UNIVERSIDADE ESTADUAL PAULISTA
FACULDADE DE MEDICINA VETERINÁRIA E ZOOTECNIA
CAMPUS DE BOTUCATU

EFEITO DE FONTES DE ENERGIA (AMIDO, PECTINA OU GORDURA) E DA SUPLEMENTAÇÃO COM CROMO, EM DIETAS COM ALTA DENSIDADE ENERGÉTICA, NAS CONCENTRAÇÕES SÉRICAS DE GLICOSE, INSULINA E ÁCIDOS GRAXOS NÃO ESTERIFICADOS EM VACAS DE LEITE LACTANTES.

Tiago Leiva

Trabalho apresentado como exigências para obtenção do título de Doutor junto ao Programa de Pós-graduação em Zootecnia.

BOTUCATU – SP
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Tiago Leiva
Zootecnista

ORIENTADOR: Prof. Ass. Dr. José Luiz Moraes Vasconcelos

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Sumario

Páginas

LISTA DE ABREVIATURAS

AGNE – Ácidos Graxos Não Esterificado

Akt – Proteína quinase B

BEN – Balanço Energético Negativo

BHBA – Beta Hidroxibutirato

ECC – Escore de Condição Corporal

GH – Hormônio do crescimento

GLUT – Proteínas transportadoras de glicose

IGF-1 – Fator de crescimento semelhante a insulina tipo 1

IL-6 – Interleucina -6

IMS – Ingestão de Matéria Seca

LH – Hormônio Luteinizante

LMWCr – Substância ligadora de cromo de baixo peso molecular

MS – Matéria seca

PC – Peso Corporal

PI3q - Fosfatidilinositol 3-quinase

TG – Triacilglicerol

TNF-α – Fator de necrose tumoral - α

TTG – Teste de Tolerância à Glicose

Listas de Tabelas

Lista de Figuras

Lista de Tabelas

Lista de Figuras

CAPÍTULO 1

CONSIDERAÇÕES INICIAIS

1. Introdução

Com o passar dos anos, a seleção genética proporcionou aumento significativo na produção de leite por vaca, tornando-se necessária a utilização de dietas de maior densidade energética, de forma a suprir as exigências nutricionais desses animais.

No entanto, nem mesmo a utilização de dietas de maior densidade energética é capaz de anular o efeito do balanço energético negativo (BEN) que acomete vacas leiteiras durante o período de transição (intervalo entre as três semanas pré-parto e três semanas pós-parto), o qual é marcado pelo aumento nas concentrações plasmáticas de ácidos graxos não esterificados (AGNE), β-hidroxibutirato (BHBA) e hormônio do crescimento (GH), e diminuição nas concentrações de insulina, glicose e fator de crescimento semelhante à insulina tipo 1 (IGF-1). Além disso, durante o período de BEN, a sensibilidade dos tecidos periféricos à insulina é diminuída (resistência à insulina; síndrome sistêmica responsável pela diminuição na captação de glicose pelas células insulinodependentes), comprometendo a utilização de glicose e desencadeando déficit energético celular (Pettersson et al., 1994), devido às baixas concentrações de insulina e das altas concentrações circulantes de GH (Butler et al., 2003).

Por outro lado, com o decorrer da lactação, o consumo excessivo de energia pode proporcionar maior ganho de peso corporal (PC), aumento no escore de condição corporal (ECC) e também aumento da predisposição dos animais a se tornarem resistentes à insulina (Gonçalves et al., 2009; Leiva et al., 2015). Todavia nesse período, a resistência à insulina é devida ao aumento circulante das concentrações de insulina, AGNE e alguns produtos oriundos da cascata inflamatória, tais como fator de necrose tumoral-α (TNF-α) e interleucina-6 (IL-6).

Estados de resistência à insulina durante o terço médio e final de lactação têm sido relacionados ao consumo excessivo de energia, conduzindo os animais a um estado

metabólico caracterizado principalmente pelo aumento das concentrações circulantes de insulina e relacionado à queda no desempenho produtivo e reprodutivo dos animais.

Em humanos, estados de resistência à insulina são caracterizados principalmente pela alimentação rica em carboidratos e açúcares, agravados pela obesidade, e responsável pelo aparecimento de muitos transtornos fisiológicos, tais como: diminuição nas atividades renais, problemas visuais e neurais, assim como diminuição da fertilidade (American Diabets Association, 2010).

Devidas às perdas produtivas causadas pela resistência à insulina, tais como diminuição da qualidade oocitária, e agravamento do BEN (Butler et al., 2003; Baruselli et al., 2016) estudos que visam encontrar estratégias para mitigar seus efeitos, tornam-se fundamentais.

A utilização de cromo tem se mostrado um método eficiente no controle da resistência à insulina, uma vez que o cromo é um mineral parte do fator de tolerância à glicose, conhecido por potencializar a ação da insulina e por manter ativo seus receptores, otimizando a captação de glicose pelas células insulinodependentes (Schwarz e Mertz, 1959). Porém, em muitos casos a suplementação com cromo se torna necessária, pois esse mineral é encontrado em baixas concentrações em ingredientes comumente utilizados em dietas de ruminantes, podendo variar de 0,06 mg/kg de matéria seca (MS) no milho em grão até 2,2 mg/kg de MS em silagem de trigo (alimento com maior proporção; Lashkari et al., 2018). Mesmo tendo o potencial de melhorar a captação de glicose pelas células insulinodependentes, estudos ainda mostram grande variabilidade nos resultados produtivos quando cromo é adicionado às dietas de ruminantes, uma vez que o nível de inclusão de cromo ainda não está totalmente estabelecido.

Sabendo que a alta concentração de insulina é outro fator que pode predispor ao agravamento ou aparecimento da resistência à insulina, a utilização de dietas de menor

potencial à promoção do aumento de insulina pode ser uma estratégia para minimizar essa síndrome.

Assim, a presente tese tem por objetivo avaliar o efeito de diferentes fontes de energia na dieta de vacas leiteiras e a suplementação de cromo nas variáveis relacionadas à resistência à insulina.

2. Revisão de Literatura

2.1 Resistência à Insulina

Resistência à insulina é definida como resposta biológica subnormal dada a uma concentração de insulina circulante (Moller e Flier, 1981), acompanhada por intolerância à glicose, resultando em diminuição da captação de glicose pelos tecidos periféricos sensitivos à insulina (Kahn, 1978).

Resistência à insulina acomete principalmente os tecidos periféricos (músculo e tecido adiposo), os quais são altamente dependentes de insulina para promover o transporte de glicose do meio extra para o meio intracelular (Bell and Bauman, 1997). Em contrapartida, a captação de glicose pelos tecidos não epiteliais pode ser mediada por diferentes tipos de GLUTs (proteínas transportadoras de glicose), com características funcionais e distribuição tecidual distintas.

GLUTs são proteínas classificadas de acordo com a ordem cronológica de descoberta e particularmente expressas em um determinado tecido. Por exemplo, dentre as mais altamente expressas, temos: GLUT-1, a mais abundante nas células vermelhas, placenta, glândula mamária, oócito e no cérebro (não insulinodependente); GLUT-2, altamente expressa em tecidos hepáticos; GLUT-3, expressa em neurônios cerebrais e músculo fetal; e a GLUT-4 que é encontrada principalmente em tecidos periféricos (insulinodependente; Klip et al., 1994; Burnol et al., 1990).

Para a presente revisão, a GLUT-4 torna-se a proteína de maior importância, uma vez que a mesma é a principal responsável pelo transporte de glicose em células do tecido periférico e totalmente dependente de insulina para sua translocação do conteúdo intracelular para a membrana celular (Wood e Trayhurn, 2003).

A sinalização intracelular da insulina inicia-se com sua ligação a um receptor específico de membrana composto por duas subunidades alfa e duas subunidades beta, denominados receptores de insulina (Moller e Flier, 1981). A ligação da insulina com seus receptores, promove a ativação dos mesmos que são fosforilados em tirosina (Pessin e Saltiel, 2000; White, 1998), criando sítios de ligação para outra proteína denominada fosfatidilinositol 3-quinase (PI3q) (Backer et al., 1992). Essa ligação promove a ativação da PI3q, que é uma importante proteína relacionada ao transporte de glicose, aumentando a fosforilação em serina da proteína quinase B (Akt), o que permite que a glicose seja transportada para o músculo e para o tecido adiposo, devida à translocação da proteína GLUT-4 para a membrana celular (Czech e Corvera, 1999). A principal causa das falhas de comunicação entre célula e insulina é devida à perda de função dos receptores de insulina presentes na membrana celular. Abaixo é mostrado um esquema reduzido de como ocorre esse processo de sinalização da insulina nas células (Figura 1).

