THE EFFECTS OF DIETARY LIPID AND RUMEN PROTECTED *CAPSICUM* OLEORESIN ON PLASMA GLUCOSE AND INSULIN KINETICS, AND FEED EFFICIENCY IN FINISHING BEEF CATTLE

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ABSTRACT

This research was conducted to evaluate the effects of dietary lipids and rumen protected *Capsicum* oleoresin (i.e. capsaicin) on finishing beef cattle feedlot performance, DMI, carcass characteristics, circulating blood metabolites and acute phase proteins, and insulin sensitivity in response to an intravenous glucose tolerance test. In the first study, the experimental design was a 2×2+1 factorial arrangement including: no additive (control; **CON**); a low or high dose of rumen protected *Capsicum* oleoresin (CapsXL; 77 mg/d for **RPLO** and 250 mg/d for **RPHI**); or a low or high dose of *Capsicum* oleoresin that was rumen protected to a greater extent (Nexulin; 100 mg/d for **HPLO** or 322 mg/d for **HPHI**). The -LO and -HI doses were formulated to provide equal total ingested *Capsicum* oleoresin for both supplements (15.5 and 49.9 mg/day, respectively). A total of 450 steers were included, with 69 days-on-feed. All steers received the same basal diet of (dry matter basis) barley grain (86.2%), barley silage (6.0%), canola meal (6.2%), and a vitamin and mineral supplement (1.6%). Including rumen protected *Capsicum* in finishing steer diet did not affect their DMI, growth, or carcass weight. However, carcass yield grade, quality grade, and marbling score were decreased ($P \leq 0.032$) by increasing the dose of *Capsicum*. In the second study, rumen protected *Capsicum* product CapsXL was fed to 12 Hereford × Simmental beef heifers arranged in a 4 × 4 Latin square design balanced for carry-over effects, with 3 replications and 28-day period length. Treatments followed a 2 × 2 factorial design utilizing a barley-based high-grain diet that included *Capsicum* oleoresin at 0 (**C**$^{-}$) or 77 (**C**$^{+}$**)** mg/d and palmitic acid to increase dietary ether extract concentrations from 3.46% **(P**$^{-}$**)** to 7.63% **(P**$^{+}$**)** on a DM basis. The base diet consisted of barley silage (6.93%; DM basis), barley grain (82.12%), mineral (5.83%), urea (0.32%), and beet pulp (5.8%). Palmitic acid replaced beet pulp in the diet, which allowed similar forage:concentrate ratio and starch concentrations among diets. Apparent total tract digestibility, DMI, baseline fed-state blood sampling, and an intravenous glucose tolerance test and corresponding muscle biopsies to assess muscle glycogen concentrations were included in data collection in the second study. Palmitic acid increased peak insulin ($P = 0.003$), insulin positive incremental area under the curve, the total area under the curve for insulin (I-AUC and AUC, respectively; $P \leq 0.003$), insulin clearance rate ($P \leq 0.015$), and decreased both the maximum concentration of glucose reached and the maximum change in glucose concentration observed ($P \leq 0.029$) during the IVGTT. *Capsicum* prevented an increase

in insulin concentration prior to the IVGTT when fed with palmitic acid (*Capsicum* × palmitic acid interaction, $P = 0.017$). Including palmitic acid to finishing diets increased insulin resistance, but *Capsicum* may help moderate insulin concentrations. However, *Capsicum* elicited no effects during IVGTT in the second study ($P \geq 0.15$). As a high dose of *Capsicum* in the first study may negatively affect quality grades and marbling score, the lack of effect of *Capsicum* in the second study suggest that its effects in finishing beef rations may be dose dependent.

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3T3-L1	Isolated fibroblast cell line from mouse embryo
AcCoA	Acetyl CoA
AMPK	Adenosine monophosphate-activated protein kinase
AHFAT	Acid hydrolysis fat
Akt/PKB	Protein kinase B
ALA	Alanine
aPKC	Atypical protein kinase C
ARF	Adenosine diphosphate ribosylation factor
ARNO	Cytohesin/adenosine diphosphate ribosylation factor nucleotide-binding site opener
AUC	Area-under-the-curve
BCA	Bicinchoninic acid
CADG	Carcass adjusted average daily gain
CGRP	Calcium gene-related peptide
CON	Control
DAG	Diacylglycerol
Erk	Extracellular signal-regulated kinase
ESC	Ethanol soluble carbohydrate
EBW	Empty body weight
Fox01	Forkhead box 01
GLUT2	Glucose transporter 2
GLU	Glucose
GLUT4	Glucose transporter 4
GLY	Glycerol
Grb-2	Growth factor receptor-bound protein 2
GSK3/β	Glycogen synthase kinase 3 and beta
GSV	GLUT4 storage vesicles
GTT	Glucose tolerance test
HEC	Hyperinsulinemic euglycemic clamp
Hp	Haptoglobin
HPHI	High dose of RPC product HP
HPLO	Low dose of RPC product HP
I-AUC	Positive incremental area under the curve
IC	Insulin challenge
ICP	Inductively coupled plasma
IRS1/2	Insulin receptor substrate 1 and 2
IS_i	Sensitivity index
IVGTT	Intravenous glucose tolerance test
k	Clearance rate
LAC	Lactate
Maximum	Maximum level of glucose

MAPK	Mitogen-activated protein kinase
MCM	Methylmalonyl-CoA mutase
MEK	Mitogen-activated protein kinase kinase
mTORC1	Mammalian target of rapamycin complex 1
ncRNA	Non-coding ribonucleic acid
nPKC	Novel protein kinase C
OAA	Oxaloacetate
PA	Phosphatidic acid
PALM	Palmitic acid
PC	Pyruvate carboxylase
PCoAC	Propionyl-CoA carboxylase
PDK	3-phosphinositide-depended protein kinase
PEP	Phosphoenolpyruvate
PEPCK	Phosphoenolpyruvate carboxykinase
PEPCK-C	Phosphoenolpyruvate carboxykinase cytosolic
PI3K	Phosphoinositide 3-kinase
PIP2	phosphoinositol biphosphate
PIP3	phosphoinositol triphosphate
PKCα/β2/ζ/ λ	Protein kinase C alpha and beta2 and zeta and lambda
PLD	Phospholipase D
PROP	Propionate
PROPCoA	Propionyl CoA
PYR	Pyruvate
Raf	Rapidly accelerated fibrosarcoma
Range	Change in glucose level
Rheb	Ras homolog enriched in brain
RPC	Rumen protected capsaicin
RPHI	High dose of RPC product RP
RPLO	Low dose of RPC product RP
SAA	Serum amyloid A
SEM	Standard error of the mean
Shc	*Src* homology 2 and collagen homology domain-containing protein
SREBP-1c	Sterol-regulatory element-binding protein-1c
$T_{1/2}$	Time to decrease a given metabolite to half maximum level
TCA	Tricarboxylic acid cycle
TiO_2	Titanium dioxide
TNFα	Tumor necrosis factor alpha
TRiP	Triose phosphates
TRPV1	Transient receptor potential cation channel subfamily V member 1
TSC1/2	Tuberous sclerosis proteins 1 and 2
UCP1/2/3	Uncoupling protein 1 and 2 and 3

1.0 GENERAL INTRODUCTION

Feedlot cattle convert high starch feed sources into saleable cuts of meat. Young cattle are received into the feedlot setting and are quickly adapted to a high grain ration. This high grain or, 'finishing' ration is formulated to balance optimum energy from the grains with optimum fibre content from forages. Whereas the grain provides highly digestible substrate for the cattle to obtain as rapid weight gain as possible, the forage encourages rumen health (Chibisa et al. 2020; Allen, 1997).

In the conventional feedlot setting, feed grade antibiotics and rumen modifiers are added at specific rates to aid in digestion of feed by modifying the rumen environment. However, in recent years the demand for beef raised through less conventional means has broadened the feeding industry and opened niches adjacent to the conventional settings. Consumer interest in nutritional value of meat as well as interest in animal welfare has created demand for programs and markets such as grass-finished beef and organic beef. What sets these programs and markets apart is their stipulation and disallowance of use of antibiotics other than coccidiostats or histomonostats (Official Journal of the European Union, 2003).

Alternative feed additives that replace feed-grade antibiotics in the feedlot setting have been researched and include plant extracts and essential oils such as *Capsicum* oleoresin. Capsaicin, the active ingredient in *Capsicum* fruits is an extract that has been researched for many years (Watanabe et al. 1987; Oh et al. 2015). In the dairy setting, *Capsicum* treatment has been found to alter insulin sensitivity, feed intake, milk production, and blood levels of neutrophils and monocytes (Oh et al. 2015; Oh et al. 2017b). In the feedlot setting, research is more finite. Recent rumen protected *Capsicum* oleoresin (RPC) research has concluded potential health benefits when newly received feedlot calves are given RPC treatment (Westphalen et al. 2020). Studies such as Fandiño et al. (2008) have investigated the effects of rumen-available capsaicin when fed to heifers fed a high concentrate diet. However, there is no research that solely evaluates RPC throughout the finishing period.

Capsicum fed in a non-rumen protected form such as *Capsicum* oil has shown potential for production benefits such as rumen fermentation modification, however results have been conflicting (Tager and Krause, 2011; Cardozo et al. 2006). In the dairy setting, post-ruminal infusion of *Capsicum* has had additional effects such as t-lymphocyte proportion alteration, and

RPC has been found to linearly increase milk production and enhance feed efficiency – as measured by the relation of milk production to feed intake (Oh et al. 2013; Oh et al. 2017b). Furthermore, capsaicin has been found to increase pancreatic lipase and trypsin activity in rats (Platel and Srinivasan 2000).

Rumen Protected *Capsicum* oleoresin treatment in the dairy setting has been found to alter insulin sensitivity by way of decreasing the insulin area under the curve during a glucose tolerance test (Oh et al. 2017b). Since insulin resistance, feed efficiency, and additional metabolic changes are observed as time in feedlot and therefore the age of cattle increases (Verde and Trenkle 1987; Shingu et al. 2001; Joy et al. 2017), RPC may be of benefit in the feedlot setting

Based on the existing literature and the performance potential of RPC shown in previous research, these studies were conducted in the feedlot setting to investigate the effects of RPC fed in concert with conventional feedlot practices. This research includes a feedlot performance study to investigate the effects of feeding one of two RPC products which differ in level of rumen protection at one of two levels of inclusion. Part two of the research includes a metabolic study of one of the RPC products used in feedlot performance study.

2.0 LITERATURE REVIEW

2.1 Feedlot diets and cattle carcass trends

North American feedlots fulfill consumer demand for high quality beef by growing calves in an intensive setting. While keeping animal health and welfare in the forefront, feedlot managers consistently search for strategies to improve efficiency and profitability. Inherent to the industry, risks such as market volatility, consumer demand/approval, and costs of production including feed and calf costs all urge feedlot managers toward improvement and excellence. Marketing and price insurance programs exist to aid managers to mitigate risk in the marketplace; however, more hands-on approaches such as proper feed formulation and understanding the metabolic needs of the animal are becoming increasingly necessary to control performance parameters such as the cost:gain ratio of feedlot cattle. Determining appropriate maturity for harvest of feedstuffs and determining dry matter (DM) content of feeds represent the attention to detail that feedlot managers put into feed formulation. This attention to detail, combined with the necessity for increased efficiency in the feedlot operation has created the opportunity for the introduction of new tools for the feedlot manager. Tools such as accurate, software-based feed formulation and delivery systems have not only increased the efficiency of use of long-used conventional feed additives such as monensin but have enabled the inclusion and research of novel plant extract feed additives such as eugenol and *Capsicum* oleoresin (Geraci et al. 2012; De Souza et al. 2018; Cardozo et al. 2006; Duffield, Merril, and Bagg, 2012).

The purpose of this review is to summarize the literature regarding the performance characteristics of feedlot cattle during the finishing phase. The beginning will review common tangible performance parameters for feedlot cattle. Performance of calves newly received into the feedlot will be discussed. However, emphasis will be given to performance and metabolic changes of animals during the final ‘finishing’ stage in the feedlot. As the review progresses, the metabolic challenge of insulin resistance in cattle will be discussed, as will insulin and glucose homeostasis in cattle. The inclusion and observed effects on insulin kinetics of plant-based additives in cattle rations will be discussed before the review finishes with emphasis on one plant extract, capsaicin.

2.2 Feed intake and ADG

Feed intake and average daily gain (ADG) are standard performance parameters for feedlot cattle. Feed intake is usually presented as dry matter intake (DMI) in the form of kilograms per day (kg/d) or can be presented as cumulative data if weight of feed mixed into ration is recorded and consistent dry matter analyses are conducted on feed ingredients (Schoonmaker et al. 2003). One benefit of the cumulative method is that it presents a value that takes into account differences in days on feed between treatments. For example, partial data from Schoonmaker et al. (2003) shows that limit feeding steers increases the time to achieve a set backfat thickness without significantly changing cumulative DMI. However, if days on feed are held constant, feed intake, ADG, backfat thickness, and carcass weight are decreased when calves were limit fed, which is to be expected.

The ADG of cattle is commonly expressed as full body weight gain and represents the deposition of fat, protein, bone, as well as internal organs (Vasconcelos et al. 2009; Eisemann et al. 1997; Owens et al. 1995). Another approach to represent ADG to estimate nutrient retention is empty body weight gain (Owens et al. 1995; Sainz et al. 1995). As done by Sainz et al. (1995), empty body weight can be calculated by subtracting gastrointestinal tract weight from fasted weight. Multiple equations exist to calculate empty body weight, a parameter which is not to be confused with other carcass parameters such as carcass weight and quality, which are more suitable when calculating production economics (Owens et al. 1995). An additional approach is to calculate carcass-adjusted gain, or carcass adjusted average daily gain (CADG). CADG is calculated by first dividing the individual hot carcass weight by the average dressing percentage of the slaughter group to yield a carcass-adjusted final shrunk bodyweight (Koenig et al. 2020). ADG is then calculated by dividing the difference between final and initial shrunk bodyweights by the number of days on feed, yielding CADG (Koenig et al. 2020).

Receiving and growing period diets have been heavily researched, with objectives such as to quantify parameters related to compensatory gain, to observe and compare effects of dietary energy concentrations (Sainz, De la Torre, and Oltjen, 1995; Tomczak et al. 2019), to evaluate differences in carcass traits (Vasconcelos et al. 2009), and to compare performance of cattle on different energy sources with different rates of growth (Schoonmaker et al. 2003). Tables 2.1, 2.2, and 2.3 provide partial findings of such studies. In part, Table 2.1 shows that feeding

differing diets such as a high fiber diet during backgrounding may cause decreased ADG compared to steers fed a high concentrate diet ad libitum (Schoonmaker et al. 2003). However, in steers finished to equal backfat thickness of 1.27 cm, limit-feeding high concentrate rations to achieve 1.2 or 0.8 kg/d ADG during the 100-day growing period did not extend the growth-curve nor did it enhance intramuscular fat deposition at slaughter when compared to steers fed ad libitum intake of a high-concentrate or high-fiber diet during the growing period (Schoonmaker et al. 2003). Total feed offered to the limit-fed calves was determined by calf individual body weight and energy required to achieve target gain as defined by NRC (1984). Calves targeted to gain 1.2 or 0.8 kg/d ADG consumed 4.6 and 3.5 kg DMI, respectively, compared to 6.5 kg DMI for calves fed ad-libitum high concentrate ration (Schoonmaker et al. 2003). Decreases in DMI and ADG observed when limit feeding during the growing period are diminished during the finishing period when all cattle are placed on ad libitum diets (Vasconcelos et al. 2009). When altering energy density of receiving diets, Tomczak et al. (2019) (data shown in Table 2.2) concluded no treatment differences in body weight, ADG, or gain to feed ratio (G:F).

Factors unrelated to ration formulation such as body-frame size and growth-related hormone concentrations have been found to impact ADG and feed intake (Verde and Trenkle, 1987; Shingu et al. 2001). Larger steers have been found to have higher rate of gain and daily feed intake, as well as higher mean concentrations of growth hormone and cortisol (Verde and Trenkle, 1987). Although growth hormone was found to decrease with age, insulin concentrations were found to increase with age and have positive correlations (R) of 0.55 with DMI and 0.81 with BW (Verde and Trenkle, 1987). In a study comparing Holstein heifers to Japanese Black heifers, the basal growth hormone level in the smaller, slower-growing Japanese heifers was lower. Additionally, basal plasma insulin was much higher in Japanese Black Heifers than Holstein heifers after sexual maturation. These two factors could also have combined to contribute to the increased marbling and smaller frame in the Japanese heifers (Shingu et al. 2001).

These findings, although not novel, are foundational to understanding the needs of cattle in order to meet the market demands. In Canadian steers, three-year average data ending in 2018 show that cold carcass weights were 407 kg, compared to three-year average ending in the year 2000, during which time carcasses averaged 363.33 kg (Agriculture and Agri-Food Canada,

Table 2.1 Summary of effects from differing growing diets on overall feedlot dry matter intake (DMI) and average daily gain (ADG).

Study	Treatment	DMI (kg/d)	ADG (kg/d)	Conclusion
Schoonmaker et al. 2003 (Exp. 1)[1,2]	High concentrate ad libitum	7.7	1.69[a]	Tendency for high concentrate to have higher ADG (Reported *P*-value: $P < 0.10$)
	High fiber ad libitum	7.8	1.57[b]	
Schoonmaker et al. 2003 (Exp. 2)[1,3]	High concentrate ad libitum	7.8	1.33	High fiber caused numerically lower ADG
	High fiber ad libitum	7.9	1.29	
Vasconcelos et al. 2009[4]	High corn ad libitum	10.9[a]	1.95[a]	Limit feeding during growing period decreases overall intake and average daily gain ($P < 0.05$)
	Low corn ad libitum	10.6[a]	1.87[a]	
	Limit fed high corn[5]	8.4[b]	1.65[b]	

[a,b,c]Within column and study, means without common superscript differ.
[1]difference in study declared at $P < 0.10$
[2]fed to backfat thickness of 1.27 cm
[3]fed to 273 days on feed
[4]difference in study declared at $P < 0.05$
[5]ration limited to provide same energy as ad libitum low corn.

Table 2.2 Summary of effects differing growing rations have on dry matter intake (DMI) and average daily gain (ADG) in growing and finishing periods.

Study	Treatment	DMI (kg/d)		ADG (kg/d)		Conclusion
		Growing	Finishing	Growing	Finishing	
Schoonmaker et al. 2003 (Exp. 1)[1]	High concentrate ad libitum	6.5	9.0	1.54[a]	1.85	High concentrate tended to have higher ADG during growing period
	High fiber ad libitum	6.3	9.4	1.16[b]	1.95	
Schoonmaker et al. 2003 (Exp. 2)[1]	High concentrate ad libitum	6.4[a]	8.6[b]	1.55[a]	1.21[b]	High fiber grower tended to increase DMI during finishing
	High fiber ad libitum	6.1[b]	9.0[a]	1.16[b]	1.37[a]	
Vasconcelos et al. 2009[2]	High corn ad libitum	10.2[a]	11.6	2.32[a]	1.70	Limit feeding high corn and feeding low corn decreases ADG during growing
	Low corn ad libitum	10.0[a]	11.2	1.86[b]	1.88	
	Limit fed high corn[3]	6.5[b]	10.3	1.51[b]	1.75	
Tomczak et al. 2019	FIN (1.39 Mcal NEg/kg)	6.07	-	1.51	-	No differences seen in this data
	FIN+hay	6.01	-	1.48	-	
	CON (0.93 Mcal NEg/kg)	6.65	-	1.62	-	

[a,b,c]Within column and study, means without common superscript letter differ.
[1]Difference in study declared at $P < 0.10$
[2]Difference in study declared at $P < 0.05$
[3]Diet limited to provide same energy as ad libitum low corn.

2021). This trend suggests increased days on feed to produce larger steers as time progresses.

2.3 Body composition and G:F

By understanding the needs of feedlot cattle, formulating rations accurately, and targeting a lower ADG (via feed restriction) during the growing period, body composition may be manipulated. The effects of feed restriction can be manipulated by the duration, severity, and age of the animal at the time of feed restriction (Schoonmaker et al. 2003). For example, an extended backgrounding period, or a backgrounding ration targeting a restricted ADG of 0.8 kg/d can result in less empty body fat (Gill et al. 1993; Schoonmaker et al. 2003).

In a review of the literature, Owens et al. (1995) explained that research data supported the notion that compositionally, body fat percentage appears to be constant at a given fraction of mature body size (defined as maximum protein mass). They continue that when protein accretion declines to zero, body fat is approximately 36% of empty body weight (EBW). The difficulty with this model is accurately estimating the point at which protein accretion becomes zero. Additionally, fat accretion during body growth was later found to be altered by diet (Vasconcelos et al. 2009), with steers fed ad libitum high concentrate diets through both backgrounding and finishing found to have highest hot carcass weight and carcass fat percentage when compared to steers limit fed a high concentrate diet during backgrounding (Schoonmaker et al. 2003). Figure 2.1 shows the relationship between protein and fat mass to empty body weight with data attained from 16 studies including 1,174 cattle (Owens et al. 1995). The data therein indicate that mass of fat increases quadratically with weight whereas mass of protein increases more linearly. Although this data may suggest overall faster accretion of fat, the authors explicitly present that in faster gaining steers (>1.3 kg/d empty body ADG), fat accretion reaches a plateau of 550 g per day (Owens et al. 1995).

From an energy standpoint, the deposition of fat and protein have different efficiencies. In fact, on a caloric basis, the accretion of fat is 1.6 times more energy efficient at 76% efficiency with 24% heat loss, than the accretion of protein at 47% efficiency with 53% heat loss (reviewed by Owens et al. 1995). However, because the deposition of protein includes more water, fat gain by weight is only one quarter as efficient as gain of lean tissue (Owens et al. 1995). It also

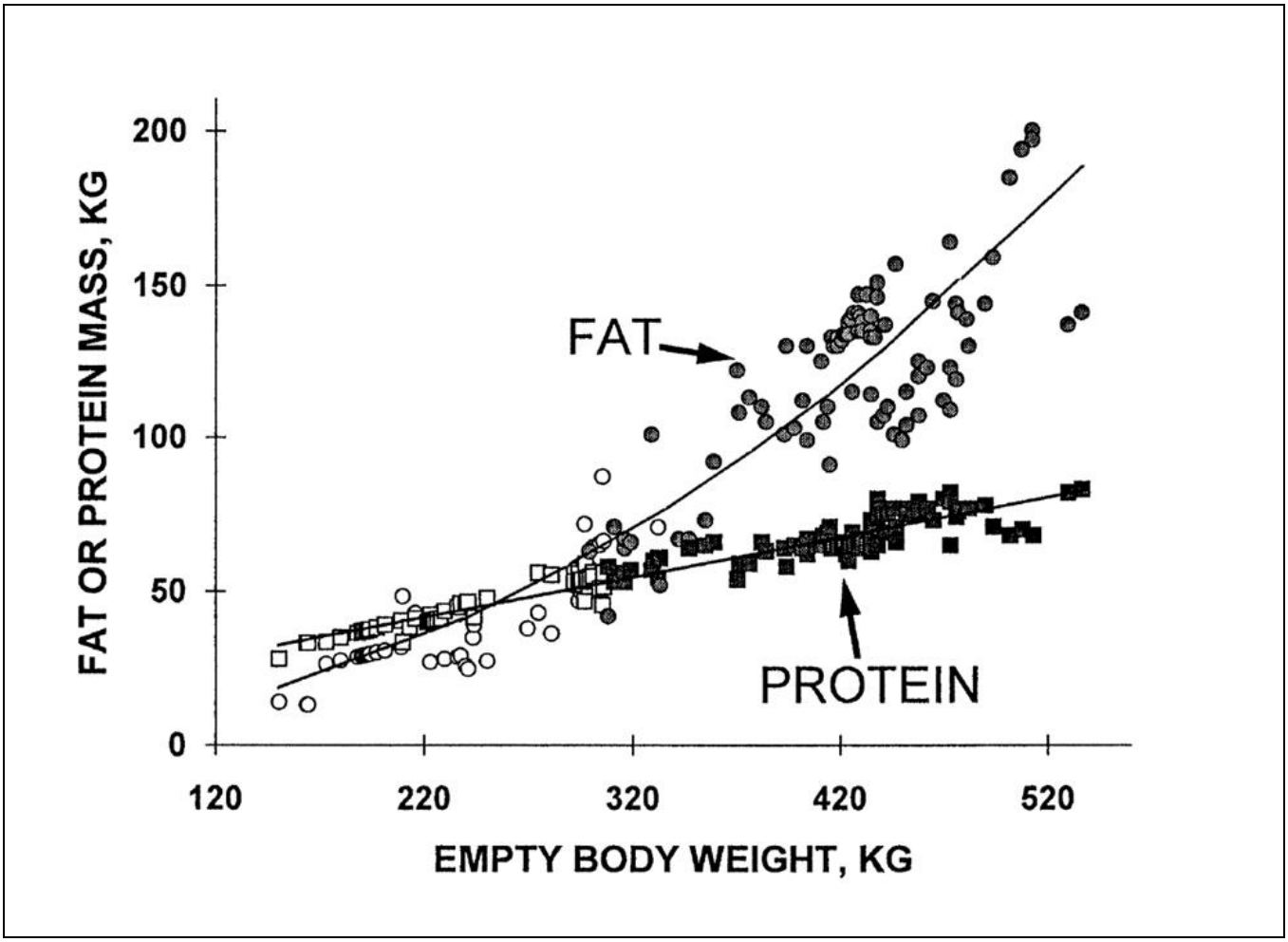

Figure 2.1 Mass of fat (circles) and protein (squares) of feedlot cattle at various empty body weights (EBW). Open symbols represent the beginning of feeding period and filled symbols represent the end (Owens et al. 1995; used with permission via Oxford University Press, license number: 5390400122476).

appears that specific substrates are required for the accretion of different tissues. For instance, glucose provides 50 to 75% of the acetyl units for intramuscular fat, whereas acetate is more valuable for subcutaneous fat as it contributes 70 to 80% of acetyl units (Smith and Crouse, 1984).

As represented by the quadratic increase in fat deposition as weight increases, calf metabolism changes as weight increases and time progresses. Eisemann et al. (1997) concluded that heavier, fatter steers are less responsive to and less sensitive to insulin. Furthermore, a positive relationship has been found between plasma insulin concentration and degree of fatness in the carcass (Trenkle and Topel, 1978). Results from other studies have strongly suggested the existence of age-related insulin resistance (Shingu et al. 2001), which relates to a decrease in feed efficiency (Joy et al. 2017).

Diet can also be used to control carcass fat deposition, with differences observed in fat thickness, yield grade, and internal fat (Sainz et al. 1995). Compared to steers fed high concentrate diet ad libitum, steers limit-fed concentrate were found to have unaffected carcass fat content, although the distribution was altered such that they had less subcutaneous fat, more internal fat, and less abdominal fat (Sainz et al. 1995). In humans, subcutaneous fat has a major metabolic buffer capacity, a role that can be lost in the presence of positive caloric balance (Patel and Abate, 2013). Not surprisingly, a prolonged positive caloric balance is observed in feedlot diets. Following this loss of buffer capacity is a deposition of triglycerides into non-adipose tissues such as skeletal muscle, the heart, and liver, commonly referred to as ectopic tissues (Patel and Abate, 2013; Snel et al. 2012).

Table 2.3 provides G:F data from the studies in Tables 2.1 and 2.2. Although Table 2.3 does not elucidate statistical difference between dietary treatment, a consistent trend of numerically decreasing feed efficiency from backgrounding to finishing is observed. The only exception of this trend is steers fed high fiber diet ad libitum in the backgrounding phase and finished on a high concentrate diet to a backfat thickness of 1.27 cm were observed having increased G:F (Schoonmaker et al. 2003). This occurrence may suggest a compensatory response of G:F, as a similar occurrence was observed by Vasconcelos et al. (2009). However, data from Vasconcelos et al. (2009) (presented in Table 2.3) do not report a numerical increase in G:F for those calves fed either a low-corn ad libitum or limit-fed high corn ration during the growing period. Sainz et

Table 2.3 Summary of effects differing growing rations have on gain to feed (G:F) ratio during backgrounding, finishing, and backgrounding and finishing periods combined.

Study	Treatment	G:F (kg/kg)			Conclusion
		Background	Finishing	Combined	
Schoonmaker et al. 2003 (Exp. 1)[1]	High concentrate ad libitum	0.237[a]	0.207	0.220[a]	High fiber had lower G:F during backgrounding and overall
	High fiber ad libitum	0.186[b]	0.209	0.201[b]	
Schoonmaker et al. 2003 (Exp. 2)[1]	High concentrate ad libitum	0.241[a]	0.140[b]	0.170[a]	High fiber lower G:F than high concentrate, overall
	High fiber ad libitum	0.189[b]	0.153[a]	0.163[b]	
Vasconcelos et al. 2009[2]	High corn ad libitum	0.228[a]	0.156[b]	0.183	Limit feeding high corn diet can numerically increase combined G:F, however bodyweight was much lower than ad lib high corn ($P < 0.01$)
	Low corn ad libitum	0.185[b]	0.177[a]	0.180	
	Limit fed high corn[3]	0.232[a]	0.179[a]	0.196	
Tomczak et al. 2019	FIN (1.39 Mcal NEg/kg)	0.247	-	-	No differences found.
	FIN+hay	0.245	-	-	
	CON (0.93 Mcal NEg/kg)	0.239	-	-	

[a,b,c]Within column and study, means without common superscript letter differ.
[1]Difference in study declared at $P < 0.10$
[2]Difference in study declared at $P < 0.05$
[3]Diet limited to provide same energy as ad libitum low corn.

al. (1995) also observed increases in EBW G:F during the finishing phase on a high concentrate diet in calves that were fed either a low concentrate diet or a high concentrate diet with limited access (to match weight gain of low concentrate calves) during the backgrounding phase. Furthermore, Sainz et al. (1995) conclude an increase in DMI plays a major role in compensatory gain, an effect also observed in Schoonmaker et al. (2003) as DMI for steers fed high fiber backgrounding diet increased ~50% during the finishing phase (Table 2.2). Data from Vasconcelos et al. (2009) indicate that source of energy during growing period does not affect overall G:F at the end of the finishing period. However, feed sources with lower digestibility can be expected to decrease G:F (Joy et al. 2017). Interestingly, Tomczak et al. (2019) observed no differences between treatments (data shown in Table 2.3). However, numerical decreases in G:F in each dietary treatment were seen even during the first 56 days in feedlot.

2.4 Cattle and development of insulin resistance

2.4.1 Insulin and glucose homeostasis

The major function of insulin is to increase glucose uptake into insulin sensitive tissues such as muscle and adipose (Tokarz et al. 2018). Not only is it imperative to meet the energetic demands of the tissue, insulin-stimulated glucose uptake is key to maintain glucose homeostasis (Tokarz et al. 2018; Manna and Jain, 2013). Further, insulin's control on muscle and adipose glucose consumption versus the hepatic output of glucose maintains glucose homeostasis (Karlsson et al, 2001). Not only a vital energy source, glucose is also a building block for macromolecules such as glycogen (Olson and Pessin, 1996). Glycogen can be stored both in skeletal muscles and the liver. However, only liver stores of glycogen can directly contribute to blood glucose level as the liver possesses glucose-6-phosphatase which converts glucose-6-phosphate to glucose (De Koster and Opsomer, 2013).

