SHORT-TERM FEED RESTRICTION IN CATTLE: MITIGATING NEGATIVE EFFECTS BY ALTERING THE FORAGE-TO-CONCENTRATE RATIO OF THE DIET

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By
Rodrigo Iván Albornoz
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ABSTRACT

The objective of this thesis was to evaluate the effects of altering the forage-to-concentrate ratio (**F:C**) of the diets fed prior-to and during a period of severe short-term feed restriction (**FR**) and the dietary F:C following FR on ruminal fermentation and short-chain fatty acid (**SCFA**) absorption. Twenty ruminally cannulated Angus × Hereford heifers were fed ad libitum either a high-forage diet (**HF**) with a F:C of 92:8 or a moderate-forage diet (**MF**) with a F:C of 60:40 during a 5-d baseline period (**BASE**), followed by 5-d of FR at 25% of their ad libitum intake (collectively represented as **PRE**). After exposure to FR, heifers were provided feed for ad libitum intake and the recovery was monitored for 3 consecutive weeks (**REC1**, **REC2** and **REC3;** collectively denoted as **POST**), with one half of the HF and MF heifers remaining on the same diet, and the other half switched to the alternative diet. Dry matter intake (**DMI**) was measured daily and ruminal pH was recorded every 2 min. Ruminal fluid was collected on d 3 of BASE and FR, and on d 5 of each REC week. Ruminal SCFA absorption was assessed in vivo using the isolated washed reticulo-rumen technique on d 5 of BASE and FR and on d 7 of REC1 and REC3. Data were analyzed as a complete randomized block design to evaluate 1) the effects of FR (BASE vs. FR) when feeding a HF or a MF diet, and 2) the effects of the F:C of the diet fed PRE and the diet fed POST on the recovery from a period of short-term FR. With respect to the effect of the F:C of the diet fed PRE on the response to FR, diet × period interactions were detected for DMI ($P = 0.030$) and ruminal SCFA concentration ($P = 0.025$), respectively. The interactions were the result of higher DMI and numerically higher SCFA concentration for MF than HF during BASE, with a reduction observed for both during FR, although diet effects were no longer present during FR. Period effects (BASE vs. FR), but not diet effects ($P > 0.05$), were detected for mean ruminal pH ($P < 0.001$) and the total SCFA absorption rate (mmol/h; $P = 0.038$). During BASE, mean pH was lower (6.44 vs. 6.86) and the SCFA absorption rate was greater relative to FR (674.5 vs. 554.8 mmol/h). It can be concluded that FR had a negative impact on ruminal SCFA absorption, while altering the diet F:C does not mitigate these effects. With respect to the recovery response, PRE × POST and PRE × POST × period interactions were tested but were not significant, thus the interactions for the PRE × period and POST × period are emphasized and main effects of diet and period are presented when the interactions were not significant. Interactions (PRE × period) were detected for DMI

($P = 0.045$) and duration (amount of time pH < 5.5; $P = 0.003$). For heifers fed HF during PRE, DMI increased from REC1 to REC2, and from REC2 to REC3, while for heifers fed MF DMI did not differ among REC periods. Duration (amount of time pH < 5.5) was numerically higher during REC1 for heifers fed HF than MF PRE (191 vs. 98 min/d), with duration decreasing from REC1 to REC2 with no further change in REC3. Total ruminal SCFA concentration and absolute absorption rate were not influenced by the diet fed PRE or period ($P > 0.05$). There were POST × period interactions for DMI ($P = 0.033$) and duration ($P < 0.001$). Dry matter intake was maximized (10.1 vs. 8.4 kg/d) without a substantial increase in duration (14 vs. 275 min/d) during REC1 when heifers were fed HF relative to those fed MF during POST, without differences thereafter between treatments, but DMI increased from REC1 to REC2 and from REC2 to REC3. The duration that ruminal pH was < 5.5 decreased from REC1 to REC2, without differences between REC2 and REC3 (145, 29 and 46 min/d, respectively). Regardless of the diet fed POST and the week of REC, total ruminal SCFA concentration and absolute absorption were not affected ($P > 0.05$). It can be concluded that severe short-term FR had a negative impact on ruminal SCFA absorption regardless the diet fed prior-to and during FR. In addition, the dietary F:C affected the recovery response with a hastened recovery when heifers were fed a diet with a low F:C prior-to and during FR and a high F:C diet post FR, without any effects on SCFA absorption.

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Head of the Department of Animal and Poultry Science

University of Saskatchewan

Saskatoon, Saskatchewan, Canada S7N 5A8

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF ABBREVIATIONS

ADF	Acid detergent fiber
ADG	Average daily gain
BASE	Baseline
BHBA	β-hydroxy butyric acid
BW	Body weight
CP	Crude protein
d	Day
DM	Dry matter
DMI	Dry matter intake
DRA	Down-regulated in adenoma
EGF	Epidermal growth factor
F:C	Forage-to-concentrate ratio
FR	Feed restriction
GC	Gas chromatography
HF	High-forage (92% forage)
IGF-I	Insulin-like growth factor I
iNDF	Indigestible neutral detergent fiber
LPS	Lipopolysaccharide
MCT	Monocarboxylate transporter
MF	Moderate-forage (60% forage)
N	Nitrogen
NDF	Neutral detergent fiber
NEFA	Non-esterified fatty acids
NHE	Na^+/H^+ exchanger
OM	Organic matter
POST	Post feed restriction
PRE	Prior-to and during feed restriction
REC	Recovery
SCFA	Short-chain fatty acid
SD	Standard deviation

SEM	Standard error of the mean
THI	Temperature-humidity index
TMR	Total mixed ration
wk	Week
WRR	Temporarily isolated and washed reticulo-rumen technique

1.0. GENERAL INTRODUCTION

Beef and dairy cattle are exposed to periods of feed restriction (**FR**) that can be classified as predictable or unexpected events. In some cases, long-term mild FR can have a beneficial effect on the efficiency of cattle performance (Rossi et al., 2001; Schmidt et al., 2005). For example, for g-rowing calves, restricting feed to approximately 90% of ad libitum intake (Clark et al., 2007) or to achieve average daily gain (**ADG**) of 90 to 95% of ad libitum fed calves (Rossi et al., 2001; Schmidt et al., 2005) has a beneficial effect on the gain:feed ratio. In addition, mild FR has a positive effect on feed digestibility (Fiems et al., 2007) and reduces the day-to-day dry matter intake (**DMI**) variation that can be associated with and a causative factor for digestive disturbances such as ruminal acidosis (Schwartzkopf-Genswein et al., 2004). Moreover, mild FR can reduce feed costs and reduce nutrient excretion in growing dairy heifers (Hoffman et al., 2007; Zanton and Heinrichs, 2008). On the other hand, moderate to severe short-term FR can predispose ruminants to metabolic disorders (Cooper et al., 1999; DeVries et al., 2008) and a negative nutrient balance (Velez and Donkin, 2005) compromising animal welfare and productive performance. This indicates that the resulting impact arising from FR depends on the severity of the FR event, duration of the FR event, and the nutritional or physiological status of the animal itself.

Short-term periods of moderate FR occur on commercial dairy operations, with a negative impact on performance and likely compromises animal health. For example, a voluntary depression in DMI of about 30% normally occurs in dairy cows during the last three weeks (**wk**) pre-partum, with 89% of that decrease occurring during the last week (Hayirli et al., 2002). Velez and Donkin (2005) reported that FR at 50% of ad libitum intake for 5 d has a negative impact on milk production and energy balance when compared to cows fed ad libitum. The same type of FR with a 1 d duration induced ruminal acidosis when cows were re-fed 4 kg of concentrate for 1 h (DeVries et al., 2008). Penner et al. (2007) has also shown that following parturition heifers are at high risk for ruminal acidosis. It should be noted that for dairy cattle, parturition represents a period where voluntary reductions in feed intake occur. Induction of acidosis was also reported for feedlot cattle fed at 80% of the previous ad libitum intake and when the amount of feed offered varied by about 18% across days (Cooper et al., 1999).

Short-term severe FR as well as complete FR (feed deprivation), can be imposed inadvertently (e.g., weaning, transportation and marketing; Cole et al., 1988; Gibb et al., 2000) or voluntarily by cattle (e.g., heat stress, metabolic and digestive disorders; Huber, 1996; Brown et al., 2000; Hansen et al., 2003). The previously mentioned situations also have negative implications for ruminant performance and welfare (Krause and Oetzel, 2006; Stookey and Watts, 2007; Arthington et al, 2008), with effects including compromised rumen epithelial barrier function and a reduction in nutrient absorptive capacity across the rumen epithelium during and post FR (Gäbel et al., 1993; Gäbel and Aschenbach, 2002; Zhang, 2012). To the author's knowledge, only one study using cattle has evaluated the negative effects of various FR events that differed in severity, on rumen function during FR and during the re-alimentation period (Zhang, 2012). In Zhang et al. (2012), a reduction on short-chain fatty acid (**SCFA**) absorption was reported when beef heifers were exposed to 5 days (**d**) of FR to 25% of ad libitum intake. However, the effects of severe FR on rumen function under different dietary scenarios has not been addressed. Previous evidence indicates that rumen epithelial function and immune status can be manipulated by modifying the nutrient source and nutrient density of the diet (Sehested et al., 2000; Rivera et al., 2005; Yang et al., 2012), allowing the use of feeding management as a mitigation strategy to minimize the negative effects caused by a short-term severe FR. In fact, it has been shown that ruminal SCFA absorption increases when diet fermentability is increased; achieved by decreasing the F:C ratio of diets (Sehested et al., 2000; Bannink et al., 2008). These mitigation strategies could be implemented at different time points relative to the time of FR (prior to FR, during FR, and following FR) to improve performance and health (Hutcheson and Cole, 1986).

According to the evidence presented, a negative effect of FR on rumen epithelial function was hypothesized, with this response being attenuated when a lower F:C diet was fed, maximizing SCFA absorption and animal energy status. Thus, to evaluate the impact of FR under different dietary scenarios, an experiment was conducted with results reported in two chapters based on experimental objectives. The first objective was to evaluate the response of rumen epithelial function and rumen fermentation when heifers were exposed to FR (25% ad libitum intake for 5 d) while being fed diets that differed in forage-to-concentrate ratio (**F:C** of 92:8 and 60:40, respectively) prior-to and during FR. The second objective was to evaluate the

carry-over effects of the diet fed prior-to and during FR, and the effects of the diet fed post FR on DMI, rumen fermentation and absorptive function during re-alimentation.

2.0. LITERATURE REVIEW

2.1. Industry Relevance of Severe Short-Term Feed Restriction and Feed Depravation

Complete removal or rejection of feed is identified as feed deprivation (i.e., fasting), while severe FR consists of reduced access to feed or a voluntary reduction in DMI. Despite the importance of FR, even though FR is a predictable occurrence in some cases, there is a paucity of data describing strategies to mitigate the effect or hasten the recovery response following a period of FR. The following text provides evidence about the industry relevance of short periods of severe FR that occur in beef and dairy cattle production systems emphasizing the effect that FR has on animal health, performance, and welfare. As episodes of FR can occur with differing severities, Table 2.1 illustrates the effect of the severity of FR and characteristics associated with FR.

2.1.1. The Weaning Period as Evidence for Short-Term Feed Restriction in the Beef Industry

Weaning is a stressful process for both the dam and the offspring (Enriquez et al., 2011; Ungerfeld et al., 2011). In response to weaning, calves spend part of the day vocalizing and walking thereby decreasing the amount of time spent eating (Price et al., 2003; Price, 2008) and ruminating (Loberg et al., 2008). The behavioural changes associated with weaning also correspond to a reduction in ADG for 3 to 5 d post weaning (Stookey and Watts, 2007). Typically, weaning is implemented with other stressful procedures such as vaccination, dehorning, and castration (Pritchard and Mendez, 1990) that could exacerbate the expected reduction in feed intake associated with weaning itself (Arthington et al., 2010; Devant et al., 2012). However, no studies to date have confirmed if weaning does reduce feed intake.

In mammals, the production of pro-inflammatory cytokines is increased under stressful situations (i.e., infection) triggering an inflammatory response (Baumann and Gauldie, 1994). In fact, it has been reported that weaning or weaning combined with confined housing in a dry lot are stressful events that can induce higher blood levels of cytokines (Kim et al., 2011; Lynch et al., 2011; O'Loughlin et al., 2011). The inflammatory response is followed by an acute phase response, with situations such as weaning and transportation increasing blood levels of acute

Table 2.1. Characteristics of the most common types of feed restriction (FR) in cattle industry.

Severity of FR	Amount fed (% of ad libitum intake)	Planned	Duration (d)	Effect on performance
Mild	75 to 95	Yes	> 30	Positive
Moderate	50 to 75	Yes	< 10	Negative
Severe	0 to 50	Yes/No	< 5	Negative
Complete	0	Yes	< 3	Negative

phase proteins such as ceruloplasmin, haptoglobin, serum amyloid A and fibrinogen (Qiu et al., 2007; Araujo et al., 2010; Lynch et al., 2011). As a consequence, the production of acute phase proteins induces changes in metabolism and negatively impacts DMI, feed efficiency, and ADG (Johnson, 1997; Klasing and Korver, 1997). Implementation of stressful procedures also predisposes calves to diseases, such as bovine respiratory disease, which has major implications for animal welfare and operation profits (Duff and Galyean, 2007).

Some weaning strategies such as 'fence-line weaning' (Church, 1997) and 'two-step weaning' (Stookey and Haley, 2001) were developed in order to minimize the stress associated with weaning on the cow and calf. Weaning strategies that reduce stress may also improve performance, DMI, and reduce the risk for disease; however, studies are needed to confirm these suggestions. With respect to behavioural changes, implementing a two-step weaning approach (calves are prevented from nursing before separation from the dam) reduced the time spent walking and increased the time spent lying and eating relative to those weaned abruptly. This approach also improved ADG for two-stage calves as ADG was greater for up to 44 d post separation from the dam, with the greatest difference during the first week post-weaning (approximately 1.2 vs. 0.4 kg/d; Haley et al., 2005). Although, there are no data available for DMI prior-to and following weaning, the growth performance data suggest that calves can face a severe FR for a short period of time (5-7 d) post weaning.

2.1.2. Transportation, Marketing and Arrival

The weaning process for beef calves is often followed by other events such as transportation, marketing, and arrival at a feedlot. The combination of these stressful events is known to cause a short period of feed deprivation coupled with low voluntary DMI for the first few weeks upon arrival (Loerch and Fluharty, 1999; Marques et al., 2012). For cattle, transportation is a stressful process known to increase the risk for disease. During transportation, cattle experience feed and water deprivation for a few hours or days depending on transportation distance and marketing system (Cole et al., 1988). Recently, González et al. (2012) collected data from commercial long-haul transport in North America and reported that for feeder cattle, transport duration was on average 14.9 h with cattle experiencing weight loss of 7.9% as a proportion of initial body weight (**BW)**. They also indicated that the amount of shrink (i.e. BW

loss) was aggravated with increasing midpoint ambient temperature and time on the truck. Collectively, transportation, marketing, and feedlot entry (i.e., co-mingling, adaptation to new feeds and the feed bunk, adaptation to a new environment) have a negative impact on feed intake, immune system, and performance post feedlot arrival (Tarrant and Grandin, 2000; Parker et al., 2003).

As previously mentioned for weaning cattle, these types of stressful situations also predispose the animal to an inflammatory response and a subsequent acute phase reaction following transportation and feedlot entry (Arthington et al., 2005; Arthington et al., 2008; Carroll et al., 2009). In addition to feed deprivation imposed by transportation, a short period with low voluntary intake could be expected post feedlot arrival due to metabolic changes (i.e., low levels of IGF-I and higher levels of glucocorticoids) imposed by the release of pro-inflammatory cytokines that are associated with an anorexic state (Weingarten, 1996; Klasing and Korver, 1997). Increasing cytokine concentrations in monogastrics increases intestinal permeability, as they have been shown to damage cellular tight junctions, affecting the intestinal barrier function increasing the risk for translocation of microorganisms and molecules (Bruewer et al., 2003; Lambert, 2009). Although the author is not aware of studies confirming this effect in ruminants, Spitz et al. (1996) demonstrated that the combination of 48 h of starvation and stress (glucocorticosteroid injection) in rats decreased tight-cell junction resistance and increased intestinal permeability, further suggesting that feed deprivation and severe FR may have an impact on epithelial tight junctions in ruminants.

Upon arrival at a feedlot calves generally experience a period of low feed intake (Loerch and Fluharty, 1999; Arthington et al., 2008) with intakes ranging between 0.5 to 1.5%, 1.5 to 2.5% and 2.5 to 3.5% of BW on the first, second and the subsequent two weeks after arrival, respectively (Hutcheson and Cole, 1986). While the amount of feed offered is not restricted, these low levels of feed intake correspond to voluntary reductions in feed consumption. Moreover, it should be acknowledged that these data were evaluated on a pen basis, and some calves may experience more severe FR as they may voluntarily fast during the first few days post arrival (Gibb et al., 2000). Hutcheson and Cole (1986) reported that upon arrival at a feedlot only 38.9% of the calves were eating on d 1 and 88.1 % were eating by d 7. This behaviour could be explained by a series of factors (Duff and Galyean, 2007), such as co-mingling (Zobel

et al., 2011), adaptation to a new feed source and feed bunk (Sowell et al., 1999), diet quality of the receiving diet (Galyean et al., 1999), exposure to infectious diseases (Chirase et al., 1991), and FR itself (Zhang et al., 2012). Collectively these factors have detrimental effects on feed intake and the recovery to normal feed intake. As previously mentioned, inflammatory responses caused by stress could also induce hypophagia during the receiving period.

Overall, the length and severity of the FR event will depend on the transportation distance (Cole et al., 1988) together with the time required for the animal to return to normal feed intake after arriving at the feedlot (Hutcheson and Cole, 1986). To model this situation, Cole and Hutcheson (1985b) imposed a series of challenges within one treatment that included feed and water deprivation for 24 h to mimic transportation followed by limited access to feed and water for 24 h to mimic marketing. Calves were then deprived of feed and water for 48 h to represent post-marketing transportation, and finally limit-fed (4.5 kg of DM/head/d) with free access to water for 2 weeks. Results from that study indicate that rumen fermentation capacity (ability of the microorganism to ferment an excess of ground substrate feed *in vitro*) decreased by 74% during deprivation when compared to pre-deprivation levels, with fermentation capacity recovering by d 7 of re-alimentation. In another experiment (Hutheson and Cole, 1986), the same deprivation and re-feeding model was used, but animals were fed ad libitum rather than being feed restricted, having access to feed during the 2 wk re-alimentation period. From this second study, it was reported that steers lost 15% of their BW during deprivation, but returned to their pre-deprivation weight by d 6 of re-alimentation. However, a delayed recovery for BW was found for calves fed a high-forage (90% forage) diet relative to those fed a moderate-forage diet (about 40% forage). In another study, Fluharty et al. (1996) subjected weaned calves to either no fasting (0 h), 48 h fasting (including 8 h of transportation) or 72 h fasting (including 8 h of transportation) prior to placing the calves in a feedlot. In the same publication, they also compared the effects of these treatments (0, 48 and 72 h fasting) but without weaning, on the same calves after spending 28 d at the feedlot. From these studies, they concluded that the recovery of DMI during re-alimentation depends on the length of the feed deprivation period and the stress experienced prior to re-alimentation (i.e., weaning and trucking), in addition to previous adaptation to feedlot conditions. In fact, DMI by d 7 of re-alimentation was greater for 0 h than 48 h fasting treatment, and also greater for 48 h than 72 h of fasting (5.8, 4.8 and 4.3

kg/d, respectively). In addition, by d 4 of re-alimentation no differences in DMI were reported between fasting duration treatments when calves were previously adapted to feedlot conditions. Studies evaluating preconditioning strategies and post feedlot arrival management suggest that nutritional strategies can be applied to accelerate the DMI recovery and ameliorate the negative impact of the reduced feed intake on animal performance (Cole and Hutcheson, 1985a; Lofgreen, 1988; Fluharty and Loerch, 1997), immune response, and animal welfare (Galyean et al., 1999; Rivera et al., 2005).