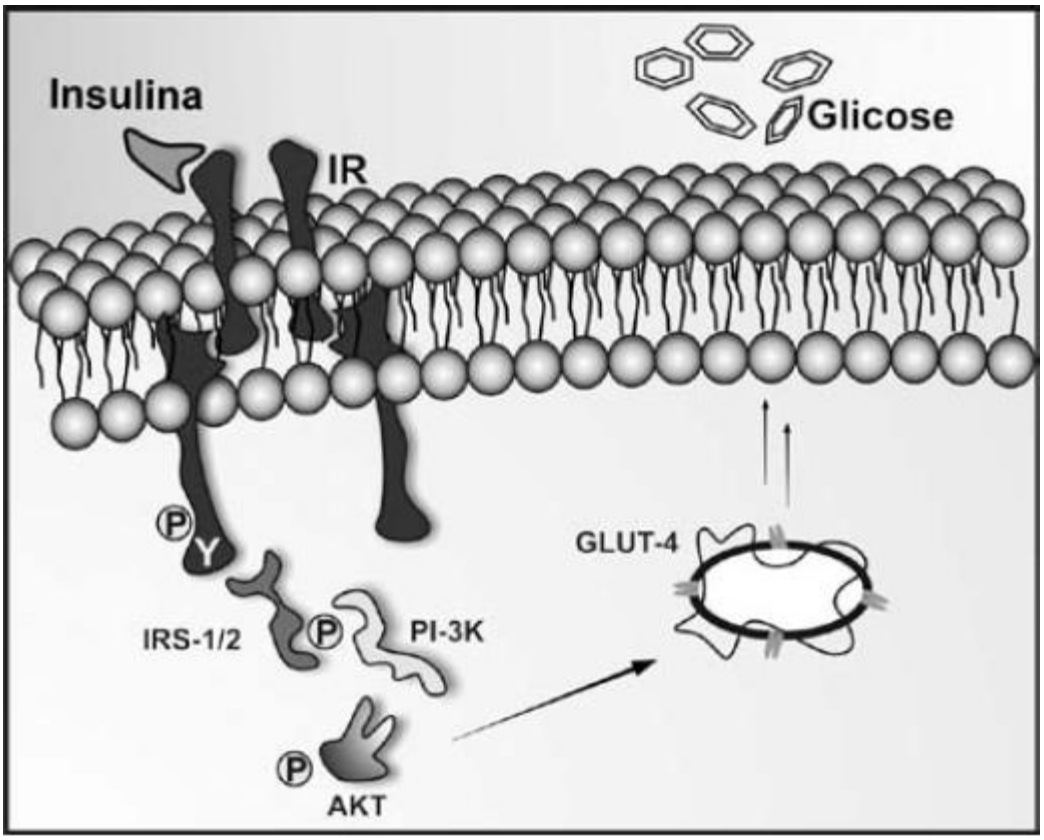

Figura 1. Via de sinalização da insulina na captação de glicose. A insulina ao se ligar ao seu receptor de membrana (IR), promove autofosforilação da subunidade beta em resíduos de tirosina (IRS) e desencadeia uma cascata de sinalização que migra para as vesículas que contém GLUT-4, promovendo seu transporte para membrana celular (Pauli et al., 2009).

A obesidade e/ou o aumento de AGNE circulantes, promovem alteração da sinalização de insulina, devida à redução da atividade dos receptores de insulina e dos PI3q na translocação dos GLUTs-4 e nas atividades das enzimas intracelulares, que podem ser consequência do acúmulo de TG e seus derivados nos músculos (Shulman, 2004).

Adicionalmente, foi descoberto que produtos dos tecidos adiposos como TNF-α e IL-6 também podem afetar a sinalização da insulina por induzirem uma resposta inflamatória, mesmo na ausência de patógenos, por serem capazes de interferir na sinalização de hormônios controladores da fome e do gasto energético (Waki e Tontonoz, 2007; Milanski et al., 2009). Esse mecanismo é decorrente da ativação de substratos intermediários da via de sinalização do TNF-α que são fosforilados em serina interferindo

na funcionalidade dos receptores de insulina, uma vez que quando fosforilados em serina a possibilidade de serem fosforilados em tirosina fica comprometida (Pauli et al., 2009).

A hiperinsulinemia é outro fator que pode comprometer a ligação da insulina com seus receptores de membrana, uma vez que, polipeptídios de amiloide ou amilina que são secretados juntamente com a insulina pelas células beta do pâncreas, são antagônicos ao transporte de glicose para os tecidos periféricos (Moller e Flier, 1981).

Além dos mecanismos citados acima, outros hormônios e metabólitos como glicocorticoides, glucagon, catecolaminas e GH podem causar resistência à insulina. Glicocorticoides podem causar resistência à insulina por diminuir o número ou a eficácia dos transportadores de insulina, e indiretamente por induzir o aumento dos níveis de glucagon e AGNE (Horner et al., 1987; Flier e Moses, 1989). Concentrações elevadas de GH diminuem a quantidade de receptores de insulina ou promovem a inibição dos transportadores de glicose por atuar na expressão dos genes desses transportadores (Flier e Moses, 1989; Tai et al., 1990). Por fim, as catecolaminas inibem a ação da insulina por estimular a gliconeogênese, glicogenólise e lipólise (Flier e Moses, 1989).

Durante o período de transição vacas leiteiras são acometidas pela diminuição da sensibilidade à insulina (resistência à insulina) nos tecidos periféricos (Lucy, 2001 e 2003), decorrente do aumento das concentrações circulantes de AGNE e GH (Grummer, 1993; Bell e Bauman, 1997; Kokkonen et al., 2005), e diminuição das concentrações circulantes de insulina (Butler et al., 2003), tipicamente observada durante o BEN.

Esse período de resistência à insulina nos tecidos periféricos é entendido como sendo um mecanismo fisiológico resultante da partição de nutrientes para dar suporte a maior demanda energética dos tecidos fetais no final da gestação e da glândula mamária no início da lactação (Bell, 1995). Contudo, os tecidos resistentes à insulina passam a utilizar acetato, derivado diretamente da fermentação ruminal, e 3-hidroxibutirato,

principalmente derivado da hidroxilação do butirato produzido no rúmem, como forma de suprir a demanda energética celular (Bell e Bauman, 1997).

Já em animais em balanço energético positivo, lactantes ou não, resistência à insulina pode se desenvolver devido aos fatores relacionados à hiperinsulenimia (Leiva et al., 2015), produtos derivados dos processos inflamatórios (IL-6 e TNF-α; Dandona et., 2004; Waki e Tontonoz, 2007, Pauli et al., 2009) e como resultado do aumento dos dias em leite (Oliveira et al., 2016), e pode se acentuar decorrente do aumento das concentrações de AGNE, que são liberados em tentativa de suprir a demanda energética (Holtenius et al., 2003; Pires et al., 2007).

Seguindo o racional descrito acima, nosso grupo de pesquisa realizou alguns estudos para investigar o efeito da densidade energética da dieta no estado de resistência à insulina em vacas leiteiras em balanço energético positivo. O primeiro estudo foi realizado em vacas secas, vazias, alimentadas com dietas a base de milho (visando o aumento das concentrações de insulina) que pretendiam atender ou exceder os requerimentos energéticos de mantença dos animais. Neste estudo foi concluído que dietas que extrapolavam as exigências energéticas das vacas induziam resistência à insulina (Leiva et al., 2014). Mais tarde, um estudo similar foi conduzido utilizando vacas lactantes (Leiva et al., 2015), no qual as mesmas eram alimentadas com dietas a base de milho, visando atender ou exceder as exigências de mantença. Os resultados deste estudo concordam com os resultados encontrados em vacas secas, dado que vacas lactantes alimentadas com dietas de alta densidade energética foram mais susceptíveis a apresentar resistência à insulina.

Com base nesses estudos concluímos que dietas de alta densidade energética que promovem hiperinsulinemia são capazes de induzir resistência à insulina em animais lactantes ou não, e nos abriu precedente para estudar se a utilização de dietas de alta

densidade energética, que não visam grandes aumentos nas concentrações de insulina, poderia mitigar esse problema.

2.2. Escore de condição corporal

Escore de condição corporal é uma ferramenta provada de avaliação do estado nutricional de vacas leiteiras (Hady et al., 1994), sendo a escala mais utilizada para sua determinação a proposta por Wildman et al., (1982), onde 1 indica vacas muito magras e 5 indica vacas obesas (escala de 1 a 5, com quebras de 0,25). Devido ao fator de enchimento do trato gastrointestinal o PC pode ser uma medida de grande variação em bovinos leiteiros, tornando assim o ECC uma avaliação mais precisa do estado nutricional do animal (West et al., 1990).

Grande parte dos artigos envolvendo ECC em vacas leiteiras são relacionados ao período de transição por se tratar de um momento crítico e altamente influenciado pelas reservas corporais dos animais. Por exemplo, alto ECC no pré-parto é associado à queda de ingestão de matéria seca (IMS) próximo ao parto (Butler, 2005) e ao aumento da ocorrência de doenças que predominam nesse período (Fronk et al., 1980; Grummer, 1993; Rukkwamsuk et al., 1999; Dann et al., 2006). Vacas com alto ECC no pré-parto apresentam maior risco de desenvolver distúrbios metabólicos como cetose e fígado gorduroso durante o pós-parto (Grummer, 1993; Smith et al., 1997). Esses distúrbios metabólicos são pertinentes a animais nessa condição corporal devido à predisposição em aumentar as concentrações de AGNE na corrente sanguínea e acumular TG no fígado (Jorritsma et al., 2001; Grummer, 2008). Além dos problemas produtivos, imunológicos e reprodutivos causados pela cetose e pelo fígado gorduroso (Bobe et al., 2004), vacas que apresentam esses distúrbios metabólicos aumentam o risco de desenvolver resistência à insulina (Ohtsuka et al., 2001), devido às altas concentrações de AGNE e GH, e às

baixas concentrações de insulina influenciarem a translocação dos GLUT-4 para membrana celular (Bossaert et al., 2008), agravando ainda mais o BEN.