Evidence suggests the secretion of insulin and its antecedent hormone, glucagon, is controlled by the nervous system. Innervation of the pancreas is such that insulin output is increased by stimulation of the parasympathetic vagus nerve; whereas, an increase in glucagon and a decrease in insulin output is observed with sympathetic splanchnic nerve innervation (Osundiji and Evans, 2013; Bergman and Miller, 1973; Bloom and Edwards, 1975). For reference, Figure 2.2 shows the travel of insulin through the body, as presented by Tokarz et al.

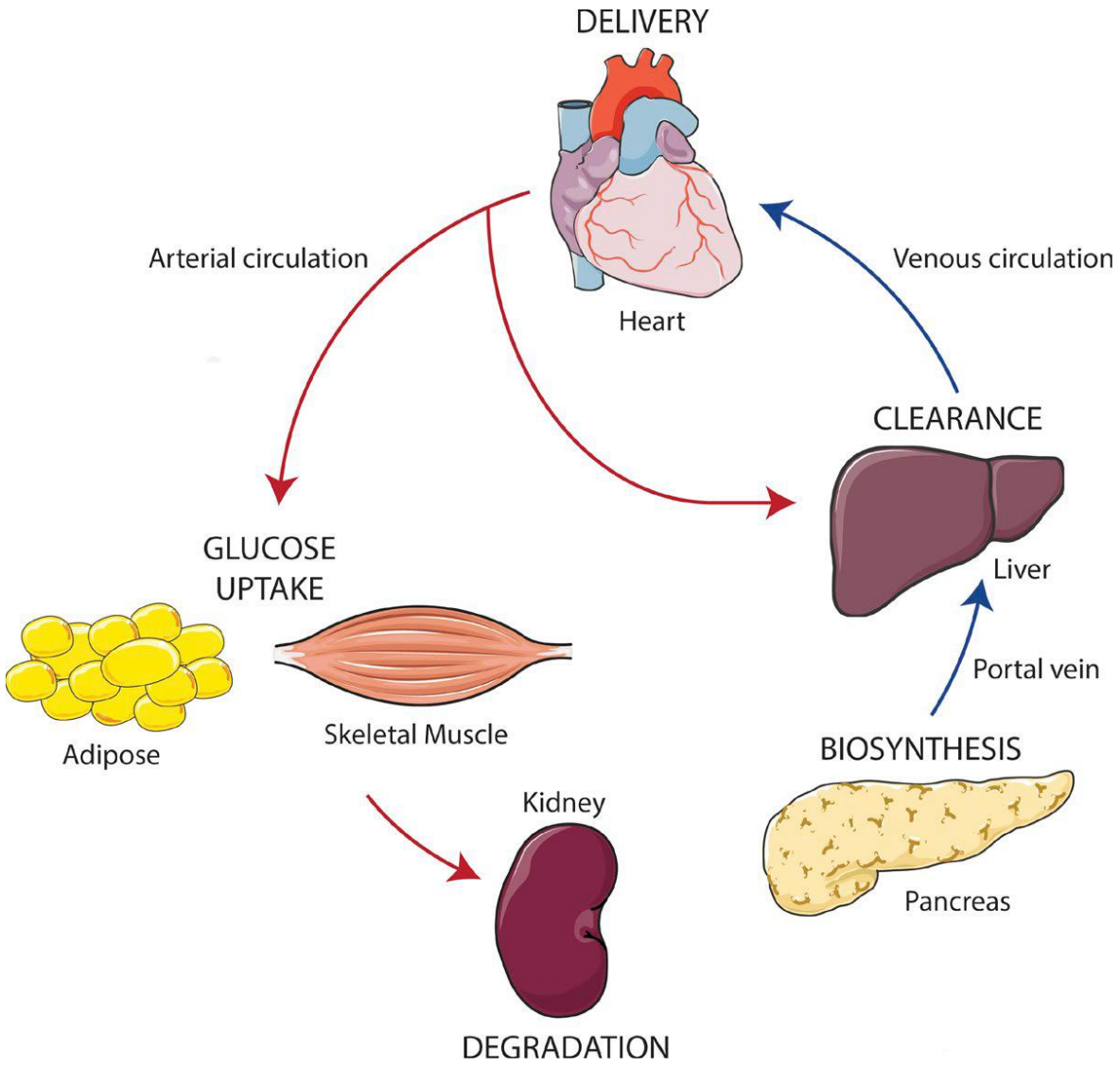

Figure 2.2 Journey of insulin in the body (Tokarz et al. 2018).

(2018). Hyperglycemia stimulates insulin secretion, which then stimulates glucose uptake into myofibers and adipocytes (Oh, 2015; Tokarz et al. 2018). To aid glucose uptake, activation of the microcirculation allows insulin delivery to tissues. To ensure proper clearance, the kidneys fulfill a critical role as they engage in insulin degradation to put an end to the hormone's action (Tokarz et al. 2018). Glucagon, conversely, is secreted to increase blood glucose level (Oh, 2015; Tokarz et al. 2018). The actions of these two hormones are coordinated to maintain a mean glucose level in beef cattle of 4.2 to 4.4 mmol/L, which can fluctuate down to 2.9 mmol/L during lactation in Shorthorn cattle (Doornenbal et al. 1988).

2.4.2 Insulin signalling and regulatory pathways

Once in the bloodstream, glucose uptake into adipocytes and muscle fibres is promoted by insulin (Tokarz et al. 2018). In addition to this vital role, insulin regulates networks of gene transcripts in muscle and liver (Batista et al. 2019). Disruptions in insulin action or an increase in insulin resistance can be detrimental and lead to complications in cattle (Nazifi et al. 2004; Taniyama et al. 1993), with effects of gene regulation surrounding proper/improper insulin action still being elucidated in recent research (Batista et al. 2019; Manna and Jain, 2013).

The insulin activation of insulin receptor tyrosine kinase which phosphorylates insulin receptor substrate (IRS) proteins and the subsequent activation of two major pathways, the phosphoinositide 3-kinase/Akt (also known as protein kinase B (PKB) (PI3K/Akt) pathway and the Ras/mitogen-activated protein kinase (MAPK) pathway, is well researched and reviewed (Batista et al. 2019; Taniguchi et al. 2006; Avruch, 1998). For visual representation of these pathways, refer to Figures 2.3 and 2.4. Insulin regulation of elements downstream of the PI3K pathway include those responsible for regulation of glucose transport, whereas the Ras-MAPK pathway is crucial for cell differentiation and mitogenesis (reviewed by Avruch, 1998). Further research by Li et al. (2010) found a branching point in the insulin signal through activation of AKT. Before reaching AKT, the insulin signal proceeds through the tyrosine phosphorylation of IRS-1 or IRS-2 (Li et al. 2010). After AKT phosphorylation, the multitude of downstream actions of insulin indicate AKT as a major amplification switch in the insulin signalling pathway (Tan et al. 2012). Shown in Figure 2.3 below, when the signal reaches AKT it may continue either toward lipogenesis (sterol-regulatory element-binding protein-1c (SREBP-1c) involved pathway), gluconeogenesis, or glycogen synthase kinase 3-β (GSK3β) which has been

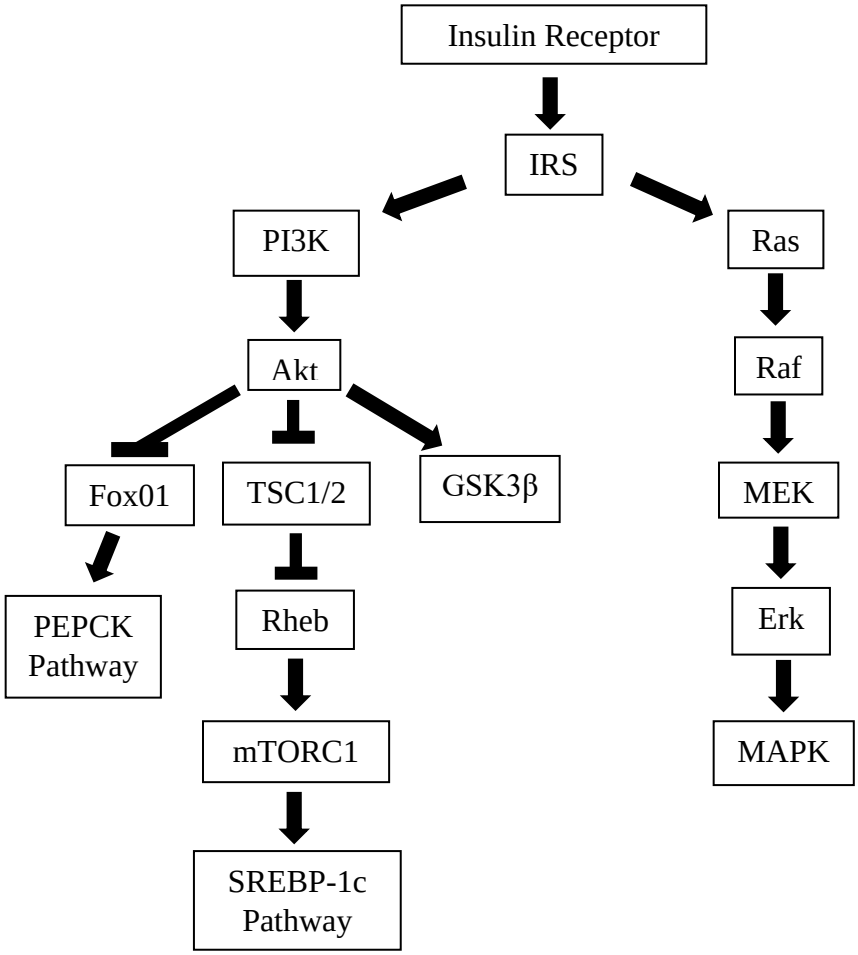

Figure 2.3 Simplified representation of insulin activated kinase cascades in the mammalian liver. Where Akt, protein kinase B; Erk, extracellular signal-regulated kinase; Fox01, forkhead box 01; GSK3β, glycogen synthase kinase-3 beta; IRS, insulin receptor substrate; MAPK, mitogen-activated protein kinase; MEK, mitogen-activated protein kinase kinase; mTORC1, mammalian target of rapamycin complex 1; PEPCK, phosphoenolpyruvate carboxykinase; PI3K, phosphoinositide 3-kinase; Raf, rapidly accelerated fibrosarcoma; Rheb, Ras homolog enriched in brain; SREBP-1c, sterol regulatory element-binding protein 1; and TSC1/2, tuberous sclerosis proteins 1 and 2.

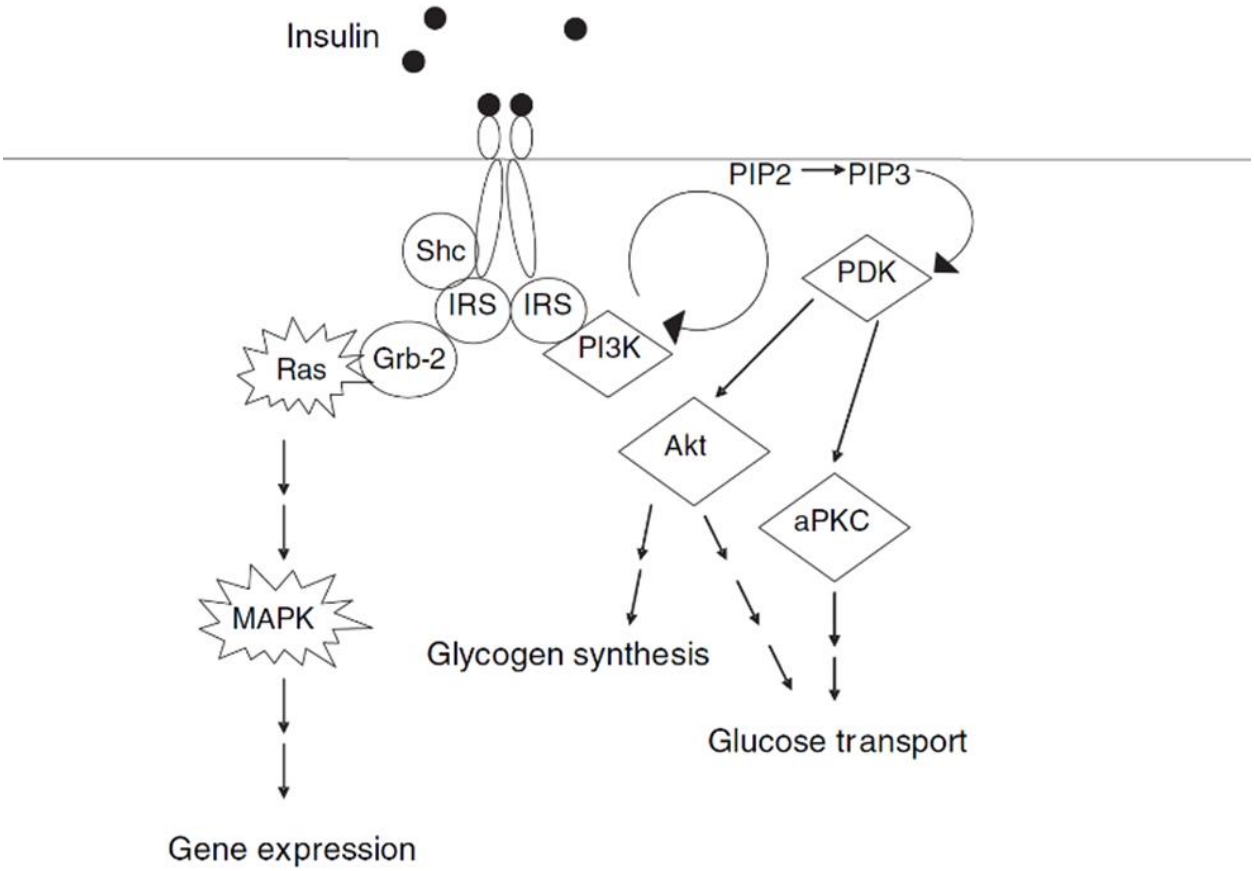

Figure 2.4 Outline of intracellular insulin activity. Where, Akt, protein kinase B; aPKC, atypical protein kinase C; Grb-2, growth factor receptor-bound protein 2; IRS, insulin receptor substrate; MAPK, mitogen activated protein kinase; PI3K, phosphoinositide 3-kinase; PIP2, phosphoinositol biphosphate; PIP3, phosphoinositol triphosphate; and Shc, *src* homology 2 and collagen homology domain-containing protein (Corcoran et al. 2007; used with permission via Oxford University Press, license number: 5390400576184).

documented in cytokine regulation during inflammatory response caused by bacteria (Li et al. 2010; Silva-Garcia et al. 2018).

Insulin's effect to increase AKT activity also appears to be correlated with serine 473 phosphorylation, which, in turn, reduces the effect of the insulin signal by uncoupling IRS-1 thus leading to reduced activation of PI3K and AKT (reviewed in Brozinick and Birnbaum, 1998; Hers et al. 2011). PI3K inhibition by wortmannin has also been found to block elevations of glucose transport and AKT activity (Brozinick Jr. and Birnbaum, 1998).

Ruminants are gluconeogenic animals and will continue to synthesize glucose when provided a diet rich in starch, with propionate accounting for 70% of the carbon supply for gluconeogenesis (Huntington and Richards, 2005). A neural signal (a combination of a decrease in sympathetic activity and an increase in parasympathetic activity, as mentioned earlier) is created upon entry of glucose into the portal vein that increases net hepatic glucose uptake, as found in dogs (Moore et al. 1996). While that study did not allow for insulin sensitivity evaluation, Moore et al. (1996) found that hepatic denervation alters net hepatic glucose uptake such that the results suggest insulin resistance, as reported by Xie et al. (1993). In the absence of hepatic anterior plexus innervation, Xie et al. (1993) found insulin's effect on glucose regulation was decreased. This suggests that hepatic nerve function is required for insulin to elicit normal effects on glucose and the authors maintain that surgical production of insulin resistance via denervation must include interruption of the hepatic parasympathetic nerves (Xie et al. 1993).

Since ruminant gluconeogenesis is so heavily dependent on propionate, a more complex view on gluconeogenesis is required. Most substrates enter gluconeogenesis via oxaloacetate (Figure 2.5); however, propionate undergoes multiple reactions before entering the tricarboxylic acid cycle via oxaloacetate (Aschenbach et al. 2010). Figure 2.5 provides a schematic of glucogenic precursor entry into gluconeogenesis in the bovine liver, as presented by Aschenbach et al. (2010). To ensure adequate glucose supply, pyruvate carboxylase and phosphoenolpyruvate carboxykinase (PEPCK-C) expression are connected to control of gluconeogenesis (Aschenbach et al. 2010). Akt also plays a role in gluconeogenesis as it could be argued it is necessary for proper insulin repression of PEPCK and glucose-6-phosphatase (Logie et al. 2007). Interestingly, only 5-10% of the maximal insulin-induced Akt (PKB) is required to fully repress these two factors,

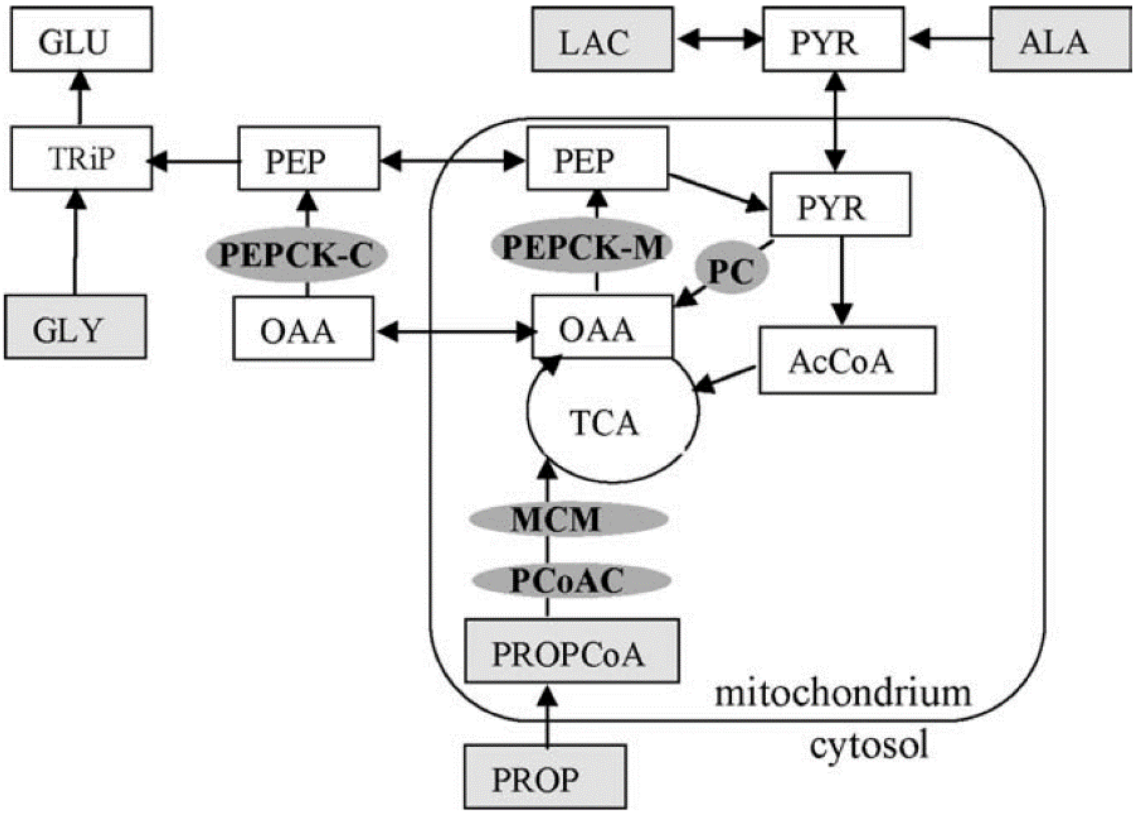

Figure 2.5 Diagram of entry of glucogenic precursors into gluconeogenesis in bovine liver. Where, AcCoA, acetyl CoA; ALA, alanine; GLU, glucose; GLY, glycerol; LAC, lactate; MCM, methylmalonyl-CoA mutase; OAA, oxaloacetate; PC, pyruvate carboxylase; PCoAC, propionyl-CoA carboxylase; PEP, phosphoenolpyruvate; PEPCK-C, phosphoenolpyruvate carboxykinase; PROP, propionate; PROPCoA, propionyl CoA; PYR, pyruvate; TCA, tricarboxylic acid cycle; and TRiP, triose phosphates (Aschenbach et al. 2010; used with permission via John Wiley and Sons, license number: 5390400973239).

establishing the importance of AKT activation in the regulation of PEPCK and glucose 6-phosphatase (Logie et al. 2007).

Sterol-regulatory element-binding protein-1c (SREBP-1c) contributes largely to the transcriptional effect of insulin. In a study investigating the effects of SREBP-1c mutant isoforms in 3T3-L1 adipocytes, Le Lay et al. (2002) found that insulin regulates genes related to both the dominant positive and dominant negative forms of SREBP-1c. In addition to this, insulin can regulate gene transcription apart from SREBP-1c. In summary, of the 47 genes studied by Le Lay et al. (2002) involved in adipose metabolism pathways, 20 were significantly affected by insulin. Nineteen of the 20 genes were sensitive to SREBP mutant overexpression, the exception being Akt/PKB. Furthermore, as concluded by Vaulont et al. (2000), there are genes such as glucokinase, and possibly the proinsulin gene in β-cells in the liver that are mainly activated by insulin.

Several members of the protein kinase C (PKC) family are diacylglycerol-activated (Protein kinase C alpha; PKCα, and protein kinase C beta2; PKCβ2) and appear to decrease insulin-stimulated glucose transport by inhibiting insulin signal feedback to pathway points such as PI3K and PKB (Letiges et al. 2002). Since PKCα and PKCβ2 activity in skeletal and adipose is increased by insulin, it appears this feedback mechanism takes place during insulin stimulation (Letiges et al. 2002). Figure 2.6 below shows the role of PKC, with additional info as presented in Corcoran et al. (2007). A third isoform, protein kinase C zeta (PKCζ) is a negative regulator of PKB and inhibits the down-stream signalling to GSK3 (Doornbos et al. 1999). To further complicate intricacies, exogenous phosphoinositol triphosphate (PIP3) has been found to increase phosphorylation of PKCζ/λ and Akt (see Figure 2.4) (Manna and Jain, 2013). Decreased PIP3 is associated with adverse metabolic instances such as downregulated PI3K, increased oxidative stress, and a diabetic state, all observed in murine differentiated adipocytes (3T3-L1 fibroblast cell line) (Manna and Jain, 2013). In human research, the amount of intramyocellular triacylglycerol may activate novel protein kinases C (nPKCs) and influence the accumulation of specific lipid metabolites that interfere with PI3K and cause phosphorylation of insulin receptor substrate (IRS) (reviewed in Corcoran et al. 2007). Figure 6, pictured below, shows PKC-mediated effects on insulin signalling as represented in Corcoran et al. (2007). The left panel

symbolizes normal insulin-mediated glucose transport protein-4 (GLUT4) transport, while the right panel symbolizes insulin resistance due to abnormal PKC activation.

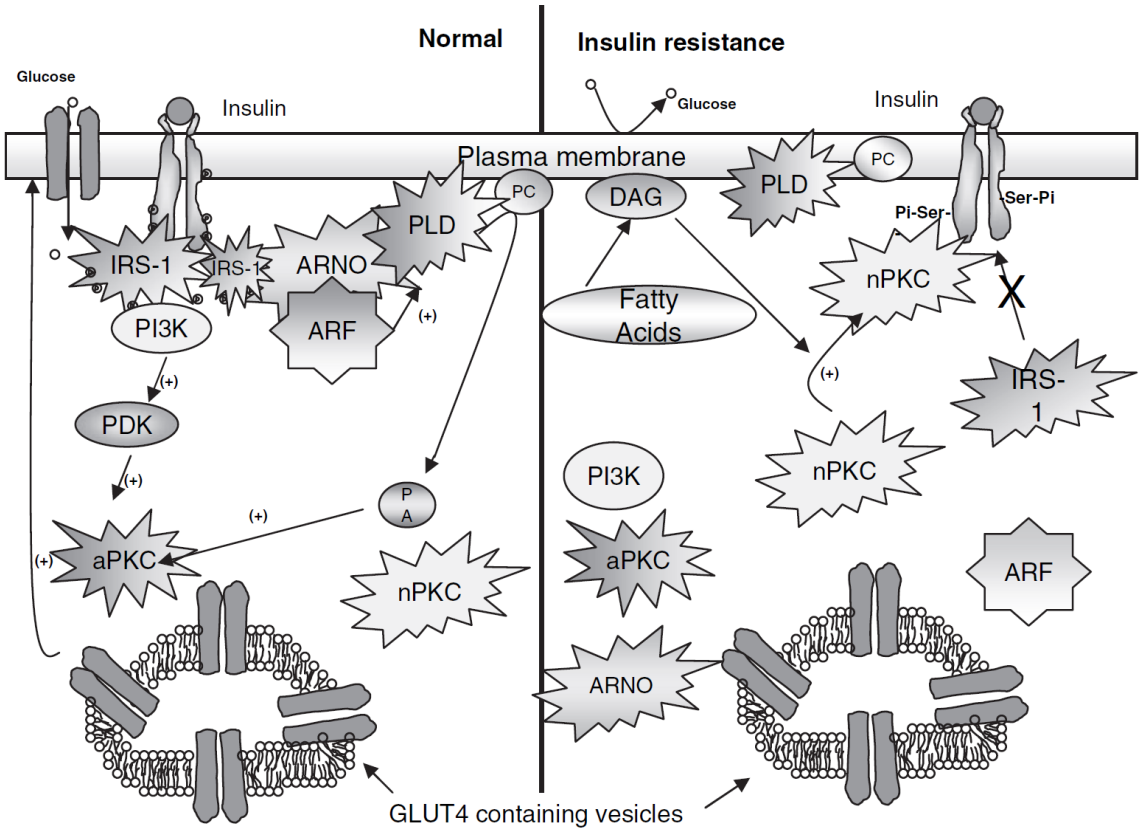

Figure 2.6 Protein kinase C (PKC)–mediated effects on insulin signaling. The left panel symbolizes normal insulin-mediated GLUT4 transport via activation of atypical PKC (aPKC). The right panel symbolizes insulin resistance due to aberrant activation of novel PKCs (nPKCs), which serine-phosphorylate the insulin receptor, thereby inhibiting the tyrosine autophosphorylation required for insulin receptor substrate (IRS) docking. Incidentally, IRSs are also serine phosphorylated by nPKCs (not shown). ARNO, cytohesin/ARF nucleotide-binding site opener; PLD, phospholipase D; PC, phosphatidylcholine; PA, phosphatidic acid; ARF, ADP ribosylation factor; PI3K, phosphoinositide 3 kinase; DAG, diacylglycerol; PDK, 3-phosphoinositide-dependent protein kinase (Corcoran et al. 2007; used with permission via Oxford University Press, license number: 5390401211674).

Found in muscle and adipose tissue, GLUT4 is a transporter that facilitates the uptake of glucose into cells in response to insulin (Tokarz et al. 2018; Chiang et al. 2001), whereas another protein, GLUT2, is found in liver and pancreatic β-cells and is vital for the absorption or removal of glucose in the liver (Olson and Pessin, 1996). Regulated by insulin signalling, GLUT4 storage vesicles (GSV) migrate to the cell periphery and release GLUT4 which then attaches to plasma membrane caveolae (Franck et al. 2007; Brewer et al. 2014; Tokarz et al. 2018; Karlsson et al. 2001). Defects in the migration of GLUT4 to the cell surface are apparent in altered metabolic states such as type II diabetes, and insulin resistance (Brewer et al. 2014). In small adipocytes, a two-fold increase in GLUT4 migration in the plasma membrane occurs following insulin stimulation, whereas there was no increase in migration in large fat cells (Franck et al. 2007). However, the absence of a decrease in activation of Akt in large fat cells suggests that insulin retains its antilipolytic control of fat cells, as a decrease in Akt may result in not only a decrease in GLUT4 translocation but also in activity of glycogen synthase (Franck et al. 2007; Corcoran et al. 2007).

2.4.3 Effects of fat inclusion on insulin responsiveness

Research has been convicting of the detrimental effects of high fat diets on insulin responsiveness. Compensation by β-cells and insulin clearing tissues may result in maintained glucose tolerance and impaired glucose tolerance was only seen when both relative β-cell function and insulin sensitivity were impaired (Mittelman et al. 2000).

Fat sources commonly used in ruminant diets include tallow, canola oil, corn oil, and palmitic acid (PALM) (Salin et al. 2012; Beauchemin and McGinn, 2006; Duckett et al. 2002; Mathews et al. 2016; Rico et al. 2016, Rico et al. 2014). The induction of a hyperlipidemic state by the infusion of triacylglycerol derived from tallow has been utilized to directly investigate the effects of elevated plasma non-esterified fatty acids (NEFA) on metabolism in nonlactating, non-gestating Holstein cows. Findings from a glucose tolerance test (GTT) and an insulin challenge (IC) performed during the infusion of triacylglycerol report the induction of insulin resistance via impaired glucose clearance during both the GTT and IC (Pires et al. 2007).

When fed in mid-lactation, PALM (C16:0) has been found to cause increased milk yield in mid-lactation dairy cows, as well as alterations in plasma NEFA that suggest insulin resistance

(Mathews et al. 2016; Rico et al. 2016). When administered to rats, PALM was found to cause levels of serum NEFA comparable to animals in a diabetic state (Reynoso et al. 2003). Palmitic acid has also been found to expand white adipose tissue and reduce insulin-stimulated tyrosine phosphorylation of IRS-2, with further data suggesting it must act through adenosine monophosphate activated protein kinase (AMPK)-mediated endoplasmic reticulum stress to cause insulin resistance (Kennedy et al. 2009, Zhu et al. 2016; Liu et al. 2010). High concentrations of PALM (0.09 g/kg) in plasma are effective in blocking the insulin signalling pathway by decreasing tyrosine phosphorylation both on insulin receptor and IRS-1 in rats (Reynoso et al. 2003).

Along with tyrosine phosphorylation, the mitogen activated protein kinase (MAPK) pathway has also been decreased following PALM treatment (Reynoso et al. 2003). Palmitate has been found to induce interleukin-6 expression in differentiated $_3T_3$-L_1 adipocytes, an effect that is not caused by all fatty acids (Ajuwon and Spurlock, 2005). Alteration in insulin signalling caused by non-esterified fatty acids and inflammatory cytokines may result in negative impacts on glucose transport (Le Marchand-Brustel et al. 2003; Hers et al. 2011). One metabolic alteration shared by NEFA and inflammatory cytokines is the induction of serine phosphorylation of IRS-1 (Le Marchand-Brustel et al. 2003). Serine phosphorylation of IRS-1 affects insulin signalling by reducing activation of PI3K and Akt (Hers et al. 2011).