While most research has focused on beef cattle, some dairy heifer rearing systems incorporate transportation to a heifer rearing facility followed by transportation back to the lactation farm when heifers approach parturition (Eicher, 2001; Wolf 2003). However, there are few studies evaluating the impact of transportation on Holstein heifers, but similar responses upon arrival as those observed for beef cattle could be expected (Stephens, 1980; Tarrant, 1990; Grandin, 1997). For example, Tyler and Cummins (2003) transported dairy heifers for a short distance (210 km for 2.5 h) to a new feeding facility and reported greater concentration of cortisol in response to shipping with values returning to pre-shipping concentration on d 7 post-arrival. The length of the journey and management during the process and post-arrival can predispose the animal to a short-term period of decreased feed intake as described previously for beef calves (Loerch and Fluharty, 1999; Arthington et al., 2008). It can also be expected that the reduction in feed intake will be prolonged upon arrival due to co-mingling (Grant and Albright, 2001) and an increase in the amount of time that the animal spends lying down post transportation (Eicher, 2001). This situation may predispose the animal to a short-term FR, and offer the potential for dietary mitigation strategies available during the relocation upon arrival (Pence et al, 2009).

2.1.3. Heat Stress

In 2003, the estimated cost of heat stress to the United States dairy industry was $897 million with an additional $369 million for the beef industry (St-Pierre et al., 2003). These estimates included effects such as decreased performance, increased mortality, and compromised reproduction (St-Pierre et al., 2003). Heat stress occurs under any combination of environmental conditions (temperature, relative humidity, solar radiation and wind speed) that increase the

effective temperature of the environment beyond the thermal neutral zone. Risk and severity of heat stress is most commonly assessed using the temperature-humidity index (THI; Hubbard et al., 1999; Davis, 2001; Mader et al., 2006). For high producing lactating dairy cattle, a THI > 72 exposes cattle to heat stress with negative implications in milk production and reproductive efficiency (Armstrong, 1994). Ominski et al. (2002) reported that in western Canada, 40% of the summer days present a THI > 72; however, the duration of exposure to heat stress in western Canada is likely mitigated by night cooling.

Exposure to heat stress triggers homeostatic mechanisms such as changes in metabolism (i.e., increased maintenance requirement, decreased somatotropin and IGF-I concentrations) and behaviour (i.e., decreased DMI, increased water intake, increased respiration rate) as an adaptive response to these conditions with negative consequences on production (reproduction, milk production, and ADG; Armstrong et al., 1999; Hahn, 1999; Rhoads et al., 2009). One of these behavioural responses is a marked reduction in feed intake by as much as 35 to 40% of the maximum daily intake in beef (NRC, 2000) and dairy cattle (NRC, 2001), respectively. Under extreme conditions, the reduction in DMI can reach up to 55% of voluntary intake in dairy cattle when compared to those in thermal neutral conditions (Huber, 1996), and when heat stress is this severe, it can lead to death due to hyperthermia in feedlot cattle (Busby and Loy, 1996; Mader, 2003).

In terms of animal health, an increased respiratory rate predisposes cattle to metabolic alkalosis due to an increase in the CO_2 expired, accompanied by an increase in the urinary output of HCO_3^- to compensate the unbalanced HCO_3^-:CO_2 ratio in blood that is normally 20:1 (Kadzere et al., 2002). Heat stress also predisposes cattle to ruminal acidosis caused by a decrease in ruminal buffer supply (loss of HCO_3^- due to altered respiration, drooling and decreased rumination) and affects feeding behavior (i.e., lower intake and feeding patterns; Kadzere et al., 2002; Bernabucci, 2012). Acidosis risk could be drastically aggravated with increasing loss of HCO_3^- as a lower HCO_3^- supply may negatively affect salivary HCO_3^- supply but also compromise SCFA absorption across the rumen epithelium in exchange for HCO_3^- (Bilk et al, 2005; Aschenbach et al., 2009). Aschenbach et al. (2011) estimated for a dairy cow that anion exchange of SCFA and HCO_3^- as an absorptive mechanism provides enough HCO_3^- to roughly match the amount of HCO_3^- supplied into the rumen through saliva intake, ranging

between 20 to 30 mol/d. As a consequence, a decrease in the HCO_3^- pool in arterial circulation could affect the activity of this absorptive mechanism, reducing the rumen buffering capacity and energy supply. Indeed, imposing FR to 25 or 50% of ad libitum intake for 5 d has been reported to increase the risk for acidosis by impairing SCFA absorption (Zhang et al., 2012). Thus, it is clear that heat stress can expose cattle to a short but severe period of FR and predispose cattle to ruminal acidosis and secondary inflammatory responses that negatively affect performance, welfare, and producer profit.

2.1.4. Parturition and Metabolic Disorders

During the last week prepartum, dairy cattle commonly voluntarily reduce feed intake by a magnitude of 30%, with DMI gradually increasing postpartum (Goff and Horst, 1997; Hayirli, 2002). As discussed above, the combination of factors such as heat stress (Grant and Albright, 1995) and metabolic disorders can aggravate the reduction in feed intake (Hansen et al., 2003). In support of this concept, Proudfoot et al. (2009) reported that for primiparous and multiparous dairy cows, a decrease in DMI by more than 5.5 kg was evident from 48 h (16.2 kg/d) to 24 h (10.9 kg/d) before parturition for healthy cows, with dystocia reducing DMI by an additional 24% (8.3 kg/d) prior to parturition. Decreased feed intake around parturition, together with an increase in nutrient requirements and metabolic changes, predisposes dairy cattle to a series of metabolic disorders (i.e., fatty liver disease, ketosis, milk fever, metritis; Drackley, 1999) that rarely occur in isolation (Curtis et al., 1985; Correa et al., 1993). In most cases, these disorders further exacerbate the negative energy balance as they are accompanied by a voluntary reduction in feed intake for a period of time that varies according to the magnitude of the health challenge (Marquardt et al., 1977). As an example, Zamet et al. (1979) reported that multiparous cows experiencing milk fever, displaced abomasum and fatty liver disease had DM and net energy intake that was 44, 46 and 70% lower during the peripartum period than healthy cows. Even though improvements in transition cow health and feeding management have taken place since Zamet's data were reported, a severe FR can still be induced and expected under these circumstances, with similar incidence levels of metabolic disease due to increasing milk production levels.

Postpartum, DMI gradually increases at the rate of 1.5 to 2.5 kg per week during the first 3 wk of lactation (Nocek et al., 1983, Bertics et al., 1992), with variation among cows (i.e., multiparous vs. primiparous) that can delay the recovery (Kertz et al., 1991; Robinson and Garrett, 1999). Understanding the impact of voluntary reductions in feed intake and strategies to accelerate the recovery response are critical to meeting the high-energy demands for lactating dairy cattle at a time where they are immuno-compromised.

2.1.5. Digestive Disorders

Ruminal acidosis is a common digestive disorder in cattle fed highly fermentable carbohydrates and is associated with negative effects on animal performance, morbidity, mortality and welfare (Owens et al., 1998; Stone, 2004; Krause and Oetzel, 2006). The impact of ruminal acidosis will depend on its severity (acute vs. subacute; Owens et al., 1988; Garrett et al., 1999; Oetzel et al., 1999) and on animal susceptibility (Bevans et al., 2005; Penner et al., 2007). It has been reported that low ruminal pH (pH of 5.4 for 4 h), decreases microbial N flow, SCFA production, and the digestibility of OM, DM and NDF measured in vitro (de Veth and Kolver, 2004). Under acidic ruminal conditions (pH 4.79 for 1 h) ion absorption (sodium, chloride, and magnesium; Gäbel et al., 1987) and SCFA absorption by ruminal epithelium (pH 5.1; Gaebel and Martens, 1988) were reduced when measured using the temporarily isolated washed reticulo-rumen technique. In response to ruminal acidosis, cattle generally voluntarily refuse feed, perhaps to limit the consumption of additional fermentable carbohydrate to aid in ruminal pH recovery (Brown et al., 2000; Krause and Oetzel, 2006). This reduction in feed intake represents another example of short-term FR. In a study by Brown et al. (2000), beef steers were exposed to an acute ruminal acidosis by restricting feed to 1, 0.5 and 0% of BW on 3 consecutive days prior to an intraruminal infusion of concentrate at 3% of BW. Feed intake decreased from 10 kg/d to 3 kg/d for 3 d following the acidosis challenge, and steers continued to consume only about 30% of the initial DMI until day 7 post-challenge when feed was offered ad libitum. In addition, animals exposed to subacute ruminal acidosis may be exposed to the translocation of LPS into the bloodstream, triggering an acute phase reaction (Gozho et al., 2005, 2007) and inflammatory induced hypophagia (Dong et al., 2011).

2.2. Feed Deprivation and Short-Term Feed Restriction Models

Previous research evaluating the effect of short-term FR or feed deprivation have utilized controlled experimental settings to address industry-relevant topics such as strategies to improve the response of highly-stressed newly-received calves (Loerch and Fluharty, 1999) and nutritional challenges associated with feed shortages (Doreau et al., 2003). Feed restriction has also been imposed to induce a negative energy balance in effort to study hepatic function under a nutrient deficiency and evaluate effects arising from ketosis (Velez and Donkin, 2005; Carlson et al., 2006), and to separate the effects of challenges such as heat stress on performance while accounting for the induced reduction in DMI (Rhoads et al., 2009). Obviously, the methodology used differs based on the objective, but collectively these studies provided foundational body of knowledge to assess the impact of the severity of feed restriction and feed deprivation on basic production outcomes.

Short periods of feed and water deprivation (ranging from 24 to 72 h) have been applied in beef cattle to simulate the restriction imposed during transportation and marketing, and to evaluate the effects on ruminal characteristics, the rate for DMI recovery, and performance during the first 2 to 4 weeks upon arrival at a feedlot (Hutcheson and Cole, 1986; Fluharty et al., 1994b; Fluharty et al., 1996). Similar feed deprivation approaches were also applied using an ovine model to evaluate the effects on rumen fermentation (Cole, 1991) and rumen absorptive (Gäbel et al., 1993) and barrier function (Gäbel and Aschenbach, 2002). Some of these authors have also evaluated dietary mitigation strategies for the negative effects caused by feed deprivation on DMI, performance and health during the re-alimentation period (Cole and Hutcheson 1985b; Hutcheson and Cole, 1986; Fluharty and Loerch, 1997).

Although, the effects of feed deprivation on sheep rumen function have been evaluated, there is still a paucity of data on the effects of a severe decrease in DMI on bovine rumen function. In addition, we have revealed situations where a severe FR can take place without imposing a complete feed deprivation in cattle. Moreover, studies evaluating mitigation strategies and the time required for recovery post FR have largely focused on performance parameters (ADG, milk yield, DMI, gain:feed), and as such, the time required for recovery of rumen function has not been addressed until recently in cattle. To our knowledge, only one

study reported results from a mild, moderate and severe short-term FR (75, 50 and 25% of ad libitum DMI for 5 d) in beef cattle looking at the negative impact on DMI, rumen function and fermentation, and the time required for recovery (Zhang, 2012). Results from Zhang et al. (2012) demonstrate the importance of FR in cattle, providing an overview of different FR severities. However, effects of a severe FR under different dietary regimens occurring in production settings have not been considered previously. As severe short-term FR periods regularly occur in cattle, a better understanding of the effects of these types of restriction under different dietary sceneries are necessary.

The lack of information on the effects caused by a severe short-term FR, make studies using feed deprivation models and moderate FR models a valuable source of information particularly when the severity of FR is as a continuum ranging from mild FR to complete feed deprivation. The following sections will describe the effects of a short-term reduction in nutrient intake on DMI, rumen fermentation, rumen absorptive function, energy balance and the risk for ruminal acidosis.

2.2.1. Effects of Imposing Short-Term Feed Deprivation and Feed Restriction on the Post-Restriction Recovery for Feed Intake

Following short-term feed deprivation (from 36 to 48 h), a lag for the recovery of DMI to initial conditions during the re-alimentation period has been reported in beef (Lofgreen et al., 1980) and dairy cattle (Agenäs et al., 2003). Following feed deprivation in beef calves, DMI gradually increased with normal feed intake only being achieved between the second to fourth week of re-alimentation (Fluharty et al., 1994a; Hutcheson and Cole, 1986; Carter et al., 2005). The rate of the recovery response appears to be dependent on the length of the feed deprivation event (Fluharty et al., 1996). In these cases, it is important to note that feed deprivation was accompanied by other stressful factors such as shipping, processing and co-mingling upon arrival. While shipping, processing, and comingling are industry relevant practices, they do not allow for the separation of factors based on their contribution to the delayed recovery response for DMI.

Studies evaluating individual factors responsible for the delayed recovery for DMI have reported that the delay is only indirectly affected by shipping, processing, and co-mingling and is

thought to be related to decreased ruminal volume, decreased rumen content weight, ruminal nutrient reserve, and a reduction in the total number of protozoa (Cole and Hutcheson, 1985a; Fluharty et al., 1996; Loerch and Fluharty, 1999). In addition, Zhang et al. (2012) evaluated the effect of the severity of FR itself showing that for beef heifers in isolation from other stressful factors, a delayed response for DMI was observed which closely resembles the recovery response observed for studies that incorporate transportation and feed restriction or deprivation in combination. In Zhang et al. (2012), DMI numerically increased from week 1 to 2, and from week 2 to 3 of re-alimentation for heifers restricted either at 50 or 25% of their ad libitum intake for 5 d. In the same study, heifers restricted at 75% of ad libitum DMI for 5 d remained on a high intake over the 3 wk of re-alimentation (average of 11.7 kg/d). This further suggests that the recovery response is dependent on the severity of the FR insult and can be independent from stress associated with transportation. A better understanding of the factors regulating the DMI recovery will help to develop strategies to improve animal performance after FR or feed deprivation.

To further understand how hepatic metabolism in dairy cattle is affected by negative energy balance several authors have utilized feed restriction models as an induction protocol. The relevance of this protocol is supported by past work with transition cows showing low feed intake prepartum (Grummer, 1995; Hayirli et al., 2002) and a rapid, but delayed increase in DMI postpartum (Zamet et al., 1979, Bertics et al., 1992; Huzzey et al., 2007). It should be acknowledged that around parturition, hormonal and metabolic changes (i.e., decreased levels of growth hormone prepartum and increase in lipolysis postpartum) take place and can be associated with a delay in the DMI recovery (Grum et al., 1996). In primiparous lactating dairy cows, a feed deprivation of 48 h decreased milk yield by 51% and increased NEFA blood levels by 20 fold, 24 h post fasting when compared to pre-fasting conditions (Agenäs et al., 2003). With respect to recovery of DMI, these same heifers required 3 d to recover DMI to initial levels. Velez and Donkin (2005) imposed FR to 50% of voluntary feed intake for 5 d reporting a negative effect on milk production and energy balance, with more than 10 d being required for feed restricted cattle to recover their DMI to values that were not different from control cows (non restricted). While the objective of these studies was not to evaluate the rate of recovery for DMI, it is clear that a short-term feed deprivation and FR have both direct and indirect effects

affecting DMI during re-alimentation. Further research on factors limiting a rapid recovery should be performed.

2.2.2. Effects of Short-Term Feed Deprivation and Short-Term Feed Restriction on Rumen Fermentation

The effect of short-term feed deprivation and short-term FR on rumen fermentation and the resulting recovery of rumen fermentation following return to ad libitum feed intake appear to be inconsistent among studies, with some reporting a rapid recovery for indicators (i.e., dry matter disappearance; NDF disappearance) of ruminal fermentation activity (Fluharty et al.,1994b), with others suggesting a delayed response for the recovery of ruminal fermentation (Cole and Hutcheson, 1981). Beef steers exposed to complete feed deprivation for 32 or 48 h responded with an increase in ruminal pH (Galyean et al., 1981; Fluharty et al., 1994b) and a decrease in total bacteria and protozoa counts. Ruminal volume, ruminal content weight, and ruminal DM have also been shown to decrease with an increase in the duration of the FR (Galyean et al., 1981; Fluharty et al., 1996). For heifers imposed to a 5-d period of FR at 25% of ad libitum intake, an increase in ruminal pH (5.34 vs. 6.51) and a decrease in total SCFA concentration (87 vs. 33 m*M*) was also reported when compared to pre-restriction conditions (Zhang et al., 2012). Exposing beef steers to 32 h of complete feed deprivation also reduced total SCFA concentration (194 vs. 40 m*M*) and the molar proportion of butyrate in ruminal fluid (8 vs. 2 mol/100 moles) when compared to the ad libitum fed control steers (Galyean et al., 1981). Despite the large difference in SCFA concentration during the feed deprivation event, differences were not observed for total SCFA concentration after 24 h of ad libitum feeding when compared to control steers. In addition, differences between control and restricted steers for total rumen bacteria and protozoa counts and rumen DM content were not detected after 72 h of re-alimentation. Clearly, the recovery of SCFA concentration, total rumen bacteria and protozoa counts and ruminal DM content occur shortly after re-alimentation and are unlikely to limit the recovery for DMI.

Baldwin (1967) and Galyean et al. (1981) reported a decrease in the total number of bacteria after 48 or 32 h of feed and water deprivation, respectively. However, Fluharty et al. (1994b) did not find differences for total and cellulolityc ruminal bacteria concentrations in beef

steers after weaning and 24 h of feed and water deprivation. It is possible that the length of the feed deprivation influenced the differential response between studies. A reduction in rumen fermentation capacity (ability of the microorganisms to ferment an excess of ground substrate feed in vitro) was also detected in Baldwin (1967) and Galyean et al. (1981) during complete feed deprivation, with fermentation capacity returning to initial levels on day 3 of re-alimentation (Galyean et al., 1981). In addition, Cole (1991) reported a decrease in rumen fermentation capacity and a delay in the return of DMI to normal levels post 72 h of feed deprivation in lambs; however, differences in rumen fermentation capacity and DMI were not detected when ruminal contents from 72 h feed deprived lambs and control lambs were exchanged. It should be acknowledged that measurements of rumen fermentation capacity were obtained using in vitro gas production based on samples of ruminal fluid and results could differ according to the substrate used for the technique, potentially explaining the differences presented by each author. Accordingly, Fluharty et al. (1994b) did not report differences for in situ dry matter disappearance from the rumen when measured before and after arrival of calves at the feedlot, also suggesting that fermentation capacity in the rumen is not affected. Despite the discrepancies among studies, collectively these data are interpreted to suggest that there is rapid recovery of microbial activity following short-term feed deprivation and likely short-term FR. This finding suggests that microbial activity is likely not the causative factor delaying the recovery for DMI. Along this line of thought, Loerch and Fluharty (1999) hypothesized that a delayed response for DMI recovery could also be related to decreased ruminal volume, decreased rumen content weight, and reduced ruminal nutrient reserve (energy, water and electrolytes).