Por outro lado, poucos são os estudos que avaliam as possíveis consequências em termos produtivos, imunológicos e reprodutivos de vacas leiteiras com alto ECC e/ou alimentadas com dietas de alta densidade energética a partir do terço médio da lactação. Para tentar entender a possível relação da obesidade (alto ECC, > 3,5) em vacas leiteiras durante a lactação com o estado de resistência à insulina, irei utilizar alguns conceitos já estabelecidos em humanos. É sabido que a obesidade é um fator predisponente ao desenvolvimento de resistência à insulina em humanos. Inicialmente a presença de AGNE circulantes era associada a uma menor fosforilação em sítios específicos e à menor ativação de proteínas-chave da via de insulina (Pauli et al., 2009). Contudo, vários fatores regulatórios produzidos por adipócitos (adipocinas) foram descritos, bem como o papel que desempenham no desenvolvimento de resistência à insulina. Outro mecanismo por meio do qual a obesidade pode causar prejuízo à ação da insulina é através da presença de radicais oriundo do estresse oxidativo. Esses radicais atuam como importantes sinalizadores intracelular capazes de modificar a função de diversas proteínas em etapas pós-transcricionais, modificando a função dos receptores de insulina (Hotamisligil, 2003).

Como em humanos, o estresse oxidativo e a produção de citocinas inflamatórias também são uma realidade em bovinos com elevado ECC, e assim esses fatores podem aumentar a susceptibilidade à doença em vacas leiteiras (O'Boyle et al., 2006). Desta forma, pelo semelhante mecanismo existente entre ambos, podemos inferir que a causa da resistência à insulina em animais com elevado ECC pode ser decorrente de fatores semelhantes aos encontrados em seres humanos.

2.3 Suplementação com Cromo

Cromo é um mineral que facilita a interação da insulina com seus receptores alvos (Mertz, 1992), potencializando a ação da insulina (Vincent et al., 2000; 2001), por fazer parte de um oligopeptídeo ligador de cromo, denominado de substância ligadora de cromo de baixo peso molecular (LMWCr) (Yamamoto et al., 1989). Este oligopeptídeo de baixo peso molecular possui 1,5 KDa e é composto por quatro resíduos de aminoácidos (glicina, cisteína, glutamato e aspartato) ligados a quatro íons de Cr^{3+} (forma mais comumente encontrada em alimentos) (Gomes et al., 2005).

O LMWCr quando ligado ao cromo também pode receber o nome de cromodulina, devido a sua semelhança em estrutura e função com a calmodulina, enquanto que na sua forma livre de minerais recebe o nome de apocromodulina (Sun et al., 2000). A cromodulina favorece a sensibilidade à insulina por estimular a atividade tirosina quinase do receptor insulínico na membrana celular. Mais especificamente, a cromodulina é estocada na forma de apocromodulina no citosol e núcleo de células sensíveis à insulina. Com o aumento da insulina ocorre maior mobilização de cromo para células alvo, mediada principalmente pela transferrina e mobilização de receptores de transferrina do meio intracelular para a membrana celular, onde ocorre a ligação da transferrina ligada ao cromo com os receptores presentes na membrana celular, que são internalizados por endocitose. No espaço intracelular os íons de cromo são liberados e se ligam à apocromodulina, tornando-a ativa (cromodulina), e por sua vez se ligam aos receptores de insulina na membrana celular amplificando o sinal dos mesmos (Figura 2; Gomes et al., 2005 - 22,27).

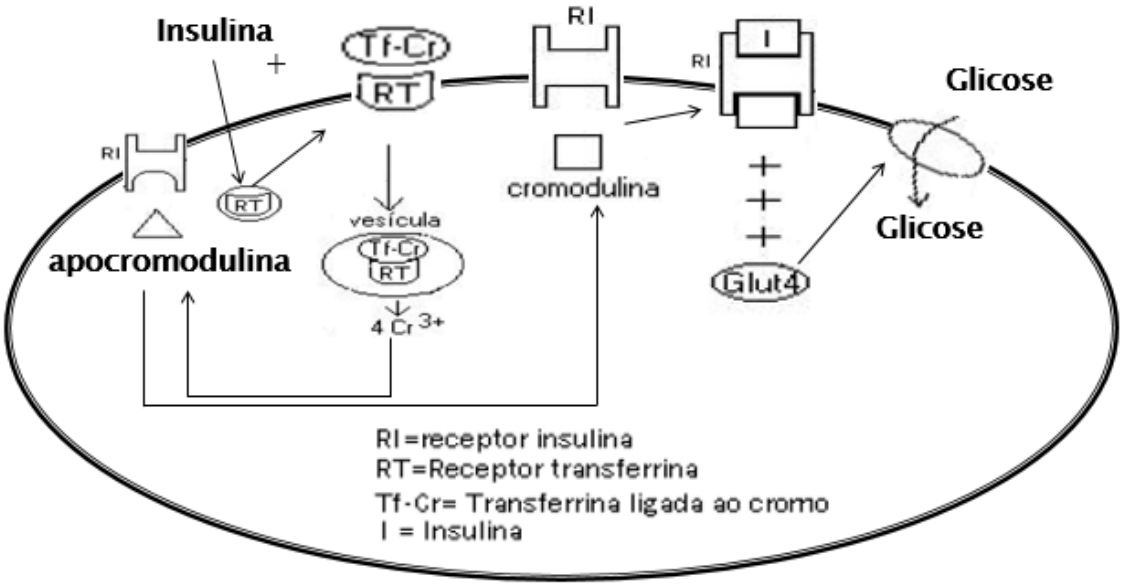

Figura 2. Modo de ação do cromo como facilitador do transporte de glicose. Adaptado de Gomes et al. (2005)

Em seres humanos e animais de laboratório, a deficiência de cromo é manifestada através de alterações no metabolismo de glicose e resistência à insulina em diversos tecidos (Mayes et al., 1993). Em ruminantes o cromo é utilizado como ferramenta para minimizar a resistência à insulina (McNamara e Valdez, 2005), devido ao seu papel de facilitador da utilização de glicose pelas células e na promoção do desempenho produtivo e reprodutivo (Westwood et al., 2002; Smith, 2004; Smith et al., 2005). Em recente estudo Pantelic et al., (2017) mostraram que a suplementação com cromo melhorou a resposta à insulina por diminuir reguladores negativos da insulina (proteínas que modificam a funcionalidade dos receptores de insulina) nos tecidos, levando a um forte impacto na cinética da insulina.

Na prática, Soltan (2009) suplementando vacas leiteiras durante o pré e pós-parto com cromo observou diminuição nas concentrações séricas de AGNE e cortisol, seguida de melhor utilização de insulina. Subiyatno et al. (1996) ao suplementarem cromo em dieta de vacas leiteiras antes e após o parto observaram redução na relação insulina:glicose e nas concentrações plasmáticas de insulina e triacilglicerol (TG). Besong et al., (1996) adicionando cromo à dieta de vacas leiteiras observaram aumento no consumo de matéria

seca (MS) e na produção de leite, e redução nas concentrações séricas de corpos cetônicos e TG.

Trabalhos do nosso grupo de pesquisadores, Leiva et al. (2014 e 2015), têm mostrado que a suplementação com propionato de cromo melhora os índices de resistência à insulina quando dietas de alta densidade energética são utilizadas em vacas secas e lactantes. No trabalho realizado no ano de 2014 com vacas secas, a suplementação com propionato de cromo diminuiu as concentrações séricas de glicose, insulina e AGNE, assim como a relação I:G durante as amostragens semanais e no teste de tolerância a glicose (TTG), mostrando sua efetividade em minimizar o estado de resistência à insulina. No trabalho realizado em 2015, com protocolo semelhante ao estudo anterior, mas utilizando vacas lactantes, observamos que a suplementação com propionato de cromo também foi efetiva em minimizar a resistência à insulina em vacas recebendo dietas de alta densidade energética.

Devido aos benefícios mostrados pela suplementação com cromo e pelas baixas concentrações de cromo presentes nos alimentos (principalmente em grãos), que compõe as dietas da maior parte das fazendas leiteiras (Spears et al., 2017), a suplementação se mostra vantajosa.

Para termos ideia da variabilidade dos dados, em um levantamento realizado pelo Committee on Animal Nutrition (1997), avaliando os resultados de trinta e quatro estudos realizados em ruminantes, foi observado que as quantidades basais de cromo nas dietas variavam de 0,79 a 1,60 mg/kg de matéria seca e a quantidade de suplementação de cromo de 5,5 a 10 mg/dia. Adicionalmente, em uma revisão realiza por Lashkari et al. (2018) foi mostrado que a quantidade de cromo pode variar de 0,06 mg/kg de matéria seca em milho grão a 2,2 mg/kg de matéria seca em silagem de trigo.