2.4.4 Determining insulin resistance

It is important to make the distinction between sensitivity and responsiveness when characterizing insulin resistance in cattle. The maximal response that can be achieved from a hormone, in this case insulin, determines the responsiveness and is typically denoted as the R_{max} (De Koster and Opsomer, 2013). Ideally, when graphed, the R_{max} value is a true plateau (Kahn, 1978). Sensitivity is determined by the concentration at which a given hormone elicits a response that is half-maximal, this is called the ED_{50} (De Koster and Opsomer, 2013; Kahn, 1978; Muniyappa et al. 2008).

Common metabolic tests for insulin and glucose kinetics include GTT or IC (commonly referred to as an insulin tolerance test, or ITT). The difference between a GTT and an IC is the resulting clearance rate in a GTT represents insulin sensitivity, whereas the glucose clearance rate in an IC represents insulin responsiveness (the maximum response to insulin) (Pires et al.

2007). A GTT is a common method to investigate individual animal glucose clearance over a set time period. A GTT involves administering glucose at a controlled rate and dose, and drawing multiple blood samples thereafter. Before administration of glucose, animals are fasted with ad libitum access to water for a little as 1 h (Fitzsimons et al. 2014). However, a more common approach is to fast animals for 12 h (Joy et al. 2017; Oh et al. 2017b). To determine amount of glucose to be administered over a 2- or 3-minute period (Joy et al. 2017; Oh et al. 2017b), common equations are 0.25 g/kg of BW (Pires et al. 2007), 0.3 g/kg of BW (Oh et al. 2017b), or 7.57 mmol/kg $BW^{0.75}$ (Joy et al. 2017). In previous research, an additional caution has been paid toward the measurement of renal glucose clearance during GTT (Gonzalez-Grajales et al. 2018). It has been purported that a glucose dose below the upper renal threshold (7.7 mmol/L) will result in increasing renal clearance of glucose in proportion to the glucose dose infused (Kaneko, 1997), with a dose of glucose infused at a rate of 0.4 g/kg BW resulting in an estimated 8% of injected glucose being cleared in the urine (Palmquist and Moser, 1981). However, following a GTT dose of 7.57 mmol/kg $BW^{0.75}$ (1.36 g/kg $BW^{0.75}$), renal clearance has been found below 2% of total dose (Joy et al. 2017).

Whereas during an IC, insulin is administered at a typical rate of 0.1 IU/kg of BW over the course of 1 minute (Pires et al. 2007; Salin et al. 2012; Rico et al. 2017). Relative to time of infusion, blood is typically sampled at (minutes relative to infusion) -10, 0, 5, 10, 15, 20, 25, 30, 45, 60, 90, 120, 180 min (Joy et al. 2017). Researchers may add additional sample times before infusion to calculate an average concentration of basal blood metabolites, or they may subtract time points such as time point 0 min (Pires et al. 2007). However, sample time point 0 min, which is typically taken immediately after infusion is completed and, in the case that one single catheter is used for both infusion and sampling, the line has been flushed of remnant glucose, is the most important to accurately record the peak glucose reached. Along with an accurate baseline, peak glucose is critical to calculating glucose clearance. Glucose clearance is typically calculated by analyzing glucose concentration at the sample point, subtracting the baseline level, and calculating the area under the curve via trapezoidal method (Pires et al. 2007; Oh et al. 2017b).

These two methods, although useful, are considered of less value to another method of determining insulin resistance called the hyperinsulinemic euglycemic clamp (HEC), also known

as the euglycemic insulin clamp test (De Koster and Opsomer, 2013). Described by Defronzo et al. (1979), the euglycemic insulin clamp involves constant infusion of insulin to reach a plateau ~100 μU/mL above basal for 120 min. To avoid the induced hyperinsulinemia from causing hypoglycemia, a glucose clamp is created by infusing glucose to maintain basal plasma glucose levels. To achieve steady insulin and glucose, there must be frequent observation of blood insulin and glucose levels throughout the test. This method of insulin sensitivity determination gains superiority by these attributes: hypoglycemia and accompanying responses are avoided by maintenance of glucose level, and clearance rate of insulin is determined by clamping insulin level (Defronzo et al. 1979).

Just as insulin resistance and sensitivity may be distinguished separately, issues regarding insulin resistance or deficient supply of insulin can be as well. The state of a subject with deficient insulin secretion caused by an autoimmune destruction of pancreatic β-cells is commonly called type 1 diabetes. This type of diabetes is insulin-dependent since it is caused by a complete deficiency in insulin secretion (De Koster and Opsomer, 2013; American Diabetes Assoc. 2004). As defined in De Koster and Opsomer (2013), insulin resistance is determined by how a normal concentration of insulin is responded to by insulin-sensitive tissues. In the case of non-insulin-dependent diabetes (type 2), the pancreatic β-cells are not destroyed, but the amount of insulin produced is insufficient. Most commonly, it is a combination of a relative deficiency of insulin secretion and insulin resistance that causes type 2 diabetes (De Koster and Opsomer, 2013; American Diabetes Assoc. 2004). In an insulin deficient state, gene expression can be affected by changes in hormones, metabolites, and other factors – diabetes itself has been found to affect ~500 genes (Yechoor et al. 2004). Among these genes may be fatty-acid metabolism-related genes, as well as lipid metabolism by non-coding ribonucleic acids (RNAs) (Yechoor et al. 2004; Batista et al. 2019).

2.4.5 Insulin resistance in finishing cattle

Apart from decreased G:F, insulin resistance increases through finishing, with plasma insulin levels increasing with age and insulin secretory function increasing after sexual maturation in high-marbling breeds such as Japanese Black heifers (Joy et al. 2017; Verde and Trenkle, 1987; Shingu et al. 2001). An interesting finding is the potential role that growth hormone (GH) may play in insulin effects. Since implanting feedlot cattle with exogenous growth promoters is

commonplace in the North American beef industry, it is necessary in this review to comment on findings related to growth hormone and insulin research scenarios (Tomczak et al. 2019; Owens et al. 1995). Multiple studies have been conducted to research the impact of exogenous GH on insulin status such as Trenkle and Topel (1978) and Verde and Trenkle (1987). The latter investigated plasma hormone concentrations in cattle with different growth potentials and found that rate of gain and daily feed intake were 45 and 51% greater in large-frame steers and that these steers also had higher mean concentrations of GH, insulin, thyroxine, and cortisol (Verde and Trenkle, 1987). The authors conclude their results suggest not only the presence of age-related insulin resistance, but also the possibility of body size-related differences in hormone concentrations rather than physiological maturity-related differences (Verde and Trenkle, 1987). This idea is upheld by Trenkle and Topel (1978) who found relatively more insulin and less GH per unit of body tissue as size and feeding time advanced. Although other research agrees with the idea of age-related insulin resistance (Shingu et al. 2001; Eisemann et al. 1997), Shingu et al. (2001) concluded no insulin sensitivity difference between small-frame (Japanese Black heifers) and large-frame (Holstein heifers) cattle. Trenkle and Topel (1978) concluded decreasing plasma concentrations of GH, pituitary GH/body weight, and secretion of GH/body weight with increasing body size. They also found carcass muscle is positively related to GH status and negatively correlated with plasma insulin concentrations, whereas carcass adipose had a negative relation to GH status and a positive correlation with plasma insulin (Trenkle and Topel, 1978).

Concurrently, growth hormone may alter insulin sensitivity as Chase et al. (2011) found GH treatment increased plasma insulin and glucose, which agrees with previous work by Feng et al. (2009). However, Feng et al. (2009) found this effect depends on the nutritional status of the animal such that the effect was observed in cattle fed a concentrate and hay diet gaining 1.1 ± 0.1 kg/d BW but not in those fed hay only and gaining 0.2 ± 0.2 kg/d BW. They conclude that GH treatment increased insulin messenger RNA (mRNA) expression in pancreatic islets and that this may mean insulin mRNA transcription was increased, or that mRNA stability was increased (Feng et al. 2009).

A decrease in insulin sensitivity in finishing cattle is directly observed through an increased fasting insulinogenic ratio as days on feed advances, a linear increase in plasma insulin level, and quadratic decrease in feed efficiency (G:F) with advancing days on feed (Joy et al. 2017).

Furthermore, Joy et al. (2017) propose the possibility that the decrease in G:F may be a result of insulin resistance causing pancreatic exhaustion as the demand for insulin is excessive and unrelenting in the late finishing stages. Supporting this claim is the conclusion that enhanced carcass growth and decreased carcass fat are coupled with a decrease in basal insulin concentration and enhanced skeletal muscle insulin sensitivity (Fitzsimons et al. 2014). Further support is provided in the positive relation of plasma insulin and glucose with fat development in bulls (Hocquette et al. 1999), and the metabolic effects on the insulin signalling pathway due to intramuscular adipose buildup as seen in monogastrics (Corcoran et al. 2007).

2.4.6 Insulin resistance in dairy cattle

Insulin resistance develops postpartum and through early lactation and is a critical adaptation that not only allows glucose partitioning to the mammary gland for milk synthesis, but also enhances adipose tissue lipolysis during early lactation (De Koster and Opsomer, 2013; Rico et al. 2017; Mann et al. 2016; Bell and Bauman, 1997). Moderate insulin resistance occurs during late pregnancy, with a decrease in insulin function causing a reduced suppression of lipolysis, resulting in increased plasma NEFA (Salin et al. 2012). Resulting effects from this elevated NEFA, in turn, indicated whole-body impairment of insulin sensitivity in Ayrshire dairy cows during late pregnancy (Salin et al. 2012). Although increased post-partum NEFA in cows that were overfed pre-partum has been speculated to be related to lower circulating glucose and insulin rather than changes in adipose tissue insulin signalling (Mann et al. 2016), insulin resistance is increased when lactating dairy cows consume a ration with excessive concentrate that exceeds their net energy for lactation requirement (Leiva et al. 2015).

2.5 Plant extracts in livestock rations

2.5.1 Common extracts

Plant extracts such as cinnamaldehyde, *Capsicum*, eugenol, anise, and alfalfa extract have been evaluated for their effects in animal production (Cardozo et al. 2006; Geraci et al. 2012; De Souza 2018; Fandiño et al. 2008; Fandiño et al. 2020). Antimicrobial activity of such plant extracts grants them an alluring factor for animal production as they may possess ability to be substitutes for conventional antimicrobial additives (Drouillard, 2018). This notion of innovating conventional means of production away from antimicrobial treatments is indicative of a refocus of the industry as consumer demand for organic products is growing (Drouillard, 2018), with

prudent use of antimicrobials increasing. Plant extracts may have a home in the North American feedlot sector should they prove to be effective and have economic benefit (Drouillard, 2018).

2.5.2 Ruminant applications and effects

Consumer perspective and preferences for the types of feed additives used and drugs administered to cattle have created market opportunities as well as restricted producer ability to operate via conventional means. Largely, impacts are realized in consumer preferences at the market and have also been accompanied by legislation such as the 2006 decision by The European Union to ban the use of antibiotics, other than coccidiostats or histomonostats, in animal feeds (Official Journal of the European Union, 2003). The presentation of such legislation urged the industry to alter course and to evaluate the efficacy of plant-based extracts in beef feed (Cardozo et al. 2006). When studied against conventional feed-grade antibiotic treatment (lasalocid + chlortetracycline) as a treatment protocol for newly received feedlot calves, a sweetener + plant extract treatment (Sucram + eugenol, cinnamaldehyde, and *Capsicum*) has been found to increase antibody response to bovine respiratory syncytial virus vaccination (De Souza et al. 2018). Although no DMI or feed efficiency differences were observed between treatments or the negative control treatment, calves receiving the sweetener + plant extract treatment had lower ADG than conventional antibiotic treatment, and was not different than control (De Souza et al. 2018).

Research prior to De Souza et al. (2018) found performance was not different between steers fed a dry rolled corn-based ration with either plant extract (cinnamaldehyde, eugenol, and *Capsicum* oleoresin), or monensin treatment (Geraci et al. 2012). Plant extracts were fed at a rate of 400 mg/d (266 mg Xtract 6965 + 133 mg Xtract 6933); whereas, monensin was included at a rate of 46.7 mg/kg of DM. Both Xtract products were products of Pancosma SA (Geneva, Switzerland), with Xtract 6965 containing 170 g/kg cinnamaldehyde and 280 g/kg eugenol encapsulated in a hydrogenated oil matrix, and Xtract 6933 (a natural *Capsicum* oleoresin) containing 12 g/kg capsaicin. In this study, plant extracts did not alter overall DMI, feed efficiency, or longissimus dorsi muscle area versus monensin treatment. A treatment × period interaction occurred during the second period (d 45 to 84) such that ADG was increased 0.20 kg/d by plant extract treatment versus monensin, however, the absence of a negative control group limits the application of this data (Geraci et al. 2012).

Additional data suggest that plant extracts may alter the acetate:propionate ratio in the rumen, DMI, water intake, and branched-chain volatile fatty acid (VFA) concentrations (Cardozo et al. 2006; Fandiño et al. 2020). Cardozo et al. (2006) fed four Holstein heifers in a 4 × 4 Latin square design in two experiments. Experiment one treatments were: alfalfa extract, a mixture of cinnamaldehyde and eugenol, a combination of alfalfa extract and cinnamaldehyde + eugenol treatment, and a control (no treatment). Experiment two treatments were: anise extract, *Capsicum* extract, a mixture of pure cinnamaldehyde and eugenol, and a control (no additives). In the study, anise oil showed the ability to alter rumen parameters as it decreased acetate to propionate ratio as well as branched-chain VFA and ammonia nitrogen concentrations compared to control. Interestingly, cinnamaldehyde + eugenol treatment altered nitrogen metabolism as rumen small-peptide plus amino acid nitrogen was increased and ammonia nitrogen concentrations were decreased (Cardozo et al. 2006). Cardozo et al. (2006) also expressed that a mixture of cinnamaldehyde and eugenol appeared to maintain or reduce DMI, whereas *Capsicum* oil caused an increase in dry matter intake. Additionally, an observed effect of increased propionate concentrations with reduced acetate and ammonia may be beneficial for beef production, suggesting anise oil or a mixture of cinnamaldehyde and eugenol may be viable additives (Cardozo et al. 2006).

More recently, Fandiño et al. (2020) researched the effects of feeding the plant extracts anise and *Capsicum* at varying ratios and total amounts. A total of 12 heifers in a 4 × 4 Latin square design were fed a 90% concentrate ration either three parts anise and one part *Capsicum*, two parts anise and two parts *Capsicum*, or one part anise and three parts *Capsicum*. These ratios were fed to meet target total doses of 100, 250, or 500 mg/d. The total VFA concentrations were not affected by treatment or dose in this study, indicating that rumen fermentation was not affected (Fandiño et al. 2020). However, the three parts anise and one part *Capsicum* treatment increased propionate and decreased the acetate:propionate ratio at the 250 and 500 mg/d doses compared to control treatment. The 500 mg/d dose of each treatment decreased the branched-chain VFA concentration, whereas the 500 mg/d dose for one part anise and three parts *Capsicum* was the only treatment to increase concentrate DMI compared to the control treatment. This effect of *Capsicum* to increase concentrate DMI at only the highest inclusion level in Fandiño et al. (2020) agrees with Cardozo et al. (2006) who saw the same effect when feeding 1000 mg/d of *Capsicum* extract by itself. However, Cardozo et al. (2006) observed an increase in

total DMI as well, whereas Fandiño et al. (2020) did not. Since both studies fed high concentrate rations, this difference in observation may suggest that the effects of anise and *Capsicum* are either neutral or antagonistic but are not additive, as indeed the authors propose (Fandiño et al. 2020), or may be related to the absence of monensin in the control treatment in Cardozo et al. (2006). Additionally, Fandiño et al. (2020) attribute the observed effects on VFA concentrations to the presence of anise since no matter the dose of *Capsicum*, effects on VFA profile only appeared when anise was fed at rates at or above a targeted 187.5 mg/d (determined when adjusting the total target mg/d with the inclusion ratio of anise). Small-peptide plus amino acid nitrogen was increased at the 500 mg/d dose for each treatment suggesting these plant extracts may decrease rumen deamination (Fandiño et al. 2020). However, it was only the three parts anise and one part *Capsicum* treatment fed at 250 mg/d that decreased ammonia-nitrogen (Fandiño et al. 2020).

2.5.3 Capsaicin

Capsaicin is the active ingredient in *Capsicum* plants that has a storied history in murine studies that goes back to 1987 and prior (Watanabe et al. 1987; Oh et al. 2015). Watanabe et al. (1987) found that capsaicin caused a dose-dependent response on epinephrine secretion that increased up to a dose rate of 600 μg/kg of capsaicin. Lee et al. (2013) found that topical application of capsaicin may increase insulin sensitivity and reduce inflammation in obese mice, and in rats given capsaicin orally, Joo et al. (2010) concluded capsaicin treatment altered thermogenesis and proteins related to lipid metabolism including heat shock protein 27 and Steap3 protein in white adipose tissue. Furthermore, mRNA levels of tumor necrosis factor α (TNFα) and leptin were reduced in capsaicin treated rats (Joo et al. 2010).

In ruminants, capsaicin treatment has been researched extensively in the dairy setting, with limited research in beef/feedlot settings. As mentioned above, studies such as Geraci et al. (2012) and De Souza et al. (2018) researched capsaicin in concert with other plant extracts. However, research regarding isolated capsaicin treatment is limited in the beef setting (Rodriguez-Prado et al. 2012; Westphalen et al. 2021). In the dairy setting, Tager and Krause (2011), Oh et al. (2015), Oh et al. (2017a, 2017b) have researched the effect of *Capsicum* at rates from 0.25g/d of Xtract 6933 (12 g/kg capsacin; Tager and Krause, 2011) to 1000 mg/d of CapsXL (1.2% capsaicinoids; Oh et al. 2015).

2.5.4 Capsaicin Method of action

Multiple methods of action have been reported for capsaicin. Several major thermogenesis and fatty acid oxidation proteins have been investigated including levels of uncoupling protein 1 (UCP1), as well as UCP2, UCP3, phosphorylated-AMPK, and phospho-acetyl-CoA carboxylase (ACC), which were all found to be increased with oral capsaicin treatment (Joo et al. 2010). Capsaicin has also been confirmed to alter release of neuropeptides such as calcium gene-related peptide (CGRP) by binding to transient receptor potential cation channel subfamily V member 1 (TRPV1), which is responsible for eliciting the burning sensation (Kang et al. 2010; reviewed in De Lourdes Reyes-Escogido et al. 2011). Capsaicin administered via topical application has been suggested to downregulate inflammatory cytokines TNFα and interleukin 6 (Lee et al. 2013).

2.5.5 Recent Use of Capsaicin in Cattle Rations

Recent research in the effects of capsaicin has been done to quantify its effects in feedlot diets on ruminal fermentation (Fandiño et al. 2008), ruminal nitrogen fractions (Cardozo et al. 2006 mentioned above), animal performance (Geraci et al. 2012), and water intake (Rodriguez-Prado et al. 2012). In dairy diets, capsaicin has been evaluated to quantify the impact on feed efficiency, blood metabolites, hormones, and certain immune responses, which reveal potential for a future of capsaicin inclusion in dairy rations (Oh et al. 2017a, 2017b). It is important to note that whereas a feedlot setting defines feed efficiency as kilograms of live weight gain per kilogram of dry matter intake, it is defined as kilograms of milk produced per kilogram of dry matter intake in dairy settings.

2.5.6 Effects on Insulin, Glucose, and other blood metabolites

Capsaicin that has been rumen protected (RPC) to limit its availability in the rumen decreases thiobarbituric acid reactive substances and haptoglobin in blood (Oh et al. 2017a). A partial summary of the effects of capsaicin treatment on glucose and insulin levels can be found in Table 2.4. Oh et al. (2017a) intravenously challenged cattle with lipopolysaccharide and found no effect on insulin or glucose concentration. During a glucose tolerance test, Oh et al. (2017b) reported that RPC had no effect on glucose concentration; however, they found area under the insulin curve was decreased by 25% with capsaicin treatment. The authors propose that the RPC may have increased calcitonin gene-related peptide, which, as mentioned above, is released as a

Table 2.4 Partial Summary of effects of capsaicin treatment on performance and blood metabolite parameters in ruminant studies.

Study	Treatment	DMI (kg/d)	ADG (kg/d)	Feed Efficiency[3]	Glucose (mg/dL)	Insulin (ng/mL)	Conclusion
Westphalen et al. (2021)	CON (no additive)	7.03	1.48	0.213	86.07	0.43	No treatment effect observed.
	15 mg/kg DM RPC[1]	7.24	1.58	0.220	87.03	0.44	
Rodriguez-Prado et al. (2012)	CON (no additive)	8.56	-	-	-	-	Linear effect on DMI observed as *Capsicum* increased ($P = 0.04$)
	125 mg/d CAP[2]	9.84	-	-	-	-	
	250 mg/d CAP[2]	8.68	-	-	-	-	
	500 mg/d CAP[2]	9.40	-	-	-	-	
Cardozo et al. (2006)	CON (No additive)	7.6	-	-	-	-	*Capsicum* extract increased DMI ($P < 0.05$).
	1 g/d *Capsicum* extract (15% capsaicin)	8.3	-	-	-	-	
Fandiño et al. (2008)	CON (no additive)	7.5	-	-	-	-	*Capsicum* oil had no effect on DMI.
	500 mg/d *Capsicum* oil	8.3	-	-	-	-	
Oh et al. (2015)	CON (no additive)	27.0	50.5	1.90	-	-	No treatment effect on DMI, Milk Yield, or Feed Efficiency.
	250 mg/d CAP[4]	27.5	51.9	1.93	-	-	
	500 mg/d CAP[4]	27.0	51.5	2.02	-	-	
	1000 mg/d CAP[4]	26.5	50.3	1.96	-	-	

Table 2.4. (continued) Partial Summary of effects of capsaicin treatment on performance and blood metabolite parameters in ruminant studies.

Study	Treatment	DMI (kg/d)	Milk Yield (kg/d)	Feed Efficiency[3]	Glucose (mg/dL)	Insulin (µIU/mL)	Conclusion
Oh et al. (2017a)	CON (no additive)	25.3	34.0	1.44	57.1	25.7	No treatment effects on these variables.
	100 mg/d RPC[1]	26.2	37.4	1.47	55.9	22.4	
	200 mg/d RPC[1]	25.5	35.8	1.56	56.2	22.7	
Oh et al. (2017b)	CON (no additive)	29.4	42.8	1.48	72.0	8.75	RPC increased feed efficiency vs CON. RPC linearly increased feed efficiency. No effect on basal glucose or insulin.
	100 mg/d RPC[1]	30.0	44.7	1.52	69.1	5.95	
	200 mg/d RPC[1]	29.2	43.9	1.57	67.8	5.81	
Tager and Krause (2011)	CON (no additive)	23.9	-	-	-	-	No treatment effect on DMI.
	250 mg/d CAP[x]	22.9	-	-	-	-	

[a,b,c]Within column and study, means without common superscript letter differ ($P < 0.05$).
[2]Xtract 6933 (6% capsaicin and dihydrocapsaicin; Pancosma, Geneva, Switzerland)
[1]Rumen protected *Capsicum* oleoresin (Nexulin, 1.1% capsaicinoids; Pancosma, Geneva, Switzerland) added to ration at rate of 15 mg/kg DM.
[3]Defined as unit of weight gained in relation to unit of weight feed intake in Westphalen et al. (2021), Rodriguez-Prado et al. (2012), Cardozo et al. (2006), and Fandiño et al. (2008), defined in Oh et al. (2015), Oh et al. (2017a), Oh et al. (2017b), and Tager and Krause (2011) as milk yield ÷ DMI.
[4]Rumen protected *Capsicum* oleoresin (CapsXL, 20% *Capsicum* oleoresin; 1.2% capsaicinoids; Pancosma, Geneva, Switzerland).

result of capsaicin binding to TRPV1 and has been found to inhibit insulin secretion (Pettersson et al. 1986; de Lourdes Reyes-Escogido et al. 2011). In the feedlot setting, Westphalen et al. (2021) found feeding RPC (Nexulin, 1.1% capsaicinoids) at a rate of 15mg/kg DM to newly received light-weight calves caused no differences in blood glucose, insulin, haptoglobin, or glutathione versus control treatment. There remains, however, no research evaluating the effect of capsaicin treatment on blood metabolite parameters of beef cattle during the finishing phase.

2.5.7 Effects on efficiency parameters and rumen fermentation

Research data on the potential for capsaicin treatment to effect efficiency parameters has been inconclusive. As shown in Table 2.4, studies have varying observations on DMI. Oh et al. (2015) concluded no effect of capsaicin on DMI, but there was a quadratic increase in milk yield with increasing capsaicin supplementation. This increase in milk production with no increase in DMI, and no effect on digestibility, resulted in a greater negative energy balance in two of the treatment groups receiving lower doses of capsaicin which may have led to the mobilization of body fat reserves (Oh et al. 2015).

Evidence of capsaicin to increase dietary digestibility are conflicting. Oh et al. (2017b) reported that RPC linearly increased digestibility of DM, organic matter (OM), and crude protein (CP), citing the increased total-tract digestibility may be caused by stimulating lipase and trypsin as suggested in rats by Platel and Srinivasan (2000). Previous work by Oh et al. (2015) found subtle or no effects on feed intake, digestibility, and antioxidant status. However, it should be noted that Oh et al. (2015) fed an unprotected, granular form of capsaicin; whereas, Oh et al. (2017b) fed capsaicin that was rumen-protected. In agreement with Oh et al. (2015), Tager and Krause (2011) previously concluded no effects on total tract digestibility of multiple parameters caused by either cinnamaldehyde with eugenol, or *Capsicum* treatment. Additionally, in the feedlot setting Cardozo et al. (2006) found *Capsicum* oil (15% capsaicin; rumen availability not specified) to increase DMI and water intake and decrease the acetate proportion in the rumen. A summary of the effects of capsaicin treatment on rumen fermentation is presented in Table 2.5. When compared to monensin, plant extract treatment which included 133 mg/d of *Capsicum* oleoresin did not alter DMI, feed efficiency, longissimus dorsi muscle area, or backfat

Table 2.5 Summary of effects of capsaicin containing treatments on rumen fermentation in ruminant studies.

Study	Treatment	VFA proportion (Mol/100 mol)			Rumen pH	Conclusion
		Acetate	Propionate	Butyrate		
Rodriguez-Prado et al. (2012)	CON (no additive)	59.6	22.3	14.8	6.03	No effects on VFA proportions or rumen pH.
	125 mg/d CAP[1]	59.5	24.2	13.6	5.84	
	250 mg/d CAP[1]	59.9	21.8	14.6	5.96	
	500 mg/d CAP[1]	55.5	27.2	13.5	5.86	
Oh et al. (2015)	CON (no additive)	50.8	31.1	12.6	5.86	No effect of CON vs. capsaicin, no effect of capsaicin treatment.
	250 mg/d CAP[2]	51.3	30.4	12.9	5.78	
	500 mg/d CAP[2]	50.5	31.4	12.3	5.76	
	1000 mg/d CAP[2]	49.8	31.9	12.5	5.78	
Fandiño et al. (2008)	CON (no additive)	55.3	25.2	13.0	5.77	*Capsicum* oil decreased acetate and increased butyrate ($P < 0.05$).
	500 mg/d *Capsicum* oil	54.0	25.6	14.1	5.78	
Cardozo et al. (2006)	CON (no additive)	56.8	26.7	11.6	6.10	*Capsicum* decreased acetate ($P < 0.05$). No effects on rumen pH, propionate, or butyrate
	1 g/d *Capsicum* extract (15% capsaicin)	53.9	29.8	12.1	6.14	
Tager and Krause (2011)	CON (no additive)	61.7	22.8	12.0	-	No effect of *Capsicum* on these parameters.
	0.25 g/d CAP[1]	61.6	22.2	12.6	-	

[a,b,c]Within column and study, means without common superscript letter differ.
[1]Xtract 6933 (6% capsaicin and dihydrocapsaicin; Pancosma, Geneva, Switzerland).
[2]Rumen protected *Capsicum* oleoresin (CapsXL, 20% *Capsicum* oleoresin; 1.2% capsaicinoids; Pancosma, Geneva, Switzerland)

deposition. However, as mentioned previously, a treatment × period interaction occurred during the second period of the study (d 45 to 84) that showed an increase in ADG in calves fed plant extract compared to those fed monensin (Geraci et al. 2012).

Further findings on rumen fermentation, as shown in Table 2.5, include that capsaicin had no effects on VFA proportions (Tager and Krause, 2011; Rodriguez-Prado et al. 2012; Oh et al. 2015), caused a decrease in acetate proportion (Cardozo et al. 2006), or caused a decrease in acetate and an increase in butyrate (Fandiño et al. 2008). One explanation for the varying observations may be the varying levels of capsaicin in the products researched. For example, the *Capsicum* product used by Cardozo et al. (2006) contained 15% capsaicin, whereas Tager and Krause (2011) and Rodriguez-Prado et al. (2012) used an extract with 6% capsaicin, and Oh et al. (2015) used a product with 1.2% capsaicinoids (CapsXL).

2.6 Summary

The ration given to feedlot animals, as well as the stage of finishing an animal is at, can affect ADG and feed efficiency of feedlot cattle. As animals progress into later stages of finishing, their feed efficiency decreases due in part to evidence that suggests age-related insulin resistance (Shingu et al. 2001; Joy et al. 2017). The general anabolic role that the hormone insulin fulfills is understood, however, a complete understanding of its intricate functions is yet to be achieved (Batista et al. 2019).

Conventional feedlot practices utilize anabolic hormone implants to achieve optimum animal ADG and minimize the required number of days on feed to reach market weight. Markets and programs that disallow the use of anabolic hormones and antibiotics have created a demand for natural feed additives that will increase animal efficiency. Capsaicin is a plant extract that has been researched as a dietary additive in animal research for several decades. Recently, studies have investigated the potential effects of capsaicin on immune response, productivity, and energy metabolism in cattle. In the dairy setting, capsaicin has shown potential to alter feed efficiency and insulin responsiveness during GTT (Oh et al. 2017b). However, its effects on parameters such as DMI and rumen fermentation have been inconsistent. In the feedlot setting capsaicin and RPC research data is very limited (Fandiño et al. 2008; Westphalen et al. 2020). There exists no research regarding the effects of RPC on blood metabolites or growth performance in finisher cattle. The occurrence of metabolic changes regarding insulin and decreased feed efficiency as

feedlot cattle progress through the finishing period provide an avenue to determine the potential effects of RPC treatment in feedlot cattle.

Acknowledging these and other findings from previous capsaicin research, a hypothesis was formulated that RPC will alter feedlot cattle metabolism and performance, that RPC effects may be modified by treatment dose and degree of rumen protection, and that RPC may alter digestibility of feedstuffs as well as alter blood metabolite levels such as insulin, glucose, and haptoglobin.

3.0 EFFECT OF RUMEN PROTECTED *CAPSICUM* OLEORESIN ON DRY MATTER INTAKE, AVERAGE DAILY GAIN, AND CARCASS CHARACTERISTICS IN FINISHING BEEF STEERS

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Author contributions: Eidsvik, Penner, McKinnon, and Moya were responsible for experimental design. Eidsvik collected and analyzed the data, wrote the first draft. All authors contributed to data interpretation and revision of the manuscript. Khelil and Blanchard contributed intellectually to the study structure and discussion.

