadratically with T1 and T2 calves showing the greatest values. With increasing number of concentrate feeders, the average time spent lying increased ($P = 0.001$), standing time decreased linearly ($P = 0.001$), and the diurnal feeding pattern changed (concentrate eating time increased but straw eating time decreased during peak feeding times, $P < 0.05$). The number of displacements from the concentrate feeders responded quadratically ($P < 0.001$) with increasing number of feeding places per pen, with T4 calves showing the lowest levels of aggression. In T1 calves, increasing ADV resulted in a linear decrease ($P = 0.03$) of ADG at wk 1 with a quadratic effect at wk 3 ($P < 0.01$). In T2 calves, increasing ADV resulted in a linear decrease ($P = 0.04$) of ADG at wk 1 but a linear increase ($P = 0.02$) at wk 3. No effect of social rank on ADG was observed in T4 calves ($P > 0.20$). Increasing social pressure at the concentrate feeders beyond the threshold of 4 heifers per feeder had a negative effect on performance. Within-pen variability in performance increased linearly as a consequence of greater effects of social dominance. Physiological indicators of welfare were not consistently affected by treatments.

INTRODUCTION

Calves start the intensive fattening period after weaning at different ages and with different physical, behavioral, and physiological conditions depending on the production system of origin. At the time of weaning, marketing, and reallocation to new facilities, calves are exposed to stress, which may be detrimental for animal welfare (Gonyou, 1986; Grandin, 1997), and negatively affect

performance and immune response (Galyean et al., 1999). The quantity and quality of resources should be optimized to reduce stress and facilitate adaptation. Many studies have investigated effects of nutrition during the arrival period, as reviewed by Galyean et al. (1999). Nevertheless, the effect of factors related to behavior, social stress, or the design of facilities has not received considerable attention during this critical period. Social stress is mainly a consequence of the rupture of social bonds, mixing of animals, and establishment of a new social hierarchy. This process is an important consideration for management (Kondo et al., 1984) and an inadequate design of facilities may negatively affect adaptation of stressed calves. Accordingly, sufficient availability of feeding space may provide uniform opportunities of access to feed among calves facing this process. Gonyou and Stricklin (1981) stated that growth of beef cattle was negatively affected during the initial 2 wk while adapting to limited feeding space. The reduction of feeding space has increased aggression (Huzzey et al., 2006) which may result from the individual's pressure in a group to earn a social rank that allows them priority to access resources (Syme, 1974). The aim of the present study was to investigate effects of increased social pressure caused by a reduced number of concentrate feeding places on performance, behavior, and welfare indicators of newly received feedlot calves.

MATERIALS AND METHODS

Animals, Treatments, and Facilities

Seventy-two female Friesian calves (104.3 ± 1.1 d of age, 102.0 ± 1.8 kg fasted BW) were purchased after weaning from a commercial farm and transported to the IRTA-PRAT Experimental Farm (Barcelona, Spain) at 1500. A factorial arrangement of treatments in a randomized complete block design with 3 treatments and 3 BW blocks was used. Calves were first distributed into 3 blocks of homogeneous 24-h fasted BW. They were then assigned to sub-groups of 3 calves with similar BW and each calf was randomly assigned to 1 of the 3 treatment pens (8 calves/pen) within a BW. Treatments consisted of 1 (**T1**), 2 (**T2**) or 4 (**T4**) feeding places/pen. Thus, the number of heifers per concentrate feeder was 8, 4 and 2 for T1, T2, and T4, respectively.

Each pen had a concrete floor and was 12.6 m long and 3.84 m wide (48.4 m^2/pen), which resulted in a space availability of 6.05 m^2/calf. Each pen had an 11.1 m^2 concrete roofed resting area bedded with wood shavings at one end and a 7.7 m^2 of a feeding area with a 2.5 m ceiling at the other end. Feeders were manufactured in steel bodies which were 1 m long, 0.40 m depth (elevated at 0.15 m from the floor) and 1.29 m tall, with a capacity of 200 L. The front of each steel body contained 1 or 2 feeding places with feed barriers (Figure 1). Therefore, only 1 feeder with 1

feeding place was set in each T1 pen, 1 feeder with 2 feeding places was set in T2 pens, and 2 feeders with 2 feeding places each were set in the T4 pens. The distance between the center points of 2 consecutive feeding places was 0.45 m. The front of the feeders had an opening for the calves' neck with a throat height of 0.50 m and was 0.15 m wide, which allowed only 1 calf to eat from each feeding place at any one time. The concentrate feeders were allocated in the front of the 3.84-m wide feeding area and 1 straw feeder was placed at each side of the concentrate feeders. Thus, the linear space available in the straw feeders was 0.34 m/calf in each T1 and T2 pen and 0.20 m/calf in the T4 pens. Concrete feeders (0.5 m at throat height) were beneath the straw feeders (1 m throat height) to avoid straw losses. One water bowl was placed at each corner of the feeding area.

A digital video-recording device was set up in a room close to the pens to record animals' behavior throughout the day (model VDVR-9, Circontrol S. A., Terrassa, Spain). A digital color/monocromo camera (model VCAM-420DNA, Circontrol S. A., Terrassa, Spain) fitted with heater resistors and autoiris vari-focal lenses (model VLEN-2812VA, 2.8 to 11.5 mm, Circontrol S. A., Terrassa, Spain) was allocated in front of the feeding area of each pen at approximately 2 m of height. An infrared light with photoelectric cells was set at each extreme of the paddock to allow video-recording at night (λ = 830 nm and 500 W; Dennard 2020, Hants, UK).

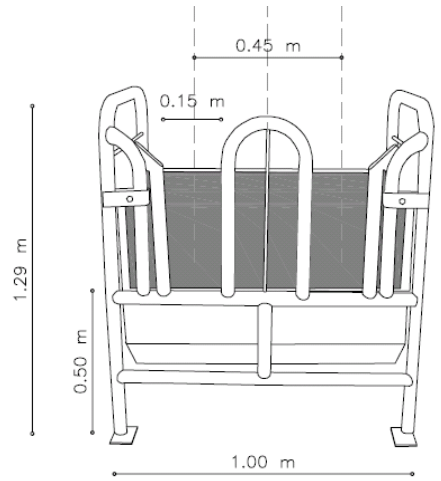

Figure 1. Design and measures of the concentrate feeder used in the experiment. One steel body was placed in T2 pens and two identical bodies were set in each T4 pen. A similar feeder was placed in T1 pens which had the same dimensions but contained only one opening for the calves' heads.

Intake and Performance. All animals received a commercial concentrate (Table 1), formulated according to NRC (1996), and barley straw (90.7 % DM, 6.72 % ash, 73.45 % NDF, 43.77 % ADF, 8.69 % ADL, 4.84 % CP, on a DM basis). Feeding management allowed ad libitum consumption of both components which were offered once a day at 0830. Fresh water was available at all times. One composited sample of the offered concentrate and straw was taken weekly for DM

determination and chemical analysis. Another sample of refusals from each pen was taken at d 7, 14, 21, and 28 for DM determination. Straw and concentrate intake was calculated for each week by weighing the amount of feed offered each day and subtracting the amount refused at the end of the wk. All calves were weighed after withdrawal of refusals on d 1 (after distribution), 7, 14, 21, and 28 at 0830 for the weekly calculation of ADG and G:F. To assess the variability of growth between calves sharing the same pen, the within-pen SD was calculated at each time point and analyzed statistically.

Physiology. Two whole blood samples from each calf were taken at 0830 by jugular venipuncture before transportation to the experimental farm (d 0), and then on d 7, 14, 21, and 28. Serum was separated within 4 h (3,000 × *g*, 20 min, 4º C) from a blood sample (10-mL Vacutainer with no additives, Plymouth, UK) and stored at -20º C until analyses of haptoglobin, β-hydroxy butyrate (β-**HBT**) and NEFA. A second unclotted blood sample (5-mL Vacutainer with EDTA, Plymouth, UK) was immediately stored at 4º C until differential cell counts were determined. Fecal samples were taken from the rectum of each calf at the same time as bleeding and kept on ice until frozen within 3 h at -20ºC for later analyses of corticosterone to assess the stress level of calves.

Maintenance Behavior. Behavior of animals was recorded for 24 h on d 2, 3, and 4 (wk 1), and 16 and 17 (wk 3). Videos were processed by continuous recording of the activities performed by all animals. Recorded activities (time spent eating concentrate and straw, drinking, lying, and standing) were registered simultaneously with their starting and ending times (to the nearest second), and the animal's identification. Several digital photographs of each calf were taken from different angles during the experiment to identify them on video by the distribution of the white and black patches on their bodies. Eating was defined as when the animal had its head into the feeder and engaged in chewing. Eating rate of concentrate and straw were calculated as the mean daily DMI of the pen divided by the corresponding mean total time spent eating by all animals in the pen. Drinking was recorded when the animal had its mouth in the water bowl. Lying was recorded as soon as the animal was not standing on its 4 legs independently of any activity the animal might perform. Time spent standing was calculated as the total duration of the observation period minus the time spent eating concentrate or straw, drinking and lying. The effect of the number of feeding places per pen on feeding patterns throughout the day was analyzed by dividing the day into 24, 1-h intervals.

Social Behavior. Displacements among calves from concentrate feeders, straw feeders, and water bowls were the events recorded. Displacements were counted at the time of occurrence and the animal's identification, the type of event, and the activity being performed when the event occurred were recorded.

Table 1. Ingredients and chemical composition of concentrate

Item	% DM
Ingredient, % DM	
Barley	31.40
Corn	28.83
Corn gluten feed	15.08
Soybean meal	9.42
Soybean hulls	6.61
Sunflower meal	3.99
Palm oil	2.07
Calcium carbonate	0.64
White salt	0.45
Sodium bicarbonate	0.89
Dicalcium phosphate	0.45
Vitamin-mineral premix [1]	0.18
Chemical composition, % DM	
DM	88.62
OM	94.04
Ash	5.96
CP	16.21
EE [2]	5.43
NDF	20.37
ADF	9.23
NFC [3]	52.02

[1] Karimix® Terneros (Laboratorios Karizoo S.A., Barcelona, Spain): vitamin and mineral premix containing, per kg DM: 3,333 kIU vitamin A, 666 kIU vitamin D_3, 2,166 IU vitamin E, 0.66 g vitamin B1, 0.66 g vitamin B2, 2 mg

vitamin B12, 26 g coline chloride, 13.4 g Zn, 3.3 g Fe, 83.3 g S, 166.6 mg Co, 3.3 g Cu, 16.6 g Mn, 16.6 g Mg, 116.6 mg I, 66.6 mg Se, 100 mg Etoxiquine, 100 mg Butilhidroxitoluene.

[2] EE: ether extract content.

[3] NFC: non-fiber carbohydrates calculated as 100 – (CP + ash + NDF + EE).

A displacement occurred when 1 of the animals ("actor") displaced a pen-mate ("reactor") that was eating or drinking and caused the reactor to completely remove its head from the container and quit the activity being performed. Displacements were further subdivided into displacements with non-physical and physical contact. However, displacements with non-physical contact constituted less than 6% of the total number of displacements and were not considered separately in any further analysis. Winner-loser relationships were not recorded when eating or drinking was not being performed by 1 of the animals involved and when the aggression did not result in the physical withdrawal of the individual from the container. These unsuccessful displacements were not considered because sometimes it was not known whether a calf right behind or beside the calf occupying the feeder was attempting to displace it, or instead was just waiting for the feeder to be free. In addition, waiting calves changed positions very quickly and displacements among them very often even occurred without the calf occupying the feeder being displaced, especially in T1 pens. Finally, there was no clear cut-off point with regard to length of time waiting, time between 2 successive attempts or how far the waiting calf moved from the feeder and then returned to be able to count it as a new failed or lost displacement. Hierarchy matrices were constructed (28 total cells or possible pairs) for each pen in each wk. Dominance order was assessed by calculating the angular dominance value (**ADV**) as the arcsine square root transformation of the average proportion of times that the individual displaced to each pen-mate (Beilharz and Zeeb, 1982):

$$ADV_i = \arcsin \sqrt{\left[\sum_{j=1\ldots N} (x_j/(x_j+y_j))/N \right]}$$

Where x is the number of times that calf i displaced calf j; y is the number of times that i was displaced by j; N is the number of calves that showed interactions with i. Therefore, the smaller the ADV, the lower the animal's social rank.

Chemical Analyses

Dry matter content of offered feed and refusals were determined by drying samples for 24 h at 103 ºC in a forced-air oven according to AOAC (1990). A composite sample of offered concentrate and straw were collected for each 2-wk period, mixed and dried in a forced air oven at 65 ºC for 48 h for later chemical analysis. Feeds were ground in a hammer mill through a 1-mm screen and

retained for analysis of DM (24 h at 103ºC) and ash (4 h at 550ºC). Organic matter was calculated as the difference between DM (AOAC, 1990; ID 950.01) and ash content (AOAC, 1990; ID 950.05). Nitrogen content was determined by the Kjeldahl procedure (AOAC, 1990; ID 976.05). Ether extract was performed according to AOAC (1990; ID 920.39). The NDF and ADF contents were determined sequentially following the procedure of Van Soest et al. (1991) using a thermostable α-amylase and sodium sulphite.

Fecal corticosterone determinations were performed in the Clinical Biochemistry Service of the Veterinary Faculty of the Universitat Autònoma de Barcelona, using the commercially available I^{125} RIA kit (Rats and Mice Corticosterone kit; ICN Pharmaceuticals; Orangeburg, NY) as described by Morrow et al. (2002). Fecal samples were first lyophilized, then extracted with methanol, and finally diluted 1:10 with the assay buffer of the kit. The intra- and inter-assay CV of the RIA was 12.21 and 15.65%, respectively. The estimated detection limit of corticosterone in feces was 5.67 ng/g DM. Haptoglobin was determined by the hemoglobin binding method with the use of a commercial haptoglobin assay (intra and interassay CV of 1.36 and 6.9%, respectively; Assay Phase Range, Tridelta Development Limited, Maynooth, Ireland); D-3-Hydroxybutyrate was determined by a kinetic enzymatic method (both intra and interassay CV of 3.7%; Ranbut D-3-Hydroxybutyrate, Randox Laboratories Ltd., Crumlin, UK) and NEFA by the colorimetric enzymatic test ACS-ACOD method (both intra- and inter-assay CV of 2.7%; NEFA C, Wako Chemicals, Neuss, Germany). Differential cell counts were carried out using an automated electronic cell-counter (ADVIA 120, Bayer, New York, USA).

Statistical Analyses

All individual data were averaged to give pen means at each sampling point over time, except for the regressions on ADV. The pen was the experimental unit for all statistical analyses (n = 3 pens/treatment). All variables expressed in percentages were previously transformed by the square root arcsine. Total white blood cells (**WBC**) count, fecal corticosterone concentration, and haptoglobin concentration were logarithmically transformed to normalize the distribution. Statistical analyses of normally distributed variables were conducted by a mixed-effects regression model for a randomized complete block design with repeated measures using the PROC MIXED of SAS (v. 9.1, SAS Inst. Inc., Cary, NC). The model contained the fixed linear and quadratic effects of the number of feeding places per pen (treatment), block, and treatment × week and block × week interactions. The random effects were modeled through the correlations among the repeated measure of time (day, week, or time interval) subjected to the pen, and through the random effect of pen. To analyze the eating patterns within the day and among weeks, double-repeated measures

were considered. Thus, the time interval of the day was subjected to the week nested within pen. In the case of a significant treatment × time interaction, the null hypothesis tested was that the linear and quadratic coefficients of regression of the number of feeding places per pen were equal to zero. The same effects were used in a Poisson regression model to analyze the number of displacements per pen and day using the GLIMMIX procedure of SAS. To assess linear and quadratic relationships between angular dominance value (**ADV**) and ADG, the correlations were modeled at 3 levels of random effects. The 3 levels were individual animal nested within the pen, repeated measures over time, and pen (with all animals within a pen being correlated). This model contained the fixed categorical effects of treatment, wk, and block plus the linear and quadratic effect of ADV and the appropriate 2- and 3-way interactions. The choice of the best covariance structure was based on fit statistics (Littell et al., 1998). Significance was declared at $P \leq 0.05$ and tendencies discussed at $P \leq 0.10$ unless otherwise noted.

RESULTS

Two calves died on d 1 of the experiment, 1 of which was in the T1 group and the other in the T4. In order to balance the experiment, 1 calf from each treatment in the low BW block was immediately replaced. During the first week of arrival 1 heifer from T4 suffered a leg injury and was treated with the recommended antibiotics and analgesics. Its blood haptoglobin level was greatly increased at the d 7 and it was not considered until it returned to normal levels at d 14.

Intake and Performance

Concentrate and total DMI responded quadratically as the number of feeding places per pen increased ($P \leq 0.01$; Table 2) with a threshold value of 2 feeding places per pen (T2), above which no positive effect was observed. However, straw DMI showed a tendency for a linear decrease as the number of feeding places increased ($P = 0.10$). Concentrate, straw, and total DMI were lowest at wk 1 ($P < 0.05$) and increased over time but the proportion of straw eaten was greatest during wk 1 ($P < 0.01$; data not shown). A week × treatment interaction was observed for concentrate DMI ($P < 0.001$) and for the proportion of straw consumed ($P = 0.01$). Increasing the number of feeding places per pen increased linearly the concentrate DMI during the first week after arrival ($P < 0.05$; Figure 2a). However, the effect was quadratic during wk 2, 3, and 4 with the lowest concentrate intake for T1 calves ($P < 0.05$). Straw DMI decreased linearly during wk 2 and 3 as the number of feeding places per pen increased ($P < 0.05$), but no treatment effect was observed during wk 1 (data not shown).

Average daily gain and BW were also affected quadratically ($P < 0.05$; Table 2) whereas there was no treatment effect on the G:F ratio. However, within-pen SD in ADG tended to decrease linearly ($P = 0.10$) and within-pen SD in BW showed a tendency for a quadratic response ($P = 0.07$) as the number of feeding places per pen increased. The treatment × week after arrival interaction was significant in ADG, intra-pen SD of ADG, G:F ratio, live BW, and within-pen SD in BW ($P < 0.10$). The ADG increased linearly during wk 1 ($P = 0.01$) after arrival at the new facilities as the number of feeding places per pen increased. However, a quadratic effect was observed at wk 3 ($P = 0.02$) and 4 ($P = 0.01$) with T2 calves gaining more than T4 and T1 (Figure 2b). Within-pen variability of ADG showed the linear decrease at wk 2 and 3 when increasing the number of feeding places per pen ($P < 0.05$; data not shown). The G:F ratio showed a linear increase in wk 1 ($P = 0.01$; data not shown). The treatment × day interaction in live BW ($P < 0.05$) was the result of a lack of treatment effect on d 0 ($P > 0.10$) but showing a linear increase in BW on d 7 and 14 ($P < 0.05$) as the number of feeding places increased. However, the effect of treatments was quadratic on d 21 and 28 with the lowest values in T1 calves ($P < 0.06$; data not shown).

Table 2. Intake and performance of Friesian calves during the 28-d period after arrival at the feedlot and received in pens with 1 (T1), 2 (T2), and 4 (T4) concentrate feeding places (8 calves/pen)

	Treatment					
Item	T1	T2	T4	SEM	L [1]	Q [1]
Concentrate, kg/d	3.23	3.66	3.64	0.049	0.05	< 0.001
Straw, kg/d	0.50	0.38	0.35	0.062	0.10	0.32
Total DMI, kg/d	3.72	4.04	4.00	0.070	0.13	0.01
Straw, kg/kg total DMI	0.137	0.096	0.088	0.016	0.08	0.24
ADG, kg/d	0.95	1.12	1.12	0.040	0.09	0.05
ADG SD, kg/d [2]	0.40	0.34	0.28	0.057	0.10	0.79
G:F, kg/kg	0.254	0.273	0.284	0.015	0.16	0.63
BW, kg/animal	121.4	125.3	126.0	0.686	0.01	0.01
BW SD, kg/animal [2]	8.62	8.64	6.90	0.493	0.10	0.07

[1] P-value of the linear (L) and quadratic (Q) effect of number of feeding places per pen.