Dado aos benefícios promovidos pela ingestão de cromo, sua suplementação torna-se uma ferramenta que pode agregar maior produtividade às granjas leiteiras

2.4 Densidade energética e fontes de energia das dietas

O consumo excessivo de energia por vacas leiteiras secas e em meio de lactação é comum e frequente em fazendas comerciais (Van Saun e Sniffen, 1996), o que promove o aumento dos casos de resistência à insulina (Leiva et al., 2014; 2015). Consequentemente, a adoção de estratégias que minimizam este problema é essencial para otimizar a produção do rebanho.

Como relatado no tópico 2.1, a hiperinsulinemia pode ser um fator agravante para desencadear o estado de resistência à insulina em vacas leiteiras. Desta forma, a adoção de técnicas nutricionais que visam minimizar a concentração de insulina circulante, tal como a diminuição de amido na dieta, pode diminuir o aparecimento deste estado em bovinos.

O principal fator pelo qual dietas a base de milho aumentam as concentrações de insulina é devido à maior produção de propionato pelas bactérias ruminais durante a digestão do amido (Hentges et al., 1996). O propionato é absorvido para corrente sanguínea e, posteriormente, captado pelo fígado e utilizado como substrato na gliconeogênese, aumentando as concentrações de glicose circulante e estimulando a síntese e liberação de insulina pelo pâncreas (Nussey e Whitehead, 2001).

No entanto, outros ingredientes, como por exemplo a polpa cítrica, podem diminuir as concentrações de insulina, uma vez que o acetato é o principal ácido graxo de cadeia curta sintetizado durante a digestão da pectina pelas bactérias ruminais, sendo oxidado ao longo dos tecidos corporais para gerar energia ou sintetizar gordura (Bergman, 1990). Cabrita et al., (2007) reportaram que o aumento na ingestão de polpa cítrica em

substituição ao milho reduziu as concentrações de insulina em vacas leiteiras. Por outro lado, Moriel et al., (2008) ao substituir milho por polpa cítrica peletizada não verificaram efeito nas concentrações séricas de insulina e justificaram que em parte este resultado poderia ser devido à relação similar de acetato:propionato proporcionada pelas dietas.

Substituição de milho por fontes de gordura também pode ser outra estratégia para tentar diminuir as concentrações circulantes de insulina sem que ocorram perdas energéticas na dieta. No entanto, alguns cuidados devem ser adotados quando se adiciona fontes de gordura às dietas de bovinos. Altos níveis de inclusão de gordura nas dietas podem causar redução na digestibilidade da forragem (Allen, 2000) e modificação na microbiota ruminal (Devendra e Lewis, 1974), principalmente quando a suplementação é feita com fontes de óleos naturais sem nenhum tipo de proteção. Isso ocorre devido à biohidrogenação das gorduras pelos microrganismos ruminais (quando ocorre a saturação dos ácidos graxos com ligação dupla adicionando hidrogênio à cadeia carbônica) ou pelo efeito físico da gordura sobre as fibras dos alimentos (Harfoot e Hazlewood, 1988). Uma estratégia utilizada para diminuir tanto os riscos de biohidrogenação quanto os ricos de interferência direta dos ácidos graxos sobre as fibras dos alimentos, é a utilização de gordura protegida contra a degradação ruminal (Emery e Herdt, 1991; Grummer e Carroll, 1991). Como exemplo da estratégia mencionada, Grummer (1988), ao suplementar vacas leiteiras com 0,680 kg/dia de sal de cálcio de ácidos graxos de palma que representava menos que 3,5% da IMS dos animais, não notou modificação nos parâmetros ruminais e produtivos de forma geral. Assim, a suplementação com gordura protegida pode ser uma ferramenta contra o desenvolvimento do estado de resistência à insulina. Garnsworthy et al. (2008) reportaram que a suplementação de gordura protegida saturada em substituição ao amido reduziu as concentrações plasmáticas de insulina em vacas leiteiras lactantes, o

que foi reconfirmado mais tarde em outro estudo realizado pelo mesmo grupo de pesquisa (Garnsworthy et al., 2009).

2.5 Resultados Reprodutivos

Índices reprodutivos em vacas leiteiras têm diminuído de maneira concomitante ao aumento da produção de leite nas últimas décadas (Butler, 2003), com taxas de prenhez no primeiro serviço de aproximadamente 40% (Walsh et al., 2011), gerando um custo por dia em aberto após o período voluntário de espera de aproximadamente USD$ 5/vaca (De Vries, 2006). Uma das possíveis razões para a diminuição da taxa de prenhez pode ser devida às mudanças hormonais e metabólicas que ocorrem durante a lactação (Webb et al., 2007). Em particular, concentrações circulantes de AGNE, glicose e insulina têm sido associadas à falha ou ao sucesso dos índices reprodutivos, uma vez que podem atuar positivamente ou negativamente sobre a pulsatilidade de hormônio luteinizante (LH), maturação oocitária, padrões de ovulação e desenvolvimento embrionário (Gong et al., 2002; Lucy, 2003; Leroy et al., 2008).

O fluído folicular é derivado dos componentes plasmáticos, tornando assim seu perfil metabólico similar ao sanguíneo (Edwards, 1974; Spicer e Echternkamp, 1995), no entanto alguns mecanismos controlados pelas células do cumulus e a presença de alguns receptores na membrana celular fazem com que as concentrações hormonais e/ou de metabólitos no folículo possam ser alteradas em relação ao sangue (Leroy et al., 2004; Nishimoto et al., 2006). Como já mencionado anteriormente, o estado de resistência à insulina é marcado por altas concentrações de AGNE e insulina que podem ou não influenciar as concentrações de glicose (Leiva et al., 2014 e 2015). Porém, o desbalanço hormonal e/ou de metabólitos podem trazer perdas reprodutivas para os animais.

Os ácidos graxos têm um importante papel na manutenção da estrutura e da função das membranas celulares, no metabolismo do colesterol, na esteriogenesis e na síntese de prostaglandinas, atuando em várias etapas do sistema reprodutivo, podendo ou não estarem relacionados ao estado energético do animal (Lucy et al., 1992). A presença de ácidos graxos circulantes também pode ter efeitos diretos na transcrição de genes que formam proteínas essenciais para a reprodução (Mattos et al., 2000). Alguns estudos têm mostrados que AGNE podem ser tóxicos para o crescimento e funcionalidade das células da granulosa de bovinos (Vanholder et al., 2005) e de humanos (Mu et al., 2001), e podem reduzir a taxa de concepção em vacas leiteiras (Beam e Butler, 1999; De Vries e Veerkamp, 2000). Um fator que pode estar relacionado a isto é a *down-regulation* dos inibidores de apoptose e a *up-regulation* dos mediadores de apoptose sobre as células reprodutivas (Leroy et al., 2008). Leroy et al. (2008) mostraram que a exposição de oócitos a ácidos graxos causaram efeitos deletérios às células da granulosa, que podem originar problemas de maturação oocitária (Tanghe et al., 2002), que consequentemente levam à redução na qualidade e criotolerância dos oócitos e embriões (Abe et al., 2002).

As concentrações de glicose no fluído folicular são consideradas um fator importante para o desenvolvimento oocitário. Em um estudo realizado por Frank et al., (2013), os autores suplementaram meios de cultura de oócitos com glicose e observaram que a expansão das células do cumulus aumentava de acordo com o aumento da glicose no meio de cultura. Por outro lado, os autores notaram que altas concentrações de glicose na pré-fertilização foram negativamente associadas ao desenvolvimento do blastocisto (Hashimoto et al., 2000). Altas concentrações de glicose no fluído folicular estão associadas às altas concentrações de espécies reativas de oxigênio e baixas concentrações de glutationa, sugerindo que a produção de blastocisto pode ser afetada devida à baixa capacidade antioxidante dos oócitos (Hashimoto et al., 2000). A propósito, mesmo sendo

a mitocôndria uma organela conhecida por produzir energia e também realizar papel antioxidante nas células, ela sofre efeito das concentrações de glicose. Ratos, que tiveram estado de resistência à insulina induzido, mostraram que altas concentrações de glicose no fluído folicular interferiram nas estruturas mitocondriais e foram associadas à disfunção das mitocôndrias e diminuição na produção de energia (Wang et al., 2009).

Evidências também associam concentrações de insulina à morfologia, proliferação e produção de hormônios pelas células do cumulus (Poretsky e Kalin, 1987). Gong et al. (2002) suplementaram vacas leiteiras em início de lactação com dieta formulada a induzir altas concentrações de insulina e observaram melhora no desempenho reprodutivo desses animais por reduzir o tempo para primeira ovulação quando comparados ao controle. Estudos *in vitro*, mostraram que concentrações de insulina não modularam as repostas dos oócitos, no entanto, a produção de blastocisto foi reduzida em altas concentrações de insulina (Laskowski et al., 2016). No mesmo estudo, embriões derivados de oócitos que foram cultivados em altas concentrações de insulina apresentaram genes ligados à estrutura de cromatina, produção de energia e metabolismo de esteroides, de colesterol e de crescimento *up-regulated*. Ademais, estudos com novilhas e vacas em meio e final de lactação alimentadas com dietas de alta densidade energética, que induziram o aumento das concentrações de insulina e da resistência à insulina, diminuíram a produção de blastocistos (Adamiak et al., 2005; Leiva et al., 2015). Em revisão, Baruselli et al., (2016) concluíram que uma das possíveis causas da baixa fertilidade em vacas com elevado dias em leite pode estar relacionada aos efeitos causados pela resistência à insulina, tipicamente observados em vacas em estágios avançados de lactação (Oliveira et al., 2016).