3.1 Abstract

This study was conducted to evaluate growth performance and carcass characteristics of finishing steers fed rumen protected *Capsicum* oleoresin supplements differing in the extent of ruminal protection and when provided at two dose rates. 450 steers were stratified by body weight (BW) into 1 of 30 pens (15 steers/pen) and pens were randomly assigned to 1 of 5 treatments in a 2×2+1 factorial arrangement including: no additive (**CON**); a low or high dose of rumen protected *Capsicum* oleoresin (77 mg/d for **RPLO** and 250 mg/d for **RPHI**); or a low or high dose of *Capsicum* oleoresin that was rumen protected to a greater extent (100 mg/d for **HPLO** or 322 mg/d for **HPHI**). Both products were delivered in the vitamin and mineral supplement. All steers received the same basal diet of (dry matter basis) barley grain (86.2%), barley silage (6.0%), canola meal (6.2%), and a vitamin and mineral supplement (1.6%). While BW at the start of the study did not differ ($P > 0.19$), there was an interaction between supplement and dose ($P = 0.034$) where increasing the dose from HPLO to HPHI numerically reduced BW while increasing dose from RPLO to RPHI numerically increased BW. Inclusion of *Capsicum*, rumen protection, and dose did not affect DMI, ADG, or G:F whether reported on a live weight or carcass-adjusted basis. Hot carcass weight, dressing percentage, and rib-eye area were not affected ($P \geq 0.11$). The proportion of carcasses grading Canadian yield grade 3 were not affected by *Capsicum* but were reduced ($P = 0.005$) for the high vs. low inclusion rates. Use of *Capsicum* did not affect quality grade but feeding the high vs. low doses decreased ($P = 0.016$) the proportion of steers grading AAA and increased ($P = 0.009$) the proportion grading AA. Marbling score was increased with the low vs. high dose of *Capsicum* ($P = 0.032$). There were no differences for liver scores. Dietary supplementation of rumen protected *Capsicum* did not affect DMI, growth, or carcass weight for finishing steers. However, increasing the dose of *Capsicum* may negatively affect carcass yield, quality grades, and marbling score.

3.2 Introduction

Feed conversion of feedlot cattle is a significant driver of profitability for the finishing sector. The feed conversion rate, measured as G:F, decreases with advancing days on feed due to increased maintenance energy requirements with increasing body weight and as the composition of gain shifts toward fat deposition (NASEM, 2016). Several studies have provided evidence that insulin responsiveness decreases with advancing days on feed (Verde and Trenkle, 1987; Kneeskern et al. 2016; Joy et al. 2017). Since glucose and amino acid uptake into insulin responsive cells is partially regulated by insulin (Tokarz et al. 2018), this decrease in insulin responsiveness questions whether insulin sensitivity contributes to the decreased G:F with advancing days on feed.

Capsaicin, the major molecule responsible for the pungent properties and sensory responses in hot peppers, and the active component in *Capsicum* oleoresin has been subject to an array of research to elucidate its effects in beef cattle nutrition (Fandiño et al. 2008; Reyes-Escogido et al. 2011; Ludy et al. 2012). In beef cattle, feeding capsaicin had antimicrobial effects in the rumen with the ability to modify ruminal fermentation (Calsamiglia et al. 2007; Rodriguez-Prado et al. 2012). Additionally, recent studies have reported increased feed efficiency, as well as increased milk production after peak lactation in dairy cows supplemented with rumen-protected *Capsicum* (Stelwagen et al. 2016; Oh et al. 2017b). Rumen protection of the capsaicin supplement decreases the likelihood of alteration in the rumen and aids in the delivery of the capsaicin to the small intestine. In dairy cattle, RPC has been reported to stimulate insulin sensitivity and increases feed efficiency (Oh et al. 2017b). However, studies evaluating the effect of the active ingredient capsaicin in finishing cattle are limited.

The current study was conducted to determine whether provision of *Capsicum* oleoresin products differing in the extent of ruminal protection and dose affect DMI, ADG, G:F and carcass characteristics of finishing beef steers.

3.3 Materials and Methods

All cattle were cared for according to the guidelines of the Canadian Council on Animal Care (Ottawa, ON, Canada) and use of steers was pre-approved by University of Saskatchewan Animal Research Ethics Board (protocol 20100021; Saskatoon, SK, Canada).

3.3.1 Experimental Design

This study was conducted at the University of Saskatchewan Livestock and Forage Centre of Excellence (Clavet, SK, Canada) as a completely randomized study with pen as the experimental unit. Four hundred fifty steers (432 ± 32 kg shrunk body weight, **BW**) were sourced from a local auction market and weighed upon arrival. Steers were treated against internal and external parasites (Solmectin; Solvet, Calgary, AB), provided an implant containing 120 mg trenbolone acetate and 24 mg estradiol (Revalor S implant; Intervet GesmbH, Austria), and were vaccinated against infectious bovine rhinotracheitis, bovine viral diarrhea types 1 and 2, bovine respiratory syncytial virus, parainfluenza 3, and *Mannheimia haemolytica* (Bovashield Gold/One Shot; Zoetis Canada Inc., Kirkland, QC) and *Clostridium chauvoei*, *C. septicum*, *C. haemoyticum*, *C. novui, C. sordellii, and C. perfringens* types B, C and D (Ultrachoice 8, Zoetis Canada Inc., Kirkland, QC). All steers were fed a receiving diet containing (DM basis) 30% barley silage, 30% grass hay, 29.52% barley grain, 10.35% canola meal, and 0.13% mineral for 12 to 33 d, dependent on timing of purchase.

Steers were weighed on 2 consecutive days and were stratified into pens based on BW. Pens were randomly assigned to 1 of 5 dietary treatments in a 2 × 2 + 1 factorial arrangement (Table 3.1; described below). Steers were then transitioned from the receiving diet to a diet containing (DM basis) barley grain (86.2%), barley silage (6.0%), canola meal (6.2%), and a vitamin and mineral supplement (1.6%) over 28 d using 7 dietary steps with 4 d/step. No treatment was applied during the diet transition. Upon completing transition protocol and provision of the finishing ration, steers were weighed on 2 consecutive days and pens were then exposed to their dietary treatments. The BW recorded at the start of treatment exposure were used to calculate the initial steer body weight for each treatment. Treatments included a negative control (**CON**), and diets that contained a low or high dose (77 mg/d for **RPLO** and 250 mg/d for **RPHI**) of rumen protected *Capsicum* oleoresin (X60-7035; 20% *Capsicum* oleoresin, 1.2% capsaicinoids with an estimated 20% bypassing the rumen; Pancosma SA., Geneva, Switzerland), or a low or high dose (100 mg/d for **HPLO** and 322mg/d for **HPHI**) of *Capsicum* oleoresin with a greater extent of rumen protection (X50-7035; 15.5% *Capsicum* oleoresin, 0.93% capsaicinoids with an estimated 40% bypassing the rumen; Pancosma SA., Geneva, Switzerland). These *Capsicum* products are rumen protected by micro-encapsulation within a fat matrix (XTRACT, 2022). These experimental *Capsicum* inclusion rates were formulated based on previous research (Oh et al.

2017a, 2017b) and with collaboration with the current study's funding body. Doses were balanced such that the total ingested *Capsicum* oleoresin (mg) was equal for both supplements

Table 3.1 Dietary ingredient inclusion rate and nutrient composition for finishing steers fed diets with rumen protected *Capsicum* oleoresin containing differing concentrations of capsaicinoids (**RP** and **HP** contained 20% *Capsicum* oleoresin with 1.2% capsaicinoids and 15.5% *Capsicum* oleoresin with 0.93% capsaicinoids, respectively) and expected extent of rumen protection at low (**LO**) or high (**HI**) doses.

	Treatment[1]				
Ingredients, % dry matter	**CON**	**HPLO**	**HPHI**	**RPLO**	**RPHI**
Barley silage	6.0	6.0	6.0	6.0	6.0
Barley grain	86.2	86.2	86.2	86.2	86.2
Canola meal	6.2	6.2	6.2	6.2	6.2
Limestone	1.4	1.4	1.4	1.4	1.4
Mineral supplement[1]	0.025	0.025	0.025	0.025	0.025
Rumen protected *Capsicum* oleoresin[2]	-	0.010	0.032	-	-
High rumen protected *Capsicum* oleoresin[2]	-	-	-	0.008	0.025
Chemical Composition, %DM[3]					
Dry matter, % as fed	83.1 ± 1.3	83.1 ± 1.3	83 ± 1.2	83.1 ± 1.3	83.1 ± 1.2
Crude protein	14.9 ± 0.2	14.9 ± 0.2	14.9 ± 0.2	14.9 ± 0.2	14.9 ± 0.2
Neutral detergent fiber	18.7 ± 1.5	18.7 ± 1.5	18.7 ± 1.5	18.7 ± 1.5	18.7 ± 1.5
Acid detergent fiber	7.9 ± 0.6	7.9 ± 0.6	7.9 ± 0.6	7.9 ± 0.6	7.9 ± 0.6
Starch	49.2 ± 0.2	49.2 ± 0.2	49.2 ± 0.2	49.2 ± 0.2	49.2 ± 0.2
Ether extract	2.3 ± 0	2.3 ± 0	2.3 ± 0	2.3 ± 0	2.3 ± 0
Organic matter	95.2 ± 0.1	95.2 ± 0.1	95.2 ± 0.1	95.2 ± 0.1	95.2 ± 0.1
Calcium	0.66 ± 0.01	0.66 ± 0.01	0.66 ± 0.01	0.66 ± 0.01	0.66 ± 0.01
Phosphorous	0.36 ± 0.00	0.36 ± 0.00	0.36 ± 0.00	0.36 ± 0.00	0.36 ± 0.00
NE_m, Mcal/kg	1.89 ± 0.00	1.89 ± 0.00	1.89 ± 0.00	1.89 ± 0.00	1.89 ± 0.00
NE_g, Mcal/kg	1.27 ± 0.00	1.27 ± 0.00	1.27 ± 0.00	1.27 ± 0.00	1.27 ± 0.00

[1]Mineral formulated to provide 33 mg/kg monensin in final diet. The mineral supplement contained, on a DM basis: 1.00% fat, 3.00% fiber, 4.00% calcium, 750.0 mg/kg cobalt, 5,000.0 mg/kg iodine, 60,000.0 mg/kg copper, 120,000 mg/kg manganese, 180,000 mg/kg zinc, 750 mg/kg selenium, 38,111.7 IU/kg vitamin A, 6,097.9 IU/kg vitamin D, and 965.0 IU/kg vitamin E.
[2]Products of Pancosma, Geneva, Switzerland. Where: amount of *Capsicum* bypassing rumen from supplement HP = 40%, and from RP = 20%.
[3]Chemical composition shown as means ± SD (n = 2).

resulting in 15.5 and 49.9 mg of *Capsicum* oleoresin for the low and high doses, respectively. Steers received their respective treatment until achieving a target live weight of 685 kg allowing for 69-d of treatment exposure.

Barley silage, barley grain, and canola meal were added to a Kuhn Knight Reel Commercial RC 260 Helix feed truck (Freightliner; Portland, OR) via a front-end loader; whereas, limestone was added using an overhead auger. The vitamin and mineral premix as well as rumen protected *Capsicum* oleoresin treatments were added through a Micromix machine (The Micro-Weigh System; Micro Beef Technologies, TX, USA). Software was used to track the amount of each ingredient added to each load and the amount of feed delivered to each pen (Read-N-Feed Bunk Reader v. 3.95.0; Micro Beef Technologies, TX, USA). Samples of barley silage, barley grain, and canola meal were collected every week and analyzed for DM content by placing samples with a known weight into a forced air oven at 55^{o}C until achieving a constant weight. Feed ingredient DM coefficients were used to ensure that the as-fed inclusion rates matched the DM formulation. In addition, dry samples of silage were ground with a Christy-Norris Hammermill (Christy & Norris 8" Lab Mill; Ipswich, United Kingdom) to pass through a 2-mm screen. Barley grain was ground using a Retsch rotary mill (Ultra Centrifugal Mill ZM 200; Retsch-Allee 1-5, Haan, Germany) to pass through a 1-mm screen. Samples were then composited by month and sent to Cumberland Valley Analytical Services (Waynesboro, PA, USA) for analysis of ash to determine the OM concentration, starch, CP, NDF, ADF, lignin, EE, and the calcium and phosphorous content. Ash was determined according to method 942.05 (AOAC, 2000) with the modification that a 1.5-g sample was used with a 4-h ashing duration and measurement using hot-weighing. Organic matter was determined by subtracting the ash concentration from 100%. Starch was determined according to the method described by Hall (2009). Crude protein was analyzed according to Official Methods of Analysis method 990.03 (AOAC, 2000) using a Leco FP-528 Nitrogen Combustion Analyzer (LECO Corp., St. Joseph, MI, USA). Neutral detergent fiber was determined according to Van Soest et al. (1991) with α-amylase (A3306, Sigma-Aldrich, St. Louis, MO), as well as sodium sulfite to increase solubilization of NDF, with the modification of Whatman 934-AH (Cytiva, Global Life Sciences Solutions USA LLC, Marlborough, MA) glass micro-fiber filters with 1.5-um particle retention. Acid detergent fiber was determined according to method 973.18 (AOAC, 2000) with the

modification of Whatman 934-AH (Cytiva, Global Life Sciences Solutions USA LLC, Marlborough, MA) glass micro-fiber filters with 1.5-µm particle retention was used in place of fritted glass crucibles. Lignin was determined using methods described by Goering and Van Soest (1970) (Forage Fiber Analysis, USDA). Ether extract was determined using AOAC method 2003.05 (AOAC, 2006). Calcium, and phosphorous concentrations were determined using AOAC method 985.01 (AOAC, 2000), with modifications of 0.35-g samples were ashed for 1 h at 535°C and then digested in open crucibles in 15% nitric acid on hotplate for 20 min. Samples were then diluted to 50 mL and analyzed on ICP (Perkin Elmer 5300 DV ICP; Perkin Elmer, CT, USA).

3.3.2 Steer Body Weight and Dry Matter Intake

All steers were weighed individually prior to feeding on two consecutive days at the start of the experiment in a squeeze chute system with suspended load cells (Avery Weigh-Tronix, Fairmont, MN, USA). The load cells were calibrated by Scale-Tech Systems (Saskatoon, SK, Canada) prior to the start of the study and calibration was verified during the study. In addition, steers were weighed, on a single day, every 2 weeks during the study and on two consecutive days at the end of the study. Live BW was corrected for gut fill (4%) and used to calculate ADG using regression (NASEM, 2016). The weight of the feed delivered daily to each pen was obtained from the feeding management software and feed bunks were manually cleaned on the same days as BW measurements. The residual feed in the bunk and their DM content were determined. Dry matter intake was calculated at a pen level by subtracting the DM weight of the refusals from the weight of the DM offered.

Upon reaching a target live unshrunk BW of 685 kg, steers were transported to a federally inspected slaughter plant (Cargill Foods; High River, AB, Canada). At the slaughter plant, hot carcass weight was determined. Dressing percentage was calculated by dividing the hot carcass weight by the shrunk (4%) live BW. Carcasses were split along the sagittal axis and cut between the 12^{th} and 13^{th} rib to allow for determination of quality and yield grades according to the 3-point yield grade system of the Canadian Beef Grading Agency (Calgary, AB, Canada). Yield grade 1, 2, and 3 categorized carcasses based on lean meat yields of 59% or more, 54 to 58%, and 53% or less, respectively. In addition, the e+v vision grading system (e+v Technology

GmbH, Germany) was used to determine yield score, fat thickness, ribeye area, and marbling score.

Livers were graded by the Canadian Food Inspection Agency (Nepean, ON, Canada) according to Elanco Liver Check System (Elanco Animal Health, Greenfield, IN, USA). Liver scores included clear (liver of normal appearance), minor (one or two small abscesses – or up to two to four well-organized abscesses that are generally under 2.54 cm diameter), or severe (one or more large abscesses as well as inflammation surrounding the abscess). Liver scoring was conducted strictly by visual observation by a trained observer, unaware of dietary treatments, at Cargill Foods (High River, AB, Canada).

3.3.3 Calculations and Statistical Analysis

All continuous data were analyzed using the PROC MIXED of SAS (version 9.4; SAS Inst. Inc., Cary, NC). Two datasets were generated with the first dataset including all 5 treatments while the second dataset only included the RPLO, RPHI, HPLO, and HPHI treatments. For the first dataset, a single polynomial contrast was used to compare the CON to all treatments containing *Capsicum* oleoresin. This model included the fixed effect of treatment with pen as a random effect. The second dataset was used to compare the effect of the supplement (RP vs. HP), dose (HI vs. LO), and the supplement × dose interaction included as fixed effects with pen as the random effect. The Tukey's post-hoc mean separation test was used to determine means that differed ($P < 0.05$).

Categorical data (yield grade, quality grade, and liver score) were analyzed using the GLIMMIX model of SAS (version 9.4) with a binomial distribution and logit data transformation. The means and the standard errors of the mean (SEM) were reverse transformed for presentation in the tables while the *P* values reported are from the transformed data analysis. Prior to analysis, the proportion of carcasses and livers within each category in a given pen were calculated. As with continuous data, the statistical approach utilized 2 datasets to compare CON to all treatments with capsaicin using a single polynomial contrast, and the second dataset was used to evaluate the effect of supplement (RP vs. HP), dose (HI vs. LO), and the supplement × dose interaction, included as fixed effects, with pen as the random effect.

3.4 Results and Discussion

Dry matter intake was not affected by inclusion of *Caspicum* oleoresin ($P = 0.30$; Table 3.2), supplement, dose, or the supplement type × dose interaction ($P \geq 0.57$; Table 3.2). Past studies have reported variable results with respect to the effect of rumen protected *Capsicum* oleoresin on DMI where several studies have observed no effect (Oh et al. 2015; 2017a; 2017b), while others have reported quadratic increases for both DMI and milk yield in responses to increasing RPC (Oh, 2015). From a finishing cattle perspective, one study has reported a linear increase in total DMI as *Capsicum* (not rumen protected) was included in levels up to 500 mg/d to fattening heifers in a Latin square design (Rodriguez-Prado et al. 2012). In the present study, there was no effect of *Capsicum* on DMI. In support of these findings, Westphalen et al. (2021) reported that feeding rumen protected *Capsicum* oleoresin at 15 mg/kg of DM (~109 mg/d) to feedlot cattle had no effect on overall DMI, G:F, or ADG.

On a shrunk live-weight basis, initial BW was not different between treatments ($P \geq 0.19$; Table 3.2), but final BW was affected by a supplement × dose interaction ($P = 0.034$). While no differences between any means were detected, the interaction was due to numerically greater BW for HPHI than HPLO, and numerically greater BW for RPLO than RPHI. There were no differences in final BW between CON and treatments containing *Capsicum* oleoresin ($P = 0.87$).

In the current experiment, ADG and G:F were not affected by the inclusion of capsicum, the supplement, dose, or interaction ($P \geq 0.42$; Table 3.2). Likewise, carcass-adjusted ADG and G:F were not affected by *Capsicum* oleoresin, supplement, dose, or the supplement × dose interaction ($P \geq 0.11$). Hot carcass weight, dressing percentage, backfat thickness, and ribeye area were not affected ($P \geq 0.11$; Table 3.3) by supplement, dose, or the interaction. The proportion of carcasses grading YG3 was greater ($P = 0.005$) when fed the low vs. high dose of *Capsicum* oleoresin, but no other yield grade effects were detected. Quality grades were not affected by inclusion of *Capsicum* oleoresin or the type of *Capsicum* oleoresin supplement; however, provision of the high dose decreased ($P = 0.016$) the proportion of carcasses grading AAA and increased ($P = 0.009$) the proportion grading AA. The change in quality grade was supported by a reduction ($P = 0.032$) in marbling score for high vs. low *Capsicum* oleoresin treatments. There were no observed effects of *Capsicum* oleoresin, supplement, dose, or their interaction on liver

Table 3.2 Dry matter intake, BW, growth, and feed conversion for finishing steers fed a barley-based diet with rumen protected *Capsicum* oleoresin containing differing concentrations of capsaicinoids (**RP** and **HP** contained 20% *Capsicum* oleoresin with 1.2% capsaicinoids and 15.5% *Capsicum* oleoresin with 0.93% capsaicinoids, respectively) and expected extent of rumen protection at low (**LO**) or high (**HI**) doses.

	Treatment[1]										
			RP[2]		**HP**[2]			***P*- value**[3]			
	CON	**SEM**	**LO**	**HI**	**LO**	**HI**	**SEM**	**CON vs. *Capsicum***	**SUPP**	**Dose**	**SUPP × Dose**
Initial BW[4], kg	571	2.7	570	569	572	574	2.8	0.80	0.19	0.83	0.63
Final BW[4,5], kg	686	3.1	691	682	685	690	3.0	0.87	0.76	0.52	0.034
DMI, kg/d	13.7	0.14	13.8	13.9	13.9	13.8	0.13	0.30	0.75	0.95	0.57
ADG, kg/d	1.77	0.038	1.80	1.75	1.75	1.75	0.037	0.92	0.51	0.51	0.51
G:F, kg/kg	0.129	0.0024	0.129	0.125	0.125	0.128	0.0024	0.42	0.89	0.73	0.20
Carcass adjusted											
ADG[6], kg/d	1.36	0.047	1.36	1.35	1.28	1.29	0.045	0.42	0.12	0.97	0.77
G:F[7], kg/kg	0.10	0.003	0.10	0.10	0.09	0.09	0.003	0.23	0.11	0.98	0.63
Final BW[4], kg	688	3.3	684	687	688	686	3.3	.62	0.75	0.88	0.50

[1]Treatment: All cattle were fed diets containing (dry matter basis) 86.2% barley grain, 6.0% barley silage, 6.2% canola meal, and 1.6% vitamin and mineral supplement, fed for the entire trial length of 69 days.
[2]Products of Pancosma, Geneva, Switzerland. Where: amount of *Capsicum* bypassing rumen from supplement HP = 40%, and from RP = 20%.
[3]*Capsicum* represents all treatments with *Capsicum* oleoresin treatment; SUPP represents the effect of RP vs. HP; Dose represents the effect of LO vs. HI dose of the *Capsicum* oleoresin supplements; and the SUPP × Dose is the interaction.
[4]Determined on a shrunk BW basis (BW × 0.96).
[5]Individual means did not differ based on the Tukey's post-hoc mean separation test.
[6]Calculated by attributing the average carcass dressing percentage to the hot carcass weight resulting in a Theoretical Final BW. The difference between the Initial BW and Theoretical Final BW is then divided by the number of days on feed to get Carcass adjusted ADG.
[7]Calculated by dividing the Carcass adjusted ADG by DMI.

Table 3.3 Carcass weight and carcass characteristics for finishing steers fed a barley-based diet with rumen protected *Capsicum* oleoresin containing differing concentrations of capsaicinoids (**RP** and **HP** contained 20% *Capsicum* oleoresin with 1.2% capsaicinoids and 15.5% *Capsicum* oleoresin with 0.93% capsaicinoids, respectively) and expected extent of rumen protection at low (**LO**) or high (**HI**) doses.

	Treatment[1]							*P*- value[2]			
			RP		HP						
	CON	SEM	LO	HI	LO	HI	SEM	CON vs. *Capsicum*	SUPP	Dose	SUPP × Dose
Hot carcass weight, kg	407	1.9	407	406	405	406	2.0	0.63	0.75	0.88	0.49
Dressing, %	59.32	0.210	58.90	59.43	59.12	58.95	0.209	0.37	0.53	0.39	0.11
Backfat thickness[3], cm	1.17	0.046	1.23	1.17	1.23	1.18	0.045	0.48	0.86	0.21	0.86
Ribeye area[3], cm^2	91.0	1.23	91.5	90.8	91.8	91.6	1.22	0.79	0.67	0.72	0.82
Yield grade[3]											
YG1, %	38.89	5.138	41.11	37.78	30.00	45.56	4.075	0.94	0.71	0.24	0.079
YG2, %	41.11	5.186	38.89	52.22	38.89	42.22	4.149	0.75	0.36	0.13	0.36
YG3, %	18.89	4.126	20.00	10.00	28.89	12.22	3.122	0.62	0.24	0.005	0.67
Yield score[4]	3.07	0.087	3.19	3.07	3.13	3.11	0.094	0.54	0.91	0.46	0.58
Quality grade[3]											
AAA, %	56.67	5.223	73.33	60.00	66.67	53.33	4.011	0.23	0.20	0.016	0.92
AA, %	40.00	5.165	26.67	37.78	25.56	43.33	3.919	0.22	0.71	0.009	0.53
A, %	1.11	1.105	0.00	0.00	3.33	2.22	0.690	0.98	0.98	1.00	1.00
Prime, %	1.11	1.105	0.00	2.22	2.22	1.11	0.843	0.98	0.98	0.98	0.97
B4, %	1.11	1.105	0.00	0.00	2.22	0.00	0.312	0.97	0.99	0.99	0.99
Marbling score[4]	405.5	6.86	421.3	412.8	429.7	403.8	7.45	0.15	0.96	0.032	0.26
Liver score[5]											
Clear, %	59.09	5.241	59.55	51.69	53.49	56.32	4.239	0.53	0.89	0.64	0.33
Minor, %	34.09	5.053	31.46	31.46	32.56	28.73	3.950	0.59	0.87	0.70	0.70
Severe, %	6.82	2.687	8.99	16.85	13.95	14.94	2.912	0.11	0.59	0.22	0.33

Where: YG1 (Canadian Yield Grade 1); YG2 (Canadian Yield Grade 2); YG3 (Canadian Yield Grade 3)

[1]Treatment: All treatments are barley-based finisher rations containing barley grain, barley silage, canola meal, and vitamin and mineral supplement, fed for the entire trial length of 69 days.

[2] *Capsicum* represents all treatments with *Capsicum* oleoresin treatment; SUPP represents the effect of RP vs. HP; Dose represents the effect of LO vs. HI dose of the *Capsicum* oleoresin supplements; and the SUPP × Dose is the interaction.

[3]According to Canadian Beef Grading Agency (Calgary, AB, Canada)

[4]Graded using e+v vision grading system (e+v Technology GmbH, Germany). Where: 600 to 699 = moderate; 500 to 599 = modest; 400 to 499 = small; 300 to 399 =slight; 200 to 299 = trace

[5]According to Elanco Liver Check System (Elanco Animal Health, Greenfield, IN). Where: Clear = no abscesses – a normal, healthy liver; Minor = one or two small abscesses, or up to two to four well-organized abscesses which are generally under one inch in diameter. The rest of the liver is healthy in appearance; Severe = one or more large abscesses present, along with inflammation of liver tissue surrounding abscess. Often, portions of the diaphragm must be trimmed to separate the liver from carcass.

abscess scores ($P \geq 0.11$). To the authors knowledge, there is no other data evaluating effects of *Capsicum* oleoresin on carcass characteristics of cattle.

With the data collected, it is not possible to confirm the underlying mechanisms that explain the change in yield grade and quality grade between the low and high doses of RPC, but it is speculated that higher doses may be related to alteration of inflammatory responses, alteration in insulin sensitivity, or by directly altering lipid metabolism. For example, while encapsulated products reduce ruminal availability, past research evaluating encapsulated *Capsicum* oleoresin has reported a tendency for a linear reduction in ruminal pH, tendency for linear increase in short-chain fatty acid concentration, and a concomitant tendency for linearly reduced acetate concentration with increasing *Capsicum* extract dose (Rodriguez-Prado et al. 2012). However, a positive linear response on DMI was also observed with increasing *Capsicum* extract dose (Rodriguez-Prado et al. 2012). Risk and severity of ruminal acidosis increases with advancing days on feed (Castillo-Lopez et al. 2014) and ruminal acidosis can lead to a local (Kent-Dennis et al. 2020) and low-grade systemic inflammatory response (Aschenbach et al. 2019) potentially mediated in part by hind-gut acidosis (Pederzolli et al. 2018). Mild inflammation has been reported to lead to insulin resistance (Horst et al. 2019) that is coupled with lipolysis in adipose tissue (Chivri et al. 2022). Additionally, low doses of RPC have been reported to reduce inflammation (Oh et al. 2015) and enhance insulin sensitivity (Oh et al. 2017b); however, the present study cannot confirm these effects. In addition to insulin resistance, there is a growing body of evidence that an activated systemic immune response markedly increases the irreversible loss of amino acids (McNeil et al. 2016) and glucose (Horst et al. 2019) likely diverting nutrients away from skeletal muscle and adipose tissue deposition (Bradford and Ylioja, 2018). As such, it is plausible that the low, but not the high dose, dampened potential inflammatory signals reducing insulin resistance and lipolysis. Conceptually, Westphalen et al. (2021) has provided evidence that RPC (1.1% capsaicinoids) fed at the rate of 15 mg/kg DM may reduce recurrent treatment of disease in feedlot cattle supporting the postulated mechanism of a dampened immune response. Future research is needed to elucidate how *Capsicum* oleoresin may alter tissue deposition, lipolysis, and carcass quality characteristics in finishing cattle.

Liver scores did not differ with the inclusion of *Capsicum* oleoresin, supplement, dose, or the supplement × dose interaction. Overall, 56.0% of the livers had no abscess, 31.7% had minor,

and 12.3% had severe abscesses. These liver scores are similar to that reported in a 2010/2011 National Auditing program led by the Canadian Beef Cattle Research Council (NBQA, 2018).

3.5 Conclusions

There were no differences observed for *Capsicum* oleoresin products expected to differ in the extent of ruminal protection. However, increasing the dose of *Capsicum* oleoresin may decrease the carcass yield grade and decrease the proportion of AAA quality grades and marbling score without affecting DMI, ADG, or dressing percentage. For yearling steers during the finishing stage, there appears to be no effect of dietary *Capsicum* oleoresin inclusion when compared to non-supplemented steers.

4.0 EFFECT OF RUMEN PROTECTED *CAPSICUM* OLEORESIN AND PALMITIC ACID ON DRY MATTER INTAKE AND BLOOD METABOLITES IN RESPONSE TO A GLUCOSE TOLERANCE TEST IN FEEDLOT CATTLE

Data availability: Data are held in the Department of Animal and Poultry Science at the University of Saskatchewan (Saskatoon, SK, Canada). Queries to access the data should be made through the corresponding author.

Author contributions: Eidsvik, Penner, McKinnon, and Moya were responsible for experimental design. Khelil and Blanchard were contributed intellectually to the study structure and discussion.