2.2.3. Effects on Short-Term Feed Deprivation and Short-Term Feed Restriction on Rumen Absorptive and Barrier Function

Previous work exposing sheep to 48 h feed deprivation has reported reductions in rumen epithelial absorptive and barrier function (Gäbel et al., 1993; Gäbel and Aschenbach, 2002). For instance, for sheep exposed to 48 h feed deprivation, in vivo transport of Na^{+}, Cl^{-}, Mg^{2+}, and the fractional absorption of SCFA through the rumen epithelium were reduced by more than 40% (Gäbel et al., 1993). The reduction in absorptive function could be considered as an advantage when considering the energetic cost for the gastrointestinal tract to maintain the absorptive function. This further suggests that the rumen epithelium may adapt to the rumen SCFA

concentration. Moreover, 48 h of feed deprivation was also shown to increase the serosal-to-mucosal flux of 3-O-methyl-α-D-glucose in vitro, suggesting that rumen barrier function was impaired (Gäbel and Aschenbach, 2002). Compromised barrier function can have a major impact on immune system stimulation if permeability to macromolecules follows the same trend (Emmanuel et al., 2007). In beef cattle, 24 h of feed and water deprivation increased plasma cortisol levels for 28 d post-deprivation and triggered an acute-phase response increasing ceruplasmin and haptoglobin levels (for 3 wk and on d 7 post-fasting, respectively) when compared to non-restricted cattle (Marques et al., 2012), further supporting that feed deprivation compromises immune status or at least presents a challenge to the immune system. Presumably, this challenge is mediated via compromised barrier function of the gastrointestinal epithelia, although other epithelial tissues (e.g., respiratory tract) should not be discounted. In support of this concept, a 5 d FR imposed at 25% of ad libitum intake in beef heifers was reported to increase the recovery of Cr-EDTA in urine, an indicator of total gastrointestinal tract barrier function (Zhang et al., 2012).

Zhang (2012) also reported a reduction in SCFA absorption in vivo during short-term FR, with the negative effect extending into the first week of re-alimentation, when compared to the third week of re-alimentation post FR (438.7, 524.4, and 747.4 mmol/h respectively). Another study (Doreau et al., 2003) reported that after a chronic FR (50% of ad libitum intake for 10 wk) approximately 2 wk is required for the recovery of the absorptive function. The implications of this delayed response for SCFA absorption are significant as SCFA contribute substantially to the metabolizable energy supply for ruminants (Bergman, 1990), and SCFA absorption is known to help to regulate rumen pH (Gäbel et al., 2002; Penner et al., 2009a; Aschenbach et al., 2011). As a consequence, a reduction in the absorptive function will have an impact on animal energy balance (Rechkemmer et al., 1995) and provoke an accumulation of organic acids in the rumen (Gäbel et al., 2002), thereby predisposing ruminants to digestive upset such as ruminal acidosis (Aschenbach et al., 2011). Thus, mitigation strategies to minimize this negative impact will likely improve animal performance and health during the FR and re-alimentation.

2.2.4. Effect of Short-Term Feed Deprivation and Feed Restriction on Energy Balance, Blood Metabolites and Hormones

Short-term exposure to FR can expose cattle to a negative energy and protein balance resulting in dramatic changes in metabolism. A restriction period of 5 d at 50% of ad libitum intake in lactating cows imposed a negative energy balance when compared to control cows (-9.11 vs. 12.93 Mcal/d), upregulating pyruvate carboxylase gene expression in hepatocytes (by about 1.5 arbitrary units) during the restriction period when compared to pre-restriction conditions (Velez and Donkin, 2005). A similar FR model in dairy cattle (5 d at 50% of ad libitum intake) also imposed a negative balance (-5.4 vs. 12.2 Mcal/d) accompanied by a negative metabolizable protein balance (-379 vs. 451 g/d) when compared to cattle fed ad libitum (Carlson et al., 2006).

Blood metabolites and insulin concentrations are also modified under feed deprivation or FR conditions. When feeding below maintenance energy requirements, tissue catabolism takes place, with increasing levels of NEFA in blood circulation due to adipose tissue mobilization (Stich and Berlan, 2004; Sejrsen et al., 2006). A 48 h period of complete feed deprivation in dairy cows increased plasma NEFA levels more than 20 times, accompanied by a decrease in plasma glucose concentration, while insulin levels dropped by about 10 mU/mL, and plasma cortisol levels dramatically increased from about 10 nmol/L to 55 nmol/L on the second day of feed deprivation (Agenäs et al., 2003). In addition, Zhang et al. (2012) reported an increase in serum NEFA concentration (184.4 vs. 495.5 µEq/L) without changes in plasma glucose levels when compared to baseline conditions on beef heifers restricted at 25% of their ad libitum intake for 3 d. As a consequence of altered metabolite and hormone concentrations, it can be expected that splanchnic tissue metabolism and energy substrate changes occur with short-term FR or complete feed deprivation.

2.2.5. Risk for Ruminal Acidosis as Influenced by Short-Term Feed Deprivation and Short-Term Feed Restriction

Ruminal acidosis has been successfully induced in acute and subacute forms by imposing a short period of feed deprivation (about 24 h; Goad et al., 1998) or by limit feeding the day prior to induction (Harmon et al., 1985; Dohme et al., 2008; Krause et al., 2009). While these

induction protocols also incorporate a grain challenge, it could be expected that the reduction in feed intake has a substantial influence on the induction of ruminal acidosis. Support for this hypothesis is based on research reporting that short-term feed deprivation and short-term FR compromise SCFA absorption across the reticulo-rumen (Gäbel et al., 1993; Zhang et al., 2012), and that ruminal pH regulation is highly dependent on SCFA absorption (Gäbel et al., 2002). Direct evidence includes results from Zhang (2012), where beef heifers consuming a moderate concentrate diet (40% concentrate diet) were restricted to 25% of their ad libitum intake for 5 d followed by provision of the same diet for ad libitum feed intake. In that study, providing heifers feed for ad libitum intake after the short-term FR induced a decrease in ruminal pH (mean pH 5.92, duration pH < 5.5 was 5.5 h/d). Moreover, slight variations in daily DMI in finishing cattle (90% of ad libitum intake for 3 d followed by 110% of ad libitum intake for 3 d) fed a high concentrate diet (92 % concentrate) can induce a depression in ruminal pH (mean pH 5.63, duration of pH < 5.5 was 11.2 h/d; Schwartzkopf-Genswein et al., 2004).

Studies evaluating abrupt dietary changes in steers (from 100% alfalfa hay to 70% concentrate diet; Coe et al., 1999) or a rapid step-up protocol (abruptly switched from 75 to 90% concentrate; Galyean et al., 1992) did not report clear effects for induced ruminal acidosis (mean pH 6.28 and 5.6 respectively). In Coe et al. (1999), steers were limit-fed at 2.5% of BW during dietary transition, with pH decreasing only from 7.20 to 6.28, while in Galyean et al. (1992) steers were fed ad libitum, but a decrease in DMI was detected on the second day when a 90% concentrate diet was fed, which could be suggestive of low ruminal pH. A decrease in DMI was reported in cattle experiencing ruminal acidosis that likely represents a feedback mechanism and strategy employed by cattle to reduce intake of fermentable carbohydrate in effort to help regulate ruminal pH (Brown et al., 2000; Krause and Oetzel, 2006). Force feeding high concentrate diets through the ruminal cannula coupled with a reduction in saliva input may explain why forcing the ruminal fermentation of highly fermentable diets may have induced situations of acute ruminal acidosis (Dougherty et al., 1975; Nagaraja et al., 1982, 1985). Although abrupt dietary transition to high grain diets can induce ruminal acidosis, more consistent responses appear to be observed when feed restriction protocols are implemented prior to induction. Thus, it appears that short periods of feed withdrawal or severe FR are a major risk factor for ruminal acidosis.

2.2.6. Dietary Forage-to-Concentrate Ratio Effects

Shifting the dietary forage-to-concentrate ratio (F:C) can modify the response of cattle to stressful situations by influencing plasma cortisol levels, DMI, milk yield, and lameness incidence (Pence et al., 2009), and rumen epithelial absorptive function (Penner et al., 2011). Particular attention has been given to the effect of increasing dietary fermentability by decreasing the F:C of diets on ruminal epithelial adaptation (Bannink et al., 2008) with recent results suggesting a marked reduction in the time required for epithelial adaptation relative to the suggestions made by Dirksen et al. (1985) and the NRC (2001). Adaptations can now be classified from functional and morphological perspectives with functional adaptation preceding morphological adaptations (Penner et al., 2011). Thus, reviewing the effects of dietary F:C on DMI, rumen fermentation and rumen absorptive function can provide valuable information when developing mitigation strategies for the negative effects caused by FR.

2.2.7. Effect of the Forage-to-Concentrate Ratio on Dry Matter Intake and Ruminal Fermentation

While decreasing the F:C is an effective approach to increase the energy density or dietary fermentability, shifting the source of concentrate to increase diet fermentability without altering the F:C can also yield a similar response. Fluharty et al. (1994a) increased the energy density in a diet with a F:C of 60:40, by decreasing the NDF content (reducing the proportion of pelleted corn cobs in the diet while increasing corn grain), thus reported that although gain:feed was greater for the high energy diet, cattle fed the low energy diet had higher DMI. In another study, Fluharty et al. (1997) evaluated diets with similar F:C (60:40) but with differing ruminal available energy by replacing part of the grain with a fat supplement; however, no improvements for DMI, ADG or gain:feed were reported. Even though in Fluharty et al. (1997) fat concentration in the diet was only 2%, previous studies have shown that a fat inclusion of 5% has an effect on ruminal fermentation, decreasing the molar proportion of propionate and increasing the molar proportion of acetate and the acetate:propionate ratio. In addition, feeding diets with the same F:C but replacing part of the main forage source with another source (15% of alfalfa silage replaced with alfalfa hay without changes on NDF content) increased chewing rate, rumination time and milk yield (Beauchemin and Buchanan-Smith, 1989). Thus, nutrient source

and feed processing should be considered when formulating diets for ruminants to modulate the response.

Decreasing the F:C in beef and dairy rations is associated with an increase in diet fermentability and increased passage rate (NRC, 2000; NRC, 2001), accompanied by a higher DMI when fed ad libitum (Macleod et al., 1983). Weiss and Shockey (1991) reported a linear increase in DMI and DM digestibility with increasing proportions of concentrate in dairy cows fed diets with a F:C of 80:20, 60:40 and 40:60. Accordingly, Macleod et al. (1983) reported a linear increase in DMI for lactating dairy cows when the F:C decreased from 80:20 to 35:65. On the other hand, when concentrate inclusion rates are greater than those used by Weiss and Shockey (1991) and Macleod et al. (1983), a detrimental effect on DMI has been reported. For example, Gill et al. (1981) fed beef steers diets with F:C of 24:76, 20:80, 16:84, 12:88 and 8:92 and reported a linear decrease in DMI with increasing concentrate proportion. Similarly, Kreikemeier et al. (1990) showed a linear decrease in DMI with increasing concentrate proportions when feeding finishing beef steers diets with F:C ranging from 15:95 to 0:100.

It should be acknowledged that DMI is not only controlled by rumen distension (Mertens, 1994), but it can be also regulated by metabolic signalling factors, nutrient levels in blood and diet characteristics (Galyean and Defoor, 2003; Allen et al., 2009). In addition to regulatory mechanisms involved through the ruminant forestomach such as osmolarity and SCFA receptors (Allen et al., 2000), other DMI regulatory factors that could be taking place have been identified (i.e., hepatic oxidation). For example, Allen et al. (2009) suggested that for stressed newly arrived cattle, stress hormones (i.e., cortisol) promote lipolysis, increasing circulating NEFA concentration, increasing NEFA uptake by the liver, and triggering hepatic oxidation, a metabolic process known as intake suppressor. In addition, diet physical and chemical characteristics can affect DMI (Galyean and Defoor, 2003; Arelovich et al., 2008). Arelovich et al. (2008) evaluated results from 29 different publications reporting the effect of dietary NDF concentration (% of DM) on beef and dairy cattle DMI. In their study, they found that dairy rations in North America have an NDF content ranging from 22.5 to 45.8%, with increases in NDF content negatively correlated with DMI. On the other hand, the NDF content in feedlot diets for beef cattle range from 7.5 to 35.3 %, with increases in NDF content positively correlated with DMI.

Ruminal microbial community composition, fermentation characteristics, and SCFA concentrations can be modified by altering the dietary F:C. Goad et al. (1998) reported that in beef steers fed a diet with a F:C of 20:80 the total bacteria, ciliated protozoa number, and the number of amylolitic bacteria were increased, while the number of fibrolytic bacteria were lower when compared to steers fed F:C of 80:20. In addition, steers fed a F:C of 20:80 had lower mean ruminal pH (6.4 vs. 6.7), molar proportion of acetate (62.7 vs. 72.8 mol/100 mol), and acetate:propionate (3.8 vs. 5.5) when compared to steers fed the 80:20 F:C diet. Even though the molar proportions of propionate and butyrate were numerically higher for steers fed the diet with a F:C of 20:80, statistical differences were not detected. Accordingly, Weiss and Shockey (1991) evaluated the effects of diets with F:C of 80:20, 60:40 and 40:60 fed to dairy cows and observed a linear increase in the concentration of acetate and butyrate, and a linear decrease in the acetate:propionate ratio with increasing proportions of concentrate in the diet.

Collectively, it is evident that not only does the F:C of the diet have an impact on ruminal fermentation and DMI, but different concentrate and forage sources, and feed processing techniques can modify fermentation parameters (Robles et al., 1981; Penner et al., 2009b; Mohammed et al., 2010). Moreover acetate, butyrate, and propionate production rates are increased, and the acetate:propionate and pH decreased with increasing dry matter fermentability (Sutton et al., 2003; Lechartier and Peyraud, 2010). On the other hand, diets with a high forage proportion have lower total production of SCFA and higher acetate:propionate than diets with higher concentrate inclusion (Van Houtert, 1993). Thus, ruminal SCFA load and individual SCFA concentrations can be modified with alterations in dietary F:C, with this approach being particularly important when developing feeding strategies to mitigate the response due to low DMI (NRC, 2001).

2.2.8. Effects of the Forage-to-Concentrate Ratio on Residence Time and Site of Digestion

Passage rate represents the flow of solid and fluid residues out of the reticulo-rumen (Wyburn, 1980). Slower passage rates are associated with reductions in DMI due to gut fill and rumen distension and higher retention times (Robles et al., 1981), while greater passage rates are associated with higher DMI and decreased ruminal feed digestion together with an increased

feed digestion in the hindgut (Robles et al., 1981; Firkins et al., 1998). Moorby et al. (2006) reported a linear increase in DMI and nutrient flow to the duodenum (kg/d; DM, OM, total N, starch, NDF and microbial N), without changes in ruminal pH and apparent ruminal digestibility (g/g consumed; DM, OM, N and starch) when increasing the proportion of concentrate in diets for lactating cows (F:C ranging from 80:20 to 35:65). Even though Moorby et al. (2006) reported an increase in microbial N flow to the duodenum, they did not detect differences in the efficiency of microbial N production (per unit of OM digested in the rumen) with increasing levels of concentrate. The lack of an improvement for microbial efficiency may be attributable to a lack of differences for ruminal OM digestion with increasing concentrate proportions. In addition, in diets with low F:C (33.5:66.5) digestive efficiency of some nutrients, such as starch and CP can be increased due to a reduction in ruminal degradation and an increase in the flow of starch and dietary CP to the small intestine when compared to diets with higher F:C (66.5:33.5; Fária-Mármol et al., 2002). However, in highly fermentable diets fed in western Canada, barley grain is the main concentrate source (Hussey, 2012) with barley having a high rate of ruminal starch digestion relative to corn grain (74.4 vs. 41.9% of starch intake), and a minor portion digested postruminally (22 vs. 49% of starch intake, respectively; Overton et al., 1995).

In summary, a higher DMI and a more active microbial community, with slight changes in feed digestibility would be expected when feeding a moderate-forage diet (~40% concentrate) versus a high or all forage diet (20 to 0% concentrate). This could be one strategy to improve nutrient provision during short-term FR and may also increase the efficiency of digestion following FR providing that increasing the dietary fermentability does not induce ruminal acidosis.

2.2.9. Absorption of Short-Chain Fatty Acids Across the Reticulo-Rumen and the Effect of the Forage-to-Concentrate Ratio on Short-Chain Fatty Acid Absorption

Ruminal SCFA production supplies about 65 to 75% of the total metabolizable energy in ruminants (Bergman, 1990). Short-chain fatty acid absorption across the reticulo-rumen epithelium accounts for about 76% of the total SCFA produced in the rumen (Weston and Hogan, 1968) with 66 of the propionate (Peters et al., 1990) and 87% of the acetate (Peters et al.,

1992) produced being removed. From this evidence, it is clear that SCFA absorption across the reticulo-rumen epithelium and the mechanisms involved play a major role in meeting the energy demand for ruminants.

Under feedlot settings in North America, cattle are fed finishing diets containing a high proportion of grain and a low inclusion of roughage. This practice results in exposure of the ruminal epithelia to high rumen fluid SCFA concentration (100 m*M*; Beauchemin et al., 2001) with this concentration being decreased under acute (< 100 m*M*) or increased under subacute (150 to 225 m*M*) ruminal acidosis conditions (Nagaraja et al., 2007). Organic acid removal from the reticulo-rumen through passive diffusion and facilitated transport mechanisms were reviewed by Aschenbach et al. (2011), emphasising the important role that these mechanisms play in terms of ruminal pH regulation and energy supply for ruminants. It has been reported that the contribution of each mechanism involved in SCFA uptake partially depends upon the dissociation state of the SCFA, with the assumption that passive diffusion rates increase when SCFA are undissociated (bound to H^+) and facilitated transport rates increased when SCFA are dissociated (Gäbel et al., 2002; Penner et al, 2009a). Bugaut (1987) calculated that at pH 5.3, about 76% of the SCFA would be in the undissociated state and over 90% at a pH of 6.8. Thus, ruminal pH plays a major role in determining whether SCFA will be in a dissociated or undissociated state, promoting different rates of passive diffusion or facilitated absorption through the reticulo-rumen wall (Dijkstra et al., 1993; Penner et al., 2011). However, the relationship between pH and absorption process has been questioned as facilitated transport for acetate uptake is greater than passive diffusion with decreasing pH (Aschenbach et al., 2009).

Passive diffusion of undissociated SCFA takes place via lipophilic diffusion and accounts for about 28 to 60% of the acetate absorption and 31 to 72% of butyrate absorption (Penner 2009a). Higher diffusion rates for butyrate are related to a higher lipophilicity and greater metabolism of this acid in the rumen epithelium that decreases the intracellular concentration thereby resulting in a favourable concentration gradient and enhancing the uptake when compared to acetate and propionate (Bergman, 1990; Kristensen et al., 2000, Kristensen and Harmon, 2004). Once the protonated SCFA is absorbed into the cell, SCFA will rapidly dissociate releasing a proton (H^+) and $SCFA^-$ in the cytosol. Mechanisms to remove the H^+ have been identified and involve the Na^+/H^+ exchanger (NHE) family (NHE1, NHE2, NHE3 and

NHE8; Graham and Simmons, 2005, Graham et al., 2007). These transporters export protons outside the cell with directional export to the apical or basolateral membranes buffering the intracellular pH (Müller et al., 2002). This Na^+/H^+ exchange is facilitated by an electrochemical gradient created by Na^+-K^+-ATPase (Graham and Simons, 2005). In addition, the cell buffering capacity is enhanced by the influx of HCO_3^- into the cell from basolateral $Na^+/HCO3^-$ cotransporters involved in cellular uptake of dissociated SCFA (Huhn et al., 2003). Yang et al. (2012) demonstrated that the mRNA expression of the isoforms NHE1 and NHE3 is positively correlated with the increase in ruminal SCFA concentration and decreased pH, achieved by feeding concentrate (400 g/d) to goats fed a base diet consisting of ad libitum peanut straw for 42 d. The increase in the mRNA of NHE1 and NHE3 in supplemented goats accounted for an increase in mRNA expression of 20 and 25%, respectively when compared to those non supplemented. Accordingly, increases in Na^+ transport were reported when supplementing concentrate (800 g/d) to sheep fed hay for 12 wk, with most of the functional adaptation (73%) occurring the first week following provision of the supplemental concentrate (Etschmann et al., 2009). Conversely, Yang et al. (2012) did not report differences in the mRNA expression of NHE1 and NHE3 in animals supplemented with concentrate versus non supplemented after a 16 h feed deprivation, suggesting that gene expression is transient within a day and likely dependent on exposure to SCFA or feeding. The above evidence suggests that the increase in NHE isoforms expression and Na^+ transport could act as an indicator of SCFA absorption, with increases in SCFA absorption occurring rapidly when feeding diets with increasing proportions of concentrate. In addition, detrimental effects on the expression of those transport proteins could rapidly occur after a short period of decreased nutrient intake.