O efeito combinatório da glicose e da insulina também é associado a maturação folicular, já que a concentração de ambos é aumentada com o crescimento folicular

(Landau et al., 2000), provavelmente para induzir a produção de estrógenos devido ao efeito gonadotrófico desses compostos (Dupont e Scaramuzzi, 2016). Em um estudo de Somchit et al. (2007), que alimentaram ovelhas com dietas que provocavam o aumento das concentrações de glicose e insulina, os autores observaram que esses animais tiveram maiores concentrações de ambos os compostos no fluído folicular, todavia não deferiram na produção de estrógenos, mas animais recebendo essas dietas tiveram maiores produções de folículos médios e pequenos.

Tendo em vista esses relatos, podemos concluir que fatores relacionados a resistência à insulina podem estar relacionados a diminuição na competência oocitária, levando a diminuição da fertilidade em vacas leiteiras, no entanto, mais estudos tornam-se necessário para o real entendimento de como esses fatores podem ser modulados.

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Resumo geral dos capítulos 2 e 3

(Overview chapter 1 and 2)

Efeito de fontes de energia (amido, pectina ou gordura) e da suplementação com cromo, em dietas com alta densidade energética, nas concentrações séricas de glicose, insulina e ácidos graxos não esterificados, em vacas de leite lactantes.

Dois experimentos foram conduzidos para avaliar o efeito de diferentes fontes de energia e da suplementação com cromo nas variáveis relacionadas a resistência à insulina, produção de leite e índices reprodutivos de vacas leiteiras lactentes consumindo excesso de energia que foram distribuídas em um arranjo fatorial 2 × 2. No primeiro estudo, vacas foram designadas a receber: 1) concentrado a base de milho moído (CRN; n = 13) ou polpa cítrica (PLP; n = 13) e 2) suplementação (n = 14) ou não (n = 12) de 10 mg/vaca/dia de propionato de cromo. No segundo estudo vacas receberam: 1) concentrado a base de milho moído (CRN; n = 20) ou com inclusão de 8% (matéria seca) de gordura de palma protegida (CSPO; n = 20), e suplementação (n = 20) ou não (n = 20) de 10 mg/dia de propionato de cromo. Para a condução de ambos os estudos, foram utilizados animais não gestantes, Holandeses × Gir, lactantes (dias de leite inicial = 80 ± 3), alimentados com silagem de milho oferecida de maneira *ad libitum* e concentrado visando atender 160% dos requerimentos energéticos de mantença. Amostras de leite e sangue foram colhidas semanalmente e produção de leite foi mensurada diariamente. Para o primeiro estudo, escore de condição corporal (ECC) e peso corporal (PC) foram avaliados semanalmente ao longo do estudo (D0 ao 182), enquanto que no segundo estudo ECC e PC foram avaliados no primeiro e no último dia (d0 e d203, respectivamente) do estudo. Ingestão de matéria seca foi avaliada apenas no segundo estudo através da diferença entre o ofertado e a sobra. Para tal, vacas foram distribuídas em cinco grupos de oito vacas (duas vacas de cada grupo experimental) e alocadas individualmente em 8 baias por 3 dias. No

final desse período os animais retornavam aos piquetes e um novo grupo de animais passavam pelo mesmo processo. Testes de tolerância a glicose (TTG; 0,5g de glicose/kg de PC) foram realizadas no d -3, 60, 120, e 180 para o primeiro estudo, e nos dias -3, 100 e 200 para o segundo estudo. Aspiração folicular para produção de embrião *in vitro* foi realizada nos d -1, 82 e 162 para o primeiro estudo e nos d -1, 98 e 198 para o segundo estudo. Os resultados obtidos no primeiro estudo foram: não foram detectadas diferenças ($P \geq 0,25$) para PC e ECC durante o experimento. Dentro das colheitas semanais de sangue, concentrações séricas de insulina e glicose assim como relação insulina:glicose foram similar entre os tratamentos ($P \geq 0,19$). Animais do grupo CRN tiveram menor ($P < 0,01$) concentrações plasmáticas de ácidos graxos não esterificados comparados com PLP (0,177 × 0,215 mmol/l, respectivamente; EMP = 0,009). Durante o TTG não foram detectadas diferenças ($P \geq 0,16$) para concentrações de glicose, taxa de desaparecimento de glicose, tempo de meia vida de glicose e relação insulina:glicose. Concentrações séricas de insulina foram menor ($P = 0,04$) em vacas CRN suplementadas com propionato de cromo compradas com vacas CRN não suplementadas com propionato de cromo (8.2 × 13,5 µUI/ml, respectivamente; EMP = 1,7), enquanto que a suplementação com propionato de cromo não impactou ($P = 0,70$) concentrações séricas de insulinas em vacas PLP. Produção de leite, gordura no leite e concentrações de sólidos totais foram similares ($P \geq 0,48$) entre os tratamentos. No entanto, CRN tiveram maior ($P < 0,01$) concentrações de proteína no leite comparadas com PLP (3,54 × 3,14%, respectivamente; EMP = 0,08). Não foram encontradas diferenças ($P \geq 0,35$) no número de oócitos coletados e embriões produzidos dentro de cada aspiração. Concluindo, fornecimento de concentrado contendo polpa cítrica peletizada para vacas leiteiras lactantes consumindo excesso de energia não melhorou as variáveis relacionadas com resistência à insulina, produção de leite e índices

reprodutivos, enquanto que suplementação com propionato de cromo apenas melhorou sensibilidade a insulina em vacas recebendo concentrado a base de milho durante o TTG.

Resultados do segundo estudo: ingestão de matéria seca, ingestão de EL_L assim como PC e mudança de ECC foram similares ($P \geq 0,22$) entre os tratamentos. Dentro das colheitas de sangue semanais, vacas CRN tiveram menor ($P \leq 0,03$) concentrações séricas de glicose, insulina, ácidos graxos e relação insulina:glicose comparadas com vacas CSPO, sugerindo aumento na sensibilidade a insulina em vacas CRN. Durante o TTG, variáveis relacionadas a sensibilidade a insulina foram maiores em vacas CRN quando comparadas a vacas CSPO. Suplementação com propionato de cromo resultou em menores ($P \leq 0,09$) concentrações séricas de insulina e relação insulina:glicose apenas em vacas CRN, indicando que suplementação com propionato de cromo melhora a sensibilidade a insulina em vacas CRN mais não em vacas CSPO. Durante o TTG, no entanto, suplementação com propionato de cromo reduziu as concentrações de insulina e a relação insulina:glicose em vacas CSPO e CRN. Produção de leite, assim como número de oócitos viáveis coletados e embriões produzidos dentro de cada aspiração não foram afetados ($P \geq 0,24$) pelos tratamentos. Em conclusão, utilização de gordura de palma protegida não melhorou a sensibilidade a insulina em vacas Holandesas x Gir consumindo excesso de energia durante o meio e o final da lactação, enquanto que suplementação com propionato de cromo foi efetivo em melhorar a sensibilidade basal da insulina em vacas CRN.

Palavras chaves: resistência à insulina, energia, cromo, vacas leiteiras

Effect of energy sources (starch, pectin or fat) and chromium supplementation, on high energy diet, on serum concentrations of glucose, insulin, and non-esterified fatty acids on lactating dairy cows.

Two experiments were conducted to evaluate the effect of different carbohydrate sources and chromium supplementation on insulin resistance parameters, milk production and reproductive outcomes in lactating dairy cows consuming high-energy diet and sorted in a 2 × 2 factorial arrangement design. In the first study, cows were distributed as follow: 1) concentrate based on ground corn (CRN; n = 13) or citrus pulp (PLP; n = 13), and 2) supplemented (n = 14) or not (n = 12) with 2.5 g/day of Cr-propionate. Cows from the second study were distributed in: 1) concentrate based on ground corn (CRN; n =20) or inclusion of 8% (DM basis) of palm oil Ca salts (CSPO; n = 20), and 2) supplemented (n =20) or not (n = 20) with 2.5 g/day of Cr-propionate. During study conduction, cows from both experiments, non-pregnant, lactating Holstein × Gir (initial days in milk = 80 ± 3) were offered corn silage for ad libitum consumption and individually received concentrate formulated to provide 160% of their daily NEL requirements. In both studies, blood and milk samples were collected weekly, and milk production was recorded daily. However, in the first study, body condition score (BCS) and body weight (BW) were recorded weekly throughout the study (day 0 to 182), whereas in the second study, BCS and BW were recorded on the first and last day (0 and 203, respectively) of the study. In the second study, dry matter intake was measured on cows from both treatments, in which they were randomly divided in 5 groups of 8 cows each and allocated to 8 individual feeding stations for 3 days. Intake was evaluated 6 times/group. Glucose tolerance tests (GTT; 0.5 g of glucose/kg of BW) were performed on day -3, 60, 120 and 180 for the first study, and on day -3, 100 and 200 for the second study. Follicle aspiration for in vitro