[2] Within-pen SD of ADG (ADG SD) and BW (BW SD).

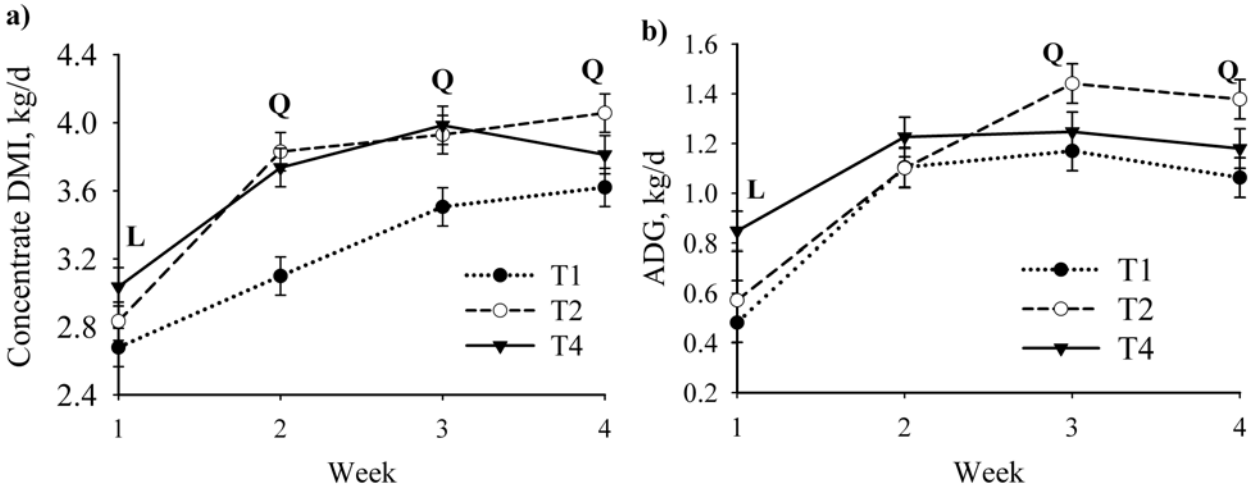

Figure 2. Concentrate DMI (a), and ADG (b) of calves throughout the 4-wk receiving period in pens with 1 (T1), 2 (T2) or 4 (T4) concentrate feeding places (8 calves/pen). $^{L, Q}$ within a week, linear or quadratic coefficients of regression of the number of feeding places was significant ($P < 0.05$).

Physiology

Fecal corticosterone concentration was not affected ($P \geq 0.54$) by the number of feeding places per pen (Table 3). Increasing the number of feeding places per pen resulted in a linear decrease in serum haptoglobin concentration ($P = 0.02$), but NEFA levels were affected quadratically with the lowest values in T4 calves ($P = 0.04$; Table 3). Treatments did not affect mean β-HBT or leukocytes. However, hematocrit percentage followed a quadratic tendency with high percentages in T1 and T2 ($P = 0.08$). A day after arrival × treatment interaction was detected ($P < 0.05$) for NEFA and β-HBT. Non-esterified fatty acids increased quadratically on d 7 and 21 with T1 and T2 calves showing the greatest levels ($P < 0.05$), but the effect was linear on d 14 (Figure 3; $P < 0.05$). A quadratic effect on β-HBT was observed on d 7 when T1 calves had the greatest concentration ($P < 0.05$; data not shown).

The main effect of day after arrival at the fattening facilities affected fecal corticosterone, haptoglobin, NEFA, β-HBT, and leukocytes ($P < 0.001$; data not shown). Fecal corticosterone was greatest on d 0 (before transport to the experimental farm) and on d 7, compared to d 14, 21, and 28 ($P \leq 0.01$). Haptoglobin and β-HBT concentrations were greatest on d 0 and remained lesser thereafter ($P < 0.10$). However, the greatest NEFA serum concentration was observed on d 7 ($P < 0.05$). Total WBC counts, neutrophils, and hematocrit percentages and the neutrophils-to-lymphocyte (**N:L**) ratio were greatest on d 7 as expected, whereas lymphocytes percentage was lowest on d 0 and 7 with increasing values over time ($P < 0.05$). All values returned to pre-transport

levels on d 28, except for lymphocytes, which remained greater, and the N:L ratio, which was lesser compared to d 0 ($P < 0.05$).

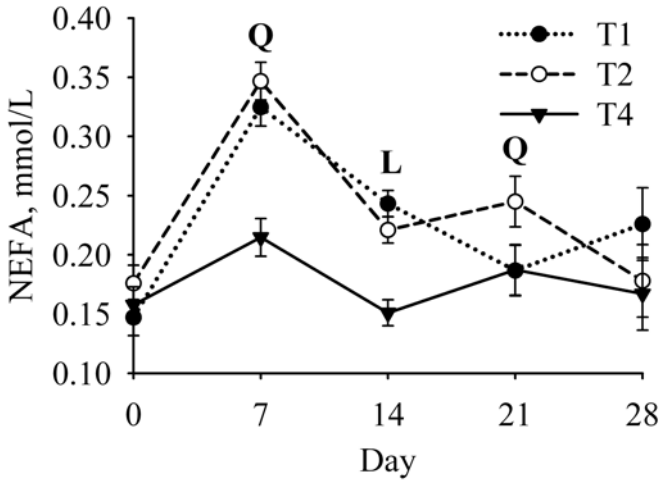

Figure 3. Serum concentration of non-sterified fatty acids (NEFA) of calves in pens with 1 (T1), 2 (T2) or 4 (T4) feeding places in the concentrate feeders (8 calves/pen) on d 0 (before transport), 7, 14, 21, and 28 after arrival at the feedlot. $^{L, Q}$ within sampling day, linear or quadratic coefficients of regression of the number of feeding places was significant ($P < 0.05$).

Daily patterns of time spent eating concentrate for wk 1 and 3 are shown in Figures 4a and 4b, respectively (treatment × week × hour, $P < 0.05$), whereas the mean pattern of time at the straw feeders is shown in Figure 4c because they did not differ between weeks (treatment × week × hour, $P > 0.10$; treatment × hour, $P < 0.05$). There were 2 major periods of concentrate feeder use, 1 after sunrise and another around sunset, and 1 minor period at midnight. In general, as the number of feeding places per pen increased, the time spent using the concentrate feeders increased at peak times during both weeks. However, quadratic effects of treatments were observed before and after the morning eating period during wk 3, when T2 calves spent more time eating concentrate. Contrarily, the time spent at the straw feeders decreased during and between both major periods of eating as the number of concentrate feeding places per pen increased. In contrast to concentrate eating pattern, a minor period of time eating straw at midnight was not observed. Time spent drinking was not affected by treatments (Table 4). Total time spent lying increased linearly ($P = 0.001$) whereas that spent in other standing activities decreased linearly when increasing the number of concentrate feeding places ($P = 0.001$; Table 4). Regardless of treatment, eating rate of concentrate and straw increased from wk 1 to 3, as well as the time spent eating barley straw ($P < 0.05$; data not shown). Lying time was lesser in wk 3 compared to wk 1 ($P < 0.05$) but no difference in standing time was observed among weeks (data not shown).

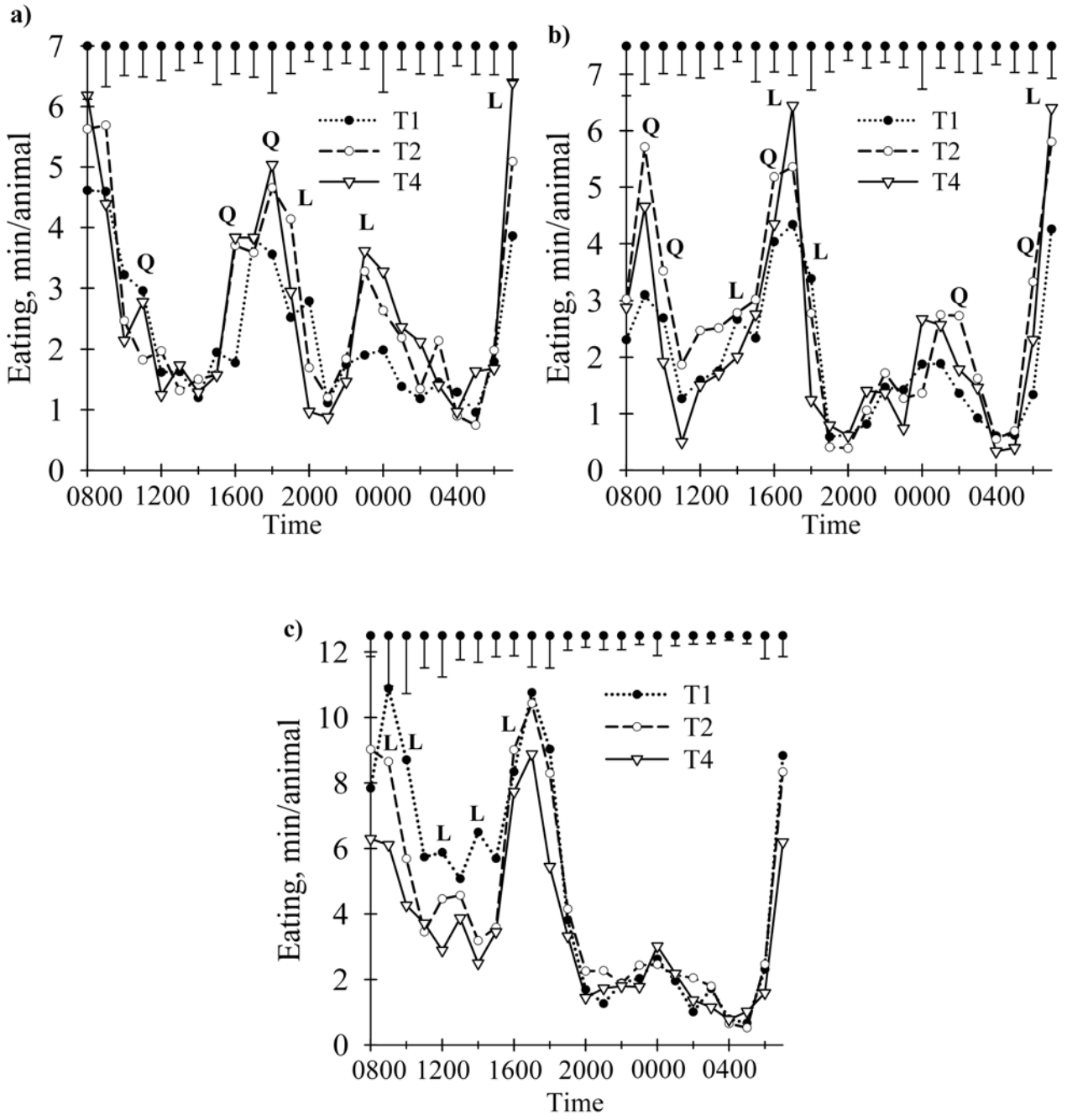

Figure 4. Concentrate eating pattern during the first (a) and the third week (b) after arrival at the feedlot, and average straw eating pattern (c) of Holstein calves in pens with 1 (T1), 2 (T2), and 4 (T4) concentrate feeding places (8 calves/pen). $^{L, Q}$ within a week, linear or quadratic coefficients of regression of the number of feeding places was significant ($P < 0.05$). The bars along the top of the graph represent 1 SEM.

Maintenance Behavior

As the number of feeding places per pen increased, the time spent using the concentrate feeders increased quadratically reflecting the greater concentrate intake in T2 and T4 ($P = 0.03$). The treatment × week interaction ($P < 0.01$) indicated that the quadratic coefficient of regression of the number of feeding places was significant in wk 3 only ($P = 0.005$; data not shown). However, time

spent at the concentrate feeders increased linearly at wk 1 ($P < 0.05$) reflecting the increase in concentrate DMI and ADG as the number of feeding places per pen increased. The intra-pen SD of the time spent using the concentrate feeders and at the straw feeders decreased linearly as the number of feeding places per pen increased ($P = 0.05$; Table 4). In agreement with the linear decrease in straw intake, time spent at the straw feeders decreased linearly as number of feeding places increased ($P = 0.02$). A treatment × week interaction was not observed in time spent in the straw feeders ($P > 0.10$).

Table 3. Mean fecal corticosterone and blood parameters of Friesian calves during 28 days after arrival at the feedlot and received in pens with 1 (T1), 2 (T2), and 4 (T4) concentrate feeding places (8 calves/pen)

	Treatment					
Item	T1	T2	T4	SEM	L [1]	Q [1]
Fecal corticosterone, ng/gDM	39.03	37.10	39.90	4.132	0.80	0.54
Haptoglobin, mg/L	305	295	284	11.4	0.02	0.95
NEFA, mmol/L	0.226	0.233	0.176	0.010	< 0.01	0.04
β-HBT, mmol/L [2]	0.183	0.177	0.182	0.005	0.98	0.37
Hematogram						
WBC, No./mL	9,950	9,889	10,177	536	0.75	0.73
Neutrophils, No./mL	3,169	3,100	3,209	284	0.77	0.50
Lymphocyte, No./mL	5,555	5,598	5,678	224	0.66	0.99
N : L	0.58	0.57	0.58	0.030	0.84	0.74
Hematocrit, %	29.93	30.02	29.56	0.515	0.52	0.08

[1] P-value of the linear (L) and quadratic (Q) effect of number of feeding places per pen.

[2] Beta-hydroxybutyrate.

[3] White blood cells.

Social Behavior

The number and distribution of displacements among calves from feed and water containers are presented in Table 5. The number of displacements from concentrate feeders responded

quadratically ($P < 0.001$) when increasing the number of concentrate feeding places per pen, being greatest in T2, intermediate in T1 and lowest in T4. The number of displacements from the straw feeders was not affected by treatments ($P = 0.11$) but the treatment × week interaction ($P < 0.01$) indicated a linear increase in the number of displacements per pen and day at wk 3 ($P = 0.01$; data not shown). The number of displacements from the water bowls showed a quadratic effect of treatment ($P = 0.006$), with T2 calves showing the greatest level of aggression in the bowls. The total number of displacements also followed a quadratic pattern ($P = 0.03$), with T2 calves showing the greatest values because of the relative weight of the displacements carried out by the calves when competing for different resources.

Table 4. Time spent on maintenance activities by Friesian calves received in pens with 1 (T1), 2 (T2) or 4 (T4) concentrate feeding places after arrival at the feedlot (8 calves/pen) [1]

	Treatment					
Item	T1	T2	T4	SEM	L [2]	Q [2]
Concentrate						
Total time, min/d	56.20	68.29	67.73	1.93	0.02	0.03
Within-pen SD, min/d [3]	23.10	14.88	11.06	3.57	0.05	0.37
Eating rate, g DM/min	55.81	49.41	52.12	2.23	0.52	0.13
Straw						
Total time, min/d	130.1	114.1	94.8	8.30	0.02	0.63
Within-pen SD, min/d [3]	42.63	32.63	23.71	5.18	0.03	0.37
Eating rate, g DM/min	3.55	3.24	3.50	0.31	0.78	0.49
Drinking, min/d	17.25	18.99	17.98	2.68	0.90	0.68
Lying, min/d	954.6	963.3	994.2	5.73	0.001	0.63
Standing, min/d	270.1	257.9	240.2	9.44	0.001	0.63

[1] Behavior was measured by continuous sampling of the activities registered for all animals during wk 1 and 3 after arrival at the feedlot.

[2] Linear (L) and quadratic (Q) effect of the number of feeding places pen.

[3] Standard deviation of time at the feeder among calves sharing the same pen.

Table 5. Number of displacements per pen and day (mean ± SEM) among Friesian calves received in pens with 1 (T1), 2 (T2), and 4 (T4) concentrate feeding places (8 calves/pen) [1]

	Treatment				
Item	T1	T2	T4	L [2]	Q [2]
Concentrate feeders	26.9 ± 1.52	40.8 ± 1.98	14.3 ± 1.04	0.10	< 0.001
Straw feeders	16.7 ± 3.55	16.5 ± 3.45	26.3 ± 4.36	0.11	0.39
Water Bowl	5.6 ± 0.62	9.4 ± 0.80	6.1 ± 0.64	0.88	0.006
Total	51.7 ± 4.48	67.0 ± 5.10	47.6 ± 4.30	0.41	0.03

[1] The number of displacements was measured by continuous sampling of all animals during wk 1 and 3 after arrival at the feedlot.

[2] Linear (L) and quadratic (Q) effect of the number of feeding places per pen**.**

The average number of displacements across treatments from the concentrate feeders increased from 20.3 ± 1.0 in wk 1 to 30.9 ± 3.0 in wk 3 ($P < 0.05$) which led to an increase in the total number of displacements from 47.6 ± 3.0 in wk 1 to 63.2 ± 4.1 in wk 3 ($P < 0.05$; data not shown). However, week after arrival did not affect the number of displacements from the straw feeders or from the water bowls.

Treatments had no effect on the average ADV which yield a mean of 0.776 ± 0.179 (linear $P = 0.63$; data not shown). When testing effects of ADV on ADG in the random regression model the treatment main effect was no longer significant and was taken out of the model. This allowed the same intercept across treatments. However, there was an interaction between the linear (ADV × week; $P = 0.03$) and quadratic effect of dominance (ADV ^{2} × week; $P = 0.08$) by week after arrival. Average daily gain tended to decrease linearly at wk 1 ($P = 0.06$) but tended to increase quadratically at wk 3 ($P = 0.07$) as the ADV increased, regardless of treatment. However, the ADV × week × treatment ($P < 0.01$) and ADV^2 × week × treatment ($P = 0.08$) triple interactions indicated that the effect of ADV on ADG was different depending on which week and treatment the coefficients of regression were measured. Increasing the ADV resulted in a negative effect on ADG within T1 (regression coefficient (***b***) = – 0.546 ± 0.247; $P = 0.03$) and T2 (b = – 0.505 ± 0.242; P = 0.04) pens at wk 1. Contrarily, the coefficient of regression of ADV in ADG was not different from zero within T4 calves at wk 1 (b = – 0.106 ± 0.258). At wk 3, the quadratic coefficient of regression

(b^2) of ADV against ADG was significant within T1 calves (b = 5.490 ± 1.828; b^2 = – 3.446 ± 1.181; $P < 0.01$). Within T2 calves at wk 3, the dominance value showed a positive linear relationship with ADG (b = 0.563 ± 0.229; P = 0.02). As in wk 1, the coefficient of regression of ADV against ADG was not different from zero within T4 pens (b = 0.303 ± 0.240; P = 0.21).