Mechanisms involved in the movement of dissociated SCFA across the rumen epithelium have been identified and are directly linked to transport proteins. One of these mechanisms involves $SCFA^-/HCO3^-$ exchange. This implies that the cellular supply of bicarbonate is not limiting and strategies to increase cellular supply of $HCO3^-$ include $Na^+/HCO3^-$ contransport and $SCFA^-/HCO3^-$ exchange (Aschenbach et al., 2011). Anion exchange mechanisms are especially important for acetate uptake, in part supported by its low lipophilicity when compared to butyrate and propionate (Huhn et al., 2003; Aschenbach et al., 2009, Penner et al., 2009a). Transport proteins catalyze the exchange of luminal $SCFA^-$ for intracellular HCO_3^-, with

estimates that about 50% of total SCFA in sheep are absorbed through this mechanism (Aschenbach et al., 2011). Bilk et al. (2005) identified 3 potential $SCFA^-/HCO_3^-$ exchangers in the rumen epithelium, two of them located on the apical membrane (down regulated-in-adenoma and putative anion transporter 1) and one of them located in the basolateral membrane (anion exchanger 2). It was previously proposed that enhancing the up-regulation of bicarbonate-dependant transporters has the potential to increase SCFA uptake from the luminal side, improving the ruminal buffering capacity (Gäbel et al., 2002). In fact, Connor et al. (2010) demonstrated that in calves fed milk replacer for 42 d and supplemented with hay or grain for another 14 d, the expression of down regulated-in-adenoma (DRA) was increased 142 fold when compared to calves fed milk replacer for 42 d. Despite the large increase in DRA, they did not evaluate functional activity and thus, it is not currently understood whether changes at the mRNA level also resulted in changes in activity.

Another group of transport proteins have been identified as HCO_3^--independent, with Ussing chamber work demonstrating that some acetate is absorbed via this mechanism when the HCO_3^--dependent pathways are inhibited (Aschenbach et al., 2009; Penner et al., 2009a). In contrast, it does not appear that butyrate absorption utilizes transporters involved in HCO_3^--independent pathways (Aschenbach et al., 2009; Penner et al., 2009a). Apical absorption of acetate has also been decreased by 55 and 34% in goat rumen epithelium when a monocarboxylate transporters1 (MCT1) inhibitor was applied either on the serosal or mucosal side of the Ussing chambers, respectively (Kirat et al., 2006). While this does not prove apical orientation of MCT it does provide justification for further research on the action of different MCT family members in the rumen epithelium. Some have suggested that MCT4 is located within the apical membrane (Kirat et al., 2007); however, they showed positive staining in the stratum corneum which lacks metabolic machinery (mitochondria) and is composed of nearly completely keratinized cells (Graham and Simmons, 2005). Thus, the physiological relevance of MCT4 localization within the stratum corneum remains as a question. Others have shown localization of MCT in the basolateral membrane (MCT1) or distributed along all the epithelial cell layers with exception of the stratum corneum (MCT2; Graham et al., 2007). The isoform MCT1 plays a major role in extruding products of butyrate and propionate intracellular metabolism (i.e., ketone bodies, lactate) into the blood (Müller et al, 2002), creating a positive

gradient (lower intracellular concentration) for the apical uptake of SCFA and regulating intracellular pH due to its proton-linked transport activity (Halestrap and Meredith, 2004). The MCT family may also be capable of transporting dissociated SCFA, but further research is required to support this suggestion (Aschenbach et al., 2011). The above mentioned studies have provided sufficient evidence emphasizing the importance of protein mediated mechanisms in the absorption of SCFA from the rumen. Moreover, it is clear that dietary manipulation can affect the mRNA expression of some of these transporters (i.e., NHE1, NHE3, downregulated-in-adenoma) providing justification that dietary strategies may be effective at manipulating SCFA absorption.

2.2.10. Promoting Short-Chain Fatty Acid Absorption Across the Reticulo-Rumen

Adaptation to highly fermentable diets involves changes in the functional and morphological structure of the ruminal epithelium (Dirksen et al., 1985, Bannink et al., 2008). Bannink et al. (2008) reported that in dairy cows, maximum papillae surface area was achieved after 3 to 4 wk or 7 to 8 wk when increasing the concentrate portion to 8 kg/d (feeding a grass and corn silage base diet) either in 1.5 wk (fast adaptation) or 3 wk (slow adaptation) postpartum, respectively. Interestingly, these adaptive responses occurred without differences in ruminal SCFA concentration between those rapidly or gradually adapted. However, they suggested that the higher SCFA load (due to higher SCFA production rates, causing a faster drop in pH) may explain the papillae response with a faster adaptation to concentrate (Bannink et al., 2008). The work of Bannink et al. (2008) represents a new line of thought suggesting that epithelial adaptation occurs more rapidly than previously suggested (Dirksen et al., 1985). Supporting this notion, Gaebel et al. (1987) reported that papillae surface area and electrolyte absorption from the rumen epithelium were increased when sheep were switched from 100% hay to 64% concentrate and when switched again to 90% concentrate with each feeding period lasting 15 wk. After feeding 90% concentrate, sheep returned to a diet consisting of 100% hay for 15 wk, with papillae surface area and absorptive capacity decreasing to values similar to the initial surface area or even below initial levels, respectively. As a comparison to initial papillae surface area, feeding a 90% concentrate diet increased the surface area by 400% (Gaebel et al., 1987). Supporting this notion, Dirksen et al. (1985) observed an increase in ruminal SCFA disappearance in non-lactating dairy cows when cows were transitioned from a low energy diet

to a high-energy diet for 7 to 14 wk. Those authors suggested that the increase in activity was attributable to an increase in ruminal papillae absorptive surface.

Increasing the proportion of concentrate in diets for ruminants is associated with changes in the concentration of SCFA, with propionate and butyrate concentration increasing and acetate concentration decreasing (Gaebel et al., 1987; Bannik et al., 2008). Increasing ruminal butyrate concentration is expected to accelerate and stimulate rumen epithelial development (Noziere et al., 2000), increasing papillae length and width (Zitnan et al., 2005). The effect of butyrate on rumen papillae proliferation is indirect, but is likely mediated via insulin and other growth factors (i.e., EGF and IGF-I) that induce proliferation (Sakata et al., 1980; Zhao and Sun, 2010). Furthermore, SCFA, especially butyrate, function as signalling molecules and may promote up-regulation of SCFA transporters (Connor et al., 2010; Penner et al., 2011) through changes in cellular signal transduction (via G-protein coupled receptors 41 and 43; Wang et al., 2012) and greater blood flow to the ruminal epithelium contributing to an increase in the SCFA uptake (Dobson, 1984). As mentioned in the previous section, increases in ruminal Na^+ absorption by increasing the energy density occur rapidly (within 7 d, Etschmann et al., 2009) which is likely related to increased SCFA absorption and the related requirement for intracellular pH regulation.

While most research has focused on accelerating the adaptive response, it is clear that a reduction in epithelial function also occurs very rapidly. Supporting this concept, Gäbel et al. (1993) observed a reduction in SCFA absorption after 2 d of feed deprivation in sheep and Zhang et al. (2012) reported a reduction in SCFA absorption after 5 d of FR at 25% of ad libitum intake in beef heifers. This suggests that, from a functional perspective, rumen epithelial adaptation occurs rapidly whereas, morphological adaptation is a long-term response.

2.3. Mitigation Strategies

Management prior to, during, and after periods of FR and the time necessary for the recovery of DMI and rumen absorptive function is not well understood. For early weaned and transported calves, 2 to 3 weeks are required for recovery based on performance indicators following arrival at a feedlot (DMI, ADG and feed efficiency; Hutcheson and Cole, 1986; Loerch and Fluharty; 1999). Similarly, DMI gradually increases post-partum in dairy cattle (1.5 to 2.5 kg/wk during the first 3 wk; Nocek et al., 1983, Bertics et al., 1992). A growing body of

evidence has suggested that numerous factors such as the diet fed pre-fasting and post-fasting (Hutcheson and Cole, 1986), rumen epithelial function (Sehested et al., 2000; Etschmann et al., 2009; Bannik et al., 2008), and energy balance (Allen et al., 2009) all affect the rate of recovery for DMI and thus performance measures (i.e., growth, milk production, etc.). Clearly, dietary-based mitigation strategies could be a viable option to ameliorate the impact of the FR and accelerate the recovery of DMI and rumen function. Strategies such as modifying the dietary CP quality and quantity, and varying the F:C have been applied, mostly in newly received cattle, to improve animal performance and health.

2.3.1. Manipulating Dietary Crude Protein

Cole and Hutcheson (1988) demonstrated that after a fasting period of 24 h, DMI in beef steers was increased when fed with a higher dietary CP content post-fasting than pre-fasting (15 vs. 8 % CP, respectively), while DMI decreased when the diet fed post-fasting had a lower CP % than the diet fed pre-fasting (10 vs. 16%, respectively) when compared to control steers (fed a 12% CP diet ad libitum without deprivation). These results are similar to those of Fluharty and Loerch (1995) showing that for newly arrived feedlot steers, diets with greater protein content post-arrival (18 vs. 12% CP) and containing spray-dried blood meal rather than soybean meal as a protein source had improved performance. During the first week of recovery, Fluharty and Loerch (1995) also showed a linear increase in ADG and feed efficiency (gain to feed) with increasing dietary CP in diets when the CP concentration ranged from 11 to 26% of DM. In all these studies, dietary treatments containing the lowest CP proportion were below the CP requirement. In fact, a past study suggested that newly received cattle should receive a diet containing 12.5% CP (Eck et al., 1988), but this study did not account for the marked depression in DMI during the first days post-arrival. These results suggest that an increase in dietary CP during receiving may compensate for the low CP intake during the first 14 d in the feedlot (Cole and Hutcheson, 1988, 1990), with this response being improved when the source of protein supplies a higher quality protein and a higher proportion of rumen undegradable protein (i.e., spray-dried blood meal; Fluharty and Loerch, 1995). Even though ruminant-origin protein sources are banned for use in ruminant production in North America (CDC, 2012), other available protein sources providing a high proportion of rumen undegradable protein could be used (i.e., fish meal). Increased feed intake was also observed for dairy cows postpartum

without differences in DMI during the prepartum period when dairy cows were fed a diet containing high levels of rumen undegradable protein during the whole transition period (35% of dietary CP; Westwood et al., 2000). Reasons for the differential response when feeding different protein sources were not provided in Fluharty and Loerch (1995), but Westwood et al. (2000) suggested that increasing the intestinal pool of amino acids when feeding higher levels of ruminal bypass protein may be leading this response.

2.3.2. Altering the Forage-to-Concentrate Ratio to Mitigate the Effect of Feed Restriction

It could be hypothesized that feeding a highly fermentable diet post FR or feed deprivation would help to compensate for low nutrient intake occurring during and immediately following short-term feed deprivation (Hutcheson and Cole; 1986) or FR (Zhang, 2012). However, a moderate or very low amount of concentrate should be provided in order to minimize the risk for ruminal acidosis following feed deprivation or FR (Brown et al., 2000). Another strategy to minimize the acidosis risk and ease the nutrient deficit could consist on feeding a moderate or high concentrate diet prior-to and during FR, to enhance the ruminal SCFA absorptive capacity (Bannik et al., 2008, Penner et al., 2011), providing at the same time a greater supply of fermentable carbohydrate relative to diets with a higher F:C. However, this positive effect on SCFA uptake could be diminished during short-term feed deprivation (Gäbel et al., 1993) or FR (Zhang et al., 2012) and even remain impaired during the re-alimentation period (Gaebel and Martens, 1988; Zhang, 2012) due to a detrimental effect of the restriction on ruminal absorptive function. Feeding a high-forage diet prior to FR would reduce digesta passage rate (Robles et al., 1981) and theoretically provide ruminants with a ruminal nutrient reserve during the restriction period (Cole and Hutcheson, 1987). In addition, feeding a high-forage diet post FR could reduce the risk for ruminal acidosis, but limit the nutrient supply during re-alimentation (low dietary nutrient density and decreased DMI), delaying the nutrient replenishment.

The effect of manipulating the dietary F:C has shown variable results, but it has been reported that a higher DMI prior to feed deprivation also promotes higher DMI post feed deprivation (Cole and Hutcheson, 1985a). Cole and Hutcheson (1987) fed diets with a F:C of

84:16, 60:40 or 35:65 pre-fasting reporting no effects on post-fasting DMI recovery when feeding a diet with a F:C of 60:40 during the re-alimentation. On the other hand, Lofgreen et al. (1980) reported an effect of dietary F:C when feeding diets with a F:C of 75:25, 50:50 or 25:75 post-fasting, with feed intake and net energy intake increasing and calf morbidity reduced when feeding a diet with a F:C 50:50. Fluharty et al. (1994a) showed that an increase in the energy density of the diet fed post feed deprivation, achieved by decreasing the NDF content of the diet (reducing the proportion of corn cobs in the diet while increasing corn grain), can have an effect on animal response. In their study, they reported that gain:feed was greater for the high energy diet, but steers fed the low energy diet had higher DMI during re-alimentation. Similarly, increasing the energy content of the receiving diet (by replacing 2% of the grain for a fat supplement) without modifications of the F:C (60:40) did not improve DMI, ADG or gain:feed (Fluharty et al.; 1997).

It should be acknowledged that both physical and chemical differences within different forages (i.e., legumes vs. grasses) can affect the intraruminal milieu, animal behaviour, and animal performance (Beauchemin and Buchanan-Smith, 1989). In fact, Lofgreen et al. (1981) reported that receiving calves with a diet composed of 100% alfalfa hay had greater DMI, ADG and feed efficiency when compared to calves fed a diet composed of 100% millet hay. Thus, nutrient content and characteristics in the diet should be taken into account when planning feeding mitigation strategies.

For dairy cattle, previous studies have investigated how changing the dietary F:C fed prior-to and post partum can be implemented to improve animal DMI, alleviate the negative energy balance and improve animal performance. In fact, DMI during the pre-partum portion of the transition period can be improved by increasing the concentrate proportion in the pre-partum diet (Hayirli et al., 2002); however this increase in pre-partum DMI did not promote a higher DMI post-partum (Halcomb et al., 2001; Rabelo et al., 2003), and cattle fed high concentrate diets had a greater depression in feed intake around calving (Hayirli et al., 2002). It should be acknowledged that during the transition period in dairy cattle, low DMI is also accompanied by metabolic and hormonal changes around parturition (Grummer et al., 2004) that could mask the effect of the F:C ratio on the increase of DMI during the post-partum phase of the transition period. In addition, previous studies also suggest that ruminal propionate levels (Oba and Allen,

2003) and NEFA blood levels (Allen et al., 2009) can also be induce hypophagia at this time. Collectively, these studies suggest that feeding strategies consisting of alterations in dietary F:C can be used to ameliorate the impact of a short-term FR and accelerate the recovery of DMI, improving animal performance and health during and post FR. However, past studies have largely focused on DMI and indicators of performance while ignoring ruminal function.

2.4. Hypothesis

The hypothesis was that short-term severe FR would have a negative effect on rumen epithelial function, with this response being attenuated when a low F:C diet is fed relative to a diet with a high F:C. A low F:C ratio diet was achieved by feeding a moderate proportion of forage in the diet (60%), with this diet not only having the potential to promote SCFA absorption, but also to minimize the risk for ruminal acidosis during re-alimentation. Furthermore, feeding a low F:C diet prior-to and during FR would not only enhance SCFA absorptive capacity, maximize nutrient absorption and energy availability during FR, but also improve the recovery response post FR. In addition, feeding a low F:C diet post FR will hasten the recovery process and minimize the negative effects caused by a FR.

2.5. Objectives

The objective of this project was to evaluate whether the dietary F:C affects ruminal fermentation and absorption of SCFA from the reticulo-rumen in response to a severe short-term FR. Moreover, the effect of the F:C of diets fed prior-to and during FR, and those fed post FR, on the recovery response was evaluated.

3.0. FEED RESTRICTION REDUCES SHORT-CHAIN FATTY ACID ABSORPTION ACROSS THE RETICULO-RUMEN OF BEEF CATTLE INDEPENDENT OF THE DIET

3.1. Abstract

The objective of this study was to evaluate the effects of altering the forage-to-concentrate ratio (**F:C**) of diets fed prior to and during short-term feed restriction (**FR**) on rumen fermentation, absorptive capacity of the reticulo-rumen, and apparent total tract digestibility. Twenty ovariectomized and ruminally cannulated Angus × Hereford heifers were blocked by BW. Heifers were individually penned in box stalls (9 m^2), having free access to water throughout the study. Heifers were randomly assigned to 1 of 2 dietary treatments, receiving either a high (**HF**; F:C of 92:8) or a moderate-forage diet (**MF**; F:C of 60:40). Diets were fed ad libitum for 14 d prior to 5 d of baseline measurements (**BASE**), followed by 5 d of FR during which heifers were restricted to 25% of ad libitum DMI relative to BASE. Dry matter intake was measured daily and ruminal pH was recorded every 2 min during the whole BASE and FR. Ruminal fluid and blood samples were collected on d 3 of BASE and FR, while short-chain fatty acid (**SCFA**) absorption was assessed *in vivo* using the isolated washed reticulo-rumen technique on d 5 of BASE and FR. Indigestible NDF was used as a marker to estimate apparent total tract digestibility. Diet × period interactions were detected for DMI ($P = 0.030$) and SCFA concentration ($P = 0.025$). The interaction was the result of higher DMI and numerically higher SCFA concentration for MF than HF during BASE, with a reduction observed for both during FR, although treatment effects were no longer present. Period effects (BASE vs. FR), but not treatment effects ($P > 0.05$), were detected for mean ruminal pH ($P < 0.001$) and the total SCFA absorption rate (mmol/h; $P = 0.038$). During BASE, mean pH was lower (6.44 vs. 6.86) and the SCFA absorption rate was greater (675 vs. 555 mmol/h) relative to FR. Diet ($P < 0.001$) and period ($P < 0.001$) effects were detected for DM and OM digestibility with greater digestibility occurring for heifers fed MF than HF (70.5 vs. 63.3% for DM and 73.0 vs. 66% for OM) and greater digestibility during FR than BASE (69.5 vs. 64.3 % for DM and 71.7 vs. 67.2% for OM). During FR, NDF digestibility was also greater than during BASE ($P < 0.001$; 62.4 vs. 55.8%). The effect of FR on serum NEFA differed by diet (diet × period, $P < 0.001$) with NEFA being higher for heifers fed HF than MF during FR (474 vs. 378 uEq/mL, respectively) with no

differences observed between HF and MF during BASE. It can be concluded that severe short-term FR had a negative impact on ruminal SCFA absorption and animal energy balance and that altering the F:C of the diet did not mitigate these effects.

3.2. Introduction

Beef and dairy cattle experience periods of short-term feed restriction (**FR**) associated with conventional management practices (e.g., weaning and transportation; Gibb et al., 2000), changes in physiological state (e.g., parturition; Hayirli et al., 2002), and as a result of adverse environmental conditions (heat stress; St-Pierre et al., 2003). Exposure to these stressors, in isolation or combination, has been shown to alter metabolism (Rhoads et al., 2009) and consequently compromise animal performance (St-Pierre et al., 2003). As such, it is important to understand how FR affects nutrient digestibility, short-chain fatty acid (**SCFA**) absorption, and nutrient utilization.