embryo production was performed via transvaginal ovum pick-up on day -1, 82 and 162 for the first study, and on day -1, 98 and 198 for the second study. Results obtained from the first study were: no treatment differences were detected ($P \geq 0.25$) for BW and BCS changes during the experiment. Within weekly blood samples, concentrations of serum insulin and glucose, as well as insulin:glucose ratio, were similar among treatments ($P \geq 0.19$), whereas CRN had lower ($P < 0.01$) non-esterified fatty acid concentrations compared to PLP (0.177 vs. 0.215 mmol/L, respectively; SEM = 0.009). During the GTT, no treatment differences were detected ($P \geq 0.16$) for serum glucose concentration, glucose clearance rate, glucose half-life and insulin:glucose ratio. Serum insulin concentrations were lower ($P = 0.04$) in CRN supplemented with Cr-propionate compared to non-supplemented CRN (8.2 vs. 13.5 μIU/mL, respectively; SEM = 1.7), whereas Cr-propionate supplementation did not affect ($P = 0.70$) serum insulin of PLP cows. Milk production, milk fat and milk solids were similar ($P \geq 0.48$) among treatments. However, CRN had greater ($P < 0.01$) milk protein levels compared to PLP (3.54 vs. 3.14 %, respectively; SEM = 0.08). No treatment differences were detected ($P \geq 0.35$) on collected number of viable oocytes and produced embryos within each aspiration. In summary, feeding a citrus pulp-based concentrate to lactating dairy cows consuming high-energy diet did not improve insulin sensitivity, milk production and reproductive outcomes, whereas Cr-propionate supplementation only enhanced insulin sensitivity on cows receiving a corn-based concentrate during GTT.

Second study results were: mean DMI, NEL intake, as well as BW and BCS changes were similar ($P \geq 0.22$) across treatments. On average, cows gained 40 kg of BW and 0.49 of BCS during the entire experiment. Within weekly blood samples, CRN cows had lower ($P \leq 0.03$) serum concentrations of glucose, insulin, fatty acids and insulin:glucose ratio compared to CSPO cows, suggesting increased insulin sensitivity in

CRN cows. During the GTT, insulin-sensitivity traits were also greater in CRN versus CSPO cows. Supplemental Cr-propionate resulted in lower ($P \leq 0.09$) serum insulin concentrations and insulin:glucose ratio within CRN cows only, indicating that Cr-propionate improved basal insulin sensitivity in CRN but not in CSPO cows. During the GTT, however, Cr-propionate supplementation reduced hyperinsulinemia and insulin:glucose ratio across CSPO and CRN cows. Milk production, as well as collected number of viable oocytes and produced embryos within each aspiration, were not affected ($P \geq 0.24$) by treatments. Hence, replacing corn by palm oil Ca salts in the concentrate did not improve insulin sensitivity of Holstein × Gir dairy cows consuming high-energy diet during mid to late lactation, whereas Cr-supplementation was effective in improving basal insulin sensitivity in cows not receiving palm oil Ca salts.

Key words: insulin resistance, energy, chromium, dairy cows

CAPÍTULO 2

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Effects of concentrate type and chromium propionate on insulin sensitivity, productive, and reproductive parameters of lactating dairy cows consuming excessive energy

T. Leiva[1], R. F. Cooke[2,a], A. P. Brandão[1,2], U. Pardelli[1], R. O. Rodrigues[1], F. N. Corrá[1], and J. L. M. Vasconcelos[1,a]

[1] São Paulo State University - Department of Animal Production, Botucatu 18168-000, Brazil

[2] Oregon State University - Eastern Oregon Agricultural Research Center, Burns, 97720, USA

[a] Corresponding authors: vasconcelos@fmvz.unesp.br and reinaldo.cooke@oregonstate.edu.

Short Title**:** Concentrate type and Cr-propionate to dairy cows

Abstract

This experiment compared insulin sensitivity parameters, milk production, and reproductive outcomes in lactating dairy cows consuming excessive energy, and receiving in a 2 × 2 factorial arrangement design: 1) concentrate based on ground corn (**CRN**; n = 13) or citrus pulp (**PLP**; n = 13), and 2) supplemented (n = 14) or not (n = 12) with 2.5 g/day of Cr-propionate. During the experiment (day 0 to 182), 26 multiparous, non-pregnant, lactating Gir × Holstein cows (initial days in milk = 80 ± 2) were offered corn silage for ad libitum consumption, and individually received concentrate formulated to allow diets to provide 160% of their daily requirements of net energy for lactation. Cow BW and body condition score (**BCS**) were recorded weekly. Milk production was recorded daily and milk samples collected weekly. Blood samples were collected weekly prior to the morning concentrate feeding. Glucose tolerance tests (**GTT;** 0.5 g of glucose/kg of BW) were performed on days -3, 60, 120, and 180. Follicle aspiration for in vitro embryo production was performed via transvaginal ovum pick-up on days -1, 82, and 162. No treatment differences were detected ($P \geq 0.25$) for BW and BCS change during the experiment. Within weekly blood samples, concentrations of serum insulin and glucose, as well as insulin:glucose ratio were similar among treatments ($P \geq 0.19$), whereas CRN had less ($P < 0.01$) non-esterified fatty acid concentrations compared with PLP (0.177 vs. 0.215 mmol/L; SEM = 0.009). During the GTT, no treatment differences were detected ($P \geq 0.16$) for serum glucose concentration, glucose clearance rate, glucose half-life, and insulin:glucose ratio. Serum insulin concentrations were less ($P = 0.04$) in CRN supplemented with Cr-propionate compared with non-supplemented CRN (8.2 vs. 13.5 µIU/mL, respectively; SEM = 1.7), whereas Cr-propionate supplementation did not impact ($P = 0.70$) serum insulin within PLP cows. Milk production, milk fat and solid concentrations were similar ($P \geq 0.48$) between treatments. However, CRN had

greater ($P < 0.01$) milk protein concentration compared with PLP (3.54 vs. 3.14 %, respectively; SEM = 0.08). No treatment differences were detected ($P \geq 0.35$) on number of viable oocytes collected and embryos produced within each aspiration. In summary, feeding a citrus pulp-based concentrate to lactating dairy cows consuming excessive energy did not improve insulin sensitivity, milk production, and reproductive outcomes, whereas Cr-propionate supplementation only enhanced insulin sensitivity in cows receiving a corn-based concentrate during a GTT.

Keywords: Chromium, concentrate type, dairy cows, energy intake, insulin sensitivity

Implications

Feeding a concentrate based on citrus pulp instead of corn to lactating dairy cows consuming excessive energy did not benefit insulin sensitivity parameters, milk production, and reproductive outcomes. Adding Cr-propionate supplementation to these diets only enhanced insulin sensitivity parameters in cows receiving the corn-based concentrate during a glucose tolerance test. Given the known negative relationship among excessive energy intake, insulin sensitivity parameters, and productive responses in lactating cows, research is still warranted to develop nutritional strategies that mitigate insulin resistance and optimize performance and welfare in dairy cattle.

Introduction

Excessive energy intake decreases insulin sensitivity and leads to insulin resistance in non-lactating and lactating dairy cows (Leiva *et al.*, 2014; Leiva *et al.*, 2015). This syndrome, characterized by persistent hyperglycemia despite increased insulin secretion, has been shown to impair welfare and reproductive parameters of dairy cattle (Adamiak *et al.*, 2005; Leiva *et al.*, 2015; Baruselli *et al.,* 2016). Given that excessive energy intake is common and often inevitable among late-lactating and non-lactating cows in commercial dairies (Van Saun and Sniffen, 1996), nutritional strategies that mitigate insulin resistance are warranted to optimize productivity and welfare of dairy cattle.

Chromium is a critical component of the glucose tolerance factor that facilitates the action of insulin on body cells (Mertz, 1992). Accordingly, Cr-propionate supplementation prevented the decrease in insulin sensitivity caused by excessive energy intake in lactating and non-lactating dairy cows (Leiva *et al.*, 2014; Leiva *et al.*, 2015). Hyperinsulinemia is also known to down-regulate insulin receptors in cells and cause insulin resistance (Moller and Flier, 1991). Hence, reducing dietary content of insulinogenic ingredients, such as starch, may also mitigate the occurrence of this syndrome. Cabrita *et al.* (2007) reported that reducing starch intake by substituting corn for citrus pulp reduced plasma insulin concentrations in lactating dairy cows, although the effects of this dietary strategy on insulin sensitivity parameters still needs investigation. Based on this information, we hypothesized that replacing corn by citrus pulp in the dietary concentrate lessens the decrease in insulin sensitivity in lactating dairy cows consuming excessive energy, and Cr-propionate supplementation is an alternative to further alleviate this outcome. Therefore, this experiment compared insulin sensitivity parameters, milk production, and

reproductive outcomes in lactating dairy cows consuming excessive energy, receiving concentrate based on corn or citrus pulp, and supplemented or not with Cr-propionate.

Materials and Methods

This experiment was conducted at the São Paulo State University – Lageado Experimental Station, in Botucatu/SP, Brazil. The animals utilized were cared for in accordance with the practices outlined and approved by the São Paulo State University Animal Ethics Committee (#17/2015).