4.1 Abstract

The objective of this study was to evaluate the effects of *Capsicum* oleoresin when fed with or without added palmitic acid on dry matter intake (DMI), basal circulating metabolite, hormone, and acute phase protein concentrations, and insulin sensitivity in response to an intravenous glucose tolerance test (**IVGTT**). A total of 12 heifers were arranged in a 4 × 4 Latin square design balanced for carry-over effects with each of the 3 squares designed to have a unique treatment sequence. Treatments were arranged in a 2 × 2 factorial design utilizing a barley-based high-grain diet that included *Capsicum* oleoresin at 0 (**C**$^{-}$) or 77 (**C**$^{+}$**)** mg/d and palmitic acid (PALM) to increase dietary ether extract concentrations from 3.46% **(P**$^{-}$**)** to 7.63% **(P**$^{+}$**)** on a DM basis. Each period of the Latin square consisted of 28 days with days 22 to 28 used for data and sample collection. Data collection included DMI, apparent total tract digestibility, blood sampling, an IVGTT, and corresponding muscle biopsies to assess muscle glycogen concentrations. Palmitic acid inclusion decreased DMI, increased organic matter digestibility, and increased baseline serum NEFA concentration ($P \leq 0.003$). During the IVGTT, PALM increased peak insulin, insulin positive incremental area under the curve, the total area under the curve for insulin (I-AUC and AUC, respectively; $P \leq 0.003$), insulin clearance rate ($P \leq 0.015$), and decreased both the maximum concentration of glucose reached and the maximum change in glucose concentration observed ($P \leq 0.029$). *Capsicum* prevented an increase in insulin concentration prior to the IVGTT when fed with PALM (*Capsicum* × PALM interaction, $P = 0.017$). The addition of PALM to finishing diets increased insulin resistance, but *Capsicum* may help moderate insulin concentrations. However, there were no effects of *Capsicum* during IVGTT in the current study ($P \geq 0.15$).

4.2 Introduction

The anabolic hormone insulin is released from pancreatic β-cells with the primary action to stimulate the uptake of glucose and amino acids from the bloodstream into insulin sensitive tissue such as skeletal muscle (Tokarz et al. 2018). While ruminants rely on gluconeogenesis, insulin resistance has been reported in dairy (De Koster and Opsomer, 2013; Rico et al. 2017) and feedlot cattle (Joy et al. 2017; de Sousa et al. 2022). Dairy cattle exhibit insulin resistance during the transition period; whereas, feedlot cattle exhibit insulin resistance as they advance in days on feed and age as they shift tissue deposition away from skeletal muscle and toward adipose tissue (Trenkle and Topel, 1978; De Koster and Opsomer, 2013; NASEM, 2016; Joy et al. 2017). For dairy cattle, insulin resistance increases lipolysis and hepatic lipid accumulation (Rico et al. 2017) and may be a homeorhetic mechanism. For feedlot cattle, less is known; however, occurrence of insulin resistance is associated with a reduction in feed efficiency (Joy et al. 2017) and systemic inflammation (de Sousa et al. 2022) questioning whether insulin resistance may contribute to altered body composition (Chapter 3).

Strategies to mitigate insulin resistance for dairy cattle include the use plant extracts as a potential feed-based option (Oh et al. 2017b). Capsaicin (*Capsicum* oleoresin) has been reported to increase feed efficiency and milk production, to reduce the area under the curve for insulin in response to a glucose tolerance test (Oh et al. 2017b), and may dampen the proinflammatory response (Oh et al. 2017a). Capsaicin is the active compound that elicits the burning sensation present in *Capsicum* fruits such as chili and red peppers (Oh et al. 2015; Bort et al. 2019. For feedlot cattle, use of *Capsicum* oleoresin has been reported to increase DMI (Rodriguez-Prado et al. 2012) and increasing the dose of *Capsicum* reduced marbling score (Chapter 3). Additionally, ruminally protected *Capsicum* oleoresin (RPC) may improve health status as indicated by a reduction in the proportion of light-weight cattle with elevated body temperature and recurrence of these fever events (Westphalen et al. 2021). While the effect of capsaicin on insulin resistance and inflammation has been evaluated in dairy cattle, at the present time there was a lack of research evaluating the effect of capsaicin in finishing cattle.

Lipid inclusion in diets for feedlot cattle is used to increase energy density of the diet beyond that achievable with increases in dietary starch (Zinn 1989). Beyond increasing energy density, the composition of the lipid supplement can exert differing biological responses. For example,

PALM has been reported to dysregulate insulin signalling, whereas unsaturated omega-3 fatty acids such as eicosapentaenoic acid and docosahexaenoic acid are anti-inflammatory fats that can decrease site-specific insulin resistance (Reynoso et al. 2003; Sears and Perry, 2015). The effects of PALM treatment include the inhibition of the insulin signalling pathway via insulin receptor and insulin receptor substrate-1, the reduction glucose uptake, and increased inflammatory response (Reynoso et al. 2003; Kennedy et al. 2009; Hernández-Cáceres et al. 2019). As such, inclusion of PALM may provide a model to induce insulin resistance in feedlot cattle.

The hypothesis for this study was that PALM inclusion would increase insulin resistance and indicators for systemic inflammation for heavy finishing beef heifers, and that RPC would mitigate inflammation and decrease insulin resistance. The objective of this study was to evaluate the effects of *Capsicum* oleoresin when fed with or without added PALM on DMI, basal circulating metabolite, hormone, and acute phase protein concentrations, and insulin sensitivity in response to an intravenous glucose tolerance test (IVGTT).

4.3 Materials and Methods

All cattle were cared for according to the guidelines of the Canadian Council on Animal Care (Ottawa, ON, Canada) and all procedures were pre-approved by University of Saskatchewan Animal Research Ethics Board (protocol 20100021; Saskatoon, SK, Canada).

4.3.1 Experimental Design

Prior to the start of the study, 12 Hereford × Simmental beef heifers were group-housed in an outdoor pen and transitioned from a barley-based (silage and grain) diet with a forage-to-concentrate ratio of 50:50 to one with a 12:88 ratio. The transition was accomplished using six diets with each fed for four days. On day 24 of the dietary transition protocol, heifers were fed in their outside group pen in the morning, moved into individual tie-stalls in the afternoon, and provided fresh feed. After two days of indoor feeding, all heifers received a common diet resembling that used in the experiment, with a forage-to-concentrate ratio of 10:90 until the start of the experiment that was initiated by square 5 to 9 d later.

Throughout the study, heifers were housed indoors in individual tie-stalls (3.14 m^2), each separated by 0.9 m and equipped with a waterbed (Pro-Line Manufacturing, Saskatoon, SK, Canada), individual water bowl, and a suspended ball (Jolly Pets, Streetsboro, OH, USA) for environmental enrichment. Daily care involved removal of wood shavings, cleaning of stalls, and

reapplication of wood shavings. On a daily basis, heifers were provided with 2 h (0730 to 0930 h) of access to an outdoor exercise yard. Heifers were provided access to exercise except during intensive collection periods or when the combined temperature and wind yielded windchill values below -30°C.

Heifers were weighed on two consecutive days before the start of experiment, prior to feeding (0800 h). The average body weight (BW) arising from the two-day weights was used to stratify heifers into one of three squares creating light (511 ± 17 kg), medium (531 ± 5 kg), and heavy-weight (559 ± 6 kg) squares with four heifers in each square. Heifers were grouped in individual tie-stalls by square. Subsequently, heifers within a square were randomly assigned to a treatment sequence in a 4 × 4 Latin square design balanced for carry-over effects with each square having a different treatment sequence. The Latin square was designed with 28 d periods including 21 d of diet adaptation and 7 d for data and sample collection. To manage sampling events, the start of squares were offset by 2 d.

Dietary treatments (Table 4.1) were arranged as a 2 × 2 factorial design with *Capsicum* oleoresin (CapsXL; Pancosma, Rolle, Switzerland) included at 0 (**C**$^{-}$) or 77 (**C**$^{+}$**)** mg/d (Chapter 3) and PALM (Energizer Rumen Protected (RP10); Scothorn Nutrition, Grand Pré, NS, Canada) was added to the basal diet (ether extract (EE) = 3.46%**; P**$^{-}$**)** to achieve 7.63% **(P**$^{+}$**)** EE on a DM basis (Table 4.1). The 77 mg/d dose rate of **RPC** was chosen based on increased marbling score and proportion of steers with AAA grading observed in (Chapter 3). CapsXL is a rumen protected *Capsicum* oleoresin product, protected by a double layer of micro-encapsulation within a fat matrix (XTRACT, 2022). Palmitic acid was added to increase insulin resistance (Zhu et al. 2016, Mathews et al. 2016; Reynoso et al. 2003). The *Capsicum* oleoresin was weighed daily (allowing for 5% target feed refusal) and mixed into a 1-kg subsample of fresh feed that was mixed into the full diet allocation for the respective heifers.

Diets consisted of barley silage (6.93%; DM basis), barley grain (82.12%), mineral (5.83%), urea (0.32%), and beet pulp (5.8%). Palmitic acid was included by replacing beet pulp, ensuring diets were similar with respect to the forage:concentrate ratio and dietary starch concentration. Urea was added to diets containing PALM to balance crude protein (CP) among all diets. Heifers were fed their diet as a total mixed ration once daily (1000 h) with the amount offered

Table 4.1 Dietary ingredient and chemical composition used to test the effect of *Capsicum* oleoresin and palmitic acid inclusion in diets for finishing heifers**.**

	Treatment[1]			
Ingredients, %DM	C+P+	C+P-	C-P+	C-P-
Barley silage	6.93	6.93	6.93	6.93
Steam rolled barley	82.12	82.12	82.12	82.12
Mineral and vitamin supplement[2]	5.83	5.83	5.83	5.83
Beet pulp	0.3	4.8	0.30	4.8
Urea	0.48	0.32	0.48	0.32
Palmitic acid[3]	4.33	-	4.33	-
CapsXL[4]	77 mg/d	77 mg/d	-	-
Nutrient Composition, %DM				
Dry matter, % as fed	82.73 ± 1.55	82.41 ± 1.57	82.73 ± 1.55	82.41 ± 1.57
OM	94.89 ± 0.33	94.59 ± 0.35	94.89 ± 0.33	94.59 ± 0.35
CP	13.11 ± 0.09	13.09 ± 0.08	13.11 ± 0.09	13.09 ± 0.08
ADF	8.23 ± 0.39	9.28 ± 0.39	8.23 ± 0.39	9.28 ± 0.39
NDF	16.38 ± 0.42	18.12 ± 0.42	16.38 ± 0.42	18.12 ± 0.42
Starch	54.88 ± 1.00	54.93 ± 1.00	54.88 ± 1.00	54.93 ± 1.00
Ethanol soluble carbohydrates	1.31 ± 0.47	1.94 ± 0.48	1.31 ± 0.47	1.94 ± 0.48
Ether extract[5]	7.63 ± 0.23	3.46 ± 0.23	7.63 ± 0.23	3.46 ± 0.23
Ca	0.74 ± 0.07	0.77 ± 0.07	0.74 ± 0.07	0.77 ± 0.07
P	0.32 ± 0.01	0.32 ± 0.01	0.32 ± 0.01	0.32 ± 0.01
Ash	5.11 ± 0.33	5.41 ± 0.35	5.11 ± 0.33	5.41 ± 0.35
NE_m, Mcal/kg	2.08 ± 0.03	1.90 ± 0.03	2.08 ± 0.03	1.90 ± 0.03
NE_g, Mcal/kg	1.32 ± 0.02	1.26 ± 0.02	1.32 ± 0.02	1.26 ± 0.02

[1]Treatments: C+P+: *Capsicum* oleoresin and palmitic acid; C+P-: *Capsicum* oleoresin; C-P+: palmitic acid; C-P-: control.

[2]Mineral formulated to provide 33 mg/kg monensin (Rumensin; Elanco Animal Health, Indianapolis, IN, USA) and 0.4 mg/day of melengestrol acetate (MGA 100 Premix) in final diet. The mineral contained, on a DM basis: 8.81% calcium (limestone; dicalcium phosphate), 0.41% phosphorous (dicalcium phosphate), 1.50% magnesium (Dynamate), 1.87% potassium (Dynamate), 3.25% sodium (white salt), 99.66 mg/kg manganese (manganese oxide), 365.68 mg/kg copper (copper oxide; Dynamate), 294.36 mg/kg zinc (zinc oxide), 11.00 mg/kg iodine (ethylenediamine dihydroiodide), 0.26 mg/kg cobalt, 1.08 mg/kg selenium (Diamond V Selenosource 2000), 39,591.17 IU/kg Vitamin A, 6,334.59 IU/kg Vitamin D, 1,002.47 IU/kg Vitamin E.

[3]Energizer Rumen Protected (RP10) (Scothorn Nutrition, Grand Pré, NS)

[4]CapsXL; Pancosma, Rolle, Switzerland

[5]Fat content as determined by acid hydrolysis

designed to achieve refusals weighing 5 to 10% (actual achieved was 8.2%) relative to the as fed weight of the diet provided. Prior to the morning feeding, refusals were collected (0730 h) and weighed. Dietary ingredients were collected once weekly and DM was determined once weekly for beet pulp, steam-rolled barley, and mineral, and twice weekly for barley silage. A single lot of dry-feed ingredients (beet pulp, steam-rolled barley, mineral, urea, PALM, and *Capsicum* oleoresin) were used throughout the study. Dry matter data were used to compile a 3-wk running average that was used to update the dietary as fed inclusion rates for individual ingredients to ensure the diet formulated and delivered were the same on a DM basis.

4.3.2 Heifer Body Weight and Dry Matter Intake

At the start (d 0 and 1) and end of the experiment (d 29 and 30), heifers were weighed on 2 consecutive days (0800 h). In addition, BW was recorded on d 26 of each period. Feed intake was determined daily based on the difference between the weight of the feed offered and feed refused. The daily samples were composited proportionally and dried in a forced-air oven at 55°C until achieving a constant weight to determine DMI. The dietary DM was determined based on the DM coefficient for each ingredient and the inclusion rate in the diet. Data for DMI were obtained during the 4-d total collection period (d 22 to 25 of each period) which corresponded to daily feed ingredient and refusal collection.

4.3.3 Apparent Total Tract Digestibility

Titanium dioxide (TiO2) was included in diets (0.3% of DM) as an external marker to enable fecal output estimation (Titgemeyer et al. 2001). Fecal samples were collected from the rectum from d 22 to d 25 with 12 h intervals between collection points within a day and a 15 h interval among days. At each sampling point, 200 g of feces were collected, placed in a common container, and stored at -20°C until end of collection period when they were dried in a forced-air oven at 55°C until achieving a constant weight. Dried samples were ground to pass through a 3-mm sieve using a hammer mill (Christy & Norris 8” Lab Mill; Ipswich, United Kingdom) and a sub-sample was sent to Cumberland Valley Analytical Services (Waynesboro, PA, USA) where they were re-ground to pass through a 1-mm sieve. Samples were also ground to pass through a 1-mm sieve using Retsch rotary mill (Ultra Centrifugal Mill ZM 200; Retsch-Allee 1-5, Haan, Germany) for TiO2 analysis (Myers et al. 2004). The difference between nutrient intake and

nutrient output relative to nutrient intake was used to determine digestibility via calculation with the measured consumption and excretion of TiO2 (Titgemeyer et al. 2001).

4.3.4 Baseline and Intravenous Glucose Tolerance Test (IVGTT) blood samples

On d 27 of each period, blood was collected at 0600, 1400, and 2200 h via jugular venipuncture to attain baseline blood samples. At each of the three time points, 6 mL of blood was collected into tubes containing 15 mg sodium fluoride and 12 mg potassium oxalate (BD Vacutainer, Becton, Dickinson and Company, Franklin Lakes, NJ, USA) for plasma, and 10 mL of blood was collected into tubes with a silicon spray coated interior (BD Vacutainer, Becton, Dickinson and Company, Franklin Lakes, NJ, USA) to allow for separation of serum. Plasma and serum samples collected on d 27 were composited on an equal volume basis for each heifer and stored at -20°C until analysis.

In preparation for the IVGTT (d 28), heifers were weighed prior to feeding on d 26 and heifers were denied access to feed starting at 2000 h on d 27 to ensure there was a 12 h fast prior to glucose infusion (Joy et al. 2015). Jugular catheters (2.1 × 133 mm BD Angiocath, Becton, Dickinson and Company, Franklin Lakes, NJ, USA) were inserted the morning of d 28. For the IVGTT, a 2.78 *M* solution of sterile dextrose (Dextrose 50%, Vetoquinol, Lavlatrie, QC, Canada) was infused at a dose rate of 7.57 mmol/kg $BW^{0.75}$ (1.36 g/kg $BW^{0.75}$) over a 2-min period (Joy et al. 2017). This dose was chosen as it resulted in a large increase in plasma glucose and insulin but did not induce renal glucose excretion for cattle in a similar physiological state as the current study (Joy et al. 2017). Catheters were flushed with 10 mL of heparinized saline (10 USP/mL) after infusing the glucose. Blood samples were collected at -10, 0 (immediately following glucose infusion and flushing the infusion line), 5, 10, 15, 20, 25, 30, 45, 60, 90, and 120 min relative to infusion. At each time point, 6 mL of blood was collected into tubes containing 15 mg sodium fluoride and 12 mg potassium oxalate (BD Vacutainer, Becton, Dickinson and Company, Franklin Lakes, NJ, USA) to allow for separation of plasma while inhibiting glycolysis. Samples were inverted to mix, placed on ice, and centrifuged at 2,500 × *g* for 15 min at 4°C. Plasma was harvested and samples were stored at -20ºC. Before freezing, samples were transferred to either a 5 mL cryovial (d 27 samples; Thermo Fisher Scientific, Waltham, Massachusetts, USA) or transferred into 1.5 mL snap-cap vials (High Performance

Microcentrifuge Tubes, VWR, Radnor, PA, USA) and saved in duplicate in order to minimize freeze-thaw events during analysis.

Plasma was used for insulin (Bovine Insulin ELISA; Mercodia AB, Uppsala, Sweden) and glucose (PGO Enzymes; Sigma-Aldrich Corp., St. Louis, MO) analysis. For the d 27 composite samples and the individual time points collected during the IVGTT, the insulin assay was conducted in duplicate, with intra- and inter-plate assay coefficients of variation (CV) of 1.75% and 2.07%, respectively. For glucose, samples were analyzed in duplicate resulting in intra- and inter-plate assay CV of 1.29 and 2.39%, respectively. Serum was used for the analysis of non-esterified fatty acids (NEFA; HR Series NEFA-HR(2); FUJIFILM Wako Diagnostics U.S.A. Corporation, Mountain View, CA, USA) haptoglobin (Hp; Bovine Haptoglobin, GenWay Biotech Inc., San Diego, CA, USA), and serum amyloid A (SAA; PHASE Cat. No. TP-802, Tridelta Development Limited, Maynooth, County Kildare, Ireland) in d 27 samples only. For NEFA analysis, samples were analyzed in triplicate with intra- and inter-plate assay CV of 2.85% and 1.16%, respectively. For haptoglobin, samples were analyzed in triplicate with the intra-plate assay CV of 2.12% and the inter-plate assay CV of 7.21%. For SAA, samples were analyzed in duplicate and the intra- and inter-plate assay CV were 6.64% and 10.49%, respectively.

The 12-h fasting plasma glucose and insulin concentrations (-10 min relative to glucose infusion), maximum concentrations, the difference between baseline and maximum (range), the lowest level observed (nadir), and the time at which insulin maximum occurred (T_{max}) during the IVGTT were determined. Glucose clearance rate, presented as %/min, was calculated as the slope of the natural logarithm of glucose (Kaneko, 1997) from 5 to 45 min using the slope function of Microsoft Excel 2016 (Microsoft® Excel for Microsoft 365 MSO, Microsoft Corporation, Redmond, WA, USA). The 5 to 45 min interval represented the exponential decline (second phase) and the main elimination phase in a 3-phase decay model of glucose concentrations over time (Pacini et al. 2009). From glucose clearance rate, the glucose half-life ($T_{1/2}$) was calculated by dividing 0.693 by the glucose clearance rate (Vasconcelos et al. 2009; Kneeskern et al. 2016; Zachut et al. 2013). Insulin clearance rate, expressed as %/min, was calculated as the slope of the natural logarithm of insulin from T_{max} until 90 min using the same procedure as described above for glucose. Insulin $T_{1/2}$ was calculated using the same calculation

as for glucose $T_{1/2}$ (Chiou 1978; Hare et al. 2022). Total glucose and insulin area under the curve (AUC) from 0 to 120 min ($AUC_{0to120min}$) were calculated by summing the trapezoidal area formed between measurement intervals (Chiou 1978). Further, glucose and insulin positive incremental AUC from 0 to 120 min ($I\text{-}AUC_{0to120min}$) were also calculated by taking baseline concentrations into account (Cardoso et al. 2011), as shown below:

$$\mathrm{I-AUC} = \frac{\left((C_a - C_{baseline}) + (C_b - C_{baseline})\right) \times (t_b - t_a)}{2}$$

Where; C_a = analyte concentration at time point t_a, C_b = analyte concentration at time point t_b, $C_{baseline}$ = baseline analyte concentration, and t_b and t_a = two discrete time points.

The insulin sensitivity index (IS_i), defined as the ability of insulin to aid glucose to influence its own disappearance (Bergman et al. 1979) was calculated according to Galvin et al. (1992), as modified by Pacini et al. (2009) to account for a dynamic insulin interval length.

The formula used to calculate the insulin sensitivity index:

$$IS_i = \frac{k_g}{\frac{1}{90}\int_0^{90}[I(t) - I_{90}]dt},$$

Where IS_i was calculated using; k_g = the glucose clearance rate from 5 to 45 min, divided by the product of the dynamic insulin I-AUC from 0 to 90 min (calculated using Eq. 1 above) and the length of the interval (90 min). Where; *dt* = represents the definite integral of the independent variable (time) from 0 minutes to 90 minutes, *I* = the function of each insulin concentration, *t* = each time point of blood sampling, and I_{90} = the function of the basal insulin concentration at 90 min.

4.3.5 Adipose and Skeletal Muscle Tissue Biopsies

As an approach to evaluate skeletal muscle insulin sensitivity, skeletal muscle biopsies were collected on d 28 at 0600 (prior to the IVGTT) and 1100 h (3 h post IVGTT). Biopsies were taken from the longissimus lumborum (caudal to 13th rib, ~10 cm ventral to spine). Briefly, a 225-cm^2 area was clipped and scrubbed (4% chlorhexidine gluconate, Becton, Dickinson, and Company, Franklin Lakes, NJ, USA) and sanitized (dovidine antiseptic skin cleanser; 7.5% povidone-iodine; Laboratoire Atlas Inc., Montreal, QC, Canada). Heifers were provided a total

of 30 mL of lidocaine (Lido-2, Rafter 8 Products, 87 Skyline Cres. N.E., Calgary, AB) to mitigate subcutaneous and deep tissue pain. Lidocaine was administered in an inverted L-pattern using 38-mm, 18-gauge needle (Monoject, Covidien, Dublin, Ireland). After tissue was numb, a sterile scalpel was used to create an 8-cm incision perpendicular to the spine. Forceps and a 6-mm disposable biopsy punch (Acupunch, Acuderm, Ft. Lauderdale, FL, USA) were then used to collect all biopsies. Sterile gauze was prepared and used as required throughout the sampling procedure. The sampling approach targeted 1.5 g of skeletal muscle during the pre-IVGTT sampling (1 hr prior to IVGTT) and 1.5 g biopsy of skeletal muscle taken during post-IVGTT (immediately after IVGTT). The post-IVGTT site was taken from an incision site 2.5 to 3.0 cm caudal to pre-IVGTT incision. After tissue collection, the incision site was pursed together and stapled shut using a single-use skin stapler (Appose ULC 35W, Covidien, Dublin, Ireland). Collected tissues were placed in 2-mL cryovials (Nalgene, VWR, Radnor, PA, USA), immediately snap frozen in liquid nitrogen, and stored at -80°C. Glycogen analysis was performed on the skeletal muscle samples using Glycogen Assay Kit (ab65620; abcam, Cambridge, MA, USA). During preparation for analysis, samples were kept under liquid nitrogen and ground in a mortar that was sitting on ice and 10 to 15 passes of the pestle. Samples were then boiled on a heating block (100^{o}C) for 30 mins before adding ethanol and placing into a centrifuge at 1000 × g for 15 mins at 4^{o}C. After the supernatant was removed, 1 mL of double-distilled water was added. The ethanol addition and centrifuge procedure were conducted twice more for a total of three times per sample. Finally, 1 mL of double-distilled water was added to the samples before they were analyzed for glycogen content in duplicate with intra- and inter-plate assay CV of 5.11% and 2.41%, respectively. The protein concentration (bicinchoninic acid) of the tissue was determined using a colorimetric reaction (Smith et al. 1985) with samples analyzed in triplicate using reagents from Sigma Aldrich (St. Louis, MO, USA). The intra- and inter-plate assay CV were 2.21% and 5.26%, respectively.

4.3.6 Chemical Analysis

All dried and ground feed ingredient, feces, and refusal samples were sent to Cumberland Valley Analytical Services (Waynesboro, PA, USA) and analyzed for DM, CP, ADF, NDF, starch, ethanol soluble carbohydrates (ESC), EE, Ca, P, ash. The net energy of maintenance (NE_m), as well as the net energy of gain (NE_g) were calculated with a summation equation (NRC, 2001). The DM was determined by methods described by Shreve et al. (2006). The CP was

determined by nitrogen combustion using a Leco FP-528 Nitrogen Combustion Analyzer (Leco, St. Joseph, MI, USA). The ADF was determined according to Association of Official Analytical Chemists method 973.18 (AOAC, 2000), with the modifications that, instead of fritted glass crucibles, Whatman 934-AH glass micro-fibre filters (Cytiva, Global Life Sciences Solutions USA LLC, Marlborough, MA, USA) with a 1.5 µm particle retention were used. Neutral detergent fiber (NDF) was determined using method of Van Soest et al. (1991) with α-amylase (A3306, Sigma-Aldrich, St. Louis, MO) and sodium sulfite utilizing the same crucible modification as described for ADF. Starch was determined according to Hall (2009). Ethanol soluble carbohydrate (ESC) concentration was determined using a colorimetric method described by Dubois et al. (1956). Acid hydrolysis was used to determine EE concentration in samples using a gravimetric method (method 954.02; AOAC, 2000). The Association of Official Analytical Chemists method 985.01 (AOAC, 2000) was used to determine calcium and phosphorous concentrations, with the modification that 0.35 g samples were ashed for 1 h at 535°C before digesting in open crucibles in 15% nitric acid on hotplate for 20 min, after which the samples were diluted to 50 mL and analyzed on ICP. Ash was determined using method 942.05 (AOAC, 2000) with the modification that a 1.5 g sample was used with a 4-h ashing duration. The titanium dioxide concentration in feed ingredients, fecal samples, and feed refusals was determined as per Myers et al. (2004) which included pre-digestion of samples (Tecator Digestor Auto 1011 3844, FOSS) with 98% sulfuric acid and a catalyzer (FisherTab™ CT- 135 37 Kjeldahl Tablets K3011000, Fisher Scientific). Digestion occurred at 420^{o}C for 2 h followed by the addition of 30% hydrogen peroxide, filtration of samples through Whatman 541 filter paper (Maidstone, United Kingdom), and measurement of absorbance at 410 nm.

4.3.7 Statistical Analysis

Statistical Analysis Software 9.4 (SAS Inst. Inc., Cary, NC, USA) was used to analyze data using the Mixed Model procedure as a 2 × 2 factorial within a replicated Latin square. For all parameters except muscle biopsies, the model included the fixed effects of PALM, *Capsicum*, and the 2-way interaction, as well as the random effect of square, period, and heifer nested within square. Data were tested for normality and when required, data were transformed. Transformation was required for composite insulin:glucose ratio, NEFA, peak insulin time, insulin $T_{1/2}$, and glucose I-AUC using square root transformation; and Hp, SAA, sensitivity index, maximum, and range using Log10 transformation. The Studentized residuals were used to

identify outliers, based on an absolute value of 3 being considered an outlier. Two outliers were removed from DMI data, one from OM digestibility data, and one from CP digestibility data. Two outliers were removed from d 27 composite insulin data, one outlier from composite insulin-to-glucose data, six from Hp data, two from SAA data, one from fasting insulin data, and one from fasting insulin-to-glucose ratio data. Two outliers were removed from insulin $T_{1/2}$ data, one from insulin sensitivity index data, one from glucose maximum data, one from glucose range data, one from glucose positive incremental AUC data, and one from glucose total AUC data. The starch digestibility during this study was calculated as either 98.9% or above. Therefore, statistical analysis on this parameter was deemed unwarranted. For data that was transformed, the original least squared means and the largest SEM are reported, whereas the reported *P*-value is from the transformed data SAS output. Significance was declared when $P < 0.05$ and tendencies were declared when $0.05 < P < 0.10$.

Muscle tissue biopsy data were analyzed using the same model with the inclusion of time as a fixed effect and repeated measure. The Mixed Model procedure analyzed a model that included fixed effects of *Capsicum*, PALM, time, the 2-way interaction of *Capsicum* × PALM, the 3-way interaction of *Capsicum* × PALM × time, as well as the random effect of square, period, and heifer nested within square. PROC UNIVARIATE was used to assess normality based on Shapiro-Wilk value of $P \geq 0.05$ signifying normal data. If data was not normal, it was transformed. Normalized data was then analyzed on the covariance structure with the determined lowest Akaike's Information Criterion and Bayesian Information Criterion. Glycogen and glycogen:BCA data required square root transformation, whereas BCA data required inverse method transformation. Outliers were evaluated as described above resulting in one data point from the glycogen-to-BCA ratio data being removed. For data that was transformed, *P*-values from the transformed data analysis were reported whereas the least squared means and the largest SEM are reported non-transformed data.

4.4 Results

There was no effect of *Capsicum*, PALM, or their interaction on BW in the current study ($P \geq 0.30$). Palmitic acid inclusion decreased DMI ($P < 0.001$; Table 4.2) by nearly 1 kg/d. However, there was no effect of *Capsicum* or treatment interaction ($P \geq 0.17$). The digestibility of DM was

Table 4.2 Effect of *Capsicum* oleoresin (77 mg/d) and palmitic acid (4.33% DM) inclusion fed to finishing heifers on BW, DMI, and apparent total tract nutrient digestibility.

	Treatment[1]					*P*-value		
Variable	C+P+	C+P-	C-P+	C-P-	SEM[2]	C	P	C × P
BW[3], kg	612	616	614	615	20.8	0.92	0.30	0.46
DMI, kg/d	8.36	9.57	8.51	9.19	0.384	0.65	<0.001	0.19
Digestibility, % DM								
DM	84.1	83.8	84.0	83.2	0.78	0.37	0.14	0.49
OM	97.7	97.4	97.7	97.4	0.19	0.79	<0.001	0.89
CP	83.3	82.1	83.0	81.2	1.15	0.19	0.002	0.44
ADF	38.7	46.8	40.1	46.8	2.63	0.72	0.001	0.75
NDF	44.7	50.0	45.8	48.4	2.25	0.85	0.003	0.27
Ether extract	82.0	73.9	82.2	71.9	1.94	0.22	<0.001	0.15

[1]The basal diet consisted of (DM basis) 82.12% steam-rolled barley, 6.93% barley silage, and 5.83% mineral and vitamin supplement. The remainder of the diet included the inclusion of beet pulp (4.80 vs. 0.30), urea (0.32 vs. 0.48%), and palmitic acid (0 vs. 4.33%) for the diets without (P-) and with palmitic acid (P+; Energizer Rumen Protected (RP10); Scothorn Nutrition, Grand Pré, NS), respectively. *Capsicum* oleoresin (CapsXL; Pancosma, Rolle, Switzerland) was included at 0 (C-) or 77 mg/d (C+).
[2]The SEM represents the largest SEM for the interaction terms.
[3]BW: shrunk bodyweight (body weight × 0.96).

unaffected by *Capsicum*, PALM, or *Capsicum* × PALM interaction ($P \geq 0.14$). Organic matter digestibility was greater for treatments with PALM ($P < 0.001$) but was not affected by *Capsicum* or the *Capsicum* × PALM interaction ($P \geq 0.79$). Crude protein digestibility was increased by PALM ($P = 0.002$) but was not affected by *Capsicum* or the *Capsicum* × PALM interaction ($P \geq 0.19$). The digestibility of both ADF and NDF were decreased by PALM ($P < 0.003$) but were not affected by *Capsicum* or the *Capsicum* × PALM interaction ($P \geq 0.27$). The digestibility of EE was decreased by PALM ($P < 0.001$), but was not affected by *Capsicum* or the *Capsicum* × PALM interaction ($P \geq 0.15$).