The severity of the FR depends on the amount of feed available (Gäbel et al., 1991; Gäbel et al., 1993), the duration of the restriction, and nutrient density in the diet offered (Harmon et al., 1991; Wertz-Lutz et al., 2008). Feed restriction ranging from 2 to 7 d can induce a negative energy and protein balance altering metabolic functions (e.g., hepatic gluconeogenesis and adipose tissue mobilization; Velez and Donkin, 2005; Carlson et al., 2006; Wertz-Lutz et al., 2008) and exposing sheep to 48 h of complete feed deprivation negatively impacts both the absorptive capacity (Gäbel et al., 1993) and barrier function (Gäbel and Aschenbach, 2002) of the ruminal epithelium. Furthermore, it has been recently shown that subjecting beef heifers to severe FR (5 d with cattle restricted to 25% of ad libitum DMI) compromises the absorptive function of the reticulo-rumen and total tract barrier function (Zhang et al., 2012). Together this implies that the nutrient deficit imposed by FR could be exacerbated by decreased nutrient absorption, and that dietary approaches to increase nutrient delivery may help to alleviate this response.

Rumen epithelial cellular absorptive function rapidly adapts with increased absorptive capability in response to increasing dietary fermentable energy (Etschmann et al., 2009), but also can regress when energy availability is limited as imposed through feed deprivation (Gäbel et al., 1993). As luminal SCFA act as direct and indirect signals stimulating SCFA absorption in the

short term (Sehested et al., 2000) and rumen epithelial development in the long term (Penner et al., 2011; Plöger et al., 2012), altering rumen fermentation characteristics by adjusting the dietary forage-to-concentrate ratio (**F:C**; Penner et al., 2009b) may have the potential to mitigate the negative effects of FR on ruminal epithelial function. Thus, it may be possible to develop nutritional programs that mitigate the effect of FR on ruminal epithelial function when the FR event is predictable.

The objective of our study was to evaluate whether the dietary F:C affects ruminal fermentation and absorption of SCFA from the reticulo-rumen in response to short-term FR. We hypothesized that FR would induce a negative effect on ruminal fermentation and SCFA absorption with the response being mitigated by supplying a diet with moderate F:C (60:40) compared to a high F:C diet (92:8).

3.3. Materials and Methods

Heifers were housed at the University of Saskatchewan Livestock Research Building in individual box stalls (9 m^2) and were cared for in accordance to the guidelines of the Canadian Council on Animal Care (2009). All experimental procedures were pre-approved by the University of Saskatchewan Animal Research Ethics Board (protocol 20100021).

3.3.1. Experimental Design and Feeding Management

Twenty ovariectomized and ruminally cannulated Angus × Hereford heifers were used in this study (May to September 2011). To facilitate a staggered sampling schedule, heifers were randomly blocked by BW into 1 of 3 blocks with 8, 4, and 8 heifers, in block 1, 2, and 3, respectively. The mean ± SD for BW was 463 ± 33, 482 ± 53, 485 ± 30 kg, for blocks 1, 2, and 3, respectively with block 1, 2 and 3 starting the study on May 2nd, June 23rd, and August 7th of 2011. Within block, heifers were randomly assigned to either a high-forage (**HF**) or moderate-forage (**MF**) diet (Table 3.1) that was fed prior-to and during FR. The HF and MF diets had a F:C of 92:8 and 60:40, respectively.

Table 3.1. Ingredient and chemical composition of the high-forage (HF) and moderate-forage (MF) diets.

	HF	MF[1]
Ingredient, % of DM		
Grass hay	46	30
Barley silage	46	30
Barley grain	0	32
Pellet[2]	8	8
Chemical composition[3], g/kg of DM ± SD		
DM	584 ± 69.7	557 ± 47.3
OM	907 ± 2.3	925 ± 1.9
CP	107 ± 5.7	111 ± 5.4
Crude fat	21 ± 0.4	19 ± 0.7
NDF	527 ± 4.6	405 ± 1.4
ADF	291 ± 5.4	209 ± 4.5

[1]Water was added to equalize DM content between diets as reported in this table.
[2]The pellet contained on a DM basis 21.9% barley grain, 29.6% beet pulp, 26.7% canola meal, 0.65% salt, 0.5% micro-mineral premix (0.14% sodium selenite, <0.01% iodine, <0.01% cobalt carbonate, 0.11% copper sulfate, 0.09% manganous oxide, 0.11% zinc oxide, 0.01% vitamin A, <0.01% vitamin D, 0.05% vitamin E), 12.8% mono-calcium phosphate, 7% limestone, 0.39% molasses and 0.13% monensin.
[3]Mean ± standard deviation of 3 composited samples.

The experiment consisted of a 14-d adaptation period to allow heifers to adapt to the housing and diet. Subsequently, heifers were exposed to 5 d for baseline (**BASE**) measurements and 5 d of FR. During adaptation and BASE, heifers were fed ad libitum (105-110% of voluntary intake) at 0800 h with feed refusals being collected and weighed prior to feeding. During FR, heifers were restricted to 25% of ad libitum intake determined during BASE. This level of restriction was predicted to equate to provision of 30 and 36% of NE_m for HF and MF heifers respectively. Heifers had free access to water throughout the study.

3.3.2. Data and Sample Collection

3.3.2.1. Feed Ingredient Sampling and Dry Matter Intake

The amount of feed offered and refused was recorded daily for determination of DMI. Feed ingredients were sampled once a week for barley grain and the mineral-vitamin pellet, and twice weekly for barley silage and hay. Dietary DM was adjusted on a weekly basis according to changes in feed ingredient DM and water was added to the MF diet to equalize the DM content of the two diets. Refusal samples were collected daily and a subsample (5% of total weight) was composited by cow within experimental period, and stored at -20ºC. Feed ingredient samples and refusal samples were dried in a forced air oven at 55°C until achieving a constant weight for DM determination. Subsequently, samples were ground using a hammer mill (Christy and Norris Ltd., Chelmsford, UK) to pass through a 1-mm screen. Feed samples were then composited on an equal weight basis to yield 1 ingredient sample for each block. Crude fat and CP analysis were performed on feed ingredient samples only. Analytical DM was determined after drying a known amount of sample in an oven at 135°C for 2 h (AOAC, 1990; method 930.15). Samples were later placed in a muffle furnace at 550°C for 5 h for ash determination (AOAC, 1990; method 942.05). Organic matter was determined as 100 − ash concentration. For NDF determination, a Fiber Analyzer (model A200, ANKOM Technology Corp., Fairport, NY) was used with inclusion of alpha amylase and sodium sulfite (Van Soest et al., 1991). Ether extraction was performed (AOAC, 2000; method 920.39) for crude fat determination and CP was analyzed using a nitrogen combustion analyzer (FP-528 analyzer, LECO, Saint Joseph, MI). Ground refusal samples were used for DM, ash and NDF analysis, as described previously.

3.3.2.2. Ruminal pH

Ruminal pH was recorded at 2 min intervals during BASE and FR using the indwelling Lethbridge Research Centre Ruminal pH Measurement System (Dascor, Escondido, CA) as described by Penner et al. (2006). Ruminal pH systems were standardized at the beginning and end of each measurement period using pH 4 and 7 buffers solutions (Fisher Chemical, Fair Lawn, NJ). Millivolt readings from the initial and final standardizations, within a period, were recorded and the drift between them was assumed to be linear. Millivolt data collected during incubation in the rumen were then converted to pH while accounting for drift. Subsequently, the minimum, mean and maximum pH values were determined for each cow by d and the mean across periods was used for statistical analysis. Furthermore, the pH threshold of 5.5 was used to indicate the severity of ruminal pH depression. Accordingly, the duration (min/d) below pH 5.5 was calculated. Ruminal pH data from d 5 of the BASE and FR were removed as the experimental procedures conducted on those days required ruminal contents to be removed for an extended period of time and thus biased the ruminal pH measurements.

3.3.2.3. Blood, Ruminal Digesta, and Fecal Samples

Blood, rumen digesta, and fecal samples were collected every 4 h over a period of 24 h on d 3 of BASE and FR. Blood samples were collected via a jugular catheter into one tube containing Na-heparin (143 IU Na-heparin, Becton Dickinson, Franklin Lakes, NJ) and a second tube with clot activator and silicone-coated interior (Becton Dickinson, Franklin Lakes, NJ). The tube containing Na-heparin was immediately placed on ice and centrifuged at 1,800 × g at 4°C for 15 min after which the plasma was harvested and stored at -20°C. Tubes used for serum were allowed to clot at room temperature for 4 h before being centrifuged and the serum harvested stored, as described for plasma. Equal volumes of plasma and serum collected at each time-point, within period, were used to prepare a composite sample for each cow. Plasma glucose was determined using an oxidative reaction in a solution containing PGO enzymes (No. P7119, Sigma Aldrich) and o-dianisidine (No. F5803, Sigma Aldrich, Oakville, ON, Canada) as a colorimetric substrate. Absorbance was determined using a plate reader (Spectramax Plus 384, Molecular Devices Corp., Sunnyvale, CA) at a wavelength of 450 nm. Plasma insulin was measured using a commercially available ELISA kit (No. 10-1201-01, Mercodia AB, Uppsala,

Sweden). The BHBA concentration in serum was measured using the enzymatic oxidation of BHBA to acetoacetate via 3-hydroxybutyrate dehydrogenase (No. H6501; Roche, Mississauga, Ontario, Canada) and the color change (measured at 350 nm) induced by the equimolar reduction of NAD (No. N7004, Sigma Aldrich, Oakville, ON, Canada). A commercial kit was used to determine serum NEFA concentration (HR Series NEFA-HR2, Wako Chemical, Atlanta, GA).

For ruminal digesta collection, 250 mL samples of mixed digesta were collected from 3 locations in the rumen (cranial central, central and caudal central) and combined before straining through 2 layers of cheesecloth. Subsequently, 10 mL of ruminal fluid was transferred to a tube containing 2 mL of metaphosphoric acid (wt/v) and stored at -20ºC. As with plasma and serum, samples of ruminal fluid from each collection time were composited by sampling period and used for analysis of SCFA using gas chromatography. Samples were centrifuged at 3,655 × g at 4ºC for 15 min twice using a Beckman Coulter Avanti J-E centrifuge (Indianapolis, IN). At the end of each centrifugation cycle, the supernatant was transferred for use and SCFA concentration determined according to the method modified from Khorasani et al. (1996). The final supernatant was transferred to a GC vial and iso-valeric acid was added as an internal standard. Samples were run in duplicate for SCFA determination using an Agilent 6890 Series Gas Chromatography System with FID (Wilmington, DE) and an Agilent 7683 Series Injector. Separation was achieved using a Zebron ZB-FFAP High Performance GC Capillary Colum (30 m × 320 µm × 0.25 µm, Phenomenex, Torrance, CA) with an injection split ratio of 17.1:1. Initial and final oven temperatures were 90ºC and 170ºC, respectively, with an increase rate of 10 ºC/min followed by a final 2 min hold.

Fecal grab samples were taken from the rectum at the same time as blood and ruminal digesta sampling. Fecal samples from each time-point were composited on an equal weight basis and stored at -20ºC. The fecal composites were dried in a forced air oven at 55ºC until achieving a constant weight and ground to pass through a 1 mm screen. Fecal samples were analyzed for DM, ash, and NDF as previously described for feed and refusal samples.

3.3.2.4. Short-Chain Fatty Acid Absorption

The temporarily isolated and washed reticulo-rumen (**WRR**) technique described by Care et al. (1994) was performed on d 5 of BASE and FR. Within block, 1 heifer from each treatment

was assigned to the WRR at 0900 h, performing the WRR for the other heifer at 1300 h. For block 2, all heifers were exposed to the WRR at 1300 h. Briefly, with the heifer restrained; the reticulo-rumen contents were completely evacuated and stored in a covered container. Once emptied, the reticulo-rumen was washed 3 times with warm tap water (5 L/wash at 38°C) followed by 4 consecutive washes with 5 L of washing buffer solution (38°C at pH of 6.2; Table 3.2). After each wash, the liquid was removed with a wet-dry vacuum. Once the reticulo-rumen was free of digesta particulate, the esophagus was occluded using a custom-built occluding device (University of Leipzig, Leipzig, Germany). The esophageal occluding device was constructed with an inflatable cuff to prevent saliva passage into the reticulo-rumen while allowing for aspiration of saliva using a vacuum pump (UN86KT.45P, KNF Neuberger, Inc., Trenton, NJ). The omasal orifice was occluded using a Foley catheter (Bardex Foley 75 cc balloon; C. R. Bard Inc., Covington, GA). Use of the occluding devices temporarily isolated the reticulo-rumen from the remainder of the gastro-intestinal tract such that movement of solvent and solutes were restricted to movement across the ruminal epithelia or through the opening in the ruminal cannula itself when sampling.

Once occluding devices were in place, the reticulo-rumen was washed again to remove salivary contamination that was produced during placement of the esophageal occluding device. After washing, occluding device placement was confirmed and 15 L of incubation buffer (38°C at pH 6.2; Table 3.2) was poured into the reticulo-rumen. The Cr-EDTA contained in the buffer was used as a volumetric marker. During incubation, the incubation buffer was mixed by gas lift with CO_2 (99.9%). Samples of the experimental buffer were taken prior-to, and at 5 and 45 min relative to pouring time into the reticulo-rumen. Samples were preserved in 25% metaphosphoric acid (wt/v) with a ratio of sample to metaphosphoric acid of 5:1, and analyzed for SCFA concentration as previously described for ruminal fluid. The rate of SCFA absorption was calculated as described by Gäbel et al. (1993). The Cr concentration in the buffer was determined using an atomic absorption spectrometer (ICE 3000 Series, Thermo Scientific, Cambridge, UK). A sub-sample (5 mL) of the incubation buffer solution was collected at each sampling time and used for determination of osmolality using an osmometer (Model 3250, Advanced Instruments, Inc., Norwood, MA). At the end of the WRR procedure, residual incubation buffer and occluding devices were removed and rumen digesta was returned.

Table 3.2. Formulated chemical composition of the washing and incubation buffers used to determine the rate of SCFA absorption across the temporarily isolated and washed reticulo-rumen.

Chemical	Washing buffer, m*M*[1]	Incubation buffer, m*M*[1,2]
$CaCl_2$	-	2
$MgCl_2$	-	2
NaCl	105	5
KCl	-	5
Na-acetate	10	30
K-acetate	-	35
Na-propionate	20	35
Na-butyrate	-	8
Butyric acid	-	7
L-lactic acid	-	5
$NaHCO_3$	25	25
Cr-EDTA	-	2

[1]Buffer pH was adjusted to 6.2.
[2]Osmolality (± SD) of the incubation buffer was 294 ± 2.4 mOsmol/kg (n = 40).

3.3.2.5. Apparent Total Tract Digestibility

Feed digestibility was evaluated using indigestible NDF (iNDF) as an external marker as described by Huhtanen et al. (1994). The concentration of iNDF in feed, feces, and refusal samples were determined by placing 3 g of ground sample into nylon bags (5 × 10 cm with a 6 µm pore size; Ankom Technology, Macedon, NY) and incubating them in the rumen of beef heifers for 12 d. Heifers used for the incubation were fed a grass hay diet ad libitum. Incubated samples were subsequently dried in a forced air oven at 55ºC for 2 d and analyzed for NDF content as described previously. The ratio between the intake of iNDF and fecal concentration was used to determine fecal output on a DM basis.

3.3.3. Statistical Analysis

Data were analyzed as a randomized complete block design using the PROC MIXED procedure of SAS (version 9.2, SAS Institute, Inc., Cary, NC) with heifer nested in block as the random effect, and block, treatment, period and the diet × period as fixed effects. Measurement period (BASE vs. FR) was used as a repeated measure and the covariance error structure that yielded the lowest Akaike's and Bayesian information criterion for each dependent variable was used. The model for the WRR data initially included the timing of the procedure (0900 vs. 1300 h), but as the time of the procedure was not significant ($P > 0.05$), it was removed from the model. When the F-test yielded a significant response ($P < 0.05$) the Tukey's post-hoc mean separation test was used to compare means among treatments and time. Tendencies are discussed when $0.05 < P \leq 0.10$. Data for the main effects of treatment and time are shown except when interactions were detected.

3.4. Results

3.4.1. Dry Matter Intake and Ruminal Fermentation

A diet × period interaction was detected for DMI ($P = 0.030$; Table 3.3), where DMI was greater for heifers fed MF than HF during BASE (11.9 kg/d vs. 10.6 kg/d), without differences between treatments during FR. During FR, heifers consumed about 25% (26% for HF and 25% for MF) of their ad libitum intake measured during BASE, indicating that we successfully achieved our FR target.

A diet × period interaction was detected for total SCFA concentration ($P = 0.025$; Table 3.3), where numerically, heifers fed MF had higher SCFA concentration than those fed HF during BASE but had numerically lower concentration during FR. The molar proportion of acetate was affected by a diet × period interaction ($P = 0.004$) with an increase in acetate from BASE to FR for each dietary treatment; however, the proportion of acetate was higher for heifers fed HF than MF. No interactions were detected for propionate or butyrate, but molar proportions of propionate ($P < 0.001$) and butyrate ($P = 0.001$) were higher during BASE than FR. Furthermore, heifers fed MF had a greater ($P < 0.001$) molar proportion of butyrate than heifers fed HF. Propionate also tended ($P = 0.063$) to be higher for heifers fed MF than HF.

Interactions between treatment and period were not detected for ruminal pH ($P > 0.05$; data not shown); thus, only the main effects of treatment and period are shown (Table 3.4). Heifers fed MF had lower minimum pH ($P = 0.022$), and a greater duration that pH was < 5.5 ($P = 0.046$) relative to heifers fed HF. Furthermore, mean pH tended ($P = 0.072$) to be lower for MF than HF heifers, but maximum pH was not different. Period effects were also detected where minimum, mean, and maximum pH ($P \leq 0.001$ for all variables) increased during FR relative to BASE.

Table 3.3. Effect of diet fed prior-to and during feed restriction on DMI, ruminal SCFA concentration, and the molar proportion of SCFA. Data presented are the means ± SEM for 10 heifers/treatment.

	Treatment							
	BASE		FR			*P* value		
Variable	HF	MF	HF	MF	SEM	Diet	Period	Diet × Period
DMI, kg/d	10.6[b]	11.9[a]	2.8[c]	3.0[c]	0.29	0.056	< 0.001	0.030
Total SCFA, m*M*	87.2[a]	95.6[a]	41.2[b]	39.0[b]	2.29	0.18	< 0.001	0.025
Acetate, mol/100 mol	65.4[b]	60.4[c]	69.5[a]	66.4[b]	0.54	< 0.001	< 0.001	0.004
Propionate, mol/100 mol	21.6	23.8	17.1	17.7	0.63	0.063	< 0.001	0.19
Butyrate, mol/100 mol	9.4	12.0	8.4	10.4	0.36	< 0.001	0.001	0.36

[abcd]Means within a row without a common superscript differ ($P \leq 0.05$).

Table 3.4. Effect of feeding a high-forage (HF) or a moderate-forage (MF) diet prior-to and during feed restriction (FR) on ruminal pH. Data presented are the means ± SEM for 10 heifers/treatment.[1]

	Diet				Period			
Variable	HF	MF	SEM	*P* value	BASE	FR	SEM	*P* value
Minimum pH	6.33	5.91	0.105	0.022	5.79	6.45	0.995	0.001
Mean pH	6.73	6.58	0.056	0.072	6.44	6.86	0.056	< 0.001
Maximum pH	7.09	7.07	0.048	0.72	6.94	7.22	0.045	0.001
Duration pH < 5.5, min/d	0.0	28.8	8.66	0.046	22.4	6.4	7.69	0.13

[1]Only main effects are shown as the diet × period interaction was not significant ($P > 0.05$).