Animals and diets

Twenty-six lactating, multiparous, non-pregnant Holstein cows (initial mean ± SE; parity = 3.3 ± 0.2 parities, BW = 574 ± 11 kg, body condition score [**BCS**] = 2.80 ± 0.04, milk yield = 25.9 ± 1.0 kg, and days in milk = 80 ± 2 d) were assigned to the experiment (day 0 to 182). On day 0, cows were ranked by days in milk, milk yield, BW and BCS (Wildman *et al.*, 1982), and assigned to 2 × 2 factorial arrangement design containing the following treatments: 1) concentrate based on ground corn (**CRN**; n = 13) or citrus pulp (**PLP**; n = 13), and 2) supplemented (n = 14) or not (n = 12) with 2.5 g/day of Cr-propionate (10 mg of Cr/cow daily; KemTrace 0.4% Cr; Kemin Agrifoods South America, Indaiatuba, São Paulo, Brazil). All treatment combinations had equivalent initial average days in milk, milk yield, BW and BCS.

Beginning on day -15 and until day 182, cows were maintained in a single drylot pen with ad libitum access to corn silage, water and a commercial mineral mix without the inclusion of Cr (Table 1). Corn silage was provided in feed bunks that allowed 1.5 m of linear bunk space/cow and offered at daily rates to result in ≥ 15% (DM basis) of non-consumed silage, whereas the maximum daily provision of corn silage during the experiment was 14.0 of DM/cow. Cows were

milked twice daily in a side-by-side milking system (0600 and 1700 h), and individually received their concentrate through self-locking head gates immediately after each milking.

From day -15 to -1 (adaptation period), cows received a concentrate containing (as-fed basis) 40% of soybean meal, 57% of ground corn, and 3.0% of the same commercial mineral mix offered for ad libitum consumption (Table 1). From day 0 to 182, cows received concentrate treatments described in Table 1. Concentrate intake was formulated to each individual cow so the diet (concentrate + corn silage) provided 100% (day -15 to -1) or 160% (day 0 to 182) of their daily net energy for lactation (**NE_L**) requirements, as previously described and accomplished by Leiva *et al.* (2015). All dietary treatments were formulated to similarly exceed crude protein, mineral, and vitamin requirements (NRC, 2001). Concentrate intake was adjusted weekly (day -15 to 182) using the Spartan Dairy Ration Evaluator/Balancer (version 3.0; Michigan State University, East Lansing, MI, USA), according to days in milk, milk yield, BW, and BCS, treatment, and corn silage intake estimated by the software.

Chromium-propionate was offered in the amount recommended by the manufacturer (2.5 g/cow daily of KemTrace; Kemin Agrifoods South America), mixed with 97.5 g of finely ground corn and top-dressed daily into the morning concentrate feeding of each supplemented cow. Finely ground corn (97.5 g/cow) was also top-dressed into the morning concentrate feeding of cows not assigned to Cr-proprionate supplementation, but without the addition of the Cr-propionate.

Sampling

Twice monthly, one sample of the offered corn silage and one sample of the offered concentrate were collected. Samples of the same feedstuff were pooled into a single sample at the end of the experiment and analyzed for nutrient content via wet chemistry procedures by a bromatology laboratory (3rlab, Belo Horizonte, Brazil). Calculations of NE_L and net energy for

maintenance (**NE_M**) used the equation proposed by the NRC (2001). Nutritive value of corn silage was 39.5% DM, 5.76 MJ/kg of NE_L, 5.76 MJ/kg of NE_M, and 7.7% crude protein (DM basis). Nutritive values of experimental concentrates are described in Table 1. Nutritive value of concentrate offered from day -15 to -1 was 90.3% DM, 8.1 MJ/kg of NE_L, 8.1 MJ/kg of NE_M, and 22.9% crude protein (DM basis). Cow BW and BCS were recorded weekly prior to (day -15 to -1) and during the experimental period (day 0 to 182). Cow milk production was recorded daily from day -15 to 182. These parameters were used to adjust concentrate intake of each cows on a weekly basis. Further, BCS was evaluated (Wildman *et al.*, 1982) by the same two evaluators throughout the experiment, and evaluators were blinded to which treatment the assessed cow was assigned to.

Milk samples were collected weekly from each cow during both milkings of the day, combined into one daily sample (50 mL from each milking), which was analyzed for fat, protein, and total solids content using infrared spectrometry (method 972.16; AOAC, 1999) by a commercial laboratory (Clínica do Leite; Universidade de São Paulo, Piracicaba, Brazil). Blood samples were collected weekly, prior to the morning concentrate feeding during the experiment for determination of serum glucose, insulin, and non-esterified fatty acids (**NEFA**) concentrations. Insulin to glucose ratio (**I:G**) was determined by dividing insulin and glucose concentrations within each sampling time (Bernhard *et al.*, 2012). Concentrations of glucose, NEFA, and insulin were used to determine pre-prandial revised quantitative insulin sensitivity check index (**RQUICKI**) using the equation described by Perseghin *et al.* (2001).

Glucose tolerance tests (**GTT**) were performed on days -3, 60, 120, and 180 by intravenously infusing cows with 0.5 g of glucose/kg of BW, following the same procedures,

sampling scheme and calculations for area under the curve (**AUC**), I:G, glucose clearance rate and half-life described by Leiva *et al.* (2015).

Laboratorial Analyses

During the weekly or GTT blood collections, samples were obtained from coccygeal vessels (Tvedten *et al.*, 2000) into commercial blood collection tubes (Vacutainer, 10 mL; Becton Dickinson, Franklin Lakes, NJ, USA), placed immediately on ice, centrifuged at 3000 × g at 4 °C for 30 min for serum collection, and stored at -20°C on the same day of collection. Glucose, insulin, and NEFA concentrations were analyzed as in Leiva *et al.* (2015). The intra- and interassay CV were, respectively, 3.8 and 5.8% for glucose, 2.8 and 1.8% for insulin, and 3.3 and 2.7% for NEFA. Assay sensitivity was 0.0005 mmol/L for glucose, 0.01 mmol/L for NEFA, and 0.1 µIU/mL for insulin.

Reproductive Management

Follicle aspiration was performed on days -1, 62, and 162 to evaluate treatment effects on production of viable oocytes, as well as subsequent in vitro embryo production. Cows were at random stages of the estrous cycle when assigned to follicle aspiration, which was performed via transvaginal ovum pick-up (**OPU**) according to the procedures described by Bilby *et al.* (2006). Oocytes were collected, processed and maturated for IVF as described by Leiva *et al.* (2015), and fertilized with semen from the same sire according to the procedures described by Bilby *et al.* (2006). Presumptive zygotes were incubated at 38.5°C in 5% O_2, 5% CO_2, in 100% humidified air for 7 days (Bilby *et al.*, 2006). After incubation, number of cleaved and viable embryos was recorded with a dissecting microscope. Variables that were utilized for the present experiment were; number of oocytes collected that were viable to IVF (Grades I, II, and III), number of

embryos produced, and ratio of embryos produced/viable oocytes collected within each sampling day.

Statistical analyses

Cow was considered the experimental unit given that concentrate type and choice of Cr-supplementation were individually applied to cows. All data were analyzed using cow(concentrate type × Cr-propionate supplementation) as random variable, with the MIXED procedure of SAS (SAS Inst., Inc., Cary, NC, USA; version 9.3) and Satterthwaite approximation to determine the denominator df for the tests of fixed effects. The model statement used for analysis of BW and BCS change, as well as initial and final BCS and BW during the experiment contained the effect of concentrate type, Cr-propionate supplementation, and the resultant interaction. The model statement used for analysis of daily concentrate and estimated silage intake, as well as weekly BW, BCS, milk yield, serum variables, and RQUICKI contained the effects of concentrate type, Cr-propionate supplementation, time (day or week), and the resultant interactions. The model statement used for serum glucose, serum insulin, and I:G obtained during the GTT contained the effects of concentrate type, Cr-propionate supplementation, day of GTT (day 60, 120, and 180), min of sampling, all resultant interactions, and mean values obtained from the GTT on day -3 as independent covariate. The model statement used for follicle aspiration and IVF outcomes, as well as glucose and insulin AUC, glucose clearance rate, and glucose half-life during the GTT contained the effects of concentrate type, Cr-propionate supplementation, day of follicle collection or GTT, all resultant interactions, and values obtained from collection on day -1 (reproductive variables) or -3 (GTT) as independent covariate. The specified term for the repeated statement was week for the weekly collections, day for intake and reproductive variables, and hour for the GTT, with cow(concentrate type, Cr-propionate supplementation) as subject. The covariance structure

utilized for all repeated statements was autoregressive, which provided the best fit for these analyses according to the Akaike information criterion. Results are reported as least square means, or covariately adjusted means for GTT and reproductive responses, and separated using PDIFF. Significance was set at $P \leq 0.05$, and tendencies were determined if $P > 0.05$ and ≤ 0.10. Results are reported according to main treatment effects (concentrate type and Cr supplementation) if no interactions were significant, or according to the highest-order significant ($P \leq 0.05$) interaction containing one of both main treatment effects.