DISCUSSION

Intake and Performance

Total DMI, ADG, and live BW responded quadratically in the overall arrival period as the number of feeding places per pen increased, with low values at the greatest level of competition. This indicates that there is a threshold value on performance at 2 concentrate feeding places below which the negative effects on performance were observed but above which no positive effects are attained. In agreement with these results, time at the concentrate feeders also showed a quadratic effect. Nevertheless, no such threshold was observed in wk 1 where the effects were linear because adaptation mechanisms were not triggered during the first week after arrival. This indicates that adaptation to the feedlot could be delayed when competition at the feeder is increased. However, T2 calves adapted to the social environment at wk 3 by spending more time at the concentrate feeders during daylight when competition was lesser (between both major peaks), while maintaining a preferred eating rate not different from T4. This modification probably allowed T2 calves to compensate intake and performance after wk 2. Although T1 and T2 calves prolonged the major peaks of eating activity, they seemed reluctant to change more drastically the concentrate eating pattern throughout the day towards nighttime when less competition was present, as observed by Gonyou and Stricklin (1981) and Olofsson (1999) in well adapted cattle. However, despite this result and a 20 % increase in the concentrate eating rate of T1 calves at wk 3 compared to the other groups, no compensation of intake was achieved. Concentrate eating rate was 46.5, 44.6, and 46.1 ± 2.36 g DM/min in wk 1, and 65.1, 54.2, and 58.1 ± 2.95 g DM/min in wk 3, for T1, T2, and T4, respectively (data not shown). Another adaptation to the social environment was an increase in the total time spent eating straw as the number of concentrate feeding places per pen decreased, particularly at preferred eating times, i.e. at major eating periods, and in the daytime between them. This resulted in a linear increase in straw intake as the number of feeding places per pen decreased.

The linear increase in the within-pen SD in ADG and live BW suggests that no such a threshold value existed in performance variability among calves sharing the same pen, with differences between pen-mates becoming larger as competition level increased. Furthermore, the within-pen variability of BW increased over the weeks for T1 and T2 but was more stable within T4 pens

(treatment × day $P = 0.03$; data not shown). Accordingly, the within-pen SD of BW at the start of the experiment was 5.66, 5.78, and 5.97 ± 0.15 kg per pen, whereas at the end of the adaptation period it was 12.4, 11.0, and 8.2 ± 1.9 kg/animal, for T1, T2, and T4 pens, respectively (linear $P = 0.007$). This greater within-pen variability was due, in part, to the effects of social dominance on ADG for T1 and T2 calves. Thus, the low number of feeding places per pen reduced the group and individual growth rates in T1, whereas in T2 only some individual's growth rate was affected and no effects were observed in T4. Our results suggest that individual variability in production parameters can be a useful indicator of animal welfare and that an increase in variability, even if the average values remain unchanged, may indicate that some animals have difficulties to cope with their environment. In consequence, their welfare is compromised. To our knowledge, there are no studies of feeding space for calves on high-concentrate diets upon arrival at the feedlot. Results concerning DMI in experiments dealing with feeding space have generally resulted in no effects on DMI (Longenbach et al., 1999; Oloffson, 1999). Time at the feed bunk was reduced in all cited studies when feeding space was reduced, resulting in increased eating rates. Longenbach et al. (1999) increased the feed bunk length from 0.15, to 0.31 or 0.47 m in 3 groups of replacement heifers with different ages and observed a trend for increased within-pen SD in ADG and BW in the 2 older groups, particularly greater at the end of each study. However, Longenbach et al. (1999) observed decreasing number of heifers able to eat simultaneously at a given linear feed bunk allowance as the heifers' age increased. Zinn (1989) did not observe any effect on performance when increasing the linear manger space from 0.15 to 0.60 m/an in lightweight steers at high growth rates. However, the number of animals able to eat together was not reported, smaller groups were used (4 steers/pen), and animals were well-adapted to the environment and the competing situation. Nevertheless, feed barrier design could be one contributing factor to differences with previous studies, apart from group size, stress level and adaptation period. For instance, Huzzey et al. (2006) reported that the negative effect of reduced feeding space on time at the feeder of dairy cows was greater when headlocks were used compared to post-and-rail. These authors stated that feeding was perhaps more comfortable when no physical barrier existed between the cows and the feed, and between adjacent cows.

Physiology

The week after arrival affected all performance and hematology variables independently of treatments. The DMI and the ADG were lowest during the first week, while NEFA, total WBC count, and the N:L ratio were greatest on d 7. However, fecal corticosterone concentration indicated that calves were as stressed on d 7 as on d 0 (data not shown). This would indicate that the first

week after arrival at the new conditions is the most stressful time for transported calves. Stressful conditions are associated with increased energy demand and decreased appetite, which could lead to depletion of energy stores. The linear treatment effect in wk 1 on concentrate DMI, ADG, and G:F indicates that during the most stressful week there are advantages to having more feeding space available and such a strategy could improve the animals' adaptation to the new conditions. Nevertheless, these treatment effects in wk 1 were not accompanied by changes in blood cells profile, haptoglobin, or fecal corticosterone concentration. However, the quadratic effect of treatments on serum NEFA showed greater values in T1 and T2 calves on d 7, and on β-HBT in T1 calves, as expected.

Release of NEFA from adipose tissue is the net result of lipolysis of adipocytes or increased energy demands, or both, whereas β-HBT is one of the ketone bodies that come from incomplete oxidation of NEFA when its hepatic oxidation limit is reached (Adewuyi et al., 2005). The hepatic oxidation of NEFA and the β-HBT use by peripheral tissues through high activity or muscular exercise seemed to reach a limit on d 7 of the present study, which led to their accumulation in blood when feeding places per pen were reduced. Catecholamines, meanwhile, are involved in the short-term emergency reaction that mobilizes resources quickly, such as NEFA (Raynaert, 1976), for the metabolic requirements of fight or flight, while corticosteroids amplify and extend the metabolic effects of catecholamines in the long-term general adaptation syndrome (Dantzer and Mormède, 1983). The high NEFA levels on d 7 for T1 and T2 calves could be the result of increased energy demands as shown by increased standing times, decreased lying time and increased aggressive interactions. However, marked increases in NEFA levels (0.5 to 1 mM) were also observed by mid-term underfeeding but they should return to pre-fasting levels at 1 to 2 d after re-feeding (Chilliard et al., 1998). The absence of a treatment effect on fecal corticosterone does not concur with the increase in NEFA observed at the 2 greatest levels of social pressure at the concentrate feeders in the present study.

Haptoglobin is an acute phase protein that increases in the blood as a consequence of inflammation, tissue damage or injury, and infection. Serum haptoglobin concentration decreased linearly as the number of feeding places per pen increased, although differences were very small. Additionally, no conclusions should be drawn from this result because serum haptoglobin concentration in the present study showed an abnormal decrease on d 7 and 14 compared to d 0 (data not shown), which is opposite to expectations and other reports in beef cattle after marketing (Berry et al., 2004). However, serum haptoglobin concentration did not always increase after short transportation (Arthington et al., 2003).

The number and proportion of leukocytes in the blood represent their state of distribution in the body and the activation of the immune system in response to stress. Rats with chronic stress showed decreased lymphocyte counts triggered by high adrenal hormones such as corticosterone (Dhabhar and McEwen, 1997). Many authors have also used the immune response, such as the N:L ratio and lymphocytes, as an indicator of stress and immunosupression in calves (Hickey et al., 2003). However, neither these variables nor fecal corticosterone were affected in the present study indicating that immune response was not compromised, at least at the group level, in agreement with Corkum et al. (1994). Only the lymphocyte count increased numerically as the number of feeding places increased, even under a greater hemoconcentration. The lack of a treatment effect on fecal corticosterone is striking because agonistic behavior, crowding and the mere presence of a dominant animal resulted in greater circulating corticosteroids in farm animals (Dantzer and Mormède, 1983). In dairy cattle, the concentration of corticosteroid metabolites in fecal samples reflects the amount produced at about 12 h (6 to 16 h) earlier but depends on the lower tract transit time (Morrow et al., 2002). Therefore, corticosterone in fecal samples in the present study could reflect blood levels at about, or after, 2030 of the previous day, just after the sunset major period of eating activity, but when one of the lowest levels of competition at the feeder was observed. It is also possible that the stress level caused by the increased and short-term competition in the present study is not marked enough to be detected in fecal samples. Serum NEFA concentration was measured during the major morning period of eating (0830) and showed increases at the 2 greatest competition levels in the present study, whereas a numerical decrease in lymphocytes was also observed. Both factors were affected under short-term stress models and triggered by corticosteroids in order to improve fitness by energy mobilization (NEFA) and redistribution of leukocytes to organs where the immune function is enhanced (Raynaert, 1976; Dhabhar and McEwen, 1997).

Maintenance Behavior

In agreement with effects observed on DMI and ADG, time at the concentrate feeders and concentrate eating rate also showed a quadratic effect over the 4-wk arrival period. Nielsen (1999) suggested that social animals, like cattle, try to attain a preferred feed intake and a preferred feeding rate while feeding at specific times of the day. In addition, groups of calves also synchronize their behavior trying to eat and rest at the same time. This means that when social pressure at the feeder increases animals may adapt to it by feeding at a faster rate than they prefer, eating less than they prefer or by feeding at less preferred times of the day. In the present study, as the number of feeding places per pen decreased, so did the time spent eating concentrate at all 3 peaks of eating activity, in

agreement with previous studies on dairy cows (DeVries et al., 2004; Huzzey et al., 2006). This fact is probably related to feeder occupancy but also to social avoidance, since feeders of T1 pens were only occupied for 6 min/h or 60% of the total available time during the major eating periods. Calves under T2 adapted to the social environment through an increase in concentrate eating time between both major peaks of eating but not even a greater eating rate of T1 calves in wk 3 was enough to compensate concentrate intake. Therefore, adaptation to the competing situation required more time and efforts by the calves to modify their behavior as competition level increased. Perhaps the reluctance of T1 calves to change more drastically the diurnal concentrate eating pattern together with enough availability of space in the straw feeders resulted in the increased straw intake. We hypothesize that a subordinate calf would eat straw rather than wait around the concentrate feeder while it is occupied in order to dissipate the tension and anxiety due to increased social pressure at the concentrate feeders, unlike TMR fed dairy cows when feeding space was reduced and no other feeding choice was available (Huzzey et al., 2006). When a calf was displaced from the concentrate feeder it usually went straight to the straw feeders. These processes were reflected in linear increases in the within-pen variation of time at the concentrate and straw feeders. This variability became particularly important in T1 pens because two calves ate only barley straw and did not approach the concentrate feeders in wk 3 ("non-eaters"). In addition, the circadian rhythm of eating has also been suggested as indicators of welfare in cattle (Gonyou, 1986). From this point of view, the calves in the present study were restricted in their natural eating behavior when the number of feeding places per pen decreased, particularly at peak eating times.

Daily time spent lying decreased linearly, whereas that spent standing increased as social pressure at the concentrate feeders increased. The increase in the time spent standing was found to be the result of a greater time spent waiting for an occupied feeder when increasing competition in dairy cows (Olofsson, 1999; Huzzey et al., 2006). However, we did not record which activity was performed during standing times.

Social Behavior

The frequency of fights has been considered another behavioral indicator of social stress (Dantzer and Mormède, 1983). The observed total number of displacements per animal and day was 6.46, 8.37, and 5.95 for T1, T2, and T4 calves, respectively. These values are similar to those found by Olofsson (1999) when using one cow per feeding station (7.2) but lower than those reported when using 3 or 4 cows per feeding station (Olofsson, 1999; Huzzey et al., 2006). The treatment differences in the number of displacements from each resource container may be greatly influenced by the design and spatial organization of the facilities in the present study. The feeding area was at

the front of each pen, with concentrate and straw feeders and water bowls being adjacent to each other. The lack of difference between T1 and T4 in the number of displacements from the concentrate feeders could have been the result of some calves in T1 pens avoiding competition for a feeding place. This may be due to the fact that the feed barriers design required great effort to displace a pen-mate and high aggression was suffered during displacement. For instance, the number of displacements was fewer with headlocks compared to post-and-rail (Huzzey et al., 2006). The greatest competition and level of aggression in concentrate feeders were observed at peak feeding times in the present study (data not shown), in agreement with Kondo et al. (1984). During the first days after arrival many unsuccessful displacements were carried out and it took some time for the calves to learn how to displace a pen-mate. Unsuccessful displacements were not recorded in the present study but a comprehensive discussion presented by Wierenga (1990) stated that this type of feeder design may either protect a cow from butting when feeding or she may feel unsafe because of the inability to defend herself or escape. The number of displacements from the concentrate feeders was greater in wk 3 compared to wk 1 when they are expected to decrease (Kondo et al., 1984). Thus, it is also likely that calves did not reach a stable social hierarchy by wk 3 and the results of the present study may be a consequence of the process of establishing dominance relationships.

The ADV × treatment × week interaction indicated that regression coefficients of ADV on ADG were different depending on when and in which treatment the dominance was measured. Indeed, the effect of dominance on ADG was negative in wk 1 and positive in wk 3 in T2 pens, negative in wk 1 and quadratic in wk 3 in T1 pens, but ADV did not have a significant effect in T4 pens. Moreover, the effect of ADV on ADG in wk 3 was greatest within T1 and decreased as competition at the concentrate feeder decreased. Although R-squares are not calculated in PROC MIXED models we ran the same model in PROC GLM, which explained 63% of the variation. Dominance was negatively related to time at the concentrate feeders within T1 and T2 pens in wk 1 ($P < 0.05$; data not shown). A possible hypothesis is that high ranking animals during wk 1 spent less time at the concentrate feeder and had low ADG because they were more active chasing calves to establish dominance and had lesser concentrate intake. Greater energetic cost could also be associated with increased aggressiveness (activity) in high ranking calves. Conversely, the efforts to establish dominance in wk 1 brought benefits in wk 3 because the relationships between ADV and ADG, and ADV and time spent eating concentrate were positive (data not shown). This is in agreement with observations made by Wierenga (1990), who reported that the correlation between dominance value in dairy cows and time spent in the free stalls or in the feed bunk were larger with increasing competition. The quadratic effect of ADV on ADG within T1 calves at wk 3 of the present study is

more difficult to explain since the two "non-eater" calves were responsible for this effect. These calves had a high ADV as a consequence of winning most of the few displacements they were involved in only at the straw feeders, and they had low ADG. If these calves are not considered in the analysis, the effect of ADV on ADG becomes linearly positive in T1 pens (b = 0.41; P = 0.07). Dominance was related to time spent eating concentrate in two different ways. The number of times a calf was displaced by another animal (Spearman Rho = -0.27, P = 0.02) and the number of times it displaced another individual (Spearman Rho = -0.25, P = 0.03) from the concentrate feeders, were both negatively correlated to the time spent eating concentrate and to ADG (Rho = -0.29 and P = 0.01 vs. Rho = -0.24 and P = 0.04). This could be due to several causes. First, calves being often displaced had more interrupted eating bouts. However, Harb et al. (1985) reported that submissive cows increased their eating rate more than dominants without any negative effect on intake when competition was increased. Therefore, more research is needed to study the relationship between social rank, feed intake and feeding behavior. New technologies such as automated feeding behavior monitoring systems could offer great possibilities. Second, the calves could also have developed fear of being aggressively displaced by dominant animals, leading to avoidance or refusal to enter the concentrate feeders. Finally, calves being often displaced had the choice to eat from the straw feeders where less competition and aggression was usually present because there was more space available, and there were no feed barriers separating two adjacent calves eating straw. This is consistent with the low number of displacements from the concentrate feeders observed in the T1 pens of the present study. Animals do not habituate to some aversive procedures (Grandin, 1997) and low ranking animals keep more distance and avoid encounters with dominant pen-mates (Manson and Appleby, 1990). Stricklin and Gonyou (1981) fed groups of 15 beef cattle from a single stall or from an open trough. They did not observe any effect of treatments or dominance on final BW. However, average weight in the single stall system did not increase during the initial two weeks of adaptation to the limited feeding space and about 10% of the steers were difficult to get on-feed during the first month (Gonyou and Stricklin, 1981). McPhee et al. (1964) reported that low ranking animals were interrupted more often while feeding and had shorter feeding times compared to dominant animals but there were no relationships between social rank and final BW in long term studies. In agreement with the present study, Harb et al. (1985) concluded that as competition level increased, so did the CV between cows in the time spent eating and in intake. Conversely, Leaver and Yarrow (1980) reported that dominant animals ate more than submissive ones. It is also likely that effects of dominance are exacerbated in the present experiment because of the stressful condition of the calves after arrival, the design of facilities, the

novel environment, the lack of previous experience in competitive situations, and the low number of animals per group.

In summary, increasing the number of calves per concentrate feeder linearly decreased performance during the first week after arrival at the feedlot, as measured by feed intake and average daily gain. However, performance subsequently recovered when there were four rather than eight animals per concentrate feeding place. The variability in performance within a pen was increased as competition level increased, reflecting the greater effect of dominance as social pressure at the feeder increased during hierarchy formation. Blood variables and fecal corticosterone did not show a consistent effect on immune response and welfare. Indicators of body fat turnover were greatest when 8 and 4 calves per feeder were used. Some behavioral variables could indicate that welfare is poorer as the number of feeding places per pen decreased. The daily time spent lying decreased, time spent standing increased and an alteration of the circadian rhythm of eating was observed as the number of feeding places per pen decreased. The number of aggressive interactions indicated that the social environment was not greatly affected only at the lowest level of social pressure (2 calves per concentrate feeder).

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Chapter V

Performance, behavior, and welfare of Friesian heifers housed in pens with 2, 4 and 8 individuals per concentrate feeding place

Chapter based on a paper sent to Journal of Animal Science

ABSTRACT

The objective of the present trial was to study the effect of increasing the number of heifers per concentrate feeding place on performance, behavior, welfare indicators, and ruminal fermentation of feedlot heifers. Seventy-two Friesian heifers were used in a factorial arrangement with 3 treatments and 3 blocks of similar BW. The treatments consisted of 2 (**T2**), 4 (**T4**), and 8 (**T8**) heifers per each place in the concentrate feeder (8 heifers/pen). Measurements started after 4 wk of adaptation to treatments. Concentrate and straw were fed at 0830 in individual feeders and allowed ad libitum consumption. During 6 periods of 28 d each, DMI and ADG were measured, and blood and rumen samples were taken. Corticosterone in fecal samples and behavior were measured at periods 1, 3 and 6. Final BW, ADG and G:F ratio were not affected by treatments. The variability in final BW between heifers sharing the same pen tended to increase ($P = 0.06$) and concentrate intake decreased linearly as competition increased. The proportion of abscessed livers responded quadratically, being 8, 4 and 20% for T2, T4 and T8, respectively. Concentrate eating time decreased and eating rate increased linearly, whereas the variability between pen-mates in concentrate eating time was greatest in T4 and T8. Increasing competition resulted in a quadratic response in daily lying time (greatest in T2), whereas standing time increased linearly. The number of displacements among pen-mates from the concentrate feeders, as well as the total sum of displacements, increased linearly with increasing competition. The pen-average fecal corticosterone was not affected by treatments but the pen's maximum concentration responded quadratically, being highest in T8, and dominant heifers being the most affected. Serum haptoglobin concentration increased linearly with competition, particularly within the most subordinate heifers. Increased competition reduced ruminal pH only in periods 1 and 2 and increased ruminal lactate. Increasing competition at the concentrate feeders increased the variability in final BW but performance was not affected. Detrimental effects on animal welfare might be deduced from the altered feeding behavior, reduced resting time, and increased aggression. However, fecal corticosterone indicated poor welfare only in some heifers. Ruminal lactate and blood haptoglobin indicate that the risk of rumen acidosis might increase with competition whereas liver abscesses increased at 8 heifers per feeder.