3.4.2. Ruminal Short-Chain Fatty Acid Absorption

Diet × period and diet effects were not detected for SCFA absorption measured using the WRR (Table 3.5). A reduction ($P = 0.038$) in the total SCFA absorption rate by nearly 120 mmol/h was induced by FR. The reduction in total SCFA absorption was primarily due to decreased acetate absorption, which tended ($P = 0.058$) to be lower during FR than BASE, whereas, propionate and butyrate were only numerically ($P > 0.10$) lower during FR. Similarly, when reported as the fractional rate of absorption (%/h), there were no effects of treatment on the rate of SCFA absorption, but tendencies for lower fractional rates for total SCFA ($P = 0.056$) and acetate ($P = 0.069$) absorption during FR than BASE were detected. The fractional rates of absorption for propionate and butyrate were not affected by treatment or period.

3.4.3. Apparent Total Tract Digestibility

Heifers fed the MF diet had higher total tract DM ($P < 0.001$; Table 3.6) and OM ($P < 0.001$) digestibility, with no difference in NDF ($P = 0.158$) digestibility when compared to those fed the HF diet. Period effects on apparent total tract digestibility were also detected ($P < 0.001$) with the digestibility increasing by 5.2, 4.5, and 6.6 percentage units for DM, OM, and NDF, respectively, during FR relative to BASE.

3.4.4. Blood Metabolites

Serum BHBA was not affected by diet, period, or their interaction, with an average value of 9.2 mg/dL (Table 3.7). Likewise, plasma glucose and insulin were not affected. However, an interaction ($P < 0.001$) between treatment and period was detected for NEFA, where concentrations were not different between treatments during BASE, but concentrations were higher for heifers fed HF than those fed MF during FR, and values were higher during FR than BASE.

Table 3.5. Effect of feeding a high-forage (HF) or moderate-forage (MF) diet prior-to and during feed restriction (FR) and the effect of measurement period on the rate of SCFA absorption from the temporarily isolated reticulo-rumen. Data presented are the means ± SEM for 10 heifers/treatment.[1]

	Diet				Period			
Variable	HF	MF	SEM	*P* value	BASE	FR	SEM	*P* value
Absolute absorption rate, mmol/h								
Total SCFA	569.2	660.1	47.9	0.20	674.5	554.8	42.9	0.038
Acetate	319.9	353.1	27.3	0.41	369.2	303.8	25.0	0.058
Propionate	172.4	210.9	17.4	0.14	209.3	173.9	17.0	0.13
Butyrate	77.9	96.1	7.7	0.12	95.6	78.4	7.6	0.12
Fractional absorption rate, %/h								
Total SCFA	36.4	41.0	2.6	0.23	41.8	35.5	2.4	0.056
Acetate	34.2	37.2	2.6	0.42	38.9	32.6	2.4	0.069
Propionate	38.5	44.7	3.2	0.18	44.4	38.8	3.0	0.19
Butyrate	42.2	50.1	3.8	0.16	49.8	42.5	3.8	0.19

[1]Only main effects are shown as the diet × period interaction was not significant ($P > 0.05$).

Table 3.6. Effect of feeding a high-forage (HF) or moderate-forage (MF) diet prior-to and during feed restriction (FR) and the effect of measurement period on apparent total tract digestibility.[1]

	Diet				Period			
Variable	HF	MF	SEM	*P* value	BASE	FR	SEM	*P* value
DM, %	63.3	70.5	0.57	< 0.001	64.3	69.5	0.51	< 0.001
OM, %	66.0	73.0	0.57	< 0.001	67.2	71.7	0.49	< 0.001
NDF, %	60.0	58.3	0.80	0.16	55.8	62.4	0.71	< 0.001

[1]Only main effects are shown as the diet × period interaction was not significant ($P > 0.05$).

Table 3.7. Interaction between diet fed prior-to and during feed restriction and the measurement period on the concentration of arterial metabolites and insulin. Data presented are the means ± SEM for 10 heifers/treatment.

	Treatment							
	BASE		FR			*P* value		
Variable	HF	MF	HF	MF	SEM	Diet	Period	Diet × Period
BHBA, mg/dL	9.0	9.8	8.9	8.9	0.39	0.37	0.17	0.31
Glucose, mg/dL	71.0	69.9	69.8	70.5	1.41	0.90	0.82	0.52
NEFA, uEq/L	164.9[c]	168.7[c]	474.4[a]	377.7[b]	23.21	0.87	< 0.001	< 0.001
Insulin, ug/L	0.55	0.72	0.56	0.63	0.083	0.28	0.52	0.38

[abc]Means within a row without a common superscript differ ($P \leq 0.05$).

3.5. Discussion

In conventional beef and dairy production, severe short-term FR can occur following weaning (Gibb et al., 2000), during transportation (González et al., 2012), upon arrival of animals at a feedlot (Hutcheson and Cole, 1986), when experiencing heat stress (Wheelock et al., 2010), and when metabolic (Hansen et al., 2003) and digestive disorders (Brown et al., 2000) occur. Furthermore, many of these stressors occur in combination (e.g., heat stress and transportation or weaning and transportation), thereby exacerbating the severity of the FR event (Grant and Albright, 1995). Thus, this study was designed to evaluate the effect of FR and whether altering the dietary F:C of the diet affects the response.

It is well documented that severe FR and complete feed deprivation have negative effects on energy balance (Carlson et al., 2006), animal welfare (Tarrant and Grandin, 2000), and animal performance (St-Pierre et al., 2003; Marques et al., 2012). Part of the negative impact of FR is likely mediated via energy and protein restriction (Rhoads et al., 2009). Thus, increasing the energy and protein supplied should help to alleviate the negative energy balance and mitigate the negative effects. A key approach to alleviate negative energy balance is to increase the dietary energy density (Duff and Galyean, 2007), either via lipid supplementation (Knapp and Grummer, 1991) or by altering the F:C (Rivera et al., 2005).

Decreasing the dietary F:C affects the ruminal microbial population (Cantalapiedra-Hijar et al., 2009), increases the extent of DM digestibility (Ramos et al., 2009), increases the concentration of total SCFA and alters the molar proportions of individual SCFA (Sutton et al., 2003), and generally, results in a reduction in ruminal pH (Penner et al., 2009b). These findings are supported by the current study where, during BASE, heifers fed the MF diet had greater total tract DM and OM digestibility, numerically higher SCFA concentration, and lower ruminal pH. Shifts in the molar proportion of SCFA were also observed, including a reduction in the proportion of acetate and an increase in butyrate; both of these represent responses commonly observed when the proportion of grain in the diet increases (Penner et al., 2009b; Ramos et al., 2009). These changes led to an improvement in the energy status during FR for heifers fed MF than HF, as suggested by higher energy intake, higher OM digestibility, and lower serum NEFA

during FR, and provides further support for the effectiveness of the model (i.e., lower F:C) used to mitigate FR in the current study.

Despite the successful improvement in energy status, feeding a greater proportion of concentrate did not affect the rate of SCFA absorption across the temporarily isolated reticulo-rumen either prior-to or during FR. Increasing the dietary inclusion of grain has been previously shown to promote epithelial proliferation (Dirksen et al., 1985; Steele et al., 2011) and absorptive function (Etschmann et al., 2009). It was expected that the changes induced through our dietary model would have enhanced epithelial function, as shown previously (Sehested et al., 2000; Etschmann et al., 2009). However, we did not observe statistical differences in SCFA absorption when comparing HF and MF heifers. Differences in SCFA concentration between dietary treatments were expected, with a higher SCFA concentration anticipated for heifers fed MF than HF which would promote SCFA absorption as previously reported (Sehested et al., 2000; Bannink et al., 2008). Accordingly, previous study using much larger differences in the F:C than used in the current study also demonstrated that SCFA absorption rate does not differ with differing F:C (Penner et al., 2009b). Furthermore, our data suggest that with severe FR, moderate increases in the proportion of concentrate in the diet are not sufficient to elevate ruminal SCFA concentration and prevent the decline in absorptive function induced with FR.

It is well documented that DMI affects SCFA concentration in the rumen (Harmon et al., 1991; Wertz-Lutz et al., 2008). In our study, FR decreased SCFA concentration in ruminal digesta by approximately 52% for HF and 59% for MF heifers on the third day of FR when compared to BASE. Zhang et al. (2012) imposed a similar FR model with similar dietary conditions and reported a decrease in SCFA concentration in ruminal fluid of 62%. Previous reviews have emphasized the importance of individual SCFA in terms of promoting ruminal epithelial function (Connor et al., 2010; Penner et al., 2011), suggesting that a decrease in the concentration of SCFA might affect their absorption rate (López et al., 2003) and may decrease the stimuli required to maintain epithelial absorptive function (Zhang et al., 2012). Supporting this notion, data obtained from sheep exposed to short-term feed deprivation (Gäbel et al., 1993; Gäbel and Aschenbach, 2002), chronic FR (Perrier et al., 1994; Doreau et al., 1997), and recent data from short-term FR in beef cattle (Zhang et al., 2012), along with the current study, have revealed a negative impact on absorption of SCFA across the reticulo-rumen. It appears that the

FR model imposed in the current study is consistent and repeatable as the extent of reduction for total SCFA absorption in the current study was similar to the reduction (reduction of 100 mmol/h) reported by Zhang et al. (2012). Others have reported greater reductions in SCFA absorption with either more severe feed restriction (i.e., 48 h feed deprivation; Gäbel et al., 1993) or chronic feed restriction (restricted to 50% of DMI; Doreau et al., 1997). For example, Gäbel et al. (1993) reported reductions in acetate, propionate and butyrate absorption by 56, 44 and 43% respectively (Gäbel et al., 1993), and Doreau et al. (1997) reported a reduction of 32% in total SCFA absorption when compared to those fed close to voluntary intake. Collectively, these data confirm that FR negatively affects total SCFA absorption across the reticulo-rumen and that the severity of the FR event affects the magnitude of the response. However, the underlying mechanisms driving the degenerative process of the rumen epithelium in response to FR are still not known.

In our study and that of Zhang et al. (2012), the majority of the decline for the reduction in total SCFA absorption was due to lower acetate absorption that equated to approximately 65 mmol/h, accounting for nearly 55% of the total decline in SCFA absorption. As acetate transport has a greater reliance on protein-mediated pathways than butyrate (Aschenbach et al., 2009; Penner et al., 2009a; Aschenbach et al., 2011), it could be expected that FR down-regulated the expression or activity of anion exchangers in the rumen epithelium; however, future studies coupled with gene and protein expression are needed to confirm this hypothesis.

In conclusion, this study proved that a severe episode of short-term FR in cattle negatively affects SCFA absorption across the reticulo-rumen epithelia, and that reducing the dietary F:C before and during the FR does not mitigate this effect. Further research is needed to identify strategies to mitigate the negative effect of short-term FR on reticulo-rumen absorptive function.

4.0. THE EFFECT OF THE FORAGE-TO-CONCENTRATE RATIO OF DIETS FED DURING FEED RESTRICTION AND RE-ALIMENTATION ON DRY MATTER INTAKE, RUMINAL FERMENTATION AND SHORT-CHAIN FATTY ACID ABSORPTION ACROSS THE RETICULO-RUMEN IN BEEF CATTLE

4.1. Abstract

The objective of this study was to determine if altering the forage-to-concentrate ratio (**F:C**) of diets fed prior-to and during feed restriction (**PRE**), and diets fed post feed restriction (**POST**) affected the recovery for DMI, rumen fermentation, and short-chain fatty acid (**SCFA**) absorption following feed restriction (**FR**). Twenty ruminally cannulated Angus × Hereford heifers were fed (ad libitum) either a high (**HF**; F:C = 92:8) or a moderate-forage diet (**MF**; F:C = 60:40) diet for 19 d. Heifers were then exposed to a 5-d FR period where feed was restricted to 25% of ad libitum intake. Heifers were then provided feed ad libitum with one half of the HF and MF heifers receiving the HF or MF diet during the 3-wk recovery period (**REC1**, **REC2**, **REC3**). This resulted in 4 treatment combinations (PRE-POST): HF-HF, HF-MF, MF-HF, and MF-MF. The PRE × POST and PRE × POST × period interactions were not significant and, thus, the effects of PRE × period and POST × period are presented. Interactions (PRE × period) were detected for DMI ($P = 0.045$) and the duration that ruminal pH < 5.5 ($P = 0.003$). For heifers fed HF during PRE, DMI increased from REC1 to REC3, while DMI did not differ among periods for heifers fed MF PRE. Duration that ruminal pH was < 5.5 was numerically higher during REC1 for heifers fed HF than MF PRE (191 vs. 98 min/d), with duration decreasing from REC1 to REC2 for heifers fed HF PRE. Total ruminal SCFA concentration and absorption rate were not affected by the diet fed PRE ($P > 0.05$) or period ($P > 0.05$). For heifers fed MF POST, DMI increased from REC1 to REC3 whereas DMI did not differ among REC periods for heifers fed HF POST (POST × period, $P = 0.033$). The duration that ruminal pH was < 5.5 was greater for heifers fed MF than HF post (14.1 vs. 274.9 min/d; POST × period, $P < 0.001$) with HF heifers decreasing duration from REC1 to REC2, whereas, duration did not differ among periods for HF. Ruminal SCFA concentration and rate of absorption were not affected ($P > 0.05$) by diet fed POST. Feeding a low F:C diet prior-to and during FR improved the recovery response for DMI but feeding this same diet after FR delayed the response for recovery of DMI and decreased ruminal pH.

4.2. Introduction

In ruminant production, limited access to feed or voluntary reductions in feed intake do occur and have been shown to negatively affect growth performance (Arthington et al, 2008; Pritchard and Mendez, 1990), and milk production (Roads et al., 2009). While severe short-term feed restriction (**FR**) is associated with a reduction in energy intake, recent studies have further demonstrated that short-term feed deprivation (Gäbel et al., 1993) and short-term FR decrease short-chain fatty acid (**SCFA**) absorption across the reticulo-rumen epithelium (Zhang et al., 2012), barrier function of the rumen epithelium (Gäbel and Aschenbach, 2002) or total digestive tract (Zhang et al., 2012), and compromises immune function (Marques et al., 2012). Although it may not be possible to prevent FR, in many cases the occurrence of FR is predictable such as for weaning (Gibb et al., 2000), transportation (Galyean et al., 1981; Pence et al., 2009), heat stress (West, 2003), parturition (Grummer et al., 2004), and during digestive (Brown et al., 2000) or metabolic disorders (Hansen et al., 2003). Thus, strategies to mitigate the negative effect of FR on absorptive function of the reticulo-rumen or to accelerate the recovery response have the potential to improve the health, welfare, and productivity of ruminants.

Absorption of SCFA across the reticulo-rumen provides the vast majority of the metabolizable energy supply for ruminants (Bergman, 1990) and plays a critical role in the regulation of ruminal pH (Penner et al., 2009; Aschenbach et al., 2011). Moreover, altering fermentability by increasing the proportion of concentrate in the diet increases SCFA absorption (Sehested et al., 2000). While the mechanisms behind this adaptive response are not fully elucidated, recent studies have shown that the adaptive response includes both functional adaptation (Etschmann et al., 2009; Penner et al., 2011) as well as an increase in the absorptive surface area (Dirksen et al., 1985; Bannink et al., 2008), both of which are indirectly and directly mediated by greater SCFA exposure (Penner et al., 2011). Thus, altering the forage-to-concentrate ratio (**F:C**) could be one practical strategy to accelerate the recovery response of the ruminal epithelia following exposure to short-term FR. The objective of the present study was to evaluate whether the F:C of diets fed prior-to and during FR, and those fed during recovery from FR affect the recovery of DMI, rumen fermentation, and SCFA absorption following exposure to short-term FR.

4.3. Materials and Methods

This chapter builds upon the experiment described in the previous chapter, but with a focus on the timeline for recovery following FR when cattle are fed diets differing in F:C. The chapter evaluates the effects of the recovery response upon return to ad libitum intake following FR, with the previous chapter (Chapter 3) evaluating the effects of FR and F:C of the diet fed prior-to and during FR on rumen fermentation and SCFA absorption. Interactions between subsequent recovery weeks and the dietary F:C fed prior-to and during FR and dietary F:C fed post FR on DMI, rumen fermentation, and SCFA absorption were evaluated. The experimental conditions and procedures have been provided in detail in Chapter 3 and thus will only be briefly described below. Throughout the study, heifers were cared for in accordance to the guidelines of the Canadian Council on Animal Care (2009), and experimental procedures were evaluated and pre-approved by the University of Saskatchewan Animal Research Ethics Board (protocol 20100021).

4.3.1. Experimental Design and Feeding Management

Twenty ovariectomized and ruminally-cannulated Angus × Hereford heifers were blocked by BW into 1 of 3 blocks, with 8, 4 and 8 heifers per block. The mean ± SD for BW were 463 ± 33, 482 ± 53, 485 ± 30 kg, for blocks 1, 2, and 3, respectively, with each block run consecutively to facilitate intensive sampling protocols. Within block, heifers were fed the high-forage diet (**HF**; F:C = 92:8; Table 3.1) or the moderate-forage diet (**MF**; F:C = 60:40; Table 3.1) prior-to (for 19 d) and during the 5-d FR period (**PRE**) as presented in Chapter 3. Following PRE, half of the heifers from each PRE treatment were assigned to the HF or MF diet for the duration of the 3-wk recovery period (designated as **REC1**, **REC2**, **REC3**, and collectively as **POST**) and were fed ad libitum. This approach resulted in 4 treatment combinations; (PRE-POST) HF-HF, HF-MF, MF-HF, and MF-MF. Throughout the experiment, heifers had free access to water and were fed once a day at 0800 h with refusals being collected and weighed prior to feeding.

4.3.2. Data and Sample Collection

4.3.2.1.Feed Sampling and Dry Matter Intake

The daily amount of feed offered and refused were recorded for DMI determination, with a 5% subsample of the daily feed refused collected and composited by cow within experimental periods. Barley grain and the mineral and vitamin supplement were sampled once a week with barley silage and grass hay sampled twice weekly. To account for changes in feed ingredient DM content, dietary DM was adjusted on a weekly basis and water was added to the MF diet to balance DM between the two diets. Methodology used to determine feed samples chemical composition was previously described in Chapter 3.

4.3.2.2. Ruminal pH

Ruminal pH was recorded every 2 min from d 1 of REC1 until the end of REC3 using the Lethbridge Research Centre Ruminal pH Measurement System (Dascor, Escondido, CA) as described in Penner et al. (2006). From these data, maximum, mean and minimum pH values were averaged for each cow for each d and then averaged across REC1, REC2 and REC3. The severity of ruminal pH depression was summarized by the duration (min/d) and area (pH × min/d) below the pH threshold 5.5. Data from d 7 of each recovery period were removed as ruminal contents were evacuated in order to measure the rate of SCFA absorption.

4.3.2.3. Blood, Ruminal Fluid, and Fecal Sampling

Blood, mixed ruminal digesta, and fecal samples were collected on d 5 of REC1 and REC3, with samples collected every 4 h over a 24-h period. Methodology used for collection and analysis of blood, ruminal fluid and feces have been provided in detail in Chapter 3.

4.3.2.4. Short-Chain Fatty Acid absorption

On d 7 of REC1 and REC3, the temporarily isolated and washed reticulo-rumen (WRR) technique was performed (Care et al., 1984; Gäbel et al., 1993; Zhang et al., 2012) as described in Chapter 3. The wash buffer and experimental buffer composition used were the same as described previously (Table 3.2), but the actual experimental buffer osmolality during REC1 and REC3 averaged 294.1 ± 1.7 mOsmol/kg.