There were no effects of *Capsicum*, PALM, or their interaction on fed-state glucose concentrations ($P \geq 0.28$; Table 4.3). However, there was a *Capsicum* × PALM interaction for fed-state insulin concentration ($P = 0.017$) such that *Capsicum* reduced insulin when fed with PALM, but the effect of *Capsicum* was not detectable when PALM was not included. Correspondingly, the calculated fed-state insulin:glucose ratio was affected by a *Capsicum* × palm interaction ($P = 0.036$) such that the ratio was greater for those fed C+P- than C+P+ with treatments C-P+ and C-P- intermediate and not different. Serum NEFA concentration was not affected by *Capsicum* or *Capsicum* × PALM interaction ($P \geq 0.45$) but were increased by PALM ($P = 0.003$). Serum levels of Hp were not affected by *Capsicum* or *Capsicum* × PALM interaction ($P \geq 0.105$), but were increased by PALM ($P = 0.035$). Circulating serum levels of SAA were not affected by *Capsicum* or *Capsicum* × PALM interaction ($P \geq 0.57$). However, PALM increased SAA ($P = 0.035$).

Fasting plasma glucose concentration was not affected by *Capsicum*, PALM, or the *Capsicum* × PALM interaction ($P \geq 0.14$; Table 4.4). There was a *Capsicum* × PALM interaction on fasted insulin concentrations ($P = 0.037$) although there were no statistical differences between individual means ($P \geq 0.12$). Nevertheless, numerically the interaction occurred as *Capsicum* reduced insulin when fed with PALM but insulin was increased when fed *Capsicum* without PALM. Likewise, there was a *Capsicum* × PALM interaction for the fasted insulin:glucose ratio ($P = 0.040$) although there were no statistical differences between means ($P \geq 0.090$).

During the IVGTT, peak insulin concentration was increased by PALM ($P = 0.003$) but was not affected by *Capsicum* or the *Capsicum* × PALM interaction ($P \geq 0.23$). Time to

Table 4.3 Effect of *Capsicum* oleoresin (77 mg/d) and palmitic acid (4.33% DM) fed to feedlot heifers on fed-state blood metabolites.

	Treatment[1]					*P- value*		
Variable[3]	C+P+	C+P-	C-P+	C-P-	SEM[2]	C	P	C × P
Glucose, mg/dL	65.1	65.0	64.8	63.7	1.17	0.28	0.45	0.51
Insulin, µg/L	1.97^{b}	2.45^{ab}	2.53^{a}	2.13^{ab}	0.312	0.49	0.82	0.017
Insulin:Glucose	0.031^{b}	0.041^{a}	0.037^{ab}	0.033^{ab}	0.0051	0.99	0.42	0.036
NEFA, µEq/L	122.8	99.2	121.0	107.4	12.46	0.52	0.003	0.45
Hp, ng/mL	5084.9	1832.5	1686.6	1558.7	987.09	0.17	0.035	0.11
SAA, ug/mL	136.5	90.3	127.5	90.9	30.31	0.57	0.035	0.67

[1]The basal diet consisted of (DM basis) 82.12% steam-rolled barley, 6.93% barley silage, and 5.83% mineral and vitamin supplement. The remainder of the diet included the inclusion of beet pulp (4.80 vs. 0.30), urea (0.32 vs. 0.48%), and palmitic acid (0 vs. 4.33%) for the diets without (P-) and with palmitic acid (P+; Energizer Rumen Protected (RP10); Scothorn Nutrition, Grand Pré, NS), respectively. *Capsicum* oleoresin (CapsXL; Pancosma, Rolle, Switzerland) was included at 0 (C-) or 77 mg/d (C+).

[2]The SEM represents the largest SEM for the interaction terms.

[3]Blood samples from day 27 of each period, composited from three time-points on an equal volume basis.

Table 4.4 Effect of *Capsicum* oleoresin (77 mg/d) and palmitic acid (4.33% DM) fed to feedlot heifers on blood metabolites sampled during intravenous glucose tolerance test

	Treatment[1]					*P- value*		
Variable	C+P+	C+P-	C-P+	C-P-	SEM[2]	C	P	C × P
IVGTT[3]								
Fasted glucose, mg/dL	72.77	74.71	72.78	74.72	1.460	0.99	0.14	1.00
Fasted insulin, µg/L	1.46	1.70	1.70	1.44	0.198	0.94	0.92	0.037
Insulin:Glucose (fasted)	0.020	0.023	0.023	0.020	0.0026	0.92	0.67	0.040
Insulin								
Peak, µg/L	17.26	14.37	18.63	15.35	1.729	0.23	0.003	0.84
Peak Time, min	15.42	11.67	15.94	15.00	1.837	0.15	0.058	0.27
Positive I-AUC[4], µg/L × min	578.11	482.92	663.62	470.94	77.815	0.40	0.002	0.26
Total AUC[5], µg/L × min	806.4	713.68	897.04	705.09	84.752	0.37	0.003	0.27
Clearance rate, %/min	3.55	2.98	3.52	2.97	0.344	0.92	0.015	0.96
$T_{1/2}$, min	21.11	24.92	23.56	22.69	2.341	0.88	0.13	0.087
Sensitivity Index × 10^4,[6] %(min^{-1}) × (µg/L×min)$^{-1}$	0.486	0.533	0.474	0.510	0.1233	0.60	0.095	0.51
Glucose								
Maximum, mg/dL	329.68	376.6	340.52	394.31	22.406	0.38	0.024	0.84
Range, mg/dL	256.91	301.89	267.17	319.27	22.171	0.40	0.029	0.87
Nadir, mg/dL	67.79	69.00	67.56	70.41	1.655	0.69	0.18	0.58
Clearance Rate, %/min	1.96	1.81	2.02	1.89	0.165	0.46	0.12	0.96
$T_{1/2}$, min	36.38	40.35	36.41	38.24	3.320	0.51	0.076	0.50
Positive I-AUC[4], mg/dL × min	5355.0	5701.4	5910.9	5839.6	576.63	0.18	0.53	0.48
Total AUC[5], mg/dL × min	13237.0	13630.0	12967.0	14278.0	1032.48	0.75	0.14	0.42

[1]The basal diet consisted of (DM basis) 82.12% steam-rolled barley, 6.93% barley silage, and 5.83% mineral and vitamin supplement. The remainder of the diet included the inclusion of beet pulp (4.80 vs. 0.30), urea (0.32 vs. 0.48%), and palmitic acid (0 vs. 4.33%) for the diets without (P-) and with palmitic acid (P+; Energizer Rumen Protected (RP10); Scothorn Nutrition, Grand Pré, NS), respectively. *Capsicum* oleoresin (CapsXL; Pancosma, Rolle, Switzerland) was included at 0 (C-) or 77 mg/d (C+).

[2]The SEM represents the largest SEM for the interaction terms.

[3]Intravenous Glucose Tolerance Test

[4]I-AUC: positive incremental area under curve, calculated using trapezoidal method $(((C_a - C_{baseline}) + (C_b - C_{baseline})) \times (t_b - t_a))/2$, as described by Cardoso et al. (2011).

[5]AUC: total area under curve calculated using trapezoidal method $((C_a + C_b) \times (t_b - t_a))/2$.

[6]Sensitivity Index: calculated using method described by Pacini et al. (2009).

peak insulin concentration was not affected by PALM, *Capsicum* or the *Capsicum* × PALM interaction ($P \geq 0.058$). The calculated I-AUC and total AUC for insulin were both increased by PALM ($P = 0.002$ and $P = 0.003$, respectively) but were unaffected by *Capsicum* and the *Capsicum* × PALM interaction ($P \geq 0.26$). The insulin clearance rate was increased by PALM inclusion ($P = 0.015$). However, there was no effect of *Capsicum* and no *Capsicum* × PALM interaction on insulin clearance rate ($P \geq 0.92$). Insulin $T_{1/2}$ was unaffected by *Capsicum*, PALM, or *Capsicum* × PALM interaction ($P \geq 0.087$). The calculated sensitivity index for insulin during the IVGTT was unaffected by *Capsicum*, PALM, or *Capsicum* × PALM interaction ($P \geq 0.37$).

Peak glucose concentration (maximum) during the IVGTT was decreased by PALM ($P = 0.024$). However, there were no effects of *Capsicum* or the *Capsicum* × PALM interaction on peak glucose ($P \geq 0.38$). The calculated difference between baseline glucose and peak glucose (range) was decreased ($P = 0.029$) by PALM treatment. However, there was no effect of *Capsicum* or the *Capsicum* × PALM interaction on range. *Capsicum*, PALM, and the *Capsicum* × PALM interaction did not affect the nadir glucose concentration during the IVGTT ($P \geq 0.18$). Glucose clearance rate and glucose was $T_{1/2}$ were also unaffected by *Capsicum*, PALM, or *Capsicum* × PALM interaction ($P \geq 0.076$). Calculated glucose positive AUC and total AUC were unaffected by *Capsicum*, PALM, or *Capsicum* × PALM interaction ($P \geq 0.14$).

Muscle glycogen concentrations (Table 4.5) were not affected by *Capsicum*, PALM, time, the *Capsicum* × PALM interaction, or the *Capsicum* × PALM × time interaction ($P \geq 0.30$). There were no affects of *Capsicum*, PALM, time, the *Capsicum* × PALM interaction, or the *Capsicum* × PALM × time interaction on concentration of BCA ($P \geq 0.57$). Finally, *Capsicum*, PALM, time, the *Capsicum* × PALM interaction, or the *Capsicum* × PALM × time interaction had no effect on the biopsy glycogen:BCA ratio concentration ($P \geq 0.25$).

4.5 Discussion

4.5.1 Effect of Capsicum oleoresin and Palmitic Acid on Insulin Resistance

The objective of this study was to examine the effects of *Capsicum* oleoresin when fed in the presence or absence of PALM treatment on DMI, circulating metabolites, acute phase protein concentrations, and insulin sensitivity in response to an IVGTT. The hypothesis was that feeding PALM would exacerbate insulin resistance (Reynoso et al. 2003; Zhu et al. 2016) experienced by finishing cattle (Joy et al. 2017), and that *Capsicum* would mitigate this insulin resistance. Carcass adiposity is positively correlated with circulating insulin (Trenkle and Topel, 1978), and

Table 4.5 Effect of *Capsicum* oleoresin (77 mg/d) and palmitic acid (4.33% DM) fed to feedlot heifers on skeletal muscle glycogen and bicinchoninic acid before and after intravenous glucose tolerance test.

		Treatment[1]					*P* - value				
Variable	T[2]	C+P+	C+P-	C-P+	C-P-	SEM[3]	C	P	T	C × P	C × P × T
Glycogen, mg/mL	1	164.98	198.43	171.62	203.42	49.496	0.65	0.30	0.85	0.32	0.59
	2	175.94	160.03	147.33	231.72						
BCA, mg/mL	1	0.944	0.965	0.975	0.966	0.0591	0.57	0.78	0.64	0.58	0.70
	2	0.976	0.998	0.951	0.932						
Glycogen:BCA, mg/mL	1	180.83	214.68	176.24	214.10	54.772	0.81	0.25	0.95	0.36	0.67
	2	190.99	188.68	148.08	262.50						

[1]The basal diet consisted of (DM basis) 82.12% steam-rolled barley, 6.93% barley silage, and 5.83% mineral and vitamin supplement. The remainder of the diet included the inclusion of beet pulp (4.80 vs. 0.30), urea (0.32 vs. 0.48%), and palmitic acid (0 vs. 4.33%) for the diets without (P-) and with palmitic acid (P+; Energizer Rumen Protected (RP10); Scothorn Nutrition, Grand Pré, NS), respectively. *Capsicum* oleoresin (CapsXL; Pancosma, Rolle, Switzerland) was included at 0 (C-) or 77mg/d (C+).

[2]Time: 1, biopsy sampled 1 hour before intravenous glucose tolerance test; 2, biopsy sampled 1 hour after intravenous glucose tolerance test.

[3]The SEM represents the largest SEM for the interaction terms.

Joy et al. (2017) reported increased fasting plasma insulin and increased plasma insulin under a fed state with advancing days on feed as indicators of decreased insulin sensitivity. In the current study, PALM increased the peak insulin concentration, insulin AUC, insulin I-AUC, and circulating NEFA which is interpreted to suggest that PALM increased insulin resistance (Mathews et al. 2016; Drackley, 1999; Pires et al. 2007; Burdick Sanchez et al. 2016). Supporting the hypothesis, there were observed interactions for non-fasted insulin, fasted insulin concentrations, and the insulin to glucose ratio suggesting that in combination with PALM, *Capsicum* may improve insulin sensitivity; but may have the opposite effect when PALM was not included. Although the difference in insulin concentrations between treatments C+P+ and C+P- was not significant, the observed increase in circulating insulin in C+P- may be explained as previous research has observed *Capsicum* to increase insulin by stimulating pancreatic duodenal homeobox-1 (PDX-1) and liver X receptor (LXR) expression (Zhang et al. 2017). The presence of PALM may have prevented the increase in insulin as it has been found to both reduce the nuclear localization of PDX-1 and insulin gene transcription (Hagman et al. 2005). Further, the tendency of PALM to increase the time of insulin peak during the IVGTT may indicate that *Capsicum* may stimulate insulin secretion at a higher rate, but that this action was both blunted and delayed by the presence of PALM. However, the lack of effect of *Capsicum* on insulin peak concentration and peak time does not support this.

The absence of effect of capsaicin on blood insulin during the IVGTT in the current study contradicts findings by Oh et al. (2017b) who reported a decrease for the insulin AUC with capsaicin in response to an IVGTT. However, other research has suggested that capsaicin may increase glucometabolic related genes in the liver which can stimulate insulin secretion, as well as increase pancreatic mRNA levels of IRS1, IRS2, transient receptor potential cation channel subfamily V member 1 (TRPV1), and glucose transporter 2 (GLUT2) (Zhang et al. 2017). It is known that TRPV1 is the channel through which capsaicin is active, and if mRNA levels of IRS1 and IRS2 and therefore activity of IRS1 and IRS2 were increased, then an increase in sensitivity could be expected since the insulin signal proceeds through these two substrates (Zhang et al. 2017; Li et al. 2010). However, the insulin AUC were not affected in the current study challenging the ability of *Capsicum* to dampen insulin resistance for feedlot cattle. In previous work, Oh et al. (2017b) observed a 25% decrease in the insulin AUC in response to RPC treatment (15.5% *Capsicum* oleoresin, 0.93% capsaicinoids), an observation that was not

shown in the current study. Although a similar *Capsicum* supplement was used in the study of Oh et al. (2017b) and the current study, the lower dose provided in the current study may have limited detection of changes in insulin sensitivity.

Taken alone, a higher insulin clearance rate observed with PALM treatment may be perceived to indicate an increase in insulin sensitivity. However, PALM also caused a higher peak insulin concentration as well as larger total calculated AUC and positive I-AUC by increasing plasma NEFA (Mathews et al. 2016; Rico et al. 2016). In response to this larger peak, it is possible that a decrease in insulin sensitivity of the insulin receptor and IRS1 resulted in a larger proportion of insulin being removed from circulation by the liver (De Koster and Opsomer, 2013) after the first pass (Tokarz et al. 2018). The effect of PALM to decrease the maximum and range for glucose concentrations during IVGTT contradicts previous research by Mathews et al. (2016) who found no effect on glucose parameters during IVGTT. However, the mid-lactation dairy cows in that study may not have been in an insulin resistant state characteristic of dairy cows in the transition period, and thus had substantial use of glucose by insulin insensitive tissues (Rico et al. 2017). Whether PALM altered blood insulin clearance by the liver or gluconeogenic potential cannot be confirmed with the data in the current study.

Skeletal muscle biopsies were analyzed to assess insulin responsiveness to clear glucose from circulation and deposit it toward muscle glycogen (Smith and Crouse, 1984). Glycogen determination has been evaluated in the longissimus muscles of beef steers with either high or low intramuscular fat (IMF) content (Underwood et al. 2007). Muscle glycogen as well as muscle marbling are affected by AMPK, which senses the cell energy status (Underwood et al. 2007; Bort et al. 2019). Steers with high IMF had lower glycogen synthase kinase-3 phosphorylation, glycogen content, and phosphorylation of AMPK than steers with low IMF, whereas activated AMPK activates the insulin receptor, stimulates glucose transport, and improves insulin sensitivity (Kahn et al. 2005; Daval et al. 2006; Underwood et al. 2007; Chopra et al. 2012). Thus, although *Capsicum* is capable of increasing AMPK phosphorylation through TRPV1 (Bort et al. 2019), the absence of glycogen storage affect in the current study suggests that *Capsicum* either did not alter AMPK phosphorylation, or that the effect was insufficient to overcome the insulin resistant state with or without the addition of PALM.

4.5.2 Effects of Palmitic Acid on Dry Matter Intake, Nutrient Digestibility, and Circulating Acute Phase Proteins

The observed decrease in DMI caused by PALM inclusion in the current study agrees with data from previous research (Rico et al. 2014) who found decreases in DMI of 7% and 9% in high- and low-producing dairy cows, respectively, when PALM was fed to increase total diet fatty acid composition to 5.6% DM. Rico et al. (2014) suggested that the greater dietary energy density or increased concentrations of gut peptides such as cholecystokinin and glucagon-like peptide 1 may cause a reduction in DMI when fat is added to the diet. Other research has also reported greater concentrations of gut-based peptides when feeding high fat diets (Choi and Palmquist, 1996; Relling and Reynolds, 2007).

The increase for CP digestibility supports results reported by Piantoni et al. (2013). However, the greater inclusion of urea in treatments containing PALM may simply suggest that CP digestibility may have been increased by a shift in CP supply from beet pulp to urea. Additionally, PALM may have caused increased rumen retention time and lower total CP intake (Choi and Palmquist, 1996; Ramirez Ramirez et al. 2016), resulting in increased CP digestibility.

Inclusion of PALM also increased apparent digestibility of OM and ether extract. The observed effect of PALM to increase OM digestibility contradicts findings of Rico et al. (2014) where no differences were found for dairy cattle when PALM was fed at 1.9% DM. However, Piantoni et al. (2013) found that substituting in 2% PALM in partial replacement of soyhulls increased OM and DM digestibility. Rico et al. (2014) also observed increased total fatty acid digestibility when feeding PALM, whereas Zinn (1989) observed linear decreases in fat digestion as fat inclusion was increased. This discrepancy could have been caused by the composition of the dietary fats. Rico et al. (2014) reported that increased fat digestibility with PALM is explained by increased C16 fatty acid digestibility; whereas, Zinn (1989) fed fat supplements that included a higher proportion of unsaturated fatty acids. Fatty acid digestibility was not evaluated, but it is possible that the increased ether extract digestibility explains much of the increased OM digestibility.

Palmitic acid decreased the digestibility of ADF and NDF, which agrees with previous research (Zinn, 1989). Devendra and Lewis (1974) as well as Zinn (1989) suggest that fat supplements may physically coat the fiber and thus prevent enzymatic and microbial digestion, resulting in decreased digestibility of fiber. However, other research suggests that modification

in rumen populations such as decrease in protozoa populations may be the cause for decreased fiber digestibility (Palmquist and Jenkins, 1980). In the current study reductions for ADF and NDF digestibility may be an artifact resulting from the removal of beet pulp from the diet, which has been shown to linearly increase NDF digestibility when replacing corn silage and barley grain in dairy rations (Heydari et al. 2021).

Blood Hp and SAA levels were highly variable, as indicated by the SEM (Pederzolli et al. 2018). Immune system challenges such as subacute ruminal acidosis, which increase abundancy of inflammatory cytokines such as IL-6 have been found to increase SAA levels (Khafipour et al. 2009). In the current study, it is likely that PALM stimulated the increase of Hp and SAA by causing increased inflammation (Alsemgeest et al. 1994; Gabay and Kushner, 1999; Heegaard et al. 2000; Orro et al. 2011).

4.5.3 Effect of Capsaicin on Dry Matter Intake, Nutrient Digestibility, and Circulating Acute Phase Proteins

There was no effect of capsaicin on DMI in the current study which agrees with other research evaluating *Capsicum* oleoresin in isolation (Chapter 3) or when fed with other plant extracts (Geraci et al. 2012). However, others have reported that *Capsicum* enhanced consumption of concentrate by 12.1% and tended to increase total DMI (Fandino et al. 2008). Additionally, when fed at 125, 250, and 500 mg/d, *Capsicum* has been found to linearly increase DMI in feedlot heifers fed a high concentrate diet (Rodriguez-Prado et al. 2012). In comparison, the target dose of *Capsicum* in the current study was much lower than Fandino et al. (2008) and Rodriguez-Prado et al. (2012). Other research by Kang et al. (2010) suggests capsaicin may suppress inflammatory response by acting through peroxisome proliferator antagonist receptor-α (PPARα) and TRPV1. Inflammatory cytokines have been reported to decrease whereas adiponectin, an insulin sensitizer, was increased in both adipose and liver (Kang et al. 2010; Kadowaki and Yamauchi, 2005). As adiponectin activates PPARα and AMPK (Kadowaki and Yamauchi, 2005), a decrease in skeletal muscle triglycerides could result from capsaicin treatment. However, the current study did not observe any effect on muscle glycogen concentrations, suggesting that the dose of capsaicin used may not have resulted in these metabolic modulations. However, the model used by the current study to measure glycogen accrual is novel and further research is suggested in order to improve this experimental model.

The absence of effect of capsaicin on digestibility of feed fractions agrees with previous dairy research supplementing 0.25 g/d of capsaicin supplement in a 42:58 forage-to-concentrate ration (Tager and Krause, 2011). However, a linear increase in DM and OM digestibility was observed by Oh et al. (2017b) when feeding RPC at 100 and 200 mg/d in dairy setting. It is possible that the different composition of *Capsicum* supplements caused the results from the current study to disagree with Oh et al. (2017b).

Although *Capsicum* has been found to decrease inflammatory response (Oh et al. 2017a) possibly through PPARα and TRPV1 (Kang et al. 2010), the acute phase protein Hp has been found linked to both B and T lymphocytes and an adaptive immune response in the monogastric model, the absence of effect of *Capsicum* on Hp in the current study agrees with research comparing the use of a plant extract blend (Xtract 7065; 17% eugenol, 11% cinnamaldehyde, and 7% capsaicin oleoresin; Pancosma, Rolle, Switzerland; Latack et al. 2021) to a conventional additive protocol (lasalocid, chlortetracycline, rumensin) when fed to newly weaned feedlot calves (de Souza et al. 2018). However, *Capsicum* fed alone has been found to decrease Hp concentrations following a lipopolysaccharide challenge in dairy cattle (Oh et al. 2017a). It is possible that a difference in dose of *Capsicum* resulted in this discrepancy as it appears this affect is modulated through the hindgut since a similar decrease in Hp was found in monogastric research which also reported increased villus height and reduced inflammatory response in the small intestine when feeding *Capsicum* (Liu et al. 2013).

4.6 Conclusion

Data from the current study show that inclusion of *Capsicum* in high concentrate feedlot rations may improve insulin sensitivity as it prevented the increase in fed- and fasted-state insulin levels caused by PALM treatment. However, *Capsicum* may increase insulin concentrations in the absence of PALM. *Capsicum* did not affect acute phase proteins Hp and SAA, suggesting that it did not affect inflammatory response. Palmitic acid inclusion magnified the insulin resistant metabolic state and increased inflammatory response by causing increases in insulin peak concentration, and insulin AUC during IVGTT, as well as Hp and SAA concentrations. Muscle glycogen abundance was not affected by PALM or *Capsicum*. Compared to other research, further research is suggested in the feedlot setting using higher doses of *Capsicum*. Additionally, further research using the model of sampling muscle tissue biopsies before and after IVGTT is suggested.

5.0 GENERAL DISCUSSION

A two-part study was conducted to test the hypothesis that *Capsicum* oleoresin, level of rumen protection, and dose rate of *Capsicum* (CAP) would affect insulin metabolism and energy deposition in feedlot beef yearlings. Part one, a larger scale RCBD 2 × 2 + 1 factorial performance experiment was conducted to examine the effect of feeding one of two *Capsicum* oleoresin supplements (X50-7035 (**HP**) or X60-7035 (**RP**)) at one of two dose rates which targeted delivery of 15.5 mg/d and 49.9 mg/d of *Capsicum* from the supplement. This study showed that a higher dose of *Capsicum* results in decreased AAA and increased AA grades ($P \leq 0.016$), decreased marbling ($P = 0.032$), and decreased carcasses grading yield grade 3 ($P = 0.005$) signifying a leaner carcass compared to the low dose of *Capsicum*. Part two, a 12-animal intensive metabolism study, followed a 4 × 4 Latin square design balanced for carry-over effects with 3 replications. Using a 2 × 2 factorial design with *Capsicum* and PALM, this study measured both basal blood metabolite level, and blood metabolite level in response to intravenous glucose tolerance test. The performance study supports the hypothesis that dose, but not level of rumen protection, may alter carcass adiposity without affecting BW or DMI. However, to the extent that the data provide, the metabolism study provided no evidence to support the hypothesis that *Capsicum* elicits these carcass energy deposition effects by either altering insulin resistance or altering metabolism.

Although cattle are gluconeogenic, they may still experience decreased IS and increased IR (Verde and Trenkle, 1987; De Koster and Opsomer, 2013; Locher et al. 2015; Joy et al. 2017). Methods to quantify IR include conducting an hyperinsulinemic euglycemic clamp test, IVGTT, or intravenous insulin tolerance test (De Koster and Opsomer, 2013). Calculations of IS and IR are achieved with methods such as the revised quantitative insulin sensitivity (RQUICKI), homeostasis model assessment (HOMA-IR), insulin sensitivity index (IS_i), and quantitative insulin sensitivity check index (QUICKI) equations (Holtenius and Holtenius, 2007; Matthews et al. 1985; Pacini et al. 2009; Katz et al. 2000). While QUICKI observes the inverse logarithm of fasted-state insulin + fasted-state glucose levels, RQUICKI takes into account the fasted-stated NEFA level in addition to insulin and glucose levels which improves its ability to detect differences in insulin sensitivity in subjects that are not in severe insulin resistant states (Perseghin et al. 2001). However, the development of QUICKI and RQUICKI utilized a clamp-

based insulin sensitivity index to determine their correlation and thus 'accuracy' at indicating differences in insulin sensitivity in subjects. Instead of using a method which is based on the accuracy of an insulin sensitivity index, this study used an insulin sensitivity index. The insulin sensitivity index (IS_i) used in the metabolic study during IVGTT (Pacini et al. 2009) takes into account the individual subject's glucose clearance rate during the IVGTT and allows for a flexible time window of calculation. The advantage of this method is the use of the original blood metabolite data, and the removal of dependency on the correlation of other calculations.

As calves advance in days on feed, insulin resistance increases with decreased G:F and increased circulating insulin (Joy et al. 2017; Shingu et al. 2001). Energy deposition shifts away from lean muscle mass and toward adipose tissue as the animal reaches mature weight (NASEM, 2016). As the shift toward adipose tissue deposition continues, feed efficiency as measured by G:F decreases (Joy et al. 2017). Although carcass adiposity as a whole has been found positively correlated with circulating insulin level (Trenkle and Topel, 1978), the site of deposition of adipose has also been found to effect insulin kinetics in the human model as Patel and Abate (2013) found that subcutaneous truncal fat has a much larger role than retroperitoneal or visceral fat in the development of obesity-induced insulin resistance. Further, the main substrate for subcutaneous adipose synthesis is acetate, whereas that for intramuscular fat is glucose (Smith and Crouse, 1984). The disposal of glucose is regulated by the insulin activated release of GLUT4 from GLUT4-storage vesicles in insulin sensitive tissues such as skeletal myocytes and adipocytes (Olson and Pessin, 1996; reviewed in Corcoran et al. 2007). Since the low dose of *Capsicum* resulted in a higher proportion of carcasses grading AAA, and since amount of visible fat in a cut of meat is a positive quality in North American markets (Hocquette et al. 2010), the effects of the low dose of CAP were further tested.

One theory explained in Owens et al. (1995) offers that the reason for the increase in fat deposition at the end of the finishing period is that cattle reach a mature weight. Their data suggest that at a mature weight, protein accretion stops, whereas fat accretion may continue. For example, at an empty body weight of 746 kg, their data suggest that protein accrual stops, and empty body fat is 36.2% (Owens et al. 1995). As body fat deposition increases, it becomes its own positive feedback cycle as production of pro-inflammatory cytokines such as TNF-α, and a decrease in adiponectin (an anti-inflammatory adipokine which acts as an insulin sensitizer)

perpetuate the IR state (Gustafson et al. 2007; Kang et al. 2010; Guo and Donner, 1996). As finishing continues, decreased AMPK phosphorylation has been observed in steers containing high intramuscular fat versus steers low intramuscular fat. A lower AMPK phosphorylation results in less phosphorylation and thus less inhibition of ACC, which promotes lipogenesis (Underwood et al. 2007; Hocquette et al. 2009).

Although the performance study exhibited the potential for *Capsicum* to affect carcass adiposity and marbling, the analysis of muscle glycogen content in Chapter 4 did not confirm this. As 77 mg/d of RP increased the proportion of AAA quality grade in the performance study, this dose was expected to cause an increase in muscle glycogen content in Chapter 4 finisher cattle such that a higher amount of energy in the form of glucose would deposited in the IMF depot (Smith and Crouse, 1984). The increase in proportion of AAA quality grade in Chapter 3 study could have occurred as a result of altered insulin kinetics as observed in previous dairy research (Oh et al. 2017b). During IVGTT, Oh et al. (2017b) offered that RPC-stimulated calcitonin gene-related peptide (CGRP) may have altered the secretion of insulin which prevented as large of a spike in circulating insulin resulting in a lower calculated insulin AUC, while not impeding the glucose clearing function.

While there were no effects of *Capsicum* on IS_i in the metabolic study, there was a CAPS × PALM interaction such that treatment C+P+ had lower circulating insulin than treatment C-P+ ($P = 0.01$), which may agree with the CGRP hypothesis of Oh et a. (2017b). Additionally, the release of CGRP is a result of capsaicin binding to TRPV1 (de Lourdes Reyes-Escogido et al. 2011), which increases TRPV1 expression (Kang et al. 2010). Another possibility is that *Capsicum* reduced inflammatory adipocytokines, which increase insulin resistance (Kang et al. 2010). Further, it is possible that the increase in IMF was made up of new, smaller adipocytes rather than expansion of larger ones (Kang et al. 2010), with these smaller adipocytes taking up more glucose than larger adipocytes in the presence of submaximal insulin (Okuno et al. 1998; Olefsky, 1976). The observed increase in AAA grading and marbling score, combined with numerically greater final bodyweight in Chapter 3 suggests that the low dose of *Capsicum* caused greater overall energy deposition in the form of glucose because, as mentioned, it is known that glucose is the preferred substrate for intramuscular fat (Smith and Crouse, 1984).