INTRODUCTION

Cattle usually synchronize their behavior under unrestricted or natural conditions because of their inherent photoperiodicity and allelomimetic behavior (Miller and Wood-Gush, 1991). However, Gonyou and Stricklin (1981) reported a complete loss of daily feeding patterns when 15 beef cattle per feeder were fed a 55% concentrate diet. Competition among pen-mates also increases

aggression, avoidance and intimidation behaviors, perhaps leading to social stress (Miller and Wood-Gush, 1991). Consequently, animal welfare and production may also decrease when competition for feed increases. Low ranking animals are more often interrupted while feeding, have shorter feeding times, and greater frequency of visits compared to high ranking animals (Stricklin and Gonyou, 1981; Huzzey et al., 2006). Eating time decreases and eating rate increases with competition (Nielsen, 1999; Olofsson, 1999) with subordinate cows exhibiting up to a three-fold increase (Harb et al., 1985). Therefore, increased competition in the feeders might disrupt the normal or preferred feeding patterns limiting the ability of cattle to self-regulate their digestive function. These observations led some researchers to suggest that reduced feeding space might be a risk factor of ruminal acidosis (Stone, 2004; Krause and Oetzel, 2006) and, consequently, of liver abscesses (Nagaraja and Cheng, 1998). Subacute ruminal acidosis (**SARA**) results in reduced and variable feed intake and performance (Britton and Stock, 1989; Schwartzkopf-Genswein et al., 2003). Ruminal pH and the profile of fermentation products are commonly used as indicators of SARA but acute phase proteins, such as haptoglobin, may also be used as markers (Ghozo et al., 2006; Nagaraja and Titgemeyer, 2007). The objective of the present study was to determine the long-term effects of increasing the number of heifers per each place in the concentrate feeders on performance, behavior, welfare and rumen function.

MATERIALS AND METHODS

Animals, Treatments and Facilities

The present study is the continuation of another that assessed the adaptability of newly received calves in the feedlot under the same treatments. The design of the facilities, animals and feeding management, and analytical methods were reported in the previous chapter. All animals remained in the same pen with the same pen-mates assigned upon arrival. However, one heifer under T8 was replaced at the end of the 4-wk arrival period by a heifer with the same BW as the mean of the pen because it had failed to adapt eating concentrate ("non-eater"). A second non-eater heifer under T8 was stimulated for a few days to eat concentrate and recovered a modest ADG after the arrival period.

Briefly, 72 Friesian heifers were assigned to a factorial arrangement of treatments in a factorial design with 3 treatments and 3 BW blocks. Treatments consisted of 2 (**T2**), 4 (**T4**) or 8 (**T8**) heifers per concentrate feeder. Thus, each of the 9 experimental pens contained 8 heifers and the number of concentrate feeding places per pen was 4, 2, and 1 for T2, T4, and T8, respectively. Details of housing conditions, feeders and video-recording system are described in Chapter IV. All

experimental procedures were approved by the Animal Care and Use Committee of the IRTA.

Performance and Intake. All measurements started after an arrival adaptation period of 4-weeks. Heifers were weighed for two consecutive days after this adaptation period and also before slaughter. The experiment consisted of 28-d periods. Intermediate weights were taken after withdrawal of refusals on d 1 of each experimental period for the calculation of ADG and G:F ratio. The within-pen standard deviation of BW was calculated to assess the variability of growth between heifers sharing the same pen. All animals were fed up to 380 kg of slaughter BW, which required 6, 7 and 8 periods for the low, medium and high BW block, respectively. Animals were slaughtered in a commercial abattoir, where hot carcass weight and the number of abscessed livers were registered.

All heifers received the same commercial concentrate and barley straw. The concentrate was formulated to meet NRC (1996) requirements and contained 90.6% DM, 16.6% CP, 19.2% NDF, 5.3% ether extract, 5.9% ash, and 53.0% non-structural carbohydrates. Ingredients of the concentrate were 31.0, 29.0, 15.0, 14.0, 6.6, 2.1, and 0.9% of barley, corn, corn gluten feed, soybean meal, soybean hulls, palm oil, and sodium bicarbonate, respectively, with the remainder being minerals and vitamins. The straw contained 90.7 % DM, 6.7 % ash, 73.4 % NDF, 43.8 % ADF, 8.7 % ADL, and 4.84 % CP, on a DM basis. Feeding management allowed ad libitum consumption of both components, which were fed once a day at 0830. Fresh water was available at all times. One composited sample of the offered concentrate and straw was taken during each experimental period for DM determination and chemical analysis. Straw and concentrate DM intakes were calculated weekly by weighing the amount of feed offered each day and subtracting the amount refused at the end of the week. However, intake data were pooled for each 28-d period for statistical analyses.

Maintenance Behavior. Behavior of the animals was recorded for 24-h on d 16 and 17 of the experimental periods 1, 3 and 6. Videotapes were processed by continuous recording of the activities performed by all animals. Recorded activities (time spent eating concentrate and straw, drinking, lying and standing) were registered together with their starting and ending times (to the nearest second) and the animal identification in the previous chapter. Eating rate of concentrate and straw were calculated as the daily average of the pen DMI during the week divided by the average sum of the time spent eating by all animals in the pen.

Social Behavior. Displacements among calves from concentrate feeders, straw feeders and water bowls were the events recorded. They were registered with the time of occurrence, the animal identification, the type of event, and the activity being performed when the event occurred. Criteria used to register aggressive behavior and construction of hierarchy matrices were reported in the previous chapter. Dominance order was assessed by calculating the angular dominance value

(**ADV**) as the arcsine square root transformation of the average proportion of times that the individual displaced each pen-mate as reported in Chapter IV.

Blood, Fecal and Rumen Fluid Sampling. Sampling was performed on d 23, 24 and 25 of each experimental period for the low, medium and high BW block, respectively. All samples were taken at 1630 (8 h post-feeding) because under this management ruminal pH usually reaches the nadir (Nagajara and Titgemeyer, 2007). One whole blood sample from each heifer was taken by jugular veni-puncture (10-mL Vacutainer, Plymouth, UK). Serum was separated within 4 h (3,000 × *g*, 20 min, 4º C) and stored at -20º C until analyses of haptoglobin, β-hydroxy butyrate (β-**HBT**) and NEFA were completed. The fecal sample was taken from the rectum of each heifer and frozen at -20ºC until analyses of corticosterone to assess the adrenal response of heifers. Approximately 10-mL of ruminal liquid were taken by rumenocentesis after skin disinfection with iodine and the use of sterile i.v. catheter needle (Abbocath-T 14G x 140 mm, Abbott, Sligo, Ireland). A 4-mL aliquot of ruminal fluid was sub-sampled and frozen at -20ºC until chemical analysis of organic acids. The pH of the remaining ruminal fluid was measured immediately with a glass electrode pHmeter (model 507, Crisson Instruments SA, Barcelona, Spain). Blood and rumen fluid analyses were conducted for all experimental periods but fecal corticosterone was only measured in periods 1, 3 and 6, when behavior was also recorded.

Chemical Analyses

One portion of the offered feed was analyzed for DM content during 24 h at 103 ºC in a forced-air oven according to AOAC (1990; ID 950.01). Another portion of feed samples was dried in a forced air oven at 65 ºC for 48 h, ground in a hammer mill through a 1-mm screen and stored for later chemical analysis. Samples were analyzed for DM and ash (AOAC, 1990; ID 950.05). Nitrogen content was determined by the Kjeldahl procedure (AOAC, 1990; ID 976.05). Ether extract was performed according to AOAC (1990; ID 920.39). The NDF and ADF contents were determined sequentially following the procedure of Van Soest et al. (1991) using a thermostable α-amylase and sodium sulphite.

Fecal corticosterone determinations were performed using the commercially available I^{125} radioimmunoassay kit (Rats and Mice Corticosterone kit; ICN Pharmaceuticals; Orangeburg, NY) as described by Morrow et al. (2002). Haptoglobin was determined by the hemoglobin binding method with the use of a commercial haptoglobin assay (Assay Phase Range, Tridelta Development Limited, Maynooth, Ireland); D-3-Hydroxybutyrate was determined by a kinetic enzymatic method (Ranbut D-3-Hydroxybutyrate, Randox Laboratories Ltd., Crumlin, UK) and NEFA by the colorimetric enzymatic test ACS-ACOD method (NEFA C, Wako Chemicals, Neuss, Germany).

Volatile fatty acids (**VFA**) and lactic acid concentrations of ruminal fluid were analyzed through a modification of the capillary GC method described by Richardson et al. (1989). Modifications consisted of sample conservation and pre-analysis treatment. To preserve the sample, 4-mL of ruminal fluid were added to 1 mL of a solution made up of 1% (wt/wt) solution of mercuric chloride, 2% (vol/vol) orthophosphoric acid and 0.2% (wt/wt) 4-methylvaleric acid as an internal standard in distilled water and frozen at -20º C. As a pre-analysis treatment, samples were thawed and centrifuged at 15,000 × *g* for 15 min and diluted 1:1 in distilled water.

Statistical analyses

All statistical analyses were carried out considering the 6 consecutive experimental periods when the 3 BW blocks were present, except for final BW which was measured previous to slaughter. A logarithmic transformation was applied to blood haptoglobin and fecal corticosterone concentrations. The same was applied to the ruminal lactic acid concentration plus one because of the presence of zero concentrations in some animals. All individual data were averaged to give pen means at each sampling point over time. Pen was the experimental unit for all statistical analysis (n = 3). Variables normally distributed were analyzed by a mixed-effects regression model using the PROC MIXED of SAS (v. 9.1, SAS Institute Inc., Cary, NC, USA). The model contained the fixed linear and quadratic regression coefficient of the number of feeding places per pen (treatment), BW block, linear and quadratic treatment × period, and block × period interactions. The random effects were modeled through the correlations among the repeated measure of time (period or time interval) subjected to the pen, and through the random effect of pen. To analyze the eating patterns within the day and among periods, double-repeated measures were considered (time interval of the day subjected to the period nested within pen). The same effects were used in a Poisson regression model to analyze the number of displacements per pen and day, and in a logistic regression model to analyze the proportion of animals showing liver abscesses and ruminal pH below the threshold of 5.6. In both cases the GLIMMIX procedure of SAS was used. An analysis of the social hierarchy was undertaken to study the trends of corticosterone and haptoglobin of each social category with increasing competition. Each heifer within a pen was classified as very dominant (the two heifers at the top of the social hierarchy showing the greatest ADV), dominant, subordinate, and very subordinate (the two heifers showing the lowest ADV and, therefore, being the most subordinate of the group). A mixed-effects regression model was also used for this purpose, where the correlations were modeled at three levels of random effects: the repeated measure of time, the individual animal nested within the pen, and the pen nested within treatment, with all animals within a pen being correlated (St-Pierre, 2007). This model contained the fixed categorical effects of period, BW block

and dominance category of each heifer, plus the linear and quadratic effect of the number of heifers per concentrate feeder and the appropriate two- and three-way interactions. In all models, the interaction between the linear and quadratic treatment by any of the categorical effects (period, time of the day, dominance category) tested the null hypothesis that regression coefficients of the number of heifers per feeder were equal among all levels of the categorical effect. If this interaction was significant, then regression coefficients at each level were estimated and tested for their difference from zero. The choice of the best covariance structure was based on fit statistics. Significance was declared at $P \leq 0.05$ and tendencies discussed at $0.05 < P \leq 0.10$ unless otherwise noted.

RESULTS

Performance and Intake

At the start of the present study, initial BW showed a quadratic response ($P < 0.01$) to increasing the number of heifers per concentrate feeder as a consequence of treatment effects during the adaptation period, being lowest in T8 (Table 1). The initial BW was used as a covariate to test for carryover effects on all variables tested but it was not significant in all the variables presented ($P > 0.10$) and was therefore eliminated. However, this statistical difference was not maintained until the end of the experiment (quadratic $P = 0.12$). The within-pen standard deviation of final BW tended to increase linearly as social pressure in the concentrate feeders increased ($P = 0.06$). There were no significant treatment effects on ADG, within-pen SD of ADG or G:F ratio ($P > 0.10$). Concentrate DMI decreased linearly ($P = 0.05$) with increasing number of heifers per concentrate feeder but no effects were observed on straw or total DMI ($P > 0.10$). There were no significant treatment × period interactions in any performance or intake characteristics ($P > 0.05$). Hot carcass weight was not affected but the percentage of abscessed livers followed a quadratic pattern as the number of heifers per feeder increased, being greatest in T8 ($P = 0.03$; Table 1).

Maintenance Behavior

The main effect of treatments on maintenance behavior is presented in Table 2. The average time spent eating concentrate per day decreased linearly ($P < 0.001$) as social pressure in the concentrate feeders increased. This resulted in a linear increase ($P = 0.05$) in concentrate eating rate as the number of heifers per feeder increased.

Table 1. Intake and performance of Friesian heifers grown in pens at a social pressure of 2 (T2), 4 (T4) and 8 (T8) heifers per concentrate feeder

	Treatment					
Item	T2	T4	T8	SEM	L [1]	Q [1]
Initial BW, kg	141.7	142.4	136.2	0.90	0.03	0.002
Initial BW SD, kg [2]	7.96	10.59	10.10	1.14	0.67	0.27
Final BW, kg	386.4	388.3	374.7	6.89	0.14	0.12
Final BW SD, kg [2]	28.82	29.51	38.13	3.42	0.06	0.12
ADG, kg/d	1.24	1.24	1.22	0.02	0.57	0.88
ADG SD, kg/d [2]	0.23	0.22	0.24	0.03	0.61	0.77
Gain:Feed, kg/kg	0.186	0.188	0.190	0.002	0.40	0.83
Concentrate, kg/d	6.12	5.98	5.80	0.068	0.05	0.72
Straw, kg/d	0.73	0.82	0.82	0.06	0.41	0.51
Total DMI, kg/d	6.86	6.80	6.62	0.15	0.40	0.63
Straw, kg/kg total DMI	0.104	0.117	0.120	0.003	0.17	0.40
Hot carcass weight, kg	202.3	198.2	196.3	4.39	0.36	0.71
Hot carcass weight SD, kg[2]	22.34	22.62	15.99	3.84	0.34	0.38
Abscessed livers, %	8 ± 4	4.2 ± 3	20.8 ± 6	-	0.05	0.03

[1] P-value of the linear (L) and quadratic (Q) effect of number of feeding places per pen.

[2] Within-pen standard deviation of BW (BW SD), average daily gain (ADG SD), and hot carcass weight (Hot carcass weight SD).

The intra-pen SD of concentrate eating time responded quadratically ($P = 0.001$), with T4 and T8 groups showing the greatest variability between heifers sharing the same pen. The average time spent eating barley straw was two-times greater than that spent eating concentrate, and showed a quadratic response ($P = 0.03$) as competition increased, with the lowest time in T2 pens. The intra-pen variation of time at the straw feeders responded quadratically ($P < 0.001$) as the number of heifers per concentrate feeder increased with the greatest homogeneity between pen-mates in T2. Regardless of treatment, time spent eating straw increased from 97.8 ± 3.6 min/d in period 1 to

115.8 ± 5.7 min/d in period 6 (data not shown). However, animals under T2 did not show a significant increase in the time spent eating straw, whereas those under T4 and T8 did from period 1 to 6 ($P < 0.05$; data not shown). Barley straw eating rate and time spent drinking water were not affected by treatments ($P > 0.10$). The time spent lying down decreased linearly ($P = 0.02$) as the number of heifers per concentrate feeder increased, although it also showed a quadratic response ($P = 0.005$). Contrarily, the time spent standing increased linearly ($P < 0.01$).

Table 2. Least square means of maintenance behavior of heifers housed in pens at 2 (T2), 4 (T4) and 8 (T8) heifers per concentrate feeding place [1]

Displacements	Treatment					
	T2	T4	T8	SEM	L [2]	Q [2]
Concentrate						
Eating time, min/d	63.39	60.92	45.56	2.28	0.001	0.71
Eating rate, g DM/min	103.24	103.03	121.91	5.94	0.05	0.54
Intra-pen SD, min/d	8.57	12.56	11.08	0.67	0.10	0.001
Barley Straw						
Eating time, min/d [3]	89.10	113.02	112.81	5.08	0.03	0.03
Eating rate, g DM/min	7.44	6.96	7.04	0.75	0.72	0.72
Intra-pen SD, min/d	22.23	28.40	27.76	2.21	0.25	<0.001
Drinking, min/d	20.15	20.92	21.78	1.53	0.42	0.91
Lying, min/d	929.5	909.8	899.9	1.86	0.02	0.005
Standing, min/d [4]	326.6	341.1	350.7	7.30	0.01	0.22

[1] Behavior was measured by continuous sampling of the activities registered for all animals during 2 d at periods 1, 3 and 6 which corresponded to 8, 16 and 28 wk after arrival at the feedlot.

[2] Lineal (L) and quadratic (Q) effect of the number of feeding places pen.

[3] Standard deviation of time at the feeder between heifers sharing the same pen.

[4] Time spent on other standing activities was calculated as the total observation daily time minus the time spent eating concentrate, barley straw, drinking and lying.

The period main effect ($P < 0.05$) indicated that concentrate eating rate, time spent eating straw,

straw eating rate, time spent drinking water, and time spent standing increased as heifer's age increased, whereas time spent eating concentrate and lying down decreased (data not shown). Finally, the period × treatment interaction for concentrate eating time ($P = 0.001$) and rate ($P = 0.05$), and time spent eating straw ($P < 0.05$) indicated that the treatment differences became greater as heifers grew (data not shown).

The mean concentrate feeding patterns throughout the day are presented in Figure 1a. The linear treatment effect ($P = 0.55$) was not significant but the linear treatment × time interval of day ($P = 0.01$) demonstrated that linear regression coefficients of competition level were different among different times of the day. This was the result of linear coefficients being different from zero ($P < 0.05$) during some time intervals but not during others ($P > 0.10$), as shown in Figure 1a. Accordingly, the time spent eating concentrate decreased linearly ($P < 0.05$) between 0700 to 0800, 0800 to 0900, 1200 to 1300, and 1700 to 1800 as the number of heifers per feeder increased. The quadratic treatment main affect ($P = 0.01$) and the quadratic treatment × time interval interaction ($P = 0.14$) indicated that, regardless of time of the day, the time spent eating concentrate responded quadratically but quadratic regression coefficients were different among time intervals. In fact, the average concentrate eating time per animal was 1.92, 2.08, and 1.82 ± 0.16 min/h for T2, T4, and T8, respectively, and the quadratic regression coefficient was significant at 1100 (Figure 1a).

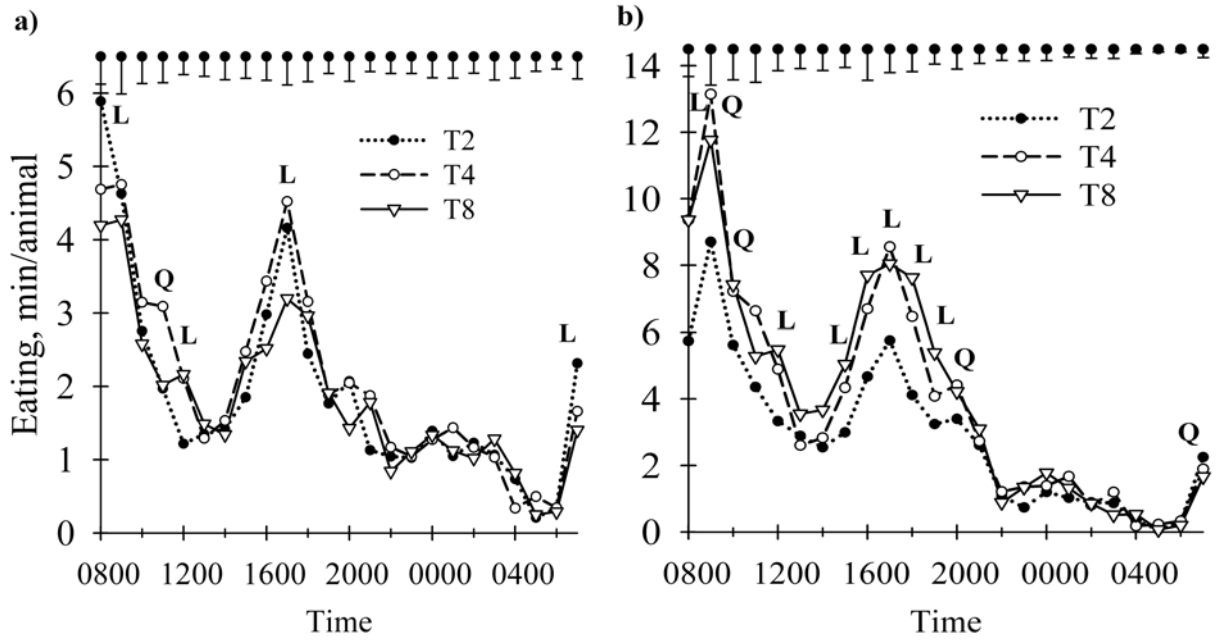

Figure 1. Average concentrate (a) and straw (b) feeding patterns of Friesian heifers housed in pens at 2 (T2), 4 (T4) and 8 (T8) heifers per concentrate feeding place. **L, Q** within a sampling time, increasing the number of heifers per concentrate feeder resulted in a significant linear or quadratic regression coefficient, respectively ($P \leq 0.05$). The bars along the top of the graph represent one SEM.