4.3.2.5.Apparent Total Tract digestibility

Indigestible NDF (iNDF) from feed, feces and feed refusal samples from REC1 and REC3 was used as an external marker to evaluate apparent total tract digestibility (Huhtanen et al., 1994). Fecal output was estimated using the ratio between the intake of iNDF and fecal concentration as described in Chapter 3.

4.3.3. Statistical Analysis

Data were analyzed as a randomized complete block design using the PROC MIXED procedure of SAS (version 9.2, SAS Institute, Inc., Cary, NC) with heifer nested in block as the random effect, and block, dietary treatments, period, treatment × period, treatment PRE × treatment POST, and the treatment PRE × treatment POST × period as fixed effects. Two data sets were used with this model to evaluate the effect of the diet fed PRE on recovery, and to evaluate the effect of the diet fed POST on recovery. Measurement periods (REC1, REC2 and REC3) were used as a repeated measure and the covariance error structure that yielded the lowest Akaike's and Bayesian Information criterion for each dependent variable was used. Significance was declared when $P \leq 0.05$, and the Tukey's post-hoc mean separation test was used to compare means among treatments and periods. Tendencies are discussed when $0.05 < P \leq 0.10$.

4.4. Results

Although the PRE × POST and PRE × POST × period interactions were tested, there were no significant PRE × POST interactions and only one PRE × POST × period interaction (molar proportion of butyrate). Thus, data were presented to highlight the effects of PRE, PRE × period, POST, POST × period, and period.

4.4.1. Effect of the Diet Fed Prior-to and During Feed Restriction (PRE) on the Recovery Response

4.4.1.1. Dry Matter Intake, Ruminal Fermentation, and Short-Chain Fatty Acid Absorption

A PRE × period ($P = 0.045$; Table 4.1) interaction was detected for DMI as heifers fed HF PRE increased DMI throughout recovery, whereas, DMI did not differ among REC1, REC2, and REC3 for heifers fed MF PRE. Minimum, mean and maximum ruminal pH during recovery were not affected by diet fed PRE ($P > 0.05$) or the PRE × period interaction ($P > 0.05$), but were affected by period ($P = 0.002$, $P < 0.001$ and $P < 0.001$, respectively; Table 4.1). Minimum and mean pH increased by 0.23 and 0.30 pH units between REC1 and REC2, but were not different thereafter. Maximum pH ($P < 0.001$) was higher in REC3 than REC1 and REC2. Duration (amount of time pH < 5.5) was numerically higher during REC1 for heifers that were fed HF than MF PRE (191 vs. 98 min/d), with both treatments having marked reductions in duration from REC1 to REC2 with no further change in REC3 (PRE × period; $P = 0.003$). A period effect ($P < 0.001$) was detected for the area below a pH threshold of 5.5 (pH × min/d), with values decreasing from REC1 to REC2, with no further change in REC3.

Total SCFA concentration and the molar proportions of acetate, propionate and butyrate in ruminal fluid were not affected by the diet fed PRE ($P < 0.05$; Table 4.2). Likewise, total SCFA concentration was not affected by period with means ranging between 96.1 and 92.7 m*M* for REC1 and REC3 respectively. The molar proportion of acetate ($P < 0.001$) increased and that of propionate ($P < 0.001$) decreased from REC1 to REC3 despite heifers being fed the same diet across REC periods. The proportion of butyrate tended ($P = 0.069$) to be higher during REC1 for heifers fed HF PRE than for those fed MF PRE, but lower for heifers fed HF PRE during REC3 than for heifers fed MF PRE.

Total absolute and fractional SCFA absorption rates were not influenced by dietary treatment or period ($P > 0.05$; Table 4.2). However, PRE × period interactions were detected for the absolute ($P = 0.050$) and fractional ($P = 0.019$) butyrate absorption rates, as well as a tendency for an interaction between PRE and period for the fractional rate of propionate absorption ($P = 0.055$). Although the post-hoc test (Tukey's) did not determine differences

among means, the absolute and fractional butyrate absorption values were higher during REC1 for heifers fed MF PRE than for those fed HF PRE but lower for heifers fed MF PRE during REC3 than heifers fed HF PRE.

4.4.1.1.Plasma and Serum Metabolites, and Insulin

The diet fed prior-to and during FR did not affect plasma glucose or serum insulin during REC ($P > 0.05$; Table 4.2). However, glucose tended (PRE × period; $P = 0.058$) to be higher for heifers fed HF PRE than for heifers fed MF PRE in REC1 only, whereas, insulin concentration tended (PRE × period; $P = 0.088$) to be highest for heifers fed MF PRE than those fed HF PRE during REC1 only. Serum BHBA was not affected by treatment, period, or their interaction ($P > 0.05$). Non-esterified fatty acids were not affected by treatment but concentrations were higher during REC1 than REC3 (period; $P = 0.022$).

4.4.1.2.Apparent Total Tract Digestibility

There were no carry-over effects of the diet fed PRE or the period of recovery ($P > 0.05$) on DM, OM, or NDF digestibility (Table 4.3). On average, DM, OM, and NDF digestibility were 66.3, 68.9 and 58.3%, respectively.

Table 4.1. Effect of the interaction between diet fed prior-to and during feed restriction (PRE) and week of recovery (REC1, REC2, and REC3) on DMI and ruminal pH. Data presented are the means ± SEM for 10 heifers/treatment.[1]

	Period									
	REC1		REC2		REC3					
	Dietary treatment fed PRE							*P* value		
Variable	HF	MF	HF	MF	HF	MF	SEM	Diet	Period	Diet × Period
DMI, kg/d	8.7^{c}	9.9^{abc}	9.8^{b}	10.0^{abc}	10.9^{a}	10.5^{ab}	0.35	0.33	< 0.001	0.045
Minimum pH	5.55^{x}	5.57^{x}	5.85^{y}	5.74^{y}	5.74^{xy}	5.69^{xy}	0.074	0.36	0.002	0.36
Mean pH	6.05^{x}	6.21^{x}	6.43^{y}	6.43^{y}	6.43^{y}	6.52^{y}	0.068	0.27	< 0.001	0.16
Maximum pH	6.83^{x}	6.96^{x}	6.90^{x}	6.91^{x}	7.19^{y}	7.20^{y}	0.083	0.53	< 0.001	0.52
Duration pH < 5.5, min/d	190.8^{a}	98.2^{ab}	22.2^{b}	36.5^{b}	45.1^{b}	46.0^{ab}	28.49	0.40	< 0.001	0.003
Area, pH < 5.5 × min/d	70.0^{y}	39.4^{y}	2.7^{x}	9.0^{x}	58.4^{xy}	13.4^{xy}	20.57	0.22	< 0.001	0.052

[1]PRE × POST and PRE × POST × period interactions were not significant ($P > 0.05$).

[abc]Means, for the interaction between treatment and period, within a row with uncommon superscripts differ ($P \leq 0.05$).

[xyz]Period means within a row with uncommon superscripts differ ($P \leq 0.05$).

Table 4.2. Effect of the interaction between diet fed prior-to and during feed restriction (PRE) and week of recovery (REC1 and REC3) on ruminal SCFA concentration, SCFA absorption, and blood metabolites and insulin. Data presented are the means ± SEM for 10 heifers/treatment.[1]

	Period							
	REC1		REC3					
	Dietary treatment fed PRE					*P* value		
Variable	HF	MF	HF	MF	SEM	Diet	Period	Diet × Period
Ruminal SCFA								
Total SCFA, m*M*	94.4	97.8	92.5	93.0	2.30	0.41	0.15	0.53
Acetate, mol/100 mol	57.5	60.0	65.3	64.5	1.49	0.17	< 0.001	0.15
Propionate, mol/100 mol	27.3	27.1	20.6	21.2	1.97	0.89	< 0.001	0.78
Butyrate, mol/100 mol[2]	10.5	9.0	9.9	10.5	0.53	0.46	0.49	0.069
Absolute absorption rate, mmol/h								
Total SCFA	602.6	655.3	615.2	561.9	64.73	0.99	0.54	0.42
Acetate	316.1	344.0	315.6	345.9	35.97	0.43	0.99	0.98
Propionate	200.7	216.4	202.6	148.8	24.84	0.47	0.19	0.17
Butyrate	86.0	95.2	97.6	67.2	9.16	0.27	0.39	0.050
Fractional absorption rate, %/h								
Total SCFA	34.5	40.1	39.2	35.0	3.39	0.83	0.97	0.16
Acetate	30.5	35.9	34.7	36.6	3.30	0.28	0.47	0.59
Propionate	39.1	44.8	43.2	31.3	4.49	0.52	0.29	0.055
Butyrate	41.7	47.9	49.9	34.3	4.42	0.34	0.52	0.019
Plasma and serum metabolites and insulin								
BHBA, mg/dL	9.3	9.4	9.3	9.2	0.64	0.98	0.84	0.84
Glucose, mg/dL	73.7	68.6	69.6	69.8	1.54	0.17	0.30	0.058
NEFA, uEq/mL	139.2	155.3	140.1	138.2	6.54	0.38	0.12	0.088
Insulin, ug/L	2.32	1.81	0.71	1.24	0.400	0.99	0.022	0.21

[1]PRE × POST interactions were not significant ($P > 0.05$). Only one PRE × POST × period interaction was significant ($P < 0.05$).
[2]A PRE × POST × period interaction was detected ($P = 0.006$) with mean values (mol/100 mol) for HF-MF during REC1 = 11.8, MF-MF during REC3 = 11.8, HF-MF during REC3 = 10.5, MF-HF during REC1 = 10.2, HF-HF during REC3 = 9.2, HF-HF during REC1 = 9.2, MF-HF during REC3 = 9.1 and MF-MF during REC1 = 7.9 with a SEM = 0.755. Only HF-MF during REC1 and MF-MF during REC3 were different from MF-MF × REC1, while none of this treatment combinations differed from the rest.

Table 4.3. Effect of the interaction between the diet fed prior-to and during feed restriction (PRE) and week of recovery (REC1 and REC3) on apparent total tract digestibility. Data presented are the means ± SEM for 10 heifers/treatment.[1]

	Period							
	REC1		REC3					
	Dietary treatment fed PRE					*P* value		
Variable	HF	MF	HF	MF	SEM	Diet	Period	Diet × Period
DM, %	68.7	63.0	68.2	65.4	0.12	0.52	0.32	0.12
OM, %	71.1	66.2	70.5	68.0	0.14	0.66	0.38	0.14
NDF, %	60.7	56.4	60.3	57.3	0.22	0.91	0.75	0.22

[1]PRE × POST and PRE × POST × Period interactions were not significant ($P > 0.05$).

4.4.2. Effect of the Diet Fed Post Feed Restriction (POST) on the Recovery Response

4.4.2.1. Dry Matter Intake, Ruminal Fermentation, and Short-Chain Fatty Acid Absorption

Dry matter intake (POST × period; $P = 0.033$) for heifers fed MF POST during REC1 was 8.4 kg/d, and represented only 79% of the intake relative to that consumed in REC3 with DMI in REC2 not differing from REC1 or REC3 (Table 4.4). For heifers fed HF POST, DMI did not differ among recovery periods.

Feeding the MF diet caused a reduction in minimum ($P < 0.001$) and mean ($P = 0.002$) ruminal pH when compared to the HF diet (Table 4.4). However, regardless of diet, minimum and mean pH were lowest during REC1 ($P = 0.002$ and $P < 0.001$, respectively) increasing to REC2 with no change thereafter. While mean pH was affected by both treatment and period, there was a tendency for an interaction between treatment and period ($P = 0.051$). The interaction showed a numerically lower mean pH for heifers fed MF than HF during REC1, with this difference diminishing thereafter. Maximum ruminal pH increased ($P < 0.001$) from REC1 and REC2 to REC3 with a tendency ($P = 0.064$) for a POST × period interaction. A POST × period interaction was detected for the duration and area that ruminal pH was < 5.5 ($P < 0.001$), with heifers fed MF having a greater duration than those fed HF during REC1. During REC2 and REC3 there were no differences between dietary treatments within periods for both area and duration, although, numerically, heifers fed MF had a greater duration than heifers fed HF POST.

Dietary treatment and recovery period did not affect ruminal SCFA concentration ($P > 0.05$; Table 4.5). However, POST × period interactions were detected for the molar proportions of acetate ($P < 0.001$) and propionate ($P = 0.001$). The molar proportion of acetate was greater for heifers fed HF than MF during REC1 and REC3 but acetate did not differ among periods for heifers fed HF, whereas the molar proportion of acetate increased for heifers fed MF from REC1 to REC3. The molar proportion of propionate decreased markedly (by 10 mol/100 mol) from REC1 to REC3 for heifers fed MF POST to the extent that differences among treatments were not present during REC3. On the other hand, no differences in propionate concentration were observed between REC1 and REC3 for heifers fed HF. The molar proportion of butyrate was

greater (P = 0.048) for heifers fed MF than HF, but recovery period did not affect the response (P > 0.05). There were no effects of the diet fed POST or recovery period on absolute SCFA absorption or fractional SCFA absorption rate (P > 0.05; Table 4.5).

4.4.2.1.Plasma and Serum Metabolites, and Insulin

Plasma glucose was not affected by diet fed POST, nor was it affected by recovery period (P > 0.05; Table 4.5). Insulin concentration was numerically higher (POST × period; P = 0.020) for MF than HF during REC1, but insulin concentration decreased markedly for MF during REC3 without differences between dietary treatments during REC3. Serum BHBA and NEFA were not affected by diet fed POST or recovery period.

4.4.2.2.Apparent Total Tract Digestibility

Dry matter, OM and NDF total tract apparent digestibility were not affected by recovery period (P > 0.05; Table 4.6) or POST in the case of DM and OM (P > 0.05). However, NDF total tract apparent digestibility was nearly 9 percentage units greater for heifers fed HF (P = 0.007) than for those fed MF.

Table 4.4. Effect of interaction between diet fed post feed restriction (POST) and week of recovery (REC1, REC2, and REC3) on DMI and ruminal pH. Data presented are the means ± SEM for 10 heifers/treatment.[1]

	Period									
	REC1		REC2		REC3					
	Dietary treatment fed POST							*P* value		
Variable	HF	MF	HF	MF	HF	MF	SEM	Diet	Period	Diet × Period
DMI, kg/d	10.1^{ab}	8.4^{c}	10.4^{ab}	9.5^{bc}	10.7^{a}	10.7^{ab}	0.35	0.038	< 0.001	0.033
Minimum pH	5.78^{x}	5.31^{x}	6.10^{y}	5.48^{y}	6.02^{xy}	5.42^{xy}	0.077	< 0.001	0.002	0.59
Mean pH	6.34^{x}	5.92^{x}	6.55^{y}	6.32^{y}	6.51^{y}	6.44^{y}	0.069	0.002	< 0.001	0.051
Maximum pH	6.99^{y}	6.82^{y}	6.91^{y}	6.91^{y}	7.13^{x}	7.26^{x}	0.066	0.80	< 0.001	0.064
Duration pH < 5.5, min/d	14.1^{b}	274.9^{a}	0.0^{b}	58.7^{b}	1.2^{b}	89.9^{ab}	27.80	< 0.001	< 0.001	< 0.001
Area, pH < 5.5 × min/d	6.5^{b}	103.0^{a}	0.0^{b}	11.8^{b}	0.1^{ab}	71.7^{ab}	20.57	0.004	< 0.001	< 0.001

[1]Only main effects are shown as the PRE × POST and PRE × POST × period interactions were not significant ($P > 0.05$).

^{abc}Means, for the interaction between treatment and period, within a row with uncommon superscripts differ ($P \leq 0.05$).

^{xyz}Period means within a row with uncommon superscripts differ ($P \leq 0.05$).

Table 4.5. Effect of the interaction between the diet fed post feed restriction (POST) and week of recovery (REC1 and REC3) on ruminal SCFA concentration, SCFA absorption, and blood metabolites and insulin. Data presented are the means ± SEM for 10 heifers/treatment.[1]

	Period							
	REC1		REC3					
	Dietary treatment fed POST					*P* value		
Variable	HF	MF	HF	MF	SEM	Diet	Period	Diet × Period
Ruminal SCFA								
Total SCFA , m*M*	98.5	93.7	93.7	91.7	2.30	0.15	0.15	0.55
Acetate, mol/100 mol	65.6^{ab}	51.9^{c}	67.2^{a}	62.7^{b}	0.48	< 0.001	< 0.001	< 0.001
Propionate, mol/100 mol	20.7^{b}	33.8^{a}	19.9^{b}	22.0^{b}	1.97	< 0.001	< 0.001	0.001
Butyrate, mol/100 mol[2]	9.7	9.9	9.1	11.2	0.53	0.048	0.49	0.100
Absolute absorption rate, mmol/h								
Total SCFA	609.3	648.7	531.7	646.1	64.73	0.26	0.54	0.57
Acetate	312.5	347.6	295.3	366.14	36.15	0.15	0.99	0.63
Propionate	207.8	209.3	160.3	191.1	24.84	0.54	0.19	0.55
Butyrate	89.3	91.9	76.0	88.8	9.16	0.42	0.39	0.59
Fractional absorption rate, %/h								
Total SCFA	37.0	37.5	33.6	40.6	3.39	0.32	0.97	0.33
Acetate	32.0	39.3	32.0	39.3	3.29	0.15	0.47	0.47
Propionate	42.9	41.0	34.0	40.6	4.47	0.63	0.29	0.33
Butyrate	46.2	43.4	39.0	45.2	4.43	0.73	0.52	0.29
Plasma and serum metabolites and insulin								
BHBA, mg/dL	9.5	9.2	9.0	9.5	0.64	0.93	0.84	0.46
Glucose, mg/dL	68.8	73.5	69.1	70.3	1.54	0.104	0.30	0.21
NEFA, uEq/mL	155.2	139.3	141	137.1	6.54	0.23	0.12	0.24
Insulin, ug/L	0.91^{ab}	3.22^{a}	0.94^{b}	1.01^{b}	0.400	0.020	0.022	0.020

[1]PRE × POST interactions were not significant ($P > 0.05$). Only one PRE × POST × period interaction was significant ($P < 0.05$).
[2]A PRE × POST × period interaction was detected ($P = 0.006$) with mean values (mol/100 mol) for HF-MF during REC1 = 11.8, MF-MF during REC3 = 11.8, HF-MF during REC3 = 10.5, MF-HF during REC1 = 10.2, HF-HF during REC3 = 9.2, HF-HF during REC1 = 9.2, MF-HF during REC3 = 9.1 and MF-MF during REC1 = 7.9 with a SEM = 0.755. Only HF-MF during REC1 and MF-MF during REC3 were different from MF-MF × REC1, while none of this treatment combinations differed from the rest.
[abc]Means within a row without a common superscript differ ($P \leq 0.05$).

Table 4.6. Effect of the interaction between diet fed post feed restriction (POST) and week of recovery (REC1 and REC3) on apparent total tract digestibility. Data presented are the means ± SEM for 10 heifers/treatment.[1]

	Period							
	REC1		REC3					
	Dietary treatment fed POST					*P* value		
Variable	HF	MF	HF	MF	SEM	Diet	Period	Diet × Period
DM, %	64.7	67.0	66.5	67.1	2.09	0.57	0.52	0.57
OM, %	67.3	70.0	68.8	69.7	1.92	0.45	0.66	0.52
NDF, %	62.9	54.3	63.4	54.2	2.36	0.007	0.91	0.90

[1]Only main effects are shown as the PRE × POST and PRE × POST × Period interactions were not significant ($P > 0.05$).