The concept that IR increases with age in feedlot cattle (Joy et al. 2017; Shingu et al. 2001) may be related to the action of other hormones such as GH and their effect on IS (Trenkle and Topel, 1978) since GH has been found to increase insulin mRNA expression in the pancreas (Feng et al. 2009). Since anabolic implants promote lean tissue deposition by stimulating factors such as IGF-1, they promote higher feed efficiency and faster deposition of feed energy into protein (Perry et al. 1991; Smith et al. 2019; Gifford et al. 2015). As feedlot calves progress in age and grow heavier, they become more insulin resistant (Eisemann et al. 1997; Joy et al. 2017). However, a comparable heifer of the same age and weight reaches slaughter weight and will 'finish' at a lighter weight compared to the steer (Zinn et al. 2008). In the performance study, steers received anabolic implants (Revalor S) that are potent for an ideal 80 to 120 days (Nichols et al. 2015), fed for 68 days on treatment, and finished to treatment average weights between 682 to 691 kg (treatments did not differ). In Chapter 4, heifers did not receive implants, received MGA treatment in the feed, experienced 28 d period length, and ended the study at average weights of 640.0, 649.5, and 694.7 kg for the low, medium, and heavy-weight blocks, respectively. The effect of the anabolic implant to increase the efficiency of gains, as well as the knowledge that heifers finish at a lower weight than steers – meaning there is a possibility that they were in a heightened state of insulin resistance compared to the steers – could be variables that affected the observations and data such the model used in Chapter 4 included animals in an inherently higher state of IR. However, the observed effects in the IVGTT or muscle glycogen data in the metabolism study did not support this. The addition of MGA without the use of steroidogenic anabolic implants has been found to increase ADG, carcass weight, longissimus muscle area, as well as marbling (Henricks et al. 1997). However, results have varied (Cook et al. 2001). Although there were treatment effects on marbling in the performance study, the absence of CAPS effect on muscle glycogen in the metabolic study should not be attributed as an effect of anabolic implants used in the performance study (Smith et al. 2007). Additionally, a major stress hormone, cortisol, is a positive reactant to the acute phase in mammals along with IL-6, TNFα, SAA, and Hp (Oh, 2015; reviewed in Gruys et al. 2005). Although cortisol has been found to be increased by inflammatory challenge such as LPS infusion, it has been found to not be significantly affected by RPC (Oh et al. 2017a). For this reason, and in effort to keep the focus of the studies on fat inclusion and insulin kinetics, cortisol determination was excluded from the current studies.

Specific gaps that remain in the research after the conclusion of these two studies include the lack of data on the potential affect that days on feed could have on the effects caused by *Capsicum*, as well as the lack of data on what the effects are when higher levels of *Capsicum* are fed to beef cattle in the feedlot setting. In the dairy setting and indeed in many other research settings, a 28 d experimental treatment period is very fitting. However, a longer experiment length for further research may be more appropriate as a feedlot nutritionist survey in 2015 shows that it takes an average 201 days on feed to finish a calf, with the average receiving and shipping weights of a calf being 272-363 kg and 590-680 kg, respectively (Samuelson et al. 2016). A 60-70 d period length in a Latin square design would not be practical, but future research may find that a larger scale randomized complete-block design metabolic study that keeps animals on one treatment the entirety of the study, and then finishes the individual animal to a specific point (ie. Backfat thickness or days on feed; Schoonmaker et al. 2003) could be a suitable way to compare effects of *Capsicum* treatment. Although this proposition is similar to the current performance study, future research would benefit from gathering blood data from this experiment regimen and needs also to focus on a larger variety of dose rates.

The second specific remaining gap in the research is the *Capsicum* dose rate. Where capsaicin products have been fed at rates up to 1000 mg/d (X60-7035; 20% *Capsicum* oleoresin, 1.2% capsaicinoids with an estimated 20% bypassing the rumen; Pancosma SA., Geneva, Switzerland) in the dairy setting (Oh et al. 2015), but the present studies incorporated only up to 250 mg/d (same X60-7035 product containing 20% *Capsicum* oleoresin used). Although the current research examined the lower level of 77 mg/d of this product in the metabolic study, there still exists the gap between the 250 mg/d and 1000 mg/d. However, the use of the 77 mg/d treatment in the current metabolism study was based on what data was available in the beef setting, and the observation that parameters such as immune cell count responded positive linear to treatment up to 1000 mg/d *Capsicum* must be acknowledged (Oh et al. 2015). This suggests that *Capsicum* may still be active at that dose rate since neutrophils are white blood cells that have a TRPV1 channel and are activated by presence of cytokines or chemokines, such as tumour necrosis factor-α, that then seek the site of infection or inflammation (Heiner et al. 2003; Hallett and Lloyds, 1995).

6.0 CONCLUSIONS

Including *Capsicum* oleoresin supplements varying in level of rumen protection at two dose rates in finisher beef diets showed the potential for the higher dose to negatively affect carcass quality and yield grade parameters, without affecting DMI, dressing percentage, or ADG. A low dose of *Capsicum* may increase marbling, and proportion of AAA quality grades. There were no effects of *Capsicum* on insulin or glucose parameters during IVGTT. However, since *Capsicum* prevented the PALM induced increase of fed- and fasted-state insulin concentrations, this shows the potential of *Capsicum* to modulate insulin sensitivity. There was no effect of *Capsicum* observed on circulating acute phase protein concentrations or muscle glycogen abundancy. The approach of collecting skeletal muscle biopsies before and after IVGTT conducted in this research is novel. Further research to improve this model of analysis is suggested.

7.0 LITERATURE CITED

AOAC (Association of Official Analytical Chemists). 2006. Official methods of analysis of AOAC International. 18th ed. Assoc. Off. Anal. Chem., Gaithersburg, MD.

AOAC (Association of Official Analytical Chemists). 2000. Official methods of analysis of AOAC International. 17th ed. Assoc. Off. Anal. Chem., Gaithersburg, MD.

Agriculture and Agri-Food Canada. 2021. Average Cattle Carcass Weights for Federal Slaughter – (in lbs.). Accessed: February, 23, 2021. https://aimis-simia.agr.gc.ca/rp/index-eng.cfm?action=gR&r=107&signature=3231D4DFD1EE94E1448DA5BB55A45A5B&pdctc=&pTpl=1#wb-cont

Ajuwon, K. M., and M. E. Spurlock. 2005. Palmitate Activates the NF-κB Transcription Factor and Induces IL-6 and TNFα Expression in 3T3-L1 Adipocytes. J. Nutr. **135**:1841-1846. doi: 10.1093/jn/135.8.1841.

Allen, M. S. 1997. Relationship between fermentation acid production in the rumen and the requirement for physically effective fiber. J. Dairy Sci. **80**:1447-1462. doi:10.3168/jds.S0022-0302(97)76074-0.

Alsemgeest S. P. M., H. C. Kalsbeek, Th. Wensing, J. P. Koeman, A. M. van Ederen, and E. Gruys. 1994. Concentrations of serum amyloid-a (SAA) and haptoglobin (HP) as parameters of inflammatory diseases in cattle. Vet. Quart. **16**:21-23. doi:10.1080/01652176.1994.9694410.

American Diabetes Association. 2004. Diagnosis and Classification of Diabetes Mellitus. Diabetes Care 27(Suppl. 1):S5-10 doi:10.2337/diacare.27.2007.S5.

Aschenbach, J. R., N. B. Kristensen, S. S. Donkin, H. M. Hammon, and G. B. Penner. 2010. Gluconeogenesis in dairy cows: The secret of making sweet milk from sour dough. IUBMB Life **62**:869–877. doi:10.1002/iub.400.

Aschenbach, J. R., Q. Zebeli, A. K. Patra, G. Greco, S. Amasheh, and G. B. Penner. 2019. Symposium review: The importance of the ruminal epithelial barrier for a healthy and productive cow. J. Dairy Sci. **102**:1866–1882. doi:10.3168/jds.2018-15243.

Avruch, J. 1998. Insulin signal transduction through protein kinase cascades. Mol. Cell. Biochem. **182**:31–48. doi:10.1023/A:1006823109415.

Batista, T. M., R. Garcia-Martin, W. Cai, M. Konishi, B. T. O'Neill, M. Sakaguchi, J. H. Kim, D. Y. Jung, J. K. Kim, and C. R. Kahn. 2019. Multi-dimensional Transcriptional Remodeling by Physiological Insulin In Vivo. Cell Rep. **26**:3429-3443.e3. doi:10.1016/j.celrep.2019.02.081.

Baumgard, L. H., E. A. Horst, E. J. Mayorga Lozano, M. A. Al-Qaisi, C. S. Shouse, S. K. Kvidera, S. Lei, J. T. Seibert, H. A. Ramirez, A. F. Keating, J. W. Ross, J. T. Selsby, R. Appuhamy, and R. P. Rhoads. 2018. Heat stress, consequences of gut barrier dysfunction. J. Anim. Sci. **96(Suppl. 2)**: 217-218. doi.org/10.1093/jas/sky073.402.

Beauchemin, K. A., and S. M. McGinn. 2006. Methane emissions from beef cattle: Effects of fumaric acid, essential oil, and canola oil. J. Anim. Sci. **84**:1489–1496. doi:10.2527/2006.8461489x.

Bergman, R. N., Y. Z. Ider, C. R. Bowden, and C. Cobelli. 1979. Quantitative estimation of insulin sensitivity. Am. J. of Physiol. 6: E667-77. doi:10.1152/ajpendo.1979.236.6.E667.

Bort, A., B. G. Sánchez, E Spínola, P. A. Mateos-Gómez, N. Rodríguez-Henche, and I. Díaz-Laviada. 2019. The red pepper's spicy ingredient capsaicin activates AMPK in HepG2 cells through CaMKKβ. PLoS ONE **14**:e0211420. doi:10.1371/journal.pone.0211420.

Boyd, J., J. W. West, J. K. Bernard, and S. S. Block. 2012. Effects of plant extracts on milk yield and apparent efficiency of lactating dairy cows during hot weather 1. Prof. Anim. Sci. **28**:338-343. doi:10.15232/S1080-7446(15)30365-X.

Brozinick, J. T., and M. J. Birnbaum. 1998. Insulin, but not contraction, activates Akt/PKB in isolated rat skeletal muscle. J. Biol. Chem. **273**:14679–14682. doi:10.1074/jbc.273.24.14679.

Burdick Sanchez, N. C., J. A. Carroll, P. R. Broadway, H. D. Hughes, S. L. Roberts, J. T. Richeson, T. B. Schmidt, and R. C. Vann. 2016. Cattle temperament influences metabolism: metabolic response to glucose tolerance and insulin sensitivity tests in beef steers. Dom. Anim. Endocrin. **56**:85-95. doi:10.1016/j.domaniend.2016.02.009.

Calsamiglia, S., M. Busquet, P. W. Cardozo, L. Castillejos, and A. Ferret. 2007. Invited review: Essential oils as modifiers of rumen microbial fermentation. J. Dairy Sci. **90**: 2580–2595. doi:10.3168/jds.2006-644.

Cardozo, P. W., Calsamiglia, S., Ferret, A., and Kamel, C. 2006. Effects of alfalfa extract, anise, capsicum, and a mixture of cinnamaldehyde and eugenol on ruminal fermentation and protein degradation in beef heifers fed a high-concentrate diet. J. Anim. Sci. **84**: 2801–2808. doi:10.2527/jas.2005-593.

Castillo-Lopez E., B. I. Wiese, S. Hendrick, J. J. McKinnon, T. A. McAllister, K. A. Beauchemin, and G. B. Penner. 2014. Incidence, prevalence, severity, and risk factors for ruminal acidosis in feedlot steers during backgrounding, diet transition, and finishing. J. Anim. Sci. **92**:3053-3063. doi:10.2527/jas2014-7599.

Chase, C. C., T. H. Elsasser, L. J. Spicer, D. G. Riley, M. C. Lucy, A. C. Hammond, T. A. Olson, and S. W. Coleman. 2011. Effect of growth hormone administration to mature miniature Brahman cattle treated with or without insulin on circulating concentrations of insulin-like growth factor-I and other metabolic hormones and metabolites. Domest. Anim. Endocrinol. **41**:1-13. doi:10.1016/j.domaniend.2011.01.006.

Chibisa, G. E., Beauchemin, K. A., Koenig, K. M., and Penner G. B. 2020. Optimum roughage proportion in barley-based feedlot cattle diets: total tract nutrient digestibility, rumination, ruminal acidosis, short-chain fatty absorption, and gastrointestinal tract barrier function. J. Anim. Sci. **98**:1-14. doi:10.1093/jas/skaa160.

Chivri, M., C.J. Rendon, M.N. Myers, C.M. Prom, S. Roy, A. Sen, A.L. Lock, and G.A. Contreras. 2022. Lipoloysaccharide induces lipolysis and insulin resistance in adipose tissue from dairy cows. J. Dairy Sci. **105**:842-855. doi: 10.3168/jds.2021-20855.

Chiou, W. L. 1978. Critical evaluation of the potential error in pharmacokinetic studies of using the linear trapezoidal rule method for the calculation of the area under the plasma level-time curve. J. of Pharm. and Biopharm. **6**:539-546. doi:10.1007/BF01062108.

Choi, B-R., and D. L. Palmquist. 1996. High fat diets increase plasma cholecystokinin and pancreatic polypeptide, and decrease plasma insulin and feed intake in lactating cows. J. Nutr. **126**:2913-2919. doi:10.1093/jn/126.11.2913.

Chopra, I., H. F. Li, H. Wang, and K. A. Webster. 2012. Phosphorylation of the insulin receptor by AMP-activated protein kinase (AMPK) promotes ligand-independent activation of the insulin signalling pathway in rodent muscle. Diabetologia **55**:783-794. doi:10.1007/s00125-011-2407-y.

Cook, R. B., J. D. Popp, T. A. McAllister, J. P. Kastelic, and R. Harland. 2001. Effects of immunization against GnRH, melengestrol acetate, and a trenbolene acetate/estradiol implant on growth and carcass characteristics of beef heifers. Theriogenology **55**:973-981. doi:10.1016/s0093-691x(01)00458-7.

Corcoran, M. P., S. Lamon-Fava, and R. A. Fielding. 2007. Skeletal muscle lipid deposition and insulin resistance: Effect of dietary fatty acids and exercise. Am. J. Clin. Nutr. **85**:662–677. doi:10.1093/ajcn/85.3.662.

Daval, M., F. Foufelle, and P. Ferré. 2006. Functions of AMP-activated protein kinase in adipose tissue. J. Physiol. **574**:55-62. doi:10.1113/jphysiol.2006.111484.

De Koster, J. D., and G. Opsomer. 2013. Insulin resistance in dairy cows. Vet. Clin. North Am. - Food Anim. Pract. **29**:299–322. doi:10.1016/j.cvfa.2013.04.002.

De Lourdes Reyes-Escogido, M., E. G. Gonzalez-Mondragon, and E. Vazquez-Tzompantzi. 2011. Chemical and pharmacological aspects of capsaicin. Molecules **16**:1253–1270. doi:10.3390/molecules16021253.

de Sousa, O. A., B. I Cappellozza, V. G. L. Fonseca, R. F. Cooke. 2022. Short communication: insulin resistance increases as days on feed advance in feedlot *Bos indicus*

beef cattle offered a high-concentrate finishing diet. J. Anim. Sci. (Accepted Manuscript) doi:10.1093/jas/skac182.

De Souza, K. A., R. F. Cooke, K. M. Schubach, A. P. Brandaõ, T. F. Schumaher, I. N. Prado, R. S. Marques, and D. W. Bohnert. 2018. Performance, health and physiological responses of newly weaned feedlot cattle supplemented with feed-grade antibiotics or alternative feed ingredients. Animal **12**:2521–2528. doi:10.1017/S1751731118000551.

Devendra, C. and D. Lewis. 1974. The interaction between dietary lipids and fibre in the sheep 2. digestibility studies. Anim. Sci. **19**:67-76. doi:10.1017/S0003356100022583.

Doornbos, R. P., M. Theelen, P. C. J. Van Der Hoeven, W. J. Van Blitterswijk, A. J. Verkleij, and P. M. P. Van Bergen En Henegouwen. 1999. Protein kinase Cζ is a negative regulator of protein kinase B activity. J. Biol. Chem. **274**:8589–8596. doi:10.1074/jbc.274.13.8589.

Doornenbal, H., A. K. Tong, and N. L. Murray. 1988. Reference values of blood parameters in beef cattle of different ages and stages of lactation. Can. J. Vet. Res. **52**:99–105. Retrieved from: https://pubmed.ncbi.nlm.nih.gov/3349406/

Drackley, J. K. 1999. Biology of dairy cows during the transition period: the final frontier? J. Dairy Sci. **82**:2259-2273. doi:10.3168/jds.S0022-0302(99)75474-3.

Drouillard, J. S. 2018. Current situation and future trends for beef production in the United States of America - A review. Asian-Australasian J. Anim. Sci. **31**:1007–1016. doi:10.5713/ajas.18.0428.

Dubois, M., K. A. Gilles, J. K. Hamilton, P. A. Rebers, and F. Smith. 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem. **28**:350. doi:10.1021/ac60111a017.

Duckett, S. K., J. G. Andrae, and F. N. Owens. 2002. Effect of high-oil corn or added corn oil on ruminal biohydrogenation of fatty acids and conjugated linoleic acid formation in beef steers fed finishing diets. J. Anim. Sci. **80**:3353–3360. doi:10.2527/2002.80123353x.

Duffield, T. F., J. K. Merril, and R. N. Bagg, 2012. Meta-analysis of the effects of monensin in beef cattle on feed efficiency, body weight gain, and dry matter intake. J. Anim. Sci. **15**:4583–4592. doi:10.2527/jas2011-5018.

Eisemann, J. H., G. B. Huntington, and D. R. Catherman. 1997. Insulin Sensitivity and Responsiveness of Portal-Drained Viscera, Liver, Hindquarters, and Whole Body of Beef Steers Weighing 275 or 490 Kilograms. J. Anim. Sci. **75**:2084–2091. doi:10.2527/1997.7582084x.

Fandiño, I., A. Ferret, and S. Calsamiglia. 2020. Dose and combinations of anise oil and capsicum oleoresin as rumen fermentation modifiers in vitro and in vivo with high concentrate diets fed to Holstein beef heifers. Anim. Feed Sci. Technol. **260**:114363. doi:10.1016/j.anifeedsci.2019.114363.

Fandiño, I., S. Calsamiglia, A. Ferret, and M. Blanch. 2008. Anise and capsicum as alternatives to monensin to modify rumen fermentation in beef heifers fed a high concentrate diet. Anim. Feed Sci. Technol. **145**:409–417. doi:10.1016/j.anifeedsci.2007.04.018.

Feng, J., Z. Gu, M. Wu, F. C. Gwazdauskas, and H. Jiang. 2009. Growth hormone stimulation of serum insulin concentration in cattle: Nutritional dependency and potential mechanisms. Domest. Anim. Endocrinol. **37**:84–92. doi:10.1016/j.domaniend.2009.03.003.

Fitzsimons, C., D. A. Kenny, S. M. Waters, B. Earley, and M. McGee. 2014. Effects of phenotypic residual feed intake on response to a glucose tolerance test and gene expression in the insulin signaling pathway in longissimus dorsi in beef cattle. J. Anim. Sci. **92**:4616–4631. doi:10.2527/jas.2014-7699.

Franck, N., K. G. Stenkula, A. Öst, T. Lindström, P. Strålfors, and F. H. Nystrom. 2007. Insulin-induced GLUT4 translocation to the plasma membrane is blunted in large compared with small primary fat cells isolated from the same individual. Diabetologia **50**:1716–1722. doi:10.1007/s00125-007-0713-1.

Gabay, C., and I. Kushner. 1999. Acute-phase proteins and other systemic responses to inflammation. New Eng. J. of Med. **340**:448-454. doi:10.1056/NEJM199902113400607.

Galvin, P., G. Ward, J. Walters, R. Pestell, M. Koschmann, A. Vaag, I. Martin, J. D. Best, and F. Alford. 1992. A simple method for quantitation of insulin sensitivity and insulin release from an intravenous glucose tolerance test. Diabet. Med. **9**:921-928. doi:10.1111/j.1464-5491.1992.tb01732.x.

Galyean, M. L., D. G. Wagner, and R. R. Johnson. 1976. Site and extent of starch digestion in steers fed processed corn rations. J. Anim. Sci. **43**:1088-1094. doi:10.2527/jas1976.4351088x.

Geraci, J. I., A. D. Garciarena, G. A. Gagliostro, K. A. Beauchemin, and D. Colombatto. 2012. Plant extracts containing cinnamaldehyde, eugenol and capsicum oleoresin added to feedlot cattle diets: Ruminal environment, short term intake pattern and animal performance. Anim. Feed Sci. Technol. **176**:123–130. doi:10.1016/j.anifeedsci.2012.07.015.

Gifford, C. A., K. A. Branham, J. O. Ellison, B. I. Gomez, C. O. Lemley, C. G. Hart, C. R. Krehbiel, B. C. Bernhard, C. L. Maxwell, C. L. Goad, D. M. Hallford, and J. A. Hernandez Gifford. 2015. Effect of anabolic implants on adrenal cortisol synthesis in feedlot beef cattle implanted early or late in the finishing phase. Phys. & Behav. **138**:118-123. doi:10.1016/j.physbeh.2014.10.025.

Gill, D. R., F. N. Owens, M. C. King, and H. G. Dolizai. 1993. Body composition of grazing or feedlot steers differing in age and background. Oklahoma Agric. Exp. Sta. Rep.: 185–190.

Goering, H. K., and P.J. Van Soest. 1970. Forage fiber analysis. USDA-ARS Handbook No. 379. USDA superintendent of documents. U.S. Government Printing Office, Washington, D.C.

Grant, R. J., and S. J. Weidner. 1992. Effect of fat from whole soybeans on performance of dairy cows fed rations differing in fiber level and particle size. J. Dairy Sci. **75**:2742-2751. doi:10.3168/jds.S0022-0302(92)78037-0.

Gruys, E., M. J. M. Toussaint, T. A. Niewold, and S. J. Koopmans. 2005. Acute phase reaction and acute phase proteins. J. Zhejiang Univ. Sci. B6:1045–1056. doi:10.1631/jzus.2005.B1045.

Guo, D., and D. B. Donner. 1996. Tumor necrosis factor promotes phosphorylation and binding of insulin receptor substrate 1 to phosphatidylinositol 3-kinase in 3T3-L1 adipocytes. J. Biol. Chem. **271**: 615–618. doi:10.1074/jbc.271.2.615.

Gustafson, B., A. Hammarstedt, C. X. Andersson, and U. Smith. 2007. Inflamed adipose tissue: a culprit underlying the metabolic syndrome and artherosclerosis. Arterioscler. Thromb. Vasc. Biol. **27**:2276-2283. doi:10.1161/ATVBAHA.107.147835.

Hagman, D. K., L. B. Hays, S. D. Parazzoli, and V. Poitout. 2005. Palmitate inhibits insulin gene expression by altering PDX-1 nuclear localization and reducing MafA expression in isolated rat islets of Langerhans. J. Biol. Chem. **37**:32413-8. doi:10.1074/jbc.M506000200.

Hall, M. B. 2009. Analysis of starch, including maltooligosaccharides, in animal feeds: a comparison of methods and a recommended method for AOAC collaborative study. JAOACI **92**:42-49.

Hallett, M. B., and D. Lloyds. 1995. Neutrophil priming: the cellular signals that say ‘amber’ but not ‘green’. Immunol. Today **16**:264-268. doi:10.1016/0167-5699(95)80178-2.

Hare, K. S., G. B. Penner, M. A. Steele, and K. M. Wood. 2022. Oversupplying metabolizable protein during late gestation to beef cattle does not influence ante- or postpartum glucose-insulin kinetics but does affect prepartum insulin resistance indices and colostrum insulin content. J. Anim. Sci. **100**:1-14. doi:10.1093/jas/skac101.

Harris, R. B., and H. Kor. 1992. Insulin insensitivity is rapidly reversed in rats by reducing dietary fat from 40 to 30% of energy. J. Nutr. **122**:1811-22. Oxford Academic. doi.org/10.1093/jn/122.9.1811

Heegaard, P. M. H., D. L. Godson, M. J. M. Toussaint, K. Tjùrnehùjd, L. E. Larsen, B. Viuff, and L. Rùnsholt. 2000. The acute phase response of haptoglobin and serum amyloid

A (SAA) in cattle undergoing experimental infection with bovine respiratory syncytial virus. Vet. Immun. and Immunopath. **77**:151-159. doi:10.1016/s0165-2427(00)00226-9.

Heiner, I., J. Eisfeld, and A. Lückhoff. 2003. Role and regulation of TRP channels in neutrophil granulocytes. Cell Calcium **33**:533–540. doi:10.1016/S0143-4160(03)00058-7.

Henricks, D. M., R. T. Brandt Jr., E. C. Titgemeyer, and C. T. Milton. 1997. Serum concentrations of trenbolone-17 beta and estradiol-17 beta and performance of heifers treated with trenbolone acetate, melengestrol acetate, or estradiol-17 beta. J. Anim. Sci. **75**:2627-2633. doi:10.2527/1997.75102627x.

Hernández-Cáceres, M. P., L. Toledo-Valenzuela, F. Díaz-Castro, Y. Ávalos, P. Burgos, C. Narro, D. Peña-Oyarzun, J. Espinoza-Caicedo, F. Cifuentes-Araneda, F. Navarro-Aguad, C. Riquelme, R. Troncoso, A. Criollo, and E. Morselli. 2019. Palmitic acid reduces the autophagic flux and insulin sensitivity through the activation of the free fatty acid receptor 1 (FFAR1) in the hypothalamic neuronal cell line N43/5. Fron. Endocrinol. (Lausanne) **10**:176. doi:10.3389/fendo.2019.00176.

Hers, I., E. E. Vincent, and J. M. Tavaré. 2011. Akt signalling in health and disease. Cell. Signal. **23**:1515–1527. doi:10.1016/j.cellsig.2011.05.004.

Heydari, M., G. R. Ghorbani, A. Sadeghi-Sefidmazgi, H. Rafiee, F. Ahamdi, and H. Saeidy. 2021. Beet pulp substituted for corn silage and barley grain in diets fed to dairy cows in the summer months: feed intake, total-tract digestibility, and milk production. Animal **15**:100063. doi:10.1016/j.animal.2020.100063.

Hocquette, J. F., F. Gondret, E. Baéza, F. Médale, C. Jurie, and D. W. Pethick. 2010. Intramuscular fat content in meat-producing animals: development, genetic and nutritional control, and identification of putative markers. Animal **4**:303-319. doi:10.1017/S1751731109991091.

Hocquette, J. F., I. Ortigues-Marty, D. Pethick, P. Herpin, and X. Fernandez. 1998. Nutritional and hormonal regulation of energy metabolism in skeletal muscles of meat-producing animals. Livest. Prod. Sci. **56**:115–143. doi:10.1016/S0301-6226(98)00187-0.

Hocquette, J. F., P. Bas, D. Bauchart, M. Vermorel, and Y. Geay. 1999. Fat partitioning and biochemical characteristics of fatty tissues in relation to plasma metabolites and hormones in normal and double-muscled young growing bulls. Comp. Biochem. Physiol. - A Mol. Integr. Physiol. **122**:127–138. doi:10.1016/S1095-6433(98)10172-1.

Holtenius, P., and K. Holtenius. 2007. A model to estimate insulin sensitivity in dairy cows. Acta Vet. Scand. **49**:29. doi:10.1186/1751-0147-49-29.

Horst, E. A., S. K. Kvidera, M. J. Dickson, H. A. Ramirez, A. F. Keating, and L. H. Baumgard. 2019. Effects of continuous and increasing lipopolysaccharide infusion on basal and stimulated metabolism in lactating Holstein cows. J. Dairy Sci. **102**:3584-3597. doi:10.3168/jds.2018.15627.

Humblet, M-F., H. Guyot, B. Boudry, F. Mbayahi, C. Hanzen, F. Rollin, and J-M Godeau. 2006. Relationship between haptoglobin, serum amyloid A, and clinical status in a survey of dairy herds during a 6-month period. Vet. Clin. Pathol. **35**:188-193. doi:10.1111/j.1939-165x.2006.tb00112.x.

Huntington, G., and C. Richards. 2005. Metabolic fate of products of starch digestion and absorption in beef and dairy cattle. Proc. Southwest Nutr. Conf.: 67–77.

Immonen, K., M. Ruusunen, K. Hissa, and E. Puolanne. 2000. Bovine muscle glycogen concentration in relation to finishing diet, slaughter and ultimate pH. Meat Sci. **55**:25-31. doi:10.1016/s0309-1740(99)00121-7.

Jamroz, D., T. Wertelecki, M. Houszka, and C. Kamel. 2006. Influence of diet type on the inclusion of plant origin active substances on morphological and histochemical characteristics of the stomach and jejunum walls in chicken. J. Anim. Physiol. Anim. Nutr. (Berl). **90**:255-268. doi:10.1111/j.1439-0396.2005.00603.x.

Jang, I. S., Y. H. Ko, S. Y. Kang, and C. Y. Lee. 2007. Effect of a commercial essential oil on growth performance, digestive enzyme activity and intestinal microflora population in broiler chickens. Anim. Feed Sci. Technol. **134**:304-315. doi:10.1016/j.anifeedsci.2006.06.009.

Joo, J. I., D. H. Kim, J. W. Choi, and J. W. Yun. 2010. Proteomic analysis for antiobesity potential of capsaicin on white adipose tissue in rats fed with a high fat diet. J. Proteome Res. **9**:2977–2987. doi:10.1021/pr901175w.

Joy, F., McKinnon, J. J., Hendrick, S., Górka, P., and Penner, G. B. 2017. Effect of dietary energy substrate and days on feed on apparent total tract digestibility, ruminal short-chain fatty acid absorption, acetate and glucose clearance, and insulin responsiveness in finishing feedlot cattle. J. Anim. Sci. **95**:5606–5616. doi:10.2527/jas2017.1817.

Kadowaki, T., and T. Yamauchi. 2005. Adiponectin and adiponectin receptors. Endo. Reviews **26**:439-451. doi:10.1210/er.2005-0005.

Kahn, R.C. 1978. Insulin resistance, insulin insensitivity, and insulin unresponsiveness: A necessary distinction. Metabolism. **27**:1893–1902. doi:10.1016/S0026-0495(78)80007-9.

Kahn, B. B., T. Alquler, D. Carling, and D. G. Hardie. 2005. AMP-activated protein kinase: ancient energy gauge provides clues to modern understanding of metabolism. Cell Metab. **1**:15-25. doi:10.1016/j.cmet.2004.12.003.