Straw eating patterns were also affected by treatment main effect (linear $P < 0.0001$; quadratic P = 0.001) and their interaction with time of the day (linear $P = 0.003$, and quadratic $P = 0.07$ treatment × time). Regardless of time of the day, the average time spent eating straw was 2.86, 3.93, and 4.01 ± 0.16 min/h for T2, T4, and T8, respectively. Figure 1b shows that the time spent eating straw increased in all daylight intervals as the number of heifers per concentrate feeder increased ($P < 0.05$).

Social Behavior

The number of displacements among pen-mates at the concentrate feeders increased linearly as the number of heifers per feeder increased (Table 3; $P < 0.001$). In contrast, the number of displacements from the straw feeders decreased linearly as the number of heifers per concentrate feeder increased ($P = 0.01$) but it also showed a tendency for a quadratic effect ($P = 0.09$). In addition, the linear ($P = 0.01$) and quadratic ($P = 0.004$) effect of treatment × period interaction indicated a linear increase in period 1 ($P = 0.01$) and quadratic responses in period 3 ($P = 0.03$) and 6 ($P = 0.08$), when T2 heifers showed the greatest number of displacements from the straw feeders (data not shown). Displacements from the water bowls followed a quadratic pattern ($P = 0.005$) as the number of heifers per feeder increased, being greatest in T4 (Table 6). The total number of displacements per pen and day increased linearly as the social pressure in the concentrate feeders increased ($P < 0.05$). Regardless of treatment, the average number of displacements from straw feeders, water bowls and the total sum increased over periods ($P < 0.05$; data not shown). However, the number of displacements from the concentrate feeders showed a non-significant numerical decrease from 36.5 at period 1 to 30.1 at period 6 ($P = 0.33$; data not shown).

The average ADV of the pen was not affected by treatments ($P > 0.10$; Table 3). As expected, there were differences among all dominance categories, with mean proportions of displacements won of 68, 55, 45, and 32%, for very dominant, dominant, subordinate and very subordinate heifers, respectively (data not shown). The quadratic treatment × ADV category ($P < 0.001$) showed that each category responded differently to treatments by changes in the average proportion of displacements won by the heifers (Table 3). Therefore, increasing the number of heifers per concentrate feeder resulted in a quadratic response ($P < 0.001$) of the ADV of very dominant and very subordinate heifers, with T4 showing the lowest ADV in very subordinate heifers but the greatest ADV in very dominant heifers.

Table 3. Number of displacements per pen and day (mean ± SE) from each resource container among heifers at 2 (T2), 4 (T4) and 8 (T8) heifers per concentrate feeding place

	Treatment				
Displacements	T2	T4	T8	L [1]	Q [1]
Concentrate Feeders	19.3 ± 1.39	33.7 ± 1.84	67.1 ± 2.61	<0.001	0.13
Straw Feeders	54.8 ± 3.51	38.3 ± 3.22	36.2 ± 2.98	0.01	0.09
Water Bowl	17.2 ± 1.26	22.3 ± 1.45	12.8 ± 1.08	0.62	0.005
Total	81.9 ± 4.48	100.6 ± 5.27	122.4 ± 6.17	0.03	0.14
ADV, arcsin $\sqrt{x}$ [2]	0.759 ± 0.018	0.767 ± 0.018	0.771 ± 0.018	0.75	0.63
Very dominant	0.938 ± 0.022	1.058 ± 0.022	0.938 ± 0.022	0.54	<0.001
Dominant	0.821 ± 0.021	0.843 ± 0.021	0.820 ± 0.021	0.85	0.17
Subordinate	0.744 ± 0.021	0.692 ± 0.021	0.741 ± 0.021	0.89	0.42
Very subordinate	0.614 ± 0.022	0.562 ± 0.022	0.645 ± 0.022	0.08	0.001

[1] Linear (L) and quadratic (Q) effect of treatments P-value.

[2] Angular dominance value (ADV) was calculated as the arcsin transformation of the average proportion of displacements won against each dyad member in the pen.

Fecal and Blood Measurements

The average fecal corticosterone concentration of the pen was not affected, whereas the individual maximum concentration of the pen responded quadratically ($P < 0.001$) as the number of heifers per concentrate feeder increased, with T4 pens showing the lowest values (Table 4). The linear treatment × dominance category interaction ($P = 0.12$) did not indicate significant linear regression coefficients but the quadratic effect of treatments × dominance class interaction ($P = 0.01$) indicated that quadratic regression coefficients were different among dominance categories. Therefore, fecal corticosterone showed a tendency for a quadratic response in dominant animals ($P = 0.06$) with T8 showing the greatest values. Serum haptoglobin concentration increased linearly ($P = 0.05$) as the number of heifers per concentrate feeder increased (Table 4). Average haptoglobin level was 219 mg/L in period 1 and 229 mg/L in period 2. Then, it increased at period 3 to 270 mg/L ($P < 0.05$) and decreased sharply from period 3 to 4 (98 mg/L) to remain at a low level thereafter, 89 and 106 ± 8 mg/L in period 5 and 6, respectively (data not shown).

Table 4. Least square means for fecal corticosterone and blood parameters, and their relationships with angular dominance value (ADV) of Friesian heifers raised at 2 (T2), 4 (T4) and 8 (T8) heifers per concentrate feeding place

	Treatment					
	T2	T4	T8	SEM	L [1]	Q [1]
Fecal corticosterone, Log[ng/gDM]						
Mean [2]	1.297	1.248	1.334	0.034	0.32	0.16
Pen's Maximum [2]	1.553	1.471	1.692	0.062	0.001	<0.001
Very dominant [3]	1.315	1.217	1.298	0.056	0.94	0.28
Dominant [3]	1.255	1.177	1.433	0.044	0.02	0.06
Subordinate [3]	1.326	1.281	1.352	0.044	0.51	0.36
Very subordinate [3]	1.302	1.329	1.263	0.057	0.53	0.73
Haptoglobin, Log[mg/L]						
Mean [2]	2.136	2.173	2.177	0.012	0.05	0.16
Very dominant [3]	2.230	2.254	2.276	0.027	0.09	0.65
Dominant [3]	2.222	2.275	2.268	0.027	0.08	0.51
Subordinate [3]	2.171	2.258	2.255	0.027	0.16	0.74
Very subordinate [3]	2.190	2.264	2.319	0.027	0.02	0.80
NEFA, mmol/L	0.097	0.098	0.097	0.004	0.93	0.83
β-HBT, mmol/L [4]	0.251	0.276	0.281	0.013	<0.001	0.36

[1] P-value of the linear (L) and quadratic (Q) effect of number of feeding places per pen.

[2] The mean and the maximum corticosterone concentration of each pen were used as the observational unit throughout 6 experimental periods.

[3] Heifers were classified in very dominant, dominant, subordinate, and very subordinate ranking based on ADV within each pen in periods 1, 3, and 6.

[4] Beta-hydroxybutyrate.

The linear treatment × dominance category interaction (P = 0.13) in blood haptoglobin

concentration indicated that there was a weak tendency of linear regression coefficients to differ among dominance categories. Therefore, increasing the number of heifers per concentrate feeder resulted in a linear increase of haptoglobin in very subordinate heifers ($P = 0.02$) and tended to increase linearly in very dominant and dominant heifers ($P < 0.10$; Table 4). Moreover, very subordinate heifers under T8 had greater haptoglobin levels than the rest of their pen-mates ($P < 0.05$), which did not happen within T2 and T4. Serum concentration of NEFA was not affected ($P > 0.10$) but β-HBT increased linearly ($P < 0.001$) as social pressure increased.

Ruminal Fermentation

Treatments did not affect the average ruminal pH at 8 h post-feeding (linear $P = 0.25$; Table 5) but the treatment × period interaction was significant (linear $P < 0.001$; quadratic $P = 0.08$), indicating that the regression coefficient of the number of heifers per feeder against ruminal pH were different among sampling periods. Indeed, ruminal pH showed a quadratic response in period 1 ($P = 0.04$; Figure 2) with T4 and T8 showing low values. In period 2, ruminal pH decreased linearly as the number of heifers per feeder increased ($P = 0.03$). Regardless of treatment, ruminal pH increased gradually from period 1 (5.37 ± 0.04) to 5 (5.92 ± 0.06) although period 6 (5.66 ± 0.10) was not different from the rest of the periods ($P > 0.05$) because a numerical decrease was observed from period 5 to 6. The proportion of heifers with ruminal pH below 5.6 tended to increase linearly ($P = 0.08$) as the number of heifers per concentrate feeder increased. The average acetate, propionate, and valerate molar proportions, as well as total VFA concentration, were not affected by treatments ($P > 0.10$; Table 5). However, butyrate molar proportion increased linearly ($P = 0.05$), whereas the branched chain VFA (BCVFA) decreased linearly ($P < 0.01$) as the number of heifers per concentrate feeder increased. The linear treatment × period interaction ($P < 0.01$) in total VFA showed a positive linear regression coefficient of the number of heifers per concentrate feeder in period 2 ($P < 0.01$), values being 167, 180 and 210 ± 10 mM for T2, T4 and T8, respectively (data not shown). Increasing the number of heifers per concentrate feeder resulted in a linear increase in ruminal lactic acid concentration ($P < 0.01$). In addition, the linear treatment × period interaction ($P = 0.07$) in ruminal lactic acid concentration indicated that regression coefficients were different among periods. Hence, linear increases were observed in periods 1, 2, and 3 ($P \leq 0.01$).

DISCUSSION

Results concerning the adaptability of these calves when received in the feedlot under the same

treatments were reported in the previous chapter. Regrouping was not carried out after the adaptation period because of the potential effects of social disorganization (Hasegawa et al., 1997) and to study if animals were able to revert those effects in the remaining fattening period.

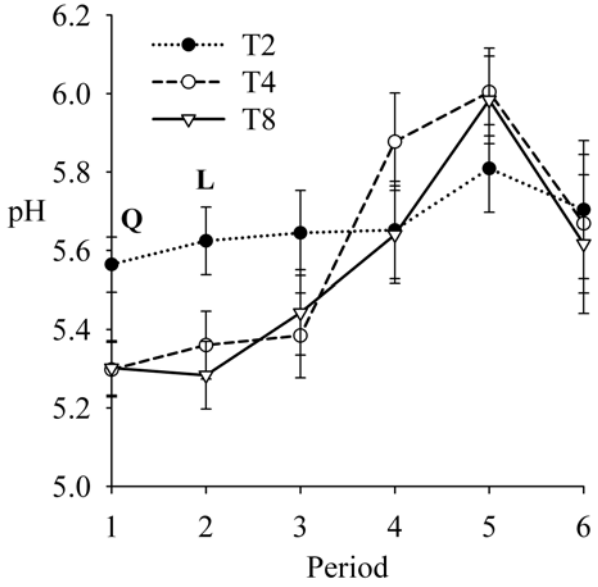

Figure 2. Ruminal pH of Friesian heifers raised at social pressures of 2 (T2), 4 (T4) and 8 (T8) heifers per concentrate feeder throughout the six 28-d periods. **L, Q** within a period, linear or quadratic regression coefficients of the number of heifer per concentrate feeder was significant ($P \leq 0.05$).

Performance and Intake

The initial BW of T8 pens was 4.3% lower than the average of the other two treatments. However, this difference was 3.4% at the time heifers were sent to the slaughterhouse. Therefore, the negative effects during the arrival period were only partially recovered. The pen average and the within-pen variability of ADG were not affected by treatments in the present study. Nevertheless, the variability of BW among heifers sharing the same pen tended to increase linearly as the number of heifers per concentrate feeder increased, confirming a lack of compensating mechanisms in the effects occurred during the arrival period. Indeed, there was a numerical linear decrease in hot carcass weight. Concentrate DMI decreased linearly as the number of heifers per feeder increased. Longenbach et al. (1999) increased the feed bunk length from 0.15, to 0.31 or 0.47 m/heifer in three groups of replacement heifers with different ages and observed a trend for increased within-pen SD in ADG and BW with diminishing feeding space in the older two groups, particularly greater at the end of each trial. In addition, Longenbach et al. (1999) observed decreasing number of heifers able to eat simultaneously at a given linear feed bunk allowance as the heifers' age increased. Thus, social pressure was not constant because body size increases with age. Finally, social pressure may not be the same for large (commercial) groups of animals compared to small experimental groups,

even if the feed bunk length allowance per animal is the same. Contrarily, the present study assessed social pressure in the concentrate feeders by controlling the number of heifers per pen able to eat simultaneously and this was maintained throughout the trial.

Table 5. Ruminal pH and fermentation profile of Friesian heifers housed in pens at a social pressure of 2 (T2), 4 (T4) and 8 (T8) heifers per concentrate feeder

	Treatment					
Item	T2	T4	T8	SEM	L [1]	Q [1]
pH	5.66	5.59	5.54	0.07	0.25	0.72
pH < 5.6, % heifers	53.5 ± 9.9	61.6 ± 9.3	73.6 ± 7.8	-	0.08	0.83
VFA, mol/100 mol [2]						
Acetate	50.58	51.38	50.18	0.73	0.51	0.29
Propionate	37.86	36.88	37.68	1.31	0.98	0.50
Butyrate	8.71	8.79	9.46	0.70	0.05	0.78
Valerate	2.10	2.28	2.11	0.17	0.69	0.71
BCFVA [3]	0.772	0.678	0.606	0.024	<0.01	0.27
Total VFA, mM	155.9	166.5	169.4	6.4	0.15	0.46
Lactate, Log(1+[mM])	0.145	0.234	0.284	0.024	0.02	0.20

[1] P-value of the linear (L) and quadratic (Q) effect of number of feeding places per pen.

[2] VFA = volatile fatty acids.

[3] Branched chain volatile fatty acids = iso-valerate + iso-butyrate.

The lack of long-term effects of increasing the social pressure at the feeders on performance in the present trial agrees with that observed by Gonyou and Stricklin (1981), Zinn et al. (1989), and Longenbach et al. (1999) in growing cattle. Contrarily, intake was not affected in those studies. Differences may be due to feeding management, group size, and feeders design. Concentrate and straw were fed in separate feeders in the present trial and concentrate feeding places were separated by barriers. Despite the fact that increased social pressure at the concentrate feeder may affect performance during stressful situations, such as after arrival, and concentrate DMI remained lower afterwards, no long-term effects were observed. Finally, the percentage of abscessed livers followed

a quadratic pattern as the number of heifers per feeder increased, with T8 showing the greatest proportion. Low ruminal pH has been regarded as one of the main predisposing factors in the etiology of liver abscesses because it produces ruminal tissue damage, allowing necrotic pathogens to enter the bloodstream (Nagaraja and Chengappa, 1998). These authors have also indicated that a change in feeding patterns, allowing cattle to become hungry, may be a predisposing factor, and sufficient bunk space was a recommendation to reduce the incidence of liver abscesses.

Maintenance Behavior

There was clear evidence that social competition for concentrate feeding increased as the number of heifers per feeder increased. Daily time spent eating concentrate decreased linearly by 28% as the number of heifers per feeding place increased from 2 to 8. However, animals partly compensated for this shorter concentrate eating time through a linear increase in the eating rate, although this increase was of a lesser magnitude (18%). This might explain the observed decrease of concentrate DMI. Olofsson (1999) observed that increasing competition from 1 to 4 cows per feeder resulted in a tendency to increase DMI in a 50% concentrate diet, a 19% decrease in eating time, a 72% increase in eating rate and a 22% increase in the number of daily meals. The quadratic effect on the within-pen SD of concentrate eating time suggests either that feeding behavior was more homogeneous among heifers sharing T2 pens or, alternatively, that feeding behavior was altered to a greater extent within T4 and T8 groups. These three variables show that both group and individual concentrate feeding behavior were altered. Harb et al. (1985) showed similar effects when increasing from 1 to 2 cows per feeder, with a positive correlation between social rank and eating time. Submissive cows in this study were probably less comfortable during feeding because their silage eating rate increased two- to three-fold. However, greater eating rate is also an adaptation mechanism to social constraints (Nielsen, 1999). Negative correlations between social rank and intake (estimated with markers) were observed by Harb et al. (1985) and Leaver and Yarrow (1980) when competition at the feeder increased.

The daily time spent eating barley straw by the heifers was lowest in T2 groups. The consequence of this result might be the lower linear straw feeding space in this treatment. The concentrate feeders of the present study were designed to be adapted to the existing experimental facilities. This shorter straw feeding space in T2 pens was a consequence of placing two concentrate feeder units in the 3.84-m feeding front, whereas only one steel unit was necessary in T4 and T8 pens. Hasegawa et al. (1997) reported variable responses in the time spent eating several feeds after mixing cows, with responses subjected to the cows' social status but the causes were not clear. In conclusion, the present study showed that increasing competition in the concentrate feeders led to

reduced eating time, and increased eating rate and number of displacements. However, increased social pressure at the straw feeders led to decreased straw eating time and increased aggressive behavior.

The daily concentrate eating patterns showed that the decrease in the time spent eating concentrate with increasing social pressure was particularly evident at peak eating times, such as after feed delivery and sunset. However, concentrate eating time at sunrise and just after feed delivery was greatest in T2, but it was numerically greatest in T4 during the afternoon and at the sunset feeding period. Meanwhile, T8 showed the lowest concentrate eating time during both sunrise and sunset feeding periods. Therefore, heifers under the greatest social pressure did not show a shift in the concentrate eating pattern towards times of the day when competition was lower. Contrarily, Gonyou and Stricklin (1981) and Oloffson (1999) reported increased eating activity during nighttime with diets containing higher forage proportions than the proportion selected by the heifers in the present trial. The availability of straw in different feeders may be the cause of this difference compared to TMR-fed cattle. This may be supported by the significant treatment effect observed in the straw eating patterns. Heifers under the greatest social pressure at the straw feeders (T2), but under the lowest at the concentrate feeders, spent the shortest time eating straw during, and between, the two major periods of eating. Therefore, reduced feeding space either in the concentrate or straw feeders may have resulted in a restriction in the heifers' natural feeding behavior, which usually leads to synchronization of feeding at peak eating times (Miller and Good-Gush, 1991). In addition, heifers showed reluctance to change both feeding patterns during less busy times of the day.

Lying time decreased whereas standing time increased with increasing number of heifers per feeder. This may suggest that subordinate animals did not greatly change their daily feeding patterns but spent more time standing without eating, perhaps waiting for lesser competition in the feeders. This was previously reported when 15 beef cattle per feeder were used (Gonyou and Stricklin, 1981) or when the number of cows per feeder increased from 1 to 4 (Oloffson, 1999). Friend (1991) concluded that deviations of behaviors such as feeding, resting or rumination patterns, and feed consumption rates are very sensitive indicators of the animal internal state. Therefore, eating patterns, and lying and standing time of the present study indicate that the welfare of the animals may decrease as feeding space is reduced. However, results depend on other factors such as feeding management and facilities design. For instance, Huzzey et al. (2006) observed that daily feeding time was greater but aggressions increased to a greater extent as competition increased using post-and-rail compared to headlocks.