4.5. Discussion

Beef and dairy cattle are exposed to periods of short-term FR that, in many cases, are predictable occurrences. Examples of predictable episodes of short-term FR include weaning (Boland et al., 2008), transportation and marketing (Loerch and Fluharty, 1999) and heat stress (Huber, 1996). In addition to decreased production (Tarrant and Grandin, 2000; Duff and Galyean, 2007), a growing body of research has demonstrated that short-term feed deprivation (Gäbel et al., 1993) and short-term FR (Zhang et al., 2012) also reduce SCFA absorption across the reticulo-rumen, and compromise barrier function of the ruminal epithelia (Gäbel and Aschenbach, 2002) and total digestive tract (Zhang et al., 2012). In fact, reported in Chapter 3 was a reduction in SCFA absorption rate occurred from BASE to FR (119.7 mmol/h). While these studies have documented reductions in absorptive and barrier function caused by a short-term reduction in feed intake, the recovery response and strategies to accelerate the recovery response have not been addressed adequately.

Increased rates of SCFA absorption (Gäbel et al., 1991; Uppal et al., 2003), enhanced barrier function (Lodemann and Martens, 2006), and increased papillae surface area (Bannink et al., 2008) are all reported outcomes from feeding diets that have a moderate-to-high fermentability relative to cattle or sheep fed diets that were high in forage or had low fermentability. These findings led to the hypothesis that the fermentability of the diet fed prior-to and during FR, and that fed following FR may accelerate the recovery response for SCFA absorption and alleviate the reduction in energy status induced by short-term FR. Although, we hypothesized that interactions between the F:C of the PRE and POST diets would be evident, we did not observe such a response and thus the discussion will focus on PRE and POST treatments effects and their interaction with week of REC.

4.5.1. Feeding a High-Forage Diet Prior-To and During FR (PRE) Negatively Affects the Recovery Response

A major finding of the present study was that feeding a HF diet prior-to and during FR negatively affected the rate of return to expected DMI, regardless of the F:C of the diet fed during recovery. It is common for newly-received feedlot cattle to have a slow rate of recovery for DMI and Hutcheson and Cole (1986) reported that 2 to 3 wk were required for newly

received cattle to achieve expected levels of feed intake. In contrast to the results of the current study, Cole and Hutcheson (1987) reported that the F:C of the diet fed prior to a feed restriction sequence (1 d of feed deprivation, 1 d of limit feeding, and 2 d of feed deprivation) did not affect DMI during the recovery period. However, it should be acknowledged that the treatments fed prior to FR were only applied for 3 d and steers were limit-fed at 1.75% of BW (Cole and Hutcheson, 1987). Thus it appears that in addition to the severity of the FR event (Zhang et al., 2012), the dietary F:C affects the rate that DMI increases following return to ad libitum intake.

Carry-over effects of the PRE diet on ruminal pH were also detected, as during REC1, low DMI occurred simultaneously with low mean ruminal pH for heifers fed HF relative to those fed MF PRE. It should be noted that the MF diets used in this study did not contain a substantial proportion of grain (< 40% on DM basis) yet the return to ad libitum intake following FR induced ruminal acidosis. Zhang (2012) used a similar experimental model for FR with a MF diet and also reported induction of ruminal acidosis with short-term FR during re-alimentation (duration that pH < 5.5 was 5.5 h/d for heifers restricted to 25% of their ad libitum intake). Further support comes from acidosis induction studies in which methodology used to induce acidosis often includes a period of FR followed by provision of highly fermentable carbohydrate (Goad et al., 1998; Krause and Oetzel, 2006; Dohme et al., 2008). For example, Goad et al. (1998) adapted steers to diets with a F:C of 80:20 or 20:80 prior to 24 h of feed deprivation. After feed deprivation, steers were fed ad libitum a 20:80 F:C diet and both dietary scenarios induced subacute ruminal acidosis (mean pH ranged between 5.0 and 5.5). Clearly, a severe short-term decrease in feed intake followed by provision of feed for ad libitum intake predisposes cattle to ruminal acidosis, even with a moderate amount of concentrate in the re-alimentation diet.

Ruminal SCFA absorption is reduced during a short period of moderate or severe FR (Zhang et al., 2012), regardless of the diet fed prior-to and during FR as reported in Chapter 3. Moreover, it appears that carry-over effects of FR on SCFA absorption are still evident 1 wk after the return to ad libitum feeding (Zhang et al., 2012). A reduction in SCFA absorption could be considered as an epithelial energy and protein conservation strategy during a period of nutrient deficit. An adaptation of epithelium metabolic activity to the low energy conditions may imply a reduction in protein synthesis (e.g., transporters) associated to SCFA absorption.

However, a reduction in SCFA absorption can also increase the risk for acid accumulation in the reticulo-rumen predisposing cattle to ruminal acidosis as shown in the current study as well as others (Gäbel et al., 2002; Penner et al., 2011; Zhang et al., 2012).

Although, in Chapter 3 we did not detect differences in SCFA absorption as affected by the F:C during the baseline or FR measurement periods, we did detect carry-over effects of the F:C of the diet fed PRE on the recovery for SCFA absorption where during REC1, cattle fed HF PRE had slower rates of butyrate absorption (mmol/h) but faster rates during REC3. With the data available it is not possible to elucidate a clear explanation for this response, especially considering that we did not observe an effect of the F:C on SCFA absorption during FR (Chapter 3). However, these data do show that carry-over effects of the diet fed prior to and during FR should be taken into account when developing dietary mitigation strategies for the negative effects of a severe short-term FR. Clear detrimental effects on DMI recovery and ruminal pH during re-alimentation can be expected when a HF diet is fed PRE, with these responses occurring independent from the diet fed POST.

4.5.2. Feeding a High-Forage Diet Following FR (POST) Improves the Recovery Response

Exposing cattle to a moderate short-term FR (50% of ad libitum intake for 5 d) has negative implications on energy balance (Velez and Donkin, 2005; Carlson et al., 2006) due to a reduction in nutrient provision but also by reducing the rate of SCFA absorption across the rumen (Zhang et al., 2012). The negative nutrient balance induced with FR may also be exacerbated by low voluntary feed intake post FR as shown in the current study and that of Zhang et al. (2012). A logical strategy to improve nutrient intake during times of low feed intake is to increase the nutrient density of the diet (NRC, 2001) by increasing the fat content (Fluharty and Loerch, 1997) or decreasing the F:C ratio.

While decreasing the F:C ratio could compensate for the decreased DMI, there is a risk for ruminal acidosis as feed intake increases (Brown et al., 2000; Penner et al., 2007), even when feeding a moderate-forage diet (F:C 60:40; Zhang et al., 2012). Accordingly, in the current study feeding MF POST increased the duration (h/d) that ruminal pH < 5.5 when compared to heifers fed HF during REC1, with a slower DMI recovery. It has been reported that subacute

ruminal acidosis induces a reduction in DMI, with DMI gradually increasing over time as mean ruminal pH increases (Brown et al., 2000; Krause and Oetzel, 2006; Zhang et al., 2012). Supporting this concept, we observed that heifers fed HF POST did not experience ruminal acidosis and had high DMI throughout REC1. The previous argument may also help to explain why NDF digestibility was higher for heifers fed HF than MF (Firkins et al., 1998; Oba and Allen, 1999) as in vitro studies have reported a reduction in NDF digestibility when ruminal pH remains at 5.4 for 4 h (de Veth and Kolver, 2004). This reduction in NDF digestibility is caused by a reduction in fibrolytic enzymes activity below ph 6 and a decrease in fibrolytic bacteria growth rate at low ruminal pH (Russell and Wilson; 1996; Calsamiglia et al., 2002; Colombatto et al., 2003)

Feeding diets with a lower F:C has been shown to accelerate adaptation of absorptive function (Etschmann et al., 2009) and barrier function (Lodemann and Martens, 2006), with proliferation of rumen papillae (Dirksen et al., 1985; Bannink et al., 2008) and stimulation of SCFA absorption (Dijkstra et al., 1993; López et al., 2003; Aschenbach et al., 2011). However, in our study feeding heifers a MF diet POST did not increase the rate of SCFA absorption after short-term FR with little to no changes occurring within the first 3 wk. It is not clear why an increase in SCFA absorption over time was not observed in our study given that Zhang et al. (2012) showed an increase in SCFA absorption after imposing a similar FR model. However, in that study, the same diet was fed prior-to and during FR. Thus, our data suggest that the effect of the PRE diet has a strong effect on the recovery response that may even outweigh the effect of the POST FR diet.

4.6. Conclusion

Results of this study suggest that reducing the F:C ratio prior to FR is a viable mitigation strategy that can be implemented when episodes of FR are predictable with observed improvements for the rate of recovery of DMI and higher ruminal pH during REC. Although, accelerating the recovery response by increasing the F:C ratio after FR improves DMI and ruminal pH, it does not affect SCFA absorption.

5.0. GENERAL DISCUSSION

5.1. Feed Restriction and Negative Implications for Ruminants

The literature reviewed in this thesis and results obtained from this project highlight the occurrence and the importance of severe FR events in conventional beef and dairy production. Severe FR is usually accompanied by other stress factors (i.e., weaning, transportation, parturition); however, it has been demonstrated that a short period of decreased feed intake alone can affect nutrient absorption across the rumen epithelium and compromise the gut barrier function (Gäbel et al, 1993; Gäbel and Aschenbach, 2002; Zhang et al, 2012). Studies evaluating mitigation strategies applied prior-to and post FR on cattle performance suggest that dietary strategies can be used to improve DMI and growth performance during recovery (Lofgreen et al., 1980; Hutcheson and Cole, 1986; Fluharty and Loerch, 1995). However, there is a paucity of data for whether the F:C can be used as a strategy to mitigate the effects arising from severe FR on rumen fermentation and absorptive function. This project provides relevant outcomes for a better understanding of the negative implications of a severe short-term FR under different dietary scenarios and evaluates the success of diets fed prior-to and during, and post FR on mitigating these negative effects. Detrimental effects on rumen epithelial absorptive function were identified regardless of the dietary F:C fed prior-to and during FR. Moreover, carry-over effects from the diet fed during this period have implications on the recovery response during re-alimentation to ad libitum feeding. In fact, feeding HF induced a slower DMI recovery and predisposed heifers to ruminal acidosis post FR. When evaluating the effects of the diet fed post FR, an improvement in DMI and ruminal pH were detected when a HF diet was fed relative to a MF diet.

5.2. Mitigating the Negative Impact of Feed Restriction and Managing the Risk for Ruminal Acidosis

Ingestion of large or moderate amounts of readily fermentable carbohydrates post FR can induce ruminal acidosis (Brown et al., 2000; Zhang, 2012), but cattle fed low amounts, such as grazing cattle are less likely to be exposed to this digestive disorder during re-alimentation. However, negative implications on SCFA absorption during FR when a high-forage diet is fed was reported in our study, with this effect most likely persisting during the recovery to ad libitum

intake (Zhang, 2012). When a HF diet was fed during re-alimentation, DMI was maximized regardless the diet fed prior-to and during FR. Decreased energy intake during FR requires metabolic adaptations for an efficient use of the nutrients available, inducing an adaptive response of the ruminal epithelium to decrease absorptive capacity to be in balance with SCFA supply. Under this scenario, a reduction in SCFA absorption during FR might in fact be beneficial as it could be expected that maintaining protein synthesis and replacement of facilitated transporters involved in SCFA absorption would be costly in terms of energy and protein. However, a rapid recovery of the absorptive capacity during re-alimentation is crucial to replenish the nutrient supply and minimize the impact of proton accumulation in the rumen.

For transported cattle, reducing the amount of time in transit reduces the impact of the FR. In fact, the recovery of pre-fasting weight and DMI post feedlot arrival is delayed with increases in the duration of the deprivation (Pate and Crockett, 1978; Fluharty et al., 1996). For transported and newly arrived feedlot cattle, morbidity and mortality rates were reduced and DMI and ADG improved post feedlot arrival when fed a diet with moderate fermentability (about 50% concentrate) prior to transportation (Hutcheson et al., 1984; Hutcheson and Cole, 1986). In addition, higher DM and energy intake, with a faster recovery of purchase weight and a reduction in morbidity were achieved when receiving cattle were fed a 50% concentrate diet (Lofgreen et al., 1980) compared with a high-forage diet. Moreover, receiving cattle fed a 75% concentrate diet increased total sick days when compared to animals fed a 50% concentrate diet (Lofgreen et al., 1980) or hay (Lofgreen et al., 1981). Increasing morbidity levels with moderate or highly fermentable diets could be associated with a reduced ruminal SCFA absorption capacity and a compromised barrier function post feed deprivation or FR (Gäbel et al, 1993; Gäbel and Aschenbach, 2002; Zhang, 2012), in addition to decreased pH and slower DMI recovery as demonstrated in the current study. Post FR, feeding a diet with moderate fermentability to receiving cattle can induce ruminal acidosis as demonstrated in this project. Ruminal acidosis coupled with compromised barrier function, could increase the translocation of pathogenic microorganisms (i.e., Fusobacterium necrophorum; Nagaraja et al., 2005) and antigens (i.e., lipopolysaccharides; Gozho et al., 2005; Plaizier et al., 2008). In accordance, Riviera et al. (2005) reported equations for bovine respiratory disease morbidity (%), DMI and ADG related to the proportion of forage inclusion in the receiving diet. According to their study,

increasing the roughage level in the diet from 40 to 100% decreased morbidity, whereas feeding a 25 to 50% roughage diet maximized DMI and ADG, compensating for the financial losses due to an increased morbidity during the first 4 wk of feedlot receiving. From a financial perspective, it seems that feeding a moderate or highly fermentable diet post FR would maximize producer profits during the first weeks post feedlot arrival. However, from an animal health and welfare standpoint, feeding a moderate-to-high concentrate level in the diet could induce ruminal acidosis within the first week of re-alimentation (Zhang; 2012) and increase the risk for infectious disease (Rivera et al., 2005). According to the findings of the current study, DMI could be maximized and risk for ruminal acidosis minimized when feeding a moderate fermentability diet prior-to FR and a HF diet for about 1 wk post FR. After 1 wk of re-alimentation to a HF diet, a transition to increasing concentrate diets could take place. For example, a preconditioning program could be applied to newly arrived feedlot cattle for about 30 d prior-to shipping and marketing, feeding a moderate fermentability diet ad libitum in a dry-lot. Post feedlot arrival, cattle would receive a HF diet (e.g., silage, hay) for 1 wk, followed by a step-up feeding program. With this approach, cattle health and welfare could be improved after a FR event.

A strategy proposed to reduce the risk for ruminal acidosis, is use of an adaptation period when introducing highly fermentable diets to cattle (Gäbel et al., 2002; Bannink et al., 2008, Penner et al., 2011). Adaptation programs to concentrate diets consist of a gradual increase of the concentrate proportion or exposure to a moderate-forage diet in order to increase SCFA concentration in rumen and enhance epithelial absorptive capacity (Sehested et al., 2000; Bannink et al., 2008). An adaptation period permits the rumen microbial bacterial community composition to evolve in accordance with the change in diet substrate. In addition, increasing the fermentability of the diet can promote the uptake of SCFA through the facilitated anion exchange of SCFA and $HCO3^-$, thereby extruding $HCO3^-$ into the rumen which helps elevate the buffering capacity of the rumen fluid (Aschenbach et al., 2011). However, in our study we reported that feeding heifers a diet with moderate fermentability prior-to and during FR was not successful for mitigating the negative impact of decreased ruminal pH when feeding the same diet post FR. This finding suggests that the ruminal adaptation did not adequately overcome the SCFA increase that occurred during re-alimentation or that the negative effects of a severe FR on

rumen epithelium absorptive capacity may persist during the re-alimentation period, as suggested by other authors (Doreau et al., 2003; Zhang, 2012).

For cattle with high metabolic demands such as lactating dairy cattle, inducing a subacute ruminal acidosis post FR can not only neatively affect cow health and welfare, but also reduce profits. For example, during the transition period, cows undergo a period of FR as parturition approaches (Hayirli et al., 2002). This effect could be exacerbated when metabolic diseases occur (Zamet et al., 1979; Hayirli, 2002). Increasing nutrient requirements postpartum due to lactation is not accompanied by a similar increase in nutrient intake, with cattle mobilizing body reserves in order to meet this deficit (Drackley, 1999). Increasing the concentrate proportion during this period could provide a higher nutrient dense diet and maximize nutrient intake; however, the risk for ruminal acidosis is problematic because it can predispose cows to disease (e.g., lameness; Nocek, 1997; Krause and Oetzel, 2006), thereby challenging the immune system and increasing economic losses due to negative effects on animal performance (e.g., milk fat depression; Stone, 1999). According to our results, DMI could be maximized and healthier ruminal pH conditions may be imposed postpartum when diet fermentability is increased prepartum and subsequently decreased postpartum. While feeding a low F:C diet is a dietary strategy already applied prepartum, feeding a diet with a high F:C postpartum would maximize DMI, with the potential to meet or increase the nutrient intake relative to those achieved by low DMI observed when a low F:C diet is fed during this period. In addition, it has the potential to reduce metabolic (e.g., displaced abomasum; Coppock, 1974) and digestive (e.g., ruminal acidosis; Penner et al., 2009b) disorders. However, further research on nutrition of transition dairy cattle are needed due to metabolic changes occurring during this period imposed by calving and the onset of milk production.

It is important to consider strategies that maximize nutrient intake and minimize the risk for ruminal acidosis after FR. Inclusion of buffers in the diet (e.g., sodium bicarbonate; Erdman, 1988) or promoting the production of endogenous buffers in saliva should be considered. Managing dietary forage particle length (Grant et al., 1990) and chemical fiber content (Beauchemin et al., 1994) can promote rumination and salivation, and consequently, increase ruminal buffering capacity. In addition, regulation of meal size and feeding frequency can also be implemented to minimize a reduction in pH and ameliorate the impacts of feeding highly

fermentable carbohydrates (Allen, 1997). Replacing the main grain source in the diet with grains with slower degradation rate (e.g., barley vs. corn; Ørskov, 1986) as well as manipulating grain processing (e.g., steam-rolled vs. dry-rolled barley; Mathison et al., 1991) can be also implemented as a strategy to decrease diet fermentation rate and minimize pH depression. Moreover, replacing the main energy source supplied by cereal grains (starch) with other energy sources (e.g., fat supplements) can also be beneficial.

5.3. Future Studies

According to the evidence presented in this thesis, further research on mitigation strategies for the negative effects of a severe FR is still needed. In order to preserve animal nutrient reserves and improve animal performance health and welfare, it is necessary to develop nutritional strategies that maximize nutrient intake post FR without causing digestive disorders. Strategies to promote SCFA absorption across the rumen epithelium have the potential to minimize the risk for ruminal acidosis and increase the uptake of metabolizable energy, promoting a more rapid recovery response post FR. Studies providing a better understanding on the mechanisms involved in the regulation of SCFA uptake under FR conditions are needed. The use of feed additives (e.g., buffer salts, rumen-bypass soluble carbohydrates and lipids) should be also considered in order to promote cattle health and performance under FR scenarios. Furthermore, developing a management system for predictable or unexpected FR events can help to mitigate or minimize the negative impact of a severe FR.

6.0. CONCLUDING REMARKS

Severe short-term FR in ruminants can occur at different stages in an animal's life under current management practices, with FR being predictable in most cases. Feed restriction has negative implications on rumen SCFA absorption and barrier function, with these two effects potentially responsible for the delayed recovery of DMI and rumen absorptive function during re-alimentation. When FR can be expected, dietary mitigation strategies, such as an increase or a moderate reduction in the F:C in the diet, can be implemented in order to attenuate the negative impact of a FR and minimize the risk for ruminal acidosis during re-alimentation. This project demonstrated that a combination of MF fed prior-to and during FR, followed by a HF diet post FR can lessen the negative effects of a FR. Further research on regulation of ruminal SCFA absorption prior to, during and post FR would be useful in promoting health and performance of ruminants.

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