Kaneko, J. J. 1997. Carbohydrate metabolism and its diseases. In: J. J. Kaneko, W. J. Harvey, and L. M. Bruss, editors, *Clinical biochemistry of domestic animals*. 6th ed. San Diego, CA: Academic Press. p. 45–81.

Kang, J. H., G. Tsuyoshi, I. S. Han, T. Kawada, Y. M. Kim, and R. Yu. 2010. Dietary capsaicin reduces obesity-induced insulin resistance and hepatic steatosis in obese mice fed a high-fat diet. Obesity **18**: 780–787. doi:10.1038/oby.2009.301.

Karlsson, M., H. Thorn, S. Parpal, P. Strålfors, and J. Gustavsson. 2001. Insulin induces translocation of glucose transporter GLUT4 to plasma membrane caveolae in adipocytes. FASEB J. **16**:249–251. doi:10.1096/fj.01-0646fje.

Katz, A., S. S. Nambi, K. Mather, A. D. Baron, D. A. Follmann, G. Sullivan, and M. J. Quon. 2000. Quantitative insulin sensitivity check index: a simple, accurate method for

assessing insulin sensitivity in humans. J. of Clin. Endocrinol. Metab. **85**:2402-2410. doi:10.1210/jcem.85.7.6661.

Kennedy, A., K. Martinez, C. C. Chuang, K. Lapoint, and M. Mcintosh. 2009. Saturated fatty acid-mediated inflammation and insulin resistance in adipose tissue: mechanisms of action and implications. J. Nutr. **139**:1–4. doi:10.3945/jn.108.098269.

Kent-Dennis, C., J. R. Aschenbach, P. J. Griebel, and G. B. Penner. 2020. Effects of lipopolysaccharide exposure in primary bovine ruminal epithelial cells. J. Dairy Sci. **103**:9587-9603. doi:10.3168/jds.2020-18652.

Khafipour, E., D. O. Krause, and J. C. Plaizier. 2009. A grain-based subacute ruminal acidosis challenge causes translocation of lipopolysaccharide and triggers inflammation. J. Dairy Sci. **92**:1060-1070. doi:10.3168/jds.2008-1389.

Kneeskern, S. G., A. C. Dilger, S. C. Loerch, D. W. Shike, and T. L. Felix. 2016. Effects of chromium supplementation to feedlot steers on growth performance, insulin sensitivity, and carcass characteristics. J. Anim. Sci. **94**:217-226. doi:10.2527/jas2015-9517.

Latack, B. C., M. F. Montano, R. A. Zinn, and J. Salinas-Chavira. 2021. Effects of a blend of cinnamaldehyde-eugenol and capsicum (Xtract® Ruminant 7065) and ionophore on performance of finishing Holstein steers and on characteristics of ruminal and total tract digestion. J. App. Anim. Res. **49**:185-193. doi:10.1080/09712119.2021.1934477.

Li, S., M. S. Brown, and J. L. Goldstein. 2010. Bifurcation of insulin signaling pathway in rat liver: mTORC1 required for stimulation of lipogenesis, but not inhibition of gluconeogenesis. Proc. Natl. Acad. Sci. U. S. A. **107**:3441–3446. doi:10.1073/pnas.0914798107.

Lippke, H. 2002. Estimation of forage intake by ruminants on pasture. Crop Science. **3**:869-872. doi:10.2135/cropsci2002.8690.

Liu, P., G. Peng, and L. Li. 2010. Endoplasmic reticulum stress mediates palmitic acid-induced insulin resistance in skeletal muscle cells. FASEB J. **24**:690.4. doi-org/10.1096/fasebj.24.1_supplement.690.4

Liu, Y., M. Song, T. M. Che, J. A. S. Almeida, J. J. Lee, D. Bravo, C.W. Maddox, and J. E. Pettigrew. 2013. Dietary plant extracts alleviate diarrhea and alter immune responses of weaned pigs experimentally infected with a pathogenic *escherichia coli*. J. Anim. Sci. **91**:5294-5306. doi:10.2527/jas2012-6194.

Le Lay, S., I. Lefrère, C. Trautwein, I. Dugail, and S. Krief. 2002. Insulin and sterol-regulatory element-binding protein-1c (SREBP-1c) regulation of gene expression in 3T3-L1 adipocytes: Identification of CCAAT/enhancer-binding protein β as an SREBP-1c target. J. Biol. Chem. **277**:35625–35634. doi:10.1074/jbc.M203913200.

Le Marchand-Brustel, Y., P. Gual, T. Grémeaux, T. Gonzalez, R. Barrès, and J. F. Tanti. 2003. Fatty acid-induced insulin resistance: Role of insulin receptor substrate 1 serine phosphorylation in the retroregulation of insulin signalling. Biochem. Soc. Trans. **31**:1152–1156. doi:10.1042/bst0311152.

Lee, G. R., M. K. Shin, D. J. Yoon, A. R. Kim, R. Yu, N. H. Park, and I. S. Han. 2013. Topical application of capsaicin reduces visceral adipose fat by affecting adipokine levels in high-fat diet-induced obese mice. Obesity **21**:115–122. doi:10.1038/oby.2012.166.

Letiges, M., M. Plomann, M. L. Standaert, G. Bandyopadhyay, M. P. Sajan, Y. Kanoh, and R. V. Farese. 2002. Knockout of PKCα enhances insulin signaling through PI3K. Mol. Endocrinol. **16**:847–858. doi:10.1210/me.16.4.847.

Leiva, T., R. F. Cooke, A. P. Brandão, U. Pardelli, R. O. Rodrigues, F. N. Corrá, and J. L. M. Vasconcelos. 2017. Effects of concentrate type and chromium propionate on insulin sensitivity, productive and reproductive parameters of lactating dairy cows consuming excessive energy. Animal **11**:436–444. doi:10.1017/S1751731116001713.

Li, S., M. S. Brown, and J. L. Goldstein. 2010. Bifurcation of insulin signaling pathway in rat liver: mTORC1 required for stimulation of lipogenesis, but not inhibition of gluconeogenesis. Proc. Natl. Acad. Sci. U. S. A. **107**:3441–3446. doi:10.1073/pnas.0914798107.

Locher, L., S. Häussler, L. Laubenthal, S. P. Singh, J. Winkler, A. Kinoshita, Á. Kenéz, J. Rehage, K. Huber, H. Sauerwein, and S. Dänicke. 2015. Effect of increasing body condition on key regulators of fat metabolism in subcutaneous adipose tissue depot and circulation of nonlactating dairy cows. J. Dairy. Sci. **98**:1057-1068. doi:10.3168/jds.2014-8710.

Logie, L., A. J. Ruiz-Alcaraz, M. Keane, Y. L. Woods, J. Bain, R. Marquez, D. R. Alessi, and C. Sutherland. 2007. Characterization of a protein kinase B inhibitor in vitro and in insulin-treated liver cells. Diabetes **56**:2218–2227. doi:10.2337/db07-0343.

Ludy, M-J., G. E. Moore, and R. D. Matters. 2012. The Effects of Capsaicin and Capsiate on Energy Balance: Critical Review and Meta-analyses of Studies in Humans. Chem. Senses **37**:103-121. doi:10.1093/chemse/bjr100.

Manna, P., and S. K. Jain. 2013. PIP3 but not PIP2 increases GLUT4 surface expression and glucose metabolism mediated by AKT/PKCζ/λ phosphorylation in 3T3L1 adipocytes. Mol Cell Biochem. **381**:291–299.

Manzanilla, E. G., J. F. Perez, M. Martin, C. Kamel, F. Baucells, and J. Gasa. 2004. Effect of plant extracts and formic acid on the intestinal equilibrium of early-weaned pigs. J. Anim. Sci. **82**:3210-3218. doi:10.2527/2004.82113210x.

Mathews, A. T., J. E. Rico, N. T. Sprenkle, A. L. Lock, and J. W. McFadden. 2016. Increasing palmitic acid intake enhances milk production and prevents glucose-stimulated fatty acid disappearance without modifying systemic glucose tolerance in mid-lactation dairy cows. J. Dairy Sci. **99**:8802–8816. doi:10.3168/jds.2016-11295.

Matthews, D. R., J. P. Hosker, A. S. Rudenski, B. A. Naylor, D. F. Treacher, and R. C. Turner. 1985. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia. **28**:412-419. doi:10.1007/BF00280883.

Mittelman, S. D., G. W. Van Citters, S. P. Kim, D. A. Davis, M. K. Dea, M. Hamilton-Wessler, and R. N. Bergman. 2000. Longitudinal compensation for fat-induced insulin

resistance includes reduced insulin clearance and enhanced β-cell response. Diabetes **49**:2116–2125. doi:10.2337/diabetes.49.12.2116.

Moore, M. C., L. Rossetti, M. J. Pagliassotti, M. Monahan, C. Venable, D. Neal, and A. D. Cherrington. 1996. Neural and pancreatic influences on net hepatic glucose uptake and glycogen synthesis. Am. J. Physiol. - Endocrinol. Metab. **271**:E215–E222. doi:10.1152/ajpendo.1996.271.2.e215.

Muniyappa, R., S. Lee, H. Chen, and M. J. Quon. 2008. Current approaches for assessing insulin sensitivity and resistance in vivo: Advantages, limitations, and appropriate usage. Am. J. Physiol. - Endocrinol. Metab. **294**:E15–E26. doi:10.1152/ajpendo.00645.2007.

Myers, W. D., P. A. Ludden, V. Nayigihugu, and B. W. Hess. 2004. Technical Note: A procedure for the preparation and quantitative analysis of samples for titanium dioxide. J. Anim. Sci. **82**:179-183. doi:10.2527/2004.821179x.

National Academy of Sciences, Engineering, and Medicine (NASEM). 2016. Nutrient requirements of beef cattle, 8th ed. Natl. Acad. Press, Washington, DC, USA. doi:10.17226/19014.

National Research Council (NRC). 2001. Nutrient requirements of dairy cattle. 7th revised edn. Washington (DC): Nat. Acad. Press. doi:10.17226/9825.

Nazifi, S., T. Karimi, and A. Rowshan Ghasrodashti. 2004. Diabetes mellitus and fatty liver in a cow: Case report. Comp. Clin. Path. **13**:82–85. doi:10.1007/s00580-004-0519-1.

NBQA, 2018. 2016/17 Plant Carcass Audit. Accessed Apr. 26, 2021. https://www.beefresearch.ca/files/pdf/NBQA-Carcass-Audit-Mar-27-2018-F.pdf.

Official Journal of the European Union. 2003. Regulation (EC) No 1831/2003 of the European parliament and the council of 22 September 2003 on additives for use in animal nutrition. Off. J. of the Eur. Union. 10/18/2003:L268/29–L268/43.

Oh, J. 2015. Effects of Capsicum oleoresin on feed intake, milk production, immune responses, blood metabolites, and hormones in lactating dairy cows. MS. College of Agricultural

Sciences. The Pennsylvania State University. ProQuest Diss. Pub. Retrieved from: https://search.proquest.com/docview/1989030832

Oh, J., A. N. Hristov, C. Lee, T. Cassidy, K. Heyler, G. A. Varga, J. Pate, S. Walusimbi, E. Brzezicka, K. Toyokawa, J. Werner, S. S. Donkin, R. Elias, S. Dowd, and D. Bravo. 2013. Immune and production responses of dairy cows to postruminal supplementation with phytonutrients. J. Dairy. Sci. **96**:7830-7843. doi:10.3168/jds.2013-7089.

Oh, J., F. Giallongo, T. Frederick, J. Pate, S. Walusimbi, R.J. Elias, E.H. Wall, D. Bravo, and A.N. Hristov. 2015. Effects of dietary Capsicum oleoresin on productivity and immune responses in lactating dairy cows. J. Dairy Sci. **98**: 6327-6339. doi:10.3168/jds.2014-9294.

Oh, J., M. Harper, F. Giallongo, D.M. Braco, E.H. Wall, and A.N. Hristov. 2017a. Effects of rumen-protected Capsicum oleoresin on immune responses in dairy cows intravenously challenged with lipopolysaccharide. J. Dairy Sci. **100**:1902-1913. doi:10.3168/jds.2016-11666.

Oh, J., Harper, M., Giallongo, F., Bravo, D. M., Wall, E. H., and Hristov, A. N. 2017b. Effects of rumen-protected Capsicum oleoresin on productivity and responses to a glucose tolerance test in lactating dairy cows. J. Dairy Sci. **100**:1888-1901. doi:10.3168/jds.2016-11665.

Okuno, A., H. Tamemoto, K. Tobe, K. Ueki, Y. Mori, K. Iwamoto, K. Umesono, Y. Akanuma, T. Fujiwara, H. Horikoshi, Y. Yazaki, and T. Kadowaki. 1998. Troglitazone increases the number of small adipocytes without the change of white adipose tissue mass in obese zucker rats. J. Clin. Invest. **101**:1354-1361. doi:10.1172/JCI1235.

Olefsky, J. M. 1976. The effects of spontaneous obesity on insulin binding, glucose transport, and glucose oxidation of isolated rat adipocytes. J. Clin. Invest. **57**:842-851. doi:10.1172/JCI108360.

Olson, A. L., and J. E. Pessin. 1996. Structure, function, and regulation of the mammalian facilitative glucose transporter gene family. Annu. Rev. Nutr. **16**:235–256. doi:10.1146/annurev.nutr.16.1.235.

Orro, T., T. Pohjanvirta, U. Rikula, A. Huovilainen, S. Alasuutari, L. Sihvonen, S. Pelkonen, and T. Soveri. 2011. Acute phase protein changes in calves during an outbreak of respiratory disease caused by bovine respiratory syncytial virus. Compar. Immun. Micro. and Infect. Dis. **34**:23-29. doi:10.1016/j.cimid.2009.10.005.

Osundiji, M. A., and M. L. Evans. 2013. Brain Control of Insulin and Glucagon Secretion. Endocrinol. Metab. Clin. North Am. **42**:1–14. doi:10.1016/j.ecl.2012.11.006.

Owens, F. N., D. R. Gill, D. S. Secrist, and S. W. Coleman. 1995. Review of some aspects of growth and development of feedlot cattle. J. Anim. Sci. **73**:3152–3172. doi:10.2527/1995.73103152x.

Pacini, G., M. Ahren, and B. Ahren. 2009. Reappraisal of the intravenous glucose tolerance index for a simple assessment of insulin sensitivity in mice. Am. J. Physio. Regul. Integr. Comp. Physiol. **296**:R1316-R1324. doi:10.1152/ajpregu.90575.2008.

Patel, P., and N. Abate. 2013. Body fat distribution and insulin resistance. Nutrients **5**:2019–2027. doi:10.3390/nu5062019.

Pederzolli, R. L. A., A. G. Van Kessel, J. Campbell, S. Hendrick, K. M. Wood, and G. B. Penner. 2018. Effect of ruminal acidosis and short-term low feed intake on indicators of gastrointestinal barrier function in Holstein steers. J. Anim. Sci. **96**:108-125. doi:10.1093/jas/skx049.

Perry, T. C., D. G. Fox, and D. H. Beermann. 1991. Effect of an implant of trenbolone acetate and estradiol on growth, feed efficiency, and carcass composition of Holstein and beef steers. J. Anim. Sci. **69**:4696-4702. doi:10.2527/1991.69124696x.

Perseghin, G., A. Caumo, M. Caloni, G. Testolin, and L. Luzi. 2001. Incorporation of the fasting plasma FFA concentration into QUICKI improves its association with insulin sensitivity in nonobese individuals. J. Clin. Endocrinol. Metab. **86**:4776-4781. doi:10.1210/jcem.86.10.7902.

Piantoni, P., A. L. Lock, and M. S. Allen. 2013. Palmitic acid increased yields of milk and milk fat and nutrient digestibility across production level of lactating cows. J. Dairy Sci. **96**:7143-7154. doi:10.3168/jds.2013-6680.

Pires, J. A. A., A. H. Souza, and R. R. Grummer. 2007. Induction of hyperlipidemia by intravenous infusion of tallow emulsion causes insulin resistance in holstein cows. J. Dairy Sci. **90**:2735–2744. doi:10.3168/jds.2006-759.

Platel, K., and K. Srinivasan. 1996. Influence of dietary spices or their active principles on digestive enzymes of small intestinal mucosa in rats. Int. J. of Food Sci. and Nut. **47**:55-59. doi:10.3109/09637489609028561.

Platel, K., and K. Srinivasan. 2000. Influence of dietary spices and their active principles on pancreatic digestive enzymes in albino rats. Nahrung **44**:42–46.

Ramirez Ramirez, H. A., K. J. Harvatine, and P. J. Kononoff. 2016. *Short communication:* Forage particle size and fat intake affect rumen passage, the fatty acid profile of milk, and milk fat production in dairy cows consuming dried distillers grains with solubles. J. Dairy Sci. **99**:392-398. doi:10.3168/jds.2015-10006.

Rehman, I., N. Mahabadi, T. Sanvictores, C. I. Rehman. 2021. Classical Conditioning. [Updated 2021 Aug 27]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK470326/

Relling, A. E., and C. K. Reynolds. 2007. Feeding rumen-inert fats differing in their degree of saturation decreases intake and increases plasma concentrations of gut peptides in lactating dairy cows. J. Dairy Sci. **90**:1506-1515. doi:10.3168/jds.S0022-0302(07)71636-3.

Reynoso, R., L. M. Salgado, and V. Calderón. 2003. High levels of palmitic acid lead to insulin resistance due to changes in the level of phosphorylation of the insulin receptor and insulin receptor substrate-1. Mol. Cell. Biochem. **246**:155–162. doi:10.1023/A:1023423005187.

Rico, D. E., Y. Ying, and K. J. Harvatine. 2014. Effect of a high-palmitic acid fat supplement on milk production and apparent total-tract digestibility in high- and low-milk yield dairy cows. J. Dairy Sci. **97**:3739-3751. https://doi.org/10.3168/jds.2013-7341.

Rico, J. E., A. T. Mathews, J. Lovett, N. J. Haughey, and J. W. McFadden. 2016. Palmitic acid feeding increases ceramide supply in association with increased milk yield, circulating nonesterified fatty acids, and adipose tissue responsiveness to a glucose challenge. J. Dairy Sci. **99**:8817-8830. https://doi.org/10.3168/jds.2016-11296.

Rico, J. E., S. S. Samii, A. T. Mathews, J. Lovett, N. J. Haughey, and J. W. McFadden. 2017. Temporal changes in sphingolipids and systemic insulin sensitivity during the transition from gestation to lactation. PLoS One **12**:1–20. doi:10.1371/journal.pone.0176787.

Rodríguez-Prado, M., A. Ferret, J. Zwieten, L. Gonzalez, D. Bravo, and S. Calsamiglia. 2012. Effects of dietary addition of capsicum extract on intake, water consumption, and rumen fermentation of fattening heifers fed a high-concentrate diet. J. Anim. Sci. **90**:1879–1884. doi:10.2527/jas.2010-3191.

Sainz, R. D., F. De la Torre, and J. W. Oltjen. 1995. Compensatory growth and carcass quality in growth-restricted and refed beef steers. J. Anim. Sci. **73**:2971–2979. doi:10.2527/1995.73102971x.

Samuelson, K. L., M. E. Hubbert, M. L. Galyean, and C. A. Loest. 2016. Nutritional recommendations of feedlot consulting nutritionists: The 2015 New Mexico State and Texas Tech University survey. Am. Soc. Of Anim. Sci. **94**:2648-2663. doi:10.2527/jas2016-0282.

Schoonmaker, J. P., M. J. Cecava, D. B. Faulkner, F. L. Fluharty, H. N. Zerby, and S. C. Loerch. 2003. Effect of source of energy and rate of growth on performance, carcass characteristics, ruminal fermentation, and serum glucose and insulin of early-weaned steers. J. Anim. Sci. **81**:843–855. doi:10.2527/2003.814843x.

Sears, B., and M. Perry. 2015. The role of fatty acids in insulin resistance. Lipids in Health and Disease **14**:121. doi:10.1186/s12944-015-0123-1.

Shingu, H., Hodate, K., Kushibiki, S., Ueda, Y., Watanabe, A., Shinoda, M., and Matsumoto, M. 2001. Profiles of growth hormone and insulin secretion, and glucose response to insulin in growing Japanese Black heifers (beef type): Comparison with Holstein heifers (dairy type). Comp. Biochem. Physiol. C. **130**:259-270. doi:10.1016/S1532-0456(01)00249-6.

Shreve, B., N. Thiex, and M. Wolf. 2006. National forage testing association reference method: NFTA Method 2.1.4 - Dry matter by oven drying for 3 hours at 105 °C. NFTA reference methods. National Forage Testing Association, Omaha, NB. www.foragetesting.org/reference-methods

Silva-García, O., R. Rico-Mata, M. C. Maldonado-Pichardo, A. Bravo-Patiño, J. J. Valdez-Alarcón, J. Aguirre-González, and V. M. Baizabal-Aguirre. 2018. Glycogen synthase kinase 3α is the main isoform that regulates the transcription factors nuclear factor-Kappa B and cAMP response element binding in bovine endothelial cells infected with Staphylococcus aureus. Front. Immunol. **9**:1–13. doi:10.3389/fimmu.2018.00092.

Smith, K. R., S. K. Duckett, M. J. Azain, R. N. Sonon Jr., and T. D. Pringle. 2007. The effect of anabolic implants on intramuscular lipid deposition in finished beef cattle. J. Anim. Sci. **85**:430-440. doi:10.2527/jas.2006-280.

Smith, P. K., R. I. Krohn, G. T. Hermanson, A. K. Mallia, F. H. Gartner, M. D. Provenzano, E. K. Fujimoto, N. M. Goeke, B. J. Olson, and D. C. Klenk. 1985. Measurement of protein using bicinchoninic acid. Anal. Biochem. **150**:76-85. doi:10.1016/0003-2697(85)90442-7.

Smith, S. B., and J. D. Crouse. 1984. Relative contributions of acetate, lactate and glucose to lipogenesis in bovine intramuscular and subcutaneous adipose tissue. J. Nutr. **114**:792–800. doi:10.1093/jn/114.4.792.

Smith, Z. K., J. Kim, and B. J. Johnson. 2019. Feedlot performance and biological responses to coated and non-coated steroidal implants containing trenbolone acetate and estradiol benzoate in finishing beef steers. J. Anim. Sci. **97**:4371-4385. doi:10.1093/jas/skz298.

Snel, M., J. T. Jonker, J. Schoones, H. Lamb, A. de Roos, H. Pijl, J. W. A. Smit, A. E. Meinders, and I. M. Jazet. 2012. Ectopic fat and insulin resistance: pathophysiology and effect of diet and lifestyle interventions. Internat. J. Endocrin. **2012**:983814. doi:10.1155/2012/983814.

Stelwagen, K., E. H. Wall, and D. M. Bravo. 2016. Effect of rumen-protected capsicum on milk production in early lactating cows in a pasture-based system. J. Anim. Sci. **94(E. Suppl. 1)**:E675.

Storlien, L. H., L. A. Baur, A. D. Kriketos, D. A. Pan, G. J. Cooney, A. B. Jenkins, G. D. Calvert, and L. V. Campbell. 1996. Dietary fats and insulin action. Diabetologia **39**:621–631. doi:10.1007/BF00418533.

Tager, L. R., and Krause, K. M. 2011. Effects of essential oils on rumen fermentation, milk production, and feeding behavior in lactating dairy cows. J. Dairy Sci. **94**: 2455–2464. doi:10.3168/jds.2010-3505.

Tan, S. X., Y. Ng, C. C. Meoli, A. Kumar, P. S. Khoo, D. J. Fazakerley, J. R. Junutula, S. Vali, D. E. James, and J. Stöckli. 2012. Amplification and demultiplexing in insulin-regulated Akt protein kinase pathway in adipocytes. J. Biol. Chem. **287**:6128–6138. doi:10.1074/jbc.M111.318238.

Taniguchi, C. M., B. Emanuelli, and C. R. Kahn. 2006. Critical nodes in signalling pathways: Insights into insulin action. Nat. Rev. Mol. Cell Biol. **7**:85–96. doi:10.1038/nrm1837.

Taniyama, H., T. Shirakawa, H. Furuoka, S. Osame, N. Kitamura, and K. Miyazawa. 1993. Spontaneous Diabetes Mellitus in Young Cattle: Histologic, Immunohistochemical, and Electron Microscopic Studies of the Islets of Langerhans. Vet. Pathol. **30**:46–54. doi:10.1177/030098589303000106.

Titgemeyer, E. C., C. K. Armendariz, D. J. Bindel, R. H. Greenwood, and C. A. Löest. 2001. Evaluation of titanium dioxide as a digestibility marker for cattle. J. Anim. Sci. **79**:1059-1063. doi:10.2527/2001.7941059x.

Tokarz, V. L., P. E. MacDonald, and A. Klip. 2018. The cell biology of systemic insulin function. J. Cell Biol. **217**:1–17. doi:10.1083/jcb.201802095.

Tomczak, D. J., C. L. Lockard, J. S. Jennings, and J. T. Richeson. 2019. Performance, rumination, and rumen pH responses to different dietary energy density and feed management strategies in auction-derived feedlot cattle. J. Anim. Sci. **97**:4682–4690. doi:10.1093/jas/skz323.

Trenkle, A., and D. G. Topel. 1978. Relationships of some endocrine measurements to growth and carcass composition of cattle. J. of Anim. Sci. **46**:1604-1609. doi:10.2527/jas1978.4661604x.

Underwood, K. R., J. Tong, M. J. Zhu, Q. W. Shen, W. J. Means, S. P. Ford, S. I. Paisley, B. W. Hess, and M. Du. 2007. Relationship between kinase phosphorylation, muscle fiber typing, and glycogen accumulation in *longissimus* muscle of beef cattle with high and low intramuscular fat. J. Agric. Food Chem. **55**:9698-9703. doi:10.1021/jf071573z.

Vasconcelos, J. T., J. E. Sawyer, L. O. Tedeschi, F. T. McCollum, and L. W. Greene. 2009. Effects of different growing diets on performance, carcass characteristics, insulin sensitivity, and accretion of intramuscular and subcutaneous adipose tissue of feedlot cattle. J. Anim. Sci. **87**:1540–1547. doi:10.2527/jas.2008-0934.

Van Soest, P. J., J. B. Robertson, and B. A. Lewis. 1991. Methods for Dietary Fiber, Neutral Detergent Fiber, and Nonstarch Polysaccharides in Relation to Animal Nutrition. J. Dairy Sci. **74**: 3583-3597. doi:10.3168/jds.S0022-0302(91)78551-2.

Vasconcelos, J. T., J. E. Sawyer, L. O. Tedeschi, F. T. McCollum, and L. W. Greene. 2009. Effects of different growing diets on performance, carcass characteristics, insulin sensitivity, and accretion of intramuscular and subcutaneous adipose tissue of feedlot cattle. J. Anim. Sci. **87**:1540-1547. doi:10.2527/jas.2008-0934.

Vaulont, S., M. Vasseur-Cognet, and A. Kahn. 2000. Glucose regulation of gene transcription. J. Biol. Chem. **275**:31555–31558. doi:10.1074/jbc.R000016200.

Verde, L. S., and Trenkle, A. 1987. Concentrations of hormones in plasma from cattle with different growth potentials. J. Anim. Sci. **64**:426–432. doi:10.2527/jas1987.642426x.

Watanabe, T., T. Kawada, M. Yamamoto, and K. Iwai. 1987. Capsaicin, a pungent principle of hot red pepper, evokes catecholamine secretion from the adrenal medulla of anesthetized rats. Biochem. Biophys. Res. Commun. **142**:259-264. doi:10.1016/0006-291x(87)90479-7

Westphalen, M. F., Carvalho, P. H. V., Oh, J., Hristov, A. N., Staniar, W. B., and Felix, T. L. 2021. Effects of feeding rumen-protected Capsicum oleoresin on growth performance, health status, and total tract digestibility of growing beef cattle. J. Anim. Feed and Sci. Tech. **271**:114778. doi:10.1016/j.anifeedsci.2020.114778.

Xie, H., V. A. Tsybenko, M. V. Johnson, and W. W. Lautt. 1993. Insulin resistance of glucose response produced by hepatic denervations. Can. J. Physiol. Pharmacol. **71**:175–178. doi:10.1139/y93-024.

XTRACT. 2022. Innovative bioactive solutions. https://service.admani.com/portal/pls/portal/docs/1/11476192.PDF (Accessed 8 July 2022).

Yechoor, V. K., M. E. Patti, K. Ueki, P. G. Laustsen, R. Saccone, R. Rauniyar, and C. R. Kahn. 2004. Distinct pathways of insulin-regulated versus diabetes-regulated gene expression: An in vivo analysis in MIRKO mice. Proc. Natl. Acad. Sci. U. S. A. **101**:16525–16530. doi:10.1073/pnas.0407574101.

Zachut, M., H. Honig, S. Striem, Y. Zick, S. Boura-Halfon, and U. Moallem. 2013. Periparturient dairy cows do not exhibit hepatic insulin resistance, yet adipose-specific insulin resistance occurs in cows prone to high weight loss. J. Dairy Sci. **96**:5656-5669. doi:10.3168/jds.2012-6142.

Zhang, S., M. Xiaohan, L. Zhang, H. Sun, and X. Liu. 2017. Capsaicin reduces blood glucose by increasing insulin levels and glycogen content better than Capsiate in streptozotocin-induced diabetic rats. J. Agric. Food Chem. **65**:2323-2330. doi:10.1021/acs.jafc.7b00132.

Zhao, C., G. Liu, X. Li, Y. Guan, Y. Wang, X. Yuan, G. Sun, Z. Wang, and X. Li. 2018. Inflammatory mechanism of rumenitis in dairy cows with subacute ruminal acidosis. BMC Vet. Res. **14**:135. doi:10.1186/s12917-018-1463-7.

Zhu, Q., R. Zhu, and J. Jin. 2016. Neutral ceramidase-enriched exosomes prevent palmitic acid-induced insulin resistance in H4IIEC3 hepatocytes. FEBS Open Bio **6**:1078-1084. doi:10.1002/2211-5463.12125.

Zinn, R. A. 1989. Influence of level and source of dietary fat on its comparative feeding value in finishing diets for feedlot steers: metabolism. J. Anim. Sci. **67**:1038-1049. doi:10.2527/jas1989.6741038x.

Zinn, R. A., A. Barreras, F. N. Owens, and A. Plascenscia. 2008. Performance by feedlot steers and heifers: daily gain, mature body weight, dry matter intake, and dietary energetics. J. Anim. Sci. **86**:2680-2689. doi:10.2527/jas.2007-0561.

8.0 APPENDIX

8.1 Dietary Adaptation Period: Step-up Protocol

	Dietary Adaptations[1]					
Ingredients, % DM	**1**	**2**	**3**	**4**	**5**	**6**
Barley silage	20.0	25.0	25.0	20.0	15.0	12.0
Pea hay	10.0					
Grass hay	20.0	15.0	5.0			
Dry rolled barley	39.52	52.27	62.08	71.98	77.1	80.2
Canola meal	10.35	6.67	6.67	6.67	6.29	6.19
Mineral	0.13	1.06	1.25	1.35	1.61	1.61

[1] Each adaptation diet was fed for four days.