Social Behavior

As expected, there was a clear positive linear relationship between the number of heifers per feeder and the number of displacements among pen-mates at the concentrate feeders. The stability over time observed in the number of displacements from the concentrate feeders may indicate that a social order for access to this resource was established and no further aggression was required. However, the stabilization of the social hierarchy required the maintenance of high levels of aggression. The number of aggressions during the present study was greater than those observed in the first and third week after arrival at the feedlot, reported in the previous chapter. This is contrary to observations made by Kondo and Kurnick (1990), who reported that the number of aggressions decreased since re-grouping and remained at low levels after a social stabilization was achieved at about 1 wk in cows and beef cattle, respectively. Nevertheless, the number of displacements from the straw feeders increased over time and treatment differences became larger as heifer age increased. This was particularly noticeable in T2 pens because of the shorter straw feeding space, which led to greater aggression. The number of displacements from the water bowls responded quadratically to increasing number of heifers per concentrate feeder and showed the greatest aggression among T4 heifers. This treatment had average social pressure at both straw and concentrates feeders. There are no obvious reasons for this result because the number and size of the water bowls were identical in all pens. Although cattle spend a small proportion of the daily time on drinking activity, Andersson et al. (1984) showed that dominance is also exerted in the water bowls and completely modifies the drinking behavior in a similar way as feeding. The number of displacements from the concentrate feeders was greatest with the maximum number of heifers per feeder (T8), and the number of displacements from straw feeders was greatest with the shortest linear space per animal (T2). Therefore, we hypothesize that T4 groups re-directed part of the aggressive interactions, needed to establish or maintain the priority of access to resources, towards the water bowls. Finally, the sum of the number of displacements from all three resource containers also followed a linear trend with increasing number of heifers per concentrate feeder, because of the greater relative importance of the displacements from the concentrate feeders. Nevertheless, the total number of displacements showed a treatment × period interaction because T2 and T8 groups showed the same number of aggressions at period 6. Moreover, aggressions in T2 were greater at the straw than at the concentrate feeders but the opposite was true for T8 groups.

No treatment effects were observed in the pen average ADV. Nevertheless, the treatment × ADV category indicated that heifers responded to treatments by modifying the aggressive behavior of the group's social categories. Therefore, T4 were subjected to the lowest social pressure at both feeders and this led to very subordinate heifers (the weakest social category) to either not needing to

be aggressive or suffering the least aggression. On the other hand, very dominant T4 heifers were able to win the greatest proportion of displacements, either by being more aggressive or suffering less aggression (fewer bidirectional displacements). Altogether, increasing the social pressure at both concentrate and straw feeders led to differences in social organization within the group and increased aggressive behaviors.

Fecal and Blood Measurements

Despite the fact that average fecal corticosterone concentration of the pen did not reach statistical significance, a quadratic response was observed in the pen's maximum fecal corticosterone concentration as the number of heifers per concentrate feeder increased. This indicates that the most stressed heifer within T8 groups showed greater adrenal activity or suffered greater stress. The untransformed maximum fecal corticosterone concentration was 38.3, 30.5 and 57.5 ± 8.8 ng/gDM, for T2, T4 and T8, respectively. These values are much greater than those reported by Morrow et al. (2002), who observed an increase from about 12 to 20 ng/g DM of feces when dairy cows were moved to a novel environment or after short transport. These most stressed heifers within a group may have suffered greater social pressure at the concentrate feeders in T8, as reflected in greater aggressions. In contrast, of the most stressed heifers in each treatment, the one in T4 showed the lowest stress because this treatment had the lowest concentrate and straw feeder social pressure combination. Glucocorticoids have extensively been measured as indicators of psychological and physical stressors. The results in corticosterone of the present study reinforce the hypothesis that increasing competition at the feeders produces social stress but only in some individuals within a group. We found no treatment effects in corticosterone during the arrival period while adapting to the new conditions. Therefore, it is suggested that social stress is the result of a long-term process, which may increase cumulatively over time in the most prone animals. Mench et al. (1990) reported increased blood cortisol in cows at 84 d after introduction of new members to the pen. Chronic or long-term stress, as measured in the present study through fecal corticosteroids over a six month period, is more likely to cause welfare and health problems than acute stress because of its effects on the central nervous system (Lane, 2006). Fecal glucocorticoids have been considered as one of the most reliable non-invasive measurements of chronic stress in animals because they do not interfere with the stress response itself (Lane, 2006). Heifers within each pen were assigned to dominance categories by their ADV to assess which social category within a group suffered the greatest stress. Only dominant heifers showed a tendency for a quadratic response when increasing the number of heifers per concentrate feeder, with those under T8 showing the greatest fecal corticosterone concentration. In addition, dominant heifers within T8 (29.9 ng/g DM)

had greater fecal corticosterone than very subordinate pen-mates (18.4 ng/gDM; $P < 0.05$), and the greatest values of the pen. Conversely, dominant heifers within T4 had lower fecal corticosterone concentration than very subordinates ($P < 0.05$), and the lowest of the pen. Within T2, there were no significant differences in fecal corticosterone between dominance categories ($P > 0.10$). Therefore, dominant heifers seem to be the most benefited social class under the lowest competition but the most stressed heifers under the greatest competition. This may suggest that, with high social pressure, dominant animals either try to ascend in the social hierarchy or they are stressed because they are pushed down in the social hierarchy. Contrarily, the fact that subordinate heifers did not show increased corticosterone may suggest that they have given up any possibility to ascend in the social hierarchy. Other studies with cows did not find relationships between dominance rank and blood corticosteroids, either when dairy cows were forced to a change in dominance rank under a competitive situation (Arave et al., 1977) or in herds composed of different breeds (Adeyemo and Heath, 1982). Contrarily to our results, Mench et al. (1990) observed greater blood cortisol level in subordinates compared to dominants at 84 d after mixing beef cows. Nonetheless, those results may have reflected differences in the response to the handling stress during bleeding. Results of the present trial suggest that social status influences how each animal copes with the social environment, with dominant heifers having more difficulties as competition increases. High glucorticoid levels have been associated with both subordinance and dominance in different species (Lane, 2006). In primate societies, the diversity in cortisol in relation to social status can be explained by different social environments experienced by individuals. Thus, subordinates show greater cortisol concentrations than dominants in overtly aggressive societies because they carry the highest rates of physical or psychological stressors, have severe resource limitations, less social support, and are minimally related to other members (Abbott et al., 2003). Conversely, these authors found that dominant counterparts show greater cortisol concentration than subordinates in non-aggressive societies, which might be the case of cattle and may even explain breed differences in response to stress. Finally, the treatment effect on the most stressed heifer within a group and on dominants highlights the importance of analyzing not only the group mean values to assess stress and welfare but also individual animals within a group.

Serum haptoglobin concentration showed a linear increase as the number of heifers per feeder increased. Skinner et al. (1991) suggested that blood haptoglobin concentrations greater than 200 mg/L are indicative of early or mild infection, a threshold exceeded from periods 1 to 3 of the present trial, coincident with the lowest ruminal pH. Blood NEFA levels were not affected by treatments but βHBT concentration increased linearly as the number of heifers per feeder increased. Both NEFA and βHBT were used as indicators of body fat metabolism because they ensure

appropriate and coordinated fuel under high energy demands or muscular activity, or when other energy sources are scarce (Adewuyi et al., 2005). Therefore, greater extent of lipolisis has been expected with increasing number of heifers per feeder because of increased energy demands needed for competition and displacements (physical activity), or because of undernutrition due to decreased DMI. In positive energy balance, ketone bodies arise mainly from the metabolism of butyrate by the rumen wall and, therefore, the increase in βHBT is not proportional to that of NEFA (Sato et al., 1999). The increase in ruminal butyrate molar proportion and concentration of the present study supports this hypothesis.

Ruminal Fermentation

Subacute ruminal acidosis is characterized by a ruminal pH below 5.6 (Britton and Stock, 1989; Owens et al., 1998) during at least 3 h/d (Gozho et al., 2006). However, acidosis encompasses an array of biochemical and physiological stresses caused by rapid production and absorption of organic acids and endotoxins, but not necessarily by low ruminal fluid pH (Britton and Stock, 1989). Therefore, the mere presence of low ruminal pH may be meaningless if it does not affect other aspects of the animal such as performance or immune response. However, when rumen pH is considered together with other indicators then SARA can be diagnosed (Nordlund et al., 2004). Nonetheless, acidosis was assessed together with other ruminal and blood measurements in the present study. The average ruminal pH throughout the 6 experimental periods was not consistently affected, but lactic acid concentration increased linearly with increasing competition. However, T4 and T8 resulted in lower ruminal pH at periods 1 and 2. Heifers under the lowest social pressure (T2) were able to maintain a more stable ruminal pH at 8 h post-feeding over periods, compared to T4 and T8. The effects observed in ruminal pH during periods 1 and 2 corresponded with greater total VFA and lactic acid concentrations. These results were due, in part, to different number of heifers showing greater than normal concentrations. Ruminal lactate does not accumulate at concentrations greater than 5 mM under normal conditions, and a concentration greater than 40 mM is indicative of severe acidosis (Owens et al., 1998; Nagaraja and Titgemeyer, 2007). Six heifers under T8, five under T4 and none under T2 had ruminal lactate concentrations greater than 5 mM during periods 1 and 2 (data not shown). Therefore, a greater proportion of heifers may be at risk, or show more severe, ruminal acidosis during these periods. In addition, the proportion of heifers with ruminal pH below 5.6 tended to increase linearly by 20 percentage units as the number of heifers per feeder increased from 2 to 8. It has been suggested that the dairy herd is at high risk of acidosis if more than 25% of the animals show ruminal pH below 5.5 (Nordlund et al., 2004) or when the average pH (rumenocentesis) across cows is below 5.6 (Stone, 2004). In the present study, this

incidence rate of ruminal pH below 5.5 was 39, 54 and 62 ± 10%, for T2, T4 and T8, respectively. However, the proportion of heifers with ruminal pH below 5.6 decreased from an average of 85.5% in period 1 to 24.3% in period 5, regardless of treatment ($P < 0.05$; data not shown). The linear increase in ruminal butyrate proportion and concentration (data not shown) with increasing number of heifers per feeder may be a result of increased lactic acid production and degradation to butyrate (Nagaraja and Titgemeyer, 2007). Branched-chain VFA come from dietary true protein degradation and microbial protein recycling, processes reduced as ruminal pH decreases (Miura et al., 1980).

Sudden drops or wide fluctuations of ruminal pH, and also a greater time under suboptimal pH increases ruminal bacteria cell lyses and, consequently, the release of endotoxins lipopolysaccharides (Nagaraja and Titgemeyer, 2007). Therefore, the acute phase response is activated and, consequently, blood haptoglobin increases (Ghozo et al., 2006). In the present study, serum haptoglobin concentration increased, on average from 157 to 178 mg/L while ruminal pH numerically decreased from 5.66 to 5.54 as the number of heifers per feeder increased from 4 to 8, respectively. When the haptoglobin concentration ([Hapto]) of the present trial was regressed against ruminal pH, the resulting equation yielded an $R^2 = 0.32$ ($P < 0.001$; pH = 5.96 (± 0.08) – 2.15 (± 0.44) [Hapto]). Therefore, despite the fact that one spot-in-time ruminal pH sample is not an accurate indicator of increased risk of SARA, ruminal lactate, total VFA concentration, branched-chain VFA, and blood haptoglobin support the hypothesis that increasing the number of heifers per concentrate feeder may be another risk factor of ruminal acidosis (Nagaraja and Titgemeyer, 2007; Ghozo et al., 2006). However, the risk seems to be high during the first 3-4 months of the growing period. Finally, the linear decrease in concentrate DMI with increasing number of heifers per feeder in the present trial may also be due to the effects of acidosis (Britton and Stock, 1989) but other factors such as social behavior may also be responsible.

Subordinate cattle may be at a greater risk of ruminal acidosis because they have to wait to access the occupied feeders causing anxiety and, therefore, meal size and eating rate might increase once the feed is reached. On the other hand, dominant animals may be more prone to over-eating because they eat first and are usually not interrupted while feeding and, therefore, may have larger meals. Disruption of the normal feeding patterns has been seen as increased risk of ruminal acidosis in individual animals within a pen because the ability to self-regulate ruminal function may be impaired (Schwartzkopf-Genswein et al., 2003). Feeding behavior in the present study showed that feeding characteristics were altered by increased competition. Larger meals and greater eating rates of highly fermentable diets may result in lower ruminal pH but individual feed intake behavior was not measured in the present study. Under the hypothesis that the increase of blood haptoglobin in the present study was due to lower ruminal pH, then very subordinate heifers should have been

more likely to suffer ruminal acidosis because their blood haptoglobin increased linearly. Ruminal pH was 5.45, 5.60, 5.53, and 5.45 for very subordinate, subordinate, dominants, and very dominants, respectively ($P = 0.09$), but not affected by treatments (data not shown).

In conclusion, increasing the number of heifers up to 8 per concentrate feeder reduces concentrate intake but does not affect performance after an adaptation period to the limited feeding space because compensating mechanisms may arise. However, an improvement in the group homogeneity of BW may be achieved with more feeding space. Heifers adapt to the increased competition by changing their behavior, though these forced changes may be detrimental for animal welfare. Fecal corticosterone concentration indicates that sufficient feeding space should be provided at both concentrate and straw feeders. Otherwise, subordinate heifers might suffer social stress. Increased competition may result in lower ruminal pH during the initial 3 or 4 months of the fattening period, which activates the acute phase response and increases liver abscesses. Four heifers per concentrate feeding place seems a reasonable upper limit from most welfare and productive viewpoints. However, more research testing the design of feeders and facilities, feeding management and cattle type are needed.

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Chapter VI

General discussion of results

1. Feeding behavior and rumen function

1.1. Effect of sodium bicarbonate on feeding behavior and rumen function. The use of sodium bicarbonate in experiment 1 (Chapter II) showed that it may alleviate the ruminal pH drop that typically occurs after feed delivery in the morning. However, this was not translated into a clear buffer effect on daily average or minimum ruminal pH neither in the number of hours in which ruminal pH remained under the suboptimal threshold value of 5.8. To further understand the effects of the buffer on ruminal pH, we conducted multiple regressions against daily average ruminal pH by considering all the parameters that were available on a daily basis. These parameters were: sodium bicarbonate content of the concentrate (%DM); the daily intakes of total feed, concentrate DM, straw DM (in kg/d, % BW, g/kg $BW^{0.75}$, % of straw in total ration); the daily intake of total OM, NDF and ADF (in kg/d, % BW, g/kg $BW^{0.75}$); daily water intake (in L/d and % BW), total daily chewing, ruminating, and eating times (expressed in h/d, min/kgDMI, min/kgOMI, min/(gDMI/kg$BW^{0.75}$); daily meal frequency and meal duration; daily average meal size and eating rate. All these parameters were included as continuous fixed variables in a mixed-effect regression model with the random effects of heifer and period. The selection of variables was done through manual 'backward' selection by successively taking out the non-significant terms ($P > 0.10$). The outcome of the multiple regression model explaining the daily average ruminal pH was the following, coefficient (SE):

$$pH = 6.331\ (0.340) + 0.109\ (0.030)\ [BICARB] - 0.124\ (0.034)\ [DMI] - 0.526\ (0.164)\ [MEAL] + 0.143\ (0.052)\ [TCT]$$

Where pH is daily average ruminal pH, the intercept was significant ($P < 0.0001$), *BICARB* is sodium bicarbonate proportion in the concentrate (in % DM; $P = 0.004$), *DMI* is the level of DMI (in kg DM/d; $P = 0.004$), *MEAL* is daily average meal size (in kg/meal; $P = 0.008$), and *TCT* is total chewing time (in h/d; $P = 0.02$).

The increase in straw intake with buffer level did not affect total time spent eating, ruminating or total chewing. It did, however, lead to increased chewing time per kg of OM and DM as detailed in Chapter II. Both were the single variables that explained the greatest proportion of the variation in daily average ruminal pH. Because chewing time is positively correlated to saliva production, its increase per unit of acid producing substrate (OM or DM) would lead to a higher buffering capacity per unit of ingested feed. Salivation is greatest during eating and ruminating (Bailey and Balch, 1961) and these measures are summarized in the total chewing time of the 4-terms model. However, total chewing time depends on level of feed intake, fiber content, and its effectiveness, as modelled

by Dijkstra et al. (1992) and Pitt et al. (1996). In the four-term model, total chewing time (min/d) contributed to a lower proportion of the explained variation (r^2 = 0.17) in mean ruminal pH compared to what happened when it was expressed in min/kg OM. There are few studies linking ruminal pH and chewing activity but not significant relationships were observed in beef cattle fed high-concentrate diets by Krause et al. (1998). The significant increase in mean ruminal pH with BICARB level may explain its action as a chemical buffer in the rumen (Kohn and Dunlap, 1998). Nonetheless, this positive BICARB effect on mean ruminal pH could be reduced as the daily average meal size increases with increasing buffer, at least numerically. It should be noted that this effect of daily average meal size on ruminal pH could be a consequence of the high relative importance of the first after-feeding meals on their daily average with increasing buffer level because the significant treatment by time of the day interaction observed for meal size as described in Chapter II. The rate and extent of SCFA production increases with meal size without a timely compensation through their absorption, inflow of saliva, and passage.

Ruminal pH in this experiment was measured at 0, 2, 4, 8, 12, 16, and 24 h after-feeding. We carried out an analysis in order to measure which variables affected ruminal pH variation between 2 consecutive sampling times. Therefore, we calculated the ruminal pH change from 0 (*b1*) to 2 (*b2*), 2 to 4, 4 to 8, 8 to 12, 12 to 16, and 16 to 24 h after-feeding as *pH change = b2 – b1*, which was used as the dependent variable. This calculation resulted in negative values when there was a decline in ruminal pH between 2 consecutive sampling points but it was positive when there was an increase. The amount of water drunk (L), the feed intake (kg), and the time spent ruminating (h) were calculated for those intervals of time. These 3 variables were considered in the analysis as continuous fixed effects but also the level of sodium bicarbonate (%DM). The model was completed with the repeated measure of time interval as a fixed categorical effect subjected to heifer by period within treatment, with a heterogeneous compound symmetry covariance structure. All two-way interactions between the continuous variables and time interval (categorical) were also included. These interactions indicated that the regression coefficients differed among time intervals for that continuous variable. Then, the level of significance from zero of the regression coefficients at each time interval was tested. The only variable that showed a significant main effect (independently of time interval) was BICARB level ($P < 0.001$) but the remaining variables did not ($P > 0.10$). In addition, all interactions between time interval and the continuous variables were significant ($P < 0.05$) indicating that the regression coefficients differed among sampling times (Table 1).

Table 1. Regression coefficients (***b***) and their standard errors (**SE**) of variables affecting ruminal pH change between time intervals after-feeding

Time interval	BICARB, %		Water intake, L		Feed intake, kg		Ruminating, h	
	b	± SE	*b*	± SE	*b*	± SE	*b*	± SE
0 – 2[1]	-0.006	0.051	0.108 [z]	0.029	-0.383 [z]	0.088	-0.980	0.648
2 – 4	0.033	0.039	-0.055	0.053	-0.432 [x]	0.201	0.040	0.326
4 – 8	-0.083	0.108	0.037	0.127	-0.362	0.866	0.115	0.422
8 – 12	-0.117 [w]	0.066	0.025	0.060	-0.535 [x]	0.218	0.212	0.477
12 – 16	0.599 [z]	0.137	0.128 [y]	0.036	1.645 [y]	0.491	0.946 [z]	0.241
16 – 24	0.439 [z]	0.111	-0.016	0.073	1.124 [x]	0.508	0.772 [y]	0.236
P [2]	< 0.001		0.07		0.004		0.05	

The regression coefficient is different than zero at: [w] $P < 0.10$, [x] $P < 0.05$, [y] $P < 0.01$, [z] $P < 0.001$.

[1] Ruminal pH change was calculated as pH at 2 h minus pH at 0 h post-feeding.

[2] Two-way interaction with time interval.

It can be concluded from Table 1 that all variables tested for their effect on ruminal pH change have different relative importance and change their trends throughout the day. In addition, these patterns were affected by buffer level during some intervals of the day as reported in Chapter II and III. Increasing the proportion of BICARB in the concentrate increased positively the ruminal pH change regardless of time interval. However, this seem to be the result of the great positive effect of BICARB on ruminal pH change during the nighttimes (from 12 to 24 h) but the reasons for these results are unknown. The negative tendency of increasing BICARB level on ruminal pH change from 8 to 12 h is also unknown. Perhaps, increasing BICARB level in the concentrate resulted in increase osmolality of rumen fluid and blood during this time, which are known to reduce salivary flow (Warner and Stacy, 1977; Carr and Titchen, 1978) and rumination (Welch, 1982). The change in ruminal pH was affected positively by the amount of water drunk in the time interval from 0 to 2 and from 12 to 16 h post-feeding. However, ruminal pH dropped between these times and, therefore, increasing water consumption attenuated the pH fall. Increasing water consumption may increase ruminal turnover rate resulting in ruminal wash out of feed particles, phosphate, ammonium, and SCFA which contribute to the removal of H^+ and substrates (Allen, 1997). Water consumption or time spent drinking increased with buffer level during these time intervals, as

reported within chapter II and III. Furthermore, this observation agrees with the theory of buffer salts mode of action on ruminal pH proposed by Russell and Chow (1993) whereby buffers increase water intake, ruminal liquid passage rate, and flow of undegraded starch from the rumen, which leads to decreased VFA production. Several authors have stated that ruminal pH decline is related to intake but no quantification or statistical analysis were reported, e.g. Allen (1997), Cooper et al. (1999), and Erickson et al. (2003). Our data indicate that 1 kg of DM eaten resulted in a drop of 0.38 pH units from 0 to 2 h but the negative effect of intake was greater in the intervals from 2 to 4 (0.43) and from 8 to 12 h (0.53) in heifers weighing from 260 to 360 kg BW. Increasing DMI, rumination time, and BICARB level resulted in a positive effect on ruminal pH change during the nighttimes but DMI and rumination were reduced by increasing BICARB level. Therefore, confounding effects may be present in these intervals. Finally, ruminating time had a positive effect on ruminal pH change only during the nighttimes when the most intense ruminating activity occurs. This highlights the importance of night recovery of ruminal conditions in order to start the next feeding cycle. In conclusion, the ruminal pH drop from 0 to 2 h post-feeding was attenuated by water consumption but it was accelerated by the amount of feed eaten. Bicarbonate concentration decreases as ruminal fluid pH decreases and is almost absent below 5.5 (Counotte et al., 1979). Thus, heifers start the feeding cycle with plenty of bicarbonate reserves in the rumen fluid but these reserves are consumed as SCFA are produced from the fermentation of food ingested. From 12 to 16 h, all independent variables had a positive effect on ruminal pH. However, these results should be taken with caution because water consumption, feed intake, and ruminating time decreased as BICARB level increased as reported in Chapter II and III.

1.2. Effect social behavior on feeding behavior and rumen function. In the second experiment presented in Chapter V, few hypotheses can be done because individual feeding behavior was not measured. However, some observations in concentrate feeding time were done in the discussion of that chapter. Indeed, both eating rate and the within-pen SD of concentrate feeding time increased with competition level, suggesting alterations in feeding behavior. The number of displacements from the concentrate feeders shows that social behavior is a factor affecting feeding behavior, which may finally affect ruminal function. Many metabolism experiments published by our group have indicated that beef cattle fed high-concentrate diets show ruminal pH with daily average values over 6.1 and daily lowest values around 5.6 (Rotger et al., 2005, 2006, 2006c; Devant et al., 2000, 2001). However, these animals were in non-competitive social environments and, therefore, they may be better able to self-regulate their ruminal function. Nevertheless, some metabolism experiments conducted within our group often result in nadir ruminal fluid pH below 5 while others

do not under similar non-competitive conditions and diets. Therefore, differences in ruminal fermentation can not only be explained only by the lack of social competition. Some experiments under similar feeding management in feedlot conditions in Barcelona (Mach et al., 2006) have consistently resulted in greater ruminal pH (pH > 6 with sampling at 4 h post-feeding) than those observed in the present thesis. Differences among experiments may be related to differences in feeding behavior as a result of differences in social and physical environment, and feeding management. As reported within Chapter V, the number of heifers with ruminal pH below 5.6 increased from 53.5 to 73.6% as competition increased. Regardless of treatment, the proportion of samples with ruminal fluid pH below 5.2 was 18.1% whereas those with ruminal pH below 5.0 were 2.43% for the feedlot experiment. Miles et al. (1998) reported that more than 80% of cattle dying from digestive disorders had ruminal pH below 5.4 whereas those dying because of non-digestive causes had ruminal fluid pH over 6. In addition, ruminal pH and the proportion of heifers with ruminal pH below 5.6 increased from period 1 to 5 but it is not known whether this is a consequence of animals' age or season of the year because these effects are confounded. During the winter months the natural cattle feeding period, feed delivery time, and personnel had the same schedules because of photoperiod. However, those factors were out of sync in the summer because feed delivery and personnel occur after the cattle have passed the major morning eating period. In conclusion, social competition, feeding management, particular characteristics of the animals, and experimental setups may influence feeding behavior and, consequently, ruminal pH.

2. Feed intake patterns

The feeding patterns of cattle follow a crepuscular rhythm under all seasons and most production systems, either under extensive (Ruckebusch and Bueno, 1978; Stricklin et al., 1976; Linnane et al., 2001) or intensive conditions (Ray and Roubicek, 1971; Hoffman and Self, 1973; Bond et al., 1978; Schwartzkopf-Genswein et al., 2003b). The daily feeding pattern is characterized by two major eating periods throughout the day: the first peak is at dawn and the other peak is at dusk (Schwartzkopf-Genswein et al., 2003b). The sunset feeding period is usually greater than the sunrise period in feedlot cattle (Schwartzkopf-Genswein et al., 2003b), loose-housed dairy cows (DeVries et al., 2003c), and cows in semi-wild conditions (Linnane et al., 2001). Very little feeding activity is observed during the night but under short photoperiods (Canada and Ireland winters) the animals have to compensate by spending more time eating during the night (Linnane et al., 2001; Gonyou and Stricklin, 1984). Unexpectedly, this small night peak was observed during the first week after arrival at the feedlot reported in Chapter IV despite the fact that photoperiod was not very short (October) but was less evident in the subsequent sampling weeks when photoperiods

were even shorter (Chapters IV and V). However, we don't know if this observation is part of the adjustment to the new environment, an alteration due to the stressful situation of calves after arrival, or age of the animals.

Nielsen (1999) concluded that animals have a preferred daily intake, a preferred eating rate, a preferred daily feeding pattern, and they also prefer to eat together with conspecifics (allelomicry). Therefore, animals will try to defend these characteristics but they will solve this equation by changing one or more of those characteristics according to the external and internal environments. In all three trials reported within this thesis intake was reduced by either increasing the bicarbonate proportion (Chapter II) of the concentrate or the competition in the concentrate feeders (Chapter IV and V). An attempt was done in order to identify the times of the day in which these reductions occurred and, therefore, the possible factors responsible of it. The unexpected result in all cases was that animals did not overcome the low intake during the day by changing the daily feeding patterns towards other times of the day when the negative factors were not supposed to be present. In Exp. 1, the addition of sodium bicarbonate significantly reduced feed intake, changed its daily pattern and all feeding characteristics throughout the day. The reduction of daily feed intake was observed in the middle of feeding cycle (from 8 to 14 h post-feeding) when diets containing sodium bicarbonate might have resulted in unpleasant or unphysiological changes as discussed in Chapter II. However, a modification of daily feeding patterns did not occur towards night or early morning feeding to compensate intake when physiological conditions are expected to be within normal ranges or at comfortable levels. Time spent feeding obtained from the videos may be assumed to follow a similar pattern than feed intake in Exp. 2 (Chapter IV and V) although not in quantity of feed consumed. Differences in eating rate should distort these patterns and greater eating rate with competition are expected to be more noticeable at peak feeding times because the reduction in concentrate feeding time was observed at all major periods of feeding (sunrise, sunset, and midnight). Again, concentrate DMI was reduced by fewer number of concentrate feeding places per pen but animals did not compensate the restriction due to feeding space through, e.g., more nighttime feeding when less competition was observed.

In conclusion, the animals in our studies were reluctant to change the daily feeding patterns and several reasons are possible. For instance, the inherent photoperiodicity and allelomimetic behavior of cattle are difficult to change since even restricting feeding to 85% of ad libitum and delivering feed at late evening did not greatly affect feeding patterns (Schwartzkopf-Genswein et al., 2003). The theory of maximization of feed intake predicts that any reduction in feed intake is the result of factors limiting feed intake. However, if this would have been the case then animals may have, for instance, fed at other times of the day in order to maximize intake. Therefore, other

theories may be more appropriate to explain the reduction in DMI such as negative reinforces or feedback signals coming from the food eaten, minimization of discomfort, or optimization mechanisms. However, several mechanisms are also possible in this scenario. For instance, greater intake during the nighttime may either cause discomfort because physiological conditions come back to uncomfortable levels, animals have to attend the feeder without a companion animal, nighttime feeding is less comfortable than daylight feeding, or simply because animals use the night to restore physiological conditions. In addition, if increasing BICARB level requires the excretion of greater amounts of Na through urine or compensation mechanisms of rumen or blood osmolality then the optimum level of feed intake would be decreased regardless of the internal or external environment during the night.

Other important issues to discuss are the different responses in eating rate and feeding patterns observed between both experiments. Animals changed eating rate accordingly to prevailing internal or external conditions in each experiment. In the first experiment, eating rate decreased but mainly at the middle of the feeding cycle when intake was also reduced (Chapter III). This suggests that physiological conditions reduced appetite or hunger as BICARB level increased, in agreement with the reduction of meal frequency. In Exp. 2 presented in Chapter IV, time spent eating concentrate decreased with more competition which resulted in greater eating rate at wk 3 and after but not at wk 1. Increasing competition reduced feeding time during the major eating periods in all weeks. In conclusion, the increased eating rate may suggest that hunger was not affected at the group level by increasing competition and it was a compensating mechanism to overcome the physical restriction, although this should be confirmed with further studies looking at individual feed intake.

Feeding patterns of the first metabolism experiment differed from those of the second feedlot experiment. The difference in daily feeding patterns between metabolism experiments (Fulton et al., 1979; Robles et al., 2007) and feedlot experiments (Schwartzkopf-Genswein et al., 2003a, 2003b) was reported in the literature. The metabolism experiment was characterized by a large amount of time spent eating during the first 2 h after feed delivery whereas the feedlot experiment is characterized by 2 major periods of eating, with the sunset period being often greater than the sunrise eating period. Although feed was offered ad libitum in both situations differences in eating patterns were large. It is likely that management of the animals has an influence on eating patterns and, therefore, it might influence ruminal fermentation patterns. Arrival of farm staff, noises, cleaning the feeders, and delivery of fresh food may increase the motivation for feeding. Furthermore, eating patterns in Exp. 1 were not greatly affected by seasons (throughout the experimental periods, data not shown) and animals have not eaten much feed until fresh feed was delivered, as evidenced from figures presented within Chapter III. As can be seen in the figures

presented within Chapter IV and V, the response to feed delivery was similar than Exp. 1 but animals started to eat earlier, and had a greater period at sunset which changed throughout seasons (data not shown). Therefore, eating patterns should be considered when comparing ruminal fermentation data.

3. Feeding behavior, ruminal fermentation, and daily intake

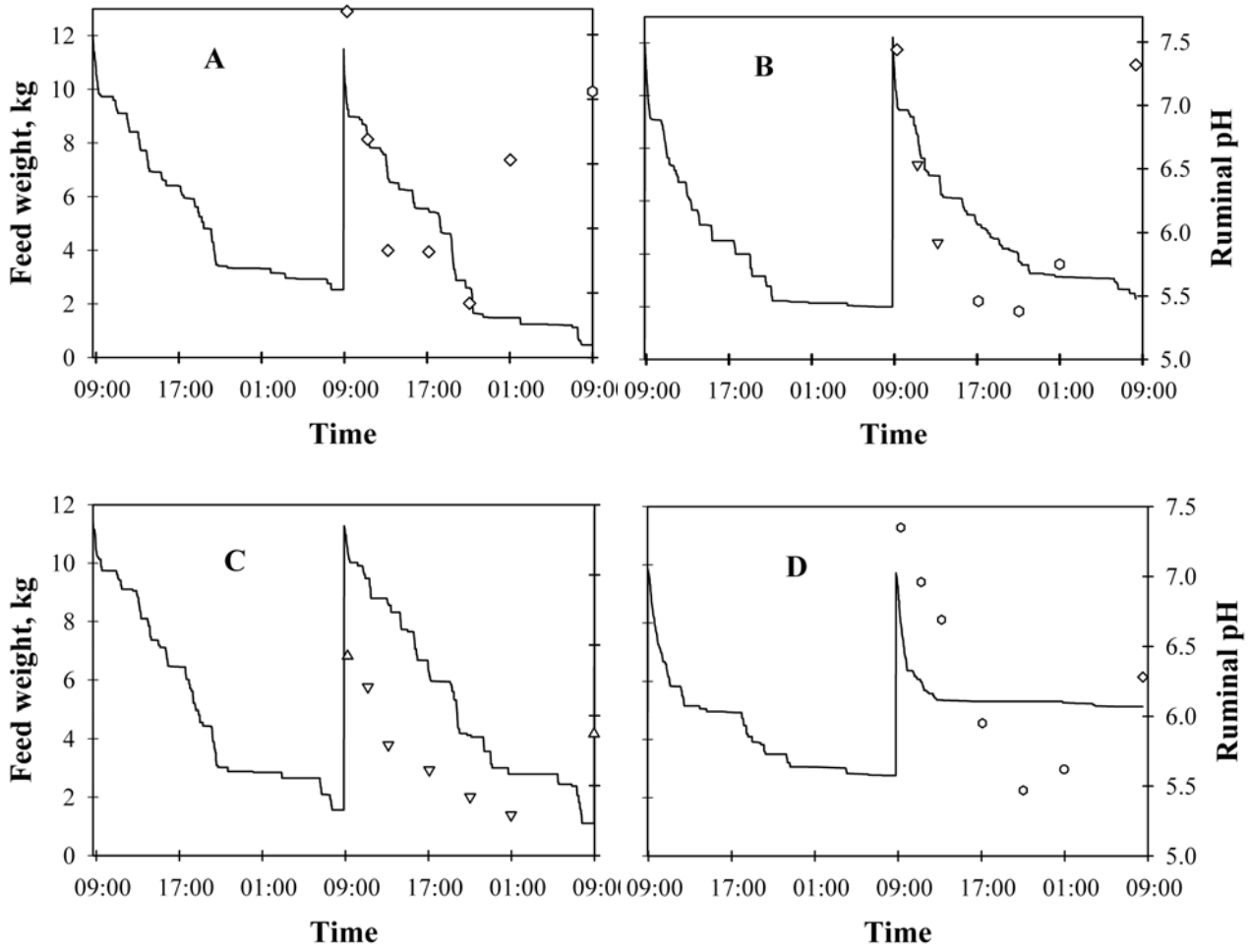
Daily DMI tended to decrease linearly with sodium bicarbonate proportion in trials presented in Chapters II and III. We discussed in those chapters the putative causes of this decrease and focused the discussion on ruminal osmolality although it was not measured. This conclusion was a consequence of the lack of effect of BICARB level on daily average ruminal pH or VFA, neither during the times of the day when intake was depressed (evening). It was concluded that different factors may predominate and contribute to the control of meal size and intake during any one 24 h period. We also emphasized the possible consequences of the large meals occurring at the beginning of the feeding cycle on the subsequent evolution of feeding patterns throughout the day. However, factors controlling the end or size of the first and largest meal did not correlated with daily intake, as suggested by Gill and Romney (1994). Based on the behavior by consequences or learning from feedbacks coming from the ingestion of food, animals are expected to learn from negative feedbacks or reinforcements and behave in consequence. In this sense, daily feeding patterns should reflect the behavior adopted and of its consequences. This is why variable feed intake is expected in acidosis situations (Schwartzkopf-Genswein et al., 2003) but Owens et al. (1998) suggested that this effect may be result of the high osmolality due to the accumulation of fermentation products. However, the accumulation of fermentation products is not the only factor responsible for the increased ruminal fluid osmolality because minerals in feed also contribute to this post-prandial increase. We present Figure 1 in order to explain the feeding behavior adopted under different buffer levels together with the ruminal pH data. Statistical analyses of these patterns were presented in Chapters II and III. Figure 1 shows the DMI pattern of one heifer when receiving the 1.25% (A) and the 5% (B) BICARB diet, and another heifer when receiving the 0% (D) and the 5% (D) BICARB diet in a different period. The heifer receiving the 1.25% BICARB (A) had 13 and 14 meals/d, 0.62 and 0.62 g DM/meal, and consumed 9.6 and 10.2 kg DM/d during d 1 and 2, respectively (Figure 1A). However, she had 10 and 8 meals/d, 1.01 and 0.76 g DM/meal, and consumed 9.6 and 9.7 kg DM/d during d 1 and 2, when receiving the 5% BICARB (Figure 1B). As can be seen in the figure, the first meal exceeded 2.0 kg DM but the second meal was smaller than 1.0 kg DM for both days when she received the 1.25% BICARB. Nevertheless, both first and second meals, which occurred within the 4 h post-feeding in both days, were larger than 2.0 kg

DM/d when the same heifer received the 5% BICARB diet. Therefore, the post-prandial ruminal pH drop was buffered with BICARB but it felt to the same nadir despite buffer level in the concentrate. It can also be observed how meals occurring after 1700 have smaller size and much lower eating rate when receiving the 5% BICARB (Figure 1B). These changes in feeding patterns are suggested to be due to prevailing physiological conditions at different times of the day. Similar conclusions may be drawn from the heifer that received the 0% (C) compared to 5% (D) BICARB diet. However, we want to emphasize the changes in feeding behavior that may lead to variable day-to-day DMI, similar to the repetitive cycles described under acidotic conditions (Schwartzkopft-Genswein et al., 2003; Bevans et al., 2005). However, ruminal pH does not seem to explain this variation among days because ruminal pH was much lower when fed the 0 (C) compared to the 5% (D) BICARB diet as suggested by the figures. No significant differences in eating patterns were found between 0 and 1.25% BICARB but water consumption during the 4 h post-feeding was greater in the 0 compared to the 1.25% BICARB, as reported in Chapter II.

We have analyzed the concentrate DMI variance during 5 consecutive days within heifer×period as affected by BICARB level to see the day-to-day variation. Concentrate DMI variance tended to increase linearly (P = 0.09) with buffer level, values being 0.39, 0.89, 0.53, and 2.02 ± 0.75 $(kg/d)^2$ for 0, 1.25, 2.5, and 5% BICARB, respectively (data not shown). We have taken a look at feeding patterns to check the possible reasons of this variation when feeding the 5% BICARB diet which are shown in Figure 1D. Feeding pattern on d 1 was similar to that described previously with a large meal after-feeding but, in addition, another relatively large meal occurred at 1800. During d 2, one large meal lasting for 235 min and a size of 3.88 kg of DM led to this heifer to stop eating for the rest of the day although she was nibbling on feed during the nighttime. This suggests that the feeding pattern of d 2 might has been a consequence of the large morning meal but also an attempt to avoid the consequences of the previous' day behavior. Therefore, she stopped eating after the first large meal and avoided the negative consequences of the evening meals. However, learning from this risky food may require a very fine 'tuning' but, even after learning to behave, the 'equilibrium' between daily intake and behavior is perhaps weak and these repetitive cycles may still occur. Another surprising result from Figure 1D is that the large unique meal in the morning led to a gradual decrease of ruminal pH to reach the nadir of 5.47 at 12 h post-feeding coincident with the greatest total VFA concentration (186 mM). This is too high a concentration of VFA considering that, for example, other heifer fed the 0% BICARB diet in the same period had a ruminal pH of around 5.5 since 4 to 12 h post-feeding and a cumulative DMI of 9 kg at 12 h but VFA remained below 180 mM. This large delay in the ruminal pH nadir and VFA peak of the first

meal may probably be due to the osmolality effects of high BICARB diets such as decrease in rumination (Welch, 1982) and in VFA absorption from the rumen (Carter and Grovum, 1990).

Figure 1. Scales feed weight patterns (continuous line) during 2 consecutive days and ruminal fluid pH (scatters) sampled at 0830, 1030, 1230, 1630, 2030, 2430, and 0830, for one heifer fed the 1.25 (**A**) and the 5% (**B**) BICARB, and another heifer fed the 0 (**C**) and the 5% (**D**) BICARB diet.



4. Low or high proportion of forage in the total ration ingested?

In dairy cows, it is common to 'hear' about the negative effects of feeding rations with insufficient fiber coming from forage. A lot of research has been done to assess which fibber is able to maintain chewing time, salivation, ruminal pH, microbial yield, and milk fat concentration (Allen, 1997). This led to more or less reliable recommendations of 'physically effective fibber' levels and its function in dairy cows in order to maintain ruminal pH and milk fat (Stone, 2004; Zebeli et al., 2006). Research in beef cattle has focused on the effects of fibber on feed conversion efficiency, liver abscesses, digestive upsets, and variability in intake and performance (Britton and

Stock, 1989; Nagaraja and Titegemeyer, 2007). In contrast to dairy rations, fibber level recommendations are more difficult to establish because ruminal pH does not seem to affect growth, or it's more difficult to measure accurately. Beef cattle fed all-concentrate diets seem to have normal intake and performance, despite the fact that ruminal fluid pH is much lower (Shain et al., 1999; Faleiro et al., 2007). However, factors other than performance should be considered. For instance, high- or all- concentrate diets may increase stereotypy behaviors such as tong rolling (Redbo et al., 1996; Redbo et al., 1997; Faleiro et al., 2007). These 'abnormal', repetitive, behaviors are seen as a result of the lack of forage to stimulate the innate high motivation for chewing in ruminants. In addition, the lack of roughages decreases ruminal pH, and seem to increase liver abscesses and ruminal epithelium damage although performance might not be affected (Harvey et al., 1968). Therefore, low forage rations may violate the third and fourth freedom of animal welfare which considers absence of pain, injury and disease, and freedom to express normal behavior, respectively (FAWC, 1997).

Provenza and Villalba (2006) described the theory of 'behavior by consequences' considering that animals continuously monitor the internal and external milieu changes. These changes are linked with behavior in order to integrate their consequences with basic metabolic processes. The consequences of behaviors may be pleasure and pain, drives and motivation, emotions and feelings. The selection of food based on learned associations or 'behavior by consequences' predicts that animals will eat as much forage as needed in order to maintain, for instance, ruminal pH within comfortable limits (Cooper et al., 1996; Kyriazakis, 1999; Forbes, 1999; Provenza and Villalba, 2006). From the welfare viewpoint, the strength of the motivation theory predicts that animals will eat as much forage as required to satisfy the essential behavior of chewing and well-being (Redbo and Nordblad, 1997; Gonyou, 1994). Some feeding managements of beef cattle in Spain, as was used in the experiments of the present thesis, allow ad libitum consumption of straw and concentrate. Therefore, if the ingestion of 'too much' concentrate results in an acidosis insult (deviation from homeostasis) or in unsatisfied behavioral need of chewing, then animals are expected to correct this situation by increasing the amount of forage eaten until this situation is repaired, discomfort is minimized or a balance between costs and benefits is optimized. Both experiments in this thesis showed that the proportion of straw selected by growing heifers is maintained within narrow limits close to 12% of the total intake, on average. Therefore, it may be suggested that eating this proportion of straw will result in a given ruminal function and well-being close to the optimum because it is the accepted by the animal, despite the fact that nadir rumen fluid pH does usually falls below 5.5 and total chewing time is as low as 400 min/d. Nevertheless, it should be pointed out that this is the situation with straw as fed in the experiments of this thesis. It

is well known that the ad libitum intake of forages and straw depends of the chemical and physical properties of the forage and the characteristics of the companion concentrate (e.g., Cooper et al., 1996).

However, there was a diet- and a social-induced situation reported in chapters II and IV, respectively, which led to increases in the forage proportion selected by our cattle. Unfortunately, ruminal osmolality was not measured in Exp. 1 but it is hypothesized that it might have been increased by addition of BICARB since other ruminal conditions were not affected. Therefore, animals may have attempted to avoid high osmolality (negative feedback) through a decrease of concentrates high in sodium bicarbonate, which would cause a further increase of osmolality. In opposition, straw may have been perceived as a 'safer' ingredient than concentrates and thus, its intake increased. In chapter IV, increasing competition for concentrate during the adaptation period led to greater aggressions and lower concentrate intake. Animals responded to this situation by increasing the intake of straw where less competition was observed. In conclusion, animals seem to be able to correct deviations from 'normality' or 'buffer' the negative effects of consuming one of the foods available through the intake ratio between concentrate and straw. Therefore, monitoring the intake or the ratio of those feed components may be a good approach to understand which or when those mechanisms are being used as a response to different diets and managements. For instance, Keunen et al. (2002) observed that the preference for long hay in dairy cows increased when SARA was induced in an attempt to attenuate it. On-farm monitoring of this intake ratio may also aid to detect errors in the design of facilities, diet formulation, or palatability. The proportion of straw selected by heifers in the second experiment increased gradually from 8.5% in period 1 to 14.2% in period 6. This may be the result of either a greater need for long forage as the rumen develops and the animal becomes a 'true' ruminant, or to a (long) learning process in order to maintain greater ruminal pH values. In fact, ruminal pH also increased throughout the second experiment.

5. Is ruminal acidosis a welfare problem?

Rations with high proportion of concentrate result in low ruminal pH, which has been linked with laminitis in dairy cows (Manson and Leaver, 1988; Nocek, 1997). However, acidosis-induced laminitis is not the only locomotory problem causing lameness (Bergsten, 2003). Galindo et al. (2000) observed that cows with severe lameness had lower dominance rank and spent more time standing and lesser resting than non-lame cows. Based on data presented within Chapter V, it may be added that low ranking animals might suffer more acidosis when feeding space is reduced in beef cattle fed high-concentrate rations. Little information on lameness in growing cattle is available but

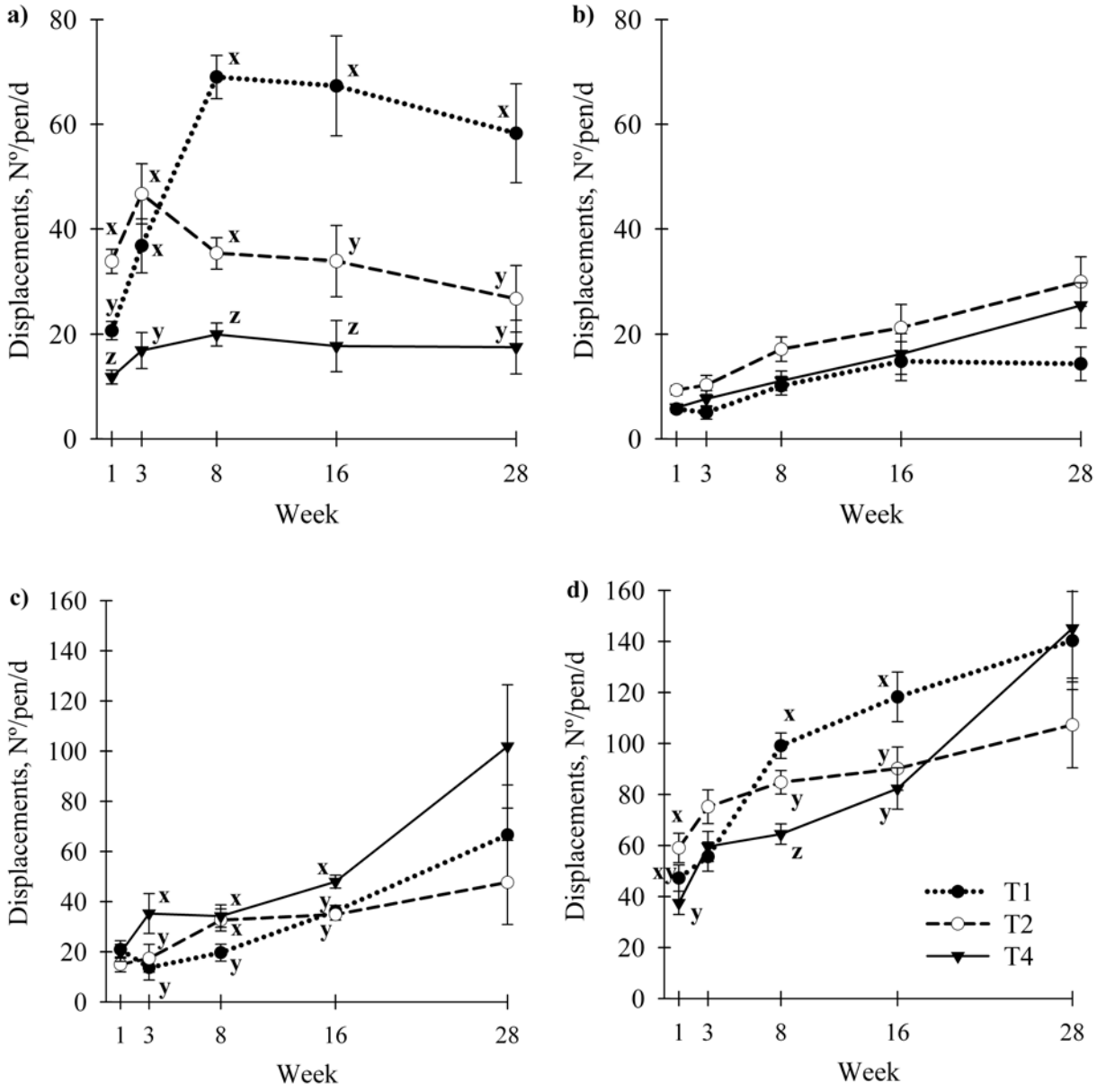
the typical signs link ruminal acidosis in feedlot cattle with locomotory problems, stiffness, and back-arched postures (Brent, 1976). Greenough et al. (1990) observed an increase incidence of sole and heel hemorrhages, foot rot, and changes in hoof structure with increasing energy-density of rations in calves and yearlings. Feedlot cattle housed in concrete floor and fed high-concentrate diets had lower foot balance and thicker sole horn than cattle on pasture (Becvar et al., 2006). Laminitis is considered a major cause of decreased welfare in modern farming due to the pain they provoke (Whay et al., 1998; FAWC, 1997). In addition, ruminal acidosis provokes the immune response through LPS or ruminal tissue damage in beef cattle (Ghozo et al., 2006; Nagaraja and Titgemeyer, 2007) violating the third freedom" of animal welfare. Ruminal acidosis may also cause rumenitis (infection, inflammation, and damage of rumen tissue), ruminal and abomasal ulcers, and liver abscesses (Slyter, 1976; Nagaraja and Chengappa, 1998). Although Mills and Jenny (1979) found that heifers fed high-concentrate diets (70% DM) had greater blood cortisol levels than those fed low-concentrate diets (20% DM) this may just be a metabolic adaptation to maintain high gluconeogenic rates in the high concentrate diets. However, more studies are needed .In addition, an important outcome from this thesis is that acidosis incidence may be increased because of inadequate design of the facilities and, therefore, may reflect welfare problems. Reduced feeding space increases competition for feed and aggressions, which may be finally linked to social stress for some individual animals within a group. Then ruminal acidosis might be another detrimental factor for animal welfare in modern farming. Nevertheless, the balance between finance and welfare is difficult to set. High-concentrate rations are necessary to sustain high levels of production and money return. On the other hand, cattle have been designed by evolution to digest forages and not concentrate, and then they did not develop a digestive system to deal with the feeding strategies used nowadays.

6. Social behavior

The number of aggressions carried out among calves from each resource container since the first week after arrival at the feedlot throughout the fattening period (Exp. 2, Chapters IV and V) were analyzed to study the treatment × week interactions on the number of displacements from the concentrate feeders ($P < 0.0001$; Figure 2a), straw feeders ($P = 0.10$; Figure 2b), water bowls ($P = 0.60$; Figure 2c), and total sum ($P = 0.01$; Figure 2d). The number of displacements from the concentrate feeders was lowest in pens with 4 feeding places per pen throughout the experiment and aggressions remained stable over time within this treatment ($P > 0.05$). Pens with 2 feeding places per pen showed an increase in the number of displacements from the concentrate feeders from wk 1 to 3 and tended to decrease thereafter. In contrast, the number of displacements at 1 concentrate

feeding place per pen increased from wk 1 to 8 and then remained unchanged until wk 28. These results indicate that within T4 (lowest competition) animals reached a relatively stable social hierarchy very quickly with no need to increase aggressions. The number of aggressions reached the maximum at wk 3 for T2 but at wk 8 after group formation for T1 (Figure 2a). Therefore, increasing competition for concentrate increased aggressions among pen-mates and they took longer time to reach the maximum level. However, physical aggressions should not be needed after stabilization of the social hierarchy (Kondo and Hurnick, 1990). Rather, the maintenance of a social order in our study required high and sustained levels of aggressions likely as a mean to maintain priority of access to concentrate feeders for some individuals within a pen. Broom and Kirkden (2004) considered this phenomenon ('heightened aggressions') as a behavioral pathology indicating poor welfare rather than a coping mechanism. Some possible causes for this result include the inability to form a stable social group or to resolve conflicts, the frustration of the motivation to feed, or the continuous violation of the individual space. Displacements from the water bowls increased over time in the same fashion for all treatments (Figure 2b). Displacements from the straw feeders increased over time for all treatments but the increase was greatest at 0.20 m/animal (T4) than at 0.34 m/animal (T2 and T1) which agreed with the increasing forage proportion selected by calves and with the increasing body size with age. Displacements from the straw feeders in T4 were four-fold greater than displacements from the concentrate feeders at wk 28, while the opposite was true for displacements from the concentrate feeders over the straw feeders in T1. This indicates that, rather than reaching a stable social hierarchy at the straw feeders, competition was becoming greater with heifers' age and contributing to unstable social environment. The greater corticosterone of dominant animals reported in Chapter V may support an unstable social environment (Creel, 2001). This is contrary to previous observations in calves (Kondo et al., 1984), steers and bulls (Tennessen et al., 1985), and dairy cows (Kondo and Hurnick, 1990). All of these studies measured agonistic interactions among group members (such as headbunt, bunt, butt, avoiding, pushing, threat, and fighting) regardless of the location or the resource competing for. It is likely that most of the aggressions recorded in those studies were not carried out to establish or maintain priority of access to resources and, therefore, they may reflect other aspects of social relationships. In fact, fights or encounters under grazing conditions are considered as 'spare' fights or play-fighting (Reinhardt and Reinhardt, 1982). In contrast, we have recorded aggressions in the way of effective displacements from resource containers only (feed and water), which may better reflect the establishment or maintenance of priority of access and aggression.

Figure 2. The number of displacements from the concentrate feeders (a), the water bowls (b), the straw feeders (c), and the total sum (d) among heifers housed in pens with 1 (T1), 2 (T2), and 4 (T4) concentrate feeding places per pen, and 0.34 (T1 and T2) or 0.20 (T4) m/animal.



Chapter VII

Final conclusions

Sodium bicarbonate, feed intake, and rumen function

Increasing the sodium bicarbonate proportion up to 5% of the concentrate dry matter:

- Decreased linearly concentrate DMI but increased straw DMI, which may negatively affect performance. It increased linearly daily water consumption only when expressed in liters per unit of DMI.
- Did not affect the daily average or minimum ruminal fluid pH. However, it alleviated the post-prandial drop of ruminal pH, whereas the daily time in which ruminal pH remained under 5.8 tended to a quadratic response with high values when no buffer was added to the concentrate.
- Increased linearly the molar proportion of acetate, butyrate, and branched-chain VFA but whereas decreased propionate proportion.

Sodium bicarbonate, feeding, and chewing behaviors

Increasing the sodium bicarbonate proportion up to 5% of the concentrate dry matter:

- Did not affect daily feeding time or average meal size, whereas meal frequency and eating rate decreased linearly.
- Increased linearly the time spent eating only when expressed in min per kg of DM or OM intake, likely as a result of the increase in the proportion of forage eaten.
- Reduced DMI from 8 to 10 and from 12 to 14 h post-feeding as well as ruminating and total chewing time but ruminal pH or the profile of fermentation products did not explain these results. However, it increased linearly the size and length of the meals occurring within the 4 h post-feeding, perhaps due to greater ruminal pH or reduction of fermentation products (mainly propionate). These interactions indicate different control mechanisms acting throughout the day.

Competition for concentrate during the arrival period at the feedlot

Increasing the social competition for concentrate during the stressful time of adaptation to the feedlot:

- Reduced linearly DMI and ADG during the first week of adaptation but the response was quadratic during weeks 3 and 4, being lowest at 8 heifers per feeding place in the concentrate feeder. These effects on ADG may be explained by the greater effects of dominance as competition increased.
- Resulted in a quadratic response in the time spent eating concentrate with low values at 8 heifers per feeding place. Daily time spent eating straw, within-pen variability of time spent eating straw and concentrate, time spent standing, and number of aggressions increased linearly with competition.

- Blood NEFA levels indicated greater body fat turnover at 4 and 8 heifers per concentrate feeding place. The profile of white blood cells and fecal corticosterone concentration suggested that the welfare of calves was not affected by competition.

Competition for concentrate during the whole fattening period

Increasing the social competition from 2 to 8 heifers per concentrate feeder:

- Decreased linearly concentrate DMI but did not affect ADG, final BW or hot carcass weight.
- Decreased linearly the time spent eating concentrate and lying down, whereas increased concentrate eating rate, number of aggressions, and time spent standing.
- Did not affect the average fecal corticosterone concentration of each pen but responded quadratically within dominant animals (greatest values at the greatest competition for concentrate).
- Reduced ruminal fluid pH during periods 1 and 2 and tended to increase linearly the proportion of heifers with ruminal pH below 5.6, as well as ruminal butyrate and lactate concentrations, and blood haptoglobin. The proportion of abscessed livers was greatest at 8 heifers per feeder. Data indicate that subacute ruminal acidosis incidence may increase with competition for concentrate.

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and

general discussion of results

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