Results and Discussion

Intake, BW, and BCS parameters

Daily concentrate intake (DM basis) was similar ($P \geq 0.57$) between PLP and CRN cows (6.1 vs 6.3 kg of DM/cow daily, respectively; SEM = 0.6), as well as between cows receiving or not Cr-propionate supplementation (6.0 vs 6.2 kg of DM/cow daily, respectively; SEM = 0.6). Estimated corn silage intake (DM basis; according to Spartan Dairy Ration Evaluator/Balancer, version 3.0, Michigan State University) was also equivalent ($P = 0.81$) among all main treatments effects (11.4, 11.2, 11.3, and 11.5 kg of DM/day for CRN, PLP, Cr-supplemented, and non-supplemented cows; SEM = 0.16), although cows were group-fed corn silage and actual corn silage intake was not evaluated. No main treatment effects were detected ($P \geq 0.25$) for final BW and BW change, as well as final BCS and BCS change (Table 2). Moreover, all cows gained (day effect, $P < 0.01$) BW (575 vs. 606 kg on day 0 and 182, respectively; SEM = 10) and BCS (2.79 vs. 3.15 of BCS on day 0 and 182, respectively; SEM = 0.05) during the experiment. These outcomes were expected and corroborates that cows across all treatment combinations similarly consumed excessive energy as designed, which was accomplished via individually-fed

concentrates formulated to result in diets providing 160% of cow daily NE_L requirements (as in Leiva *et al.*, 2015).

Serum variables evaluated weekly

No main treatment effects ($P \geq 0.57$) were detected for serum glucose concentrations (Table 3). Starch is the major dietary precursor for glucose in ruminants (Huntington, 1997); hence, it would be expected that CRN cows had greater plasma glucose concentration compared to PLP cows. Cabrita *et al.* (2007) reported less plasma glucose concentration in lactating dairy cows receiving a concentrate based on citrus-pulp compared with cohorts receiving corn-based concentrate. However, these authors formulated their experimental diets to meet NE_L requirements, whereas cows from the present experiment were fed excessive energy, which may have contributed to the lack of differences in serum glucose between CRN and PLP cows. In addition, Huntington (1997) reported that cattle are capable of synthesizing glucose from other non-structural carbohydrates such as pectin; the predominant carbohydrate of citrus pulp (NRC, 2001). Regarding the lack of Cr supplementation effects on serum glucose concentrations, Leiva *et al.* (2015) also reported a similar outcome in lactating dairy cows consuming excessive energy and receiving or not Cr-propionate supplementation. Lack of concentrate type and Cr-propionate supplementation effects on serum glucose concentrations can also be associated with the fact that serum glucose is stable in ruminants due to its homeostatic regulation, particularly in lactating cattle due to glucose uptake by the mammary gland (Bickerstaffe *et al.*, 1974).

No main treatment effects were detected ($P \geq 0.48$) for serum insulin concentrations (Table 3). These outcomes were unexpected because starch is classified as an insulinogenic nutrient (Cabrita *et al.*, 2007), and Cr-propionate supplementation reduced serum insulin concentrations in lactating dairy cows consuming excessive energy from a corn-based concentrate (Leiva *et al.*,

2015). The reason for such inconsistency in Cr-propionate effects on serum insulin between experiments is unknown, particularly because the concentrate formulation and energy feeding level used herein were similar to those used by Leiva *et al.* (2015). Nevertheless, overall lack of treatment differences on serum insulin is coherent with design of dietary treatments and results reported for serum glucose, given that circulating insulin concentrations are mainly regulated by nutrient intake and blood glucose (Nussey and Whitehead, 2001).

Chromium-propionate supplementation did not impact (P = 0.79) serum NEFA concentrations (Table 3), as similarly reported by Leiva *et al.* (2015). Accordingly, Cr supplementation has been shown to modulate circulating NEFA concentrations in periparturient cows (Hayirli *et al.*, 2001), but not in cattle with positive energy balance (Bunting *et al.*, 1994) such as cows utilized herein and by Leiva *et al.* (2015). Conversely, mean serum NEFA concentration was greater ($P < 0.01$) in PLP cows compared with CRN cohorts (Table 3). Circulating NEFA concentrations are negatively associated with energy intake and used as indicator of lipolysis in lactating dairy cattle (Grummer, 1995), whereas PLP and CRN cows similarly consumed excessive energy and gained BW and BCS during this experiment. Hence, differences detected for serum NEFA may be resultant from increased fat synthesis instead of lipolysis in PLP cows, given that diets rich in citrus pulp are known to favor ruminal acetate production (NRC, 2001), which is utilized as substrate for lipogenesis in body tissues (Bergman, 1990). Accordingly, Belibasakis and Tsirgogianni (1996) reported greater serum cholesterol concentrations in dairy cows receiving citrus pulp-based concentrate compared with cohorts fed corn-based concentrate, and attributed this outcome to increased lipogenesis in citrus pulp-fed cows.

No main treatment effects were detected ($P \geq 0.19$) for serum I:G and RQUICKI (Table 3). These variables have been used as indicators of insulin sensitivity and resistance in cattle (Hayirli *et al.*, 2001; Grünberg *et al.*, 2011). Hence, lack of main treatment effects on serum glucose, insulin, I:G, and RQUICKI suggest that neither concentrate type or Cr-propionate supplementation impacted insulin sensitivity parameters during routine management in lactating dairy cows consuming excessive energy. These outcomes also contradict Leiva *et al.* (2015), where Cr-propionate supplementation reduced serum I:G in lactating dairy cows consuming excessive energy from a corn-based concentrate. One can speculate that cows from this experiment did not gain as much BCS compared with the cows utilized by Leiva *et al.*, (2015); therefore, the decrease in insulin sensitivity caused by excessive energy intake herein was not as substantial compared to Leiva *et al.* (2015), and hindered the detection of main treatment effects on these parameters. Nevertheless, Leiva *et al.* (2015) also failed to detected Cr-propionate supplementation on RQUICKI, but suggested that RQUICKI is not a viable indicator of insulin sensitivity in lactating dairy cows in positive energy balance. To our knowledge, no other research has compared insulin sensitivity parameters in lactating dairy cows consuming excessive energy from corn-based or citrus pulp-based concentrate. Collectively, these outcomes do not support our hypothesis and indicate that replacing corn by citrus pulp or providing Cr-propionate supplementation failed to modulate insulin sensitivity parameters during routine management in lactating dairy cows consuming excessive energy.

Serum variables evaluated during the GTT

No main treatment effects were detected ($P \geq 0.16$) for serum glucose, glucose AUC, glucose clearance rate, and glucose half-life (Table 3), which corroborates the results from weekly samples. However, concentrate type × Cr-propionate supplementation interactions were detected

($P \leq 0.04$) for serum insulin concentrations, serum insulin AUC, and serum I:G during the GTT (Table 4). Serum insulin concentrations and AUC were less ($P \leq 0.05$) in CRN cows supplemented with Cr-propionate compared with non-supplemented CRN cohorts, whereas Cr-propionate supplementation did not impact ($P \geq 0.70$) serum insulin parameters within PLP cows (Table 4). Serum I:G tended to be less ($P = 0.09$) in CRN cows supplemented with Cr-propionate compared with non-supplemented CRN cohorts, and similar ($P = 0.96$) in PLP cows receiving or no Cr-propionate supplementation (Table 4). Supporting these outcomes, Leiva *et al.* (2015) also reported that Cr-propionate supplementation reduced serum insulin concentrations and I:G during a GTT in lactating dairy cows consuming excessive energy from a corn-based concentrate. It is important to note that main concentrate type effects were not detected for serum insulin and I:G (Table 3), and the tendencies ($P \leq 0.10$) detected for main Cr-propionate supplementation effects on serum insulin (Table 3) were mainly driven by its effects within CRN cows (Table 4). Although the concentrate type × Cr-propionate supplementation × time interaction was not detected during the GTT ($P \geq 0.34$), differences among treatment combinations were only detected from 10 to 120 min relative to glucose infusion for serum insulin concentrations (Figure 1), and from 60 to 120 min relative to glucose infusion for serum I:G (Figure 2). Hence, Cr-propionate supplementation reduced serum insulin concentrations and I:G within CRN cows only, lessening these variables to the levels observed within PLP cows.

Collectively, serum insulin and I:G results during the GTT suggest that Cr-propionate supplementation alleviated hyperinsulinemia and improved insulin sensitivity caused by GTT within CRN cows. The same Cr-propionate effect was not detected within PLP cows, perhaps due to the fact that the GTT had less impact on serum insulin and I:G within PLP cows (Figures 1 and 2). Supporting these outcomes, previous research from our and other research groups reported that

supplemental Cr enhanced insulin sensitivity parameters in lactating cattle receiving corn-based diets during a GTT (Hayirli *et al.*, 2001; Leiva *et al.*, 2015). Chromium is a critical component of the glucose tolerance factor that facilitates the action of insulin on body cells (Mertz, 1992), and Cr supplementation has been shown to enhance glucose metabolism in ruminants (Sumner *et al.*, 2007). More specifically, Cr modifies glucose metabolism through chromodulin, an oligopeptide that binds with high affinity to four chromic ions and enables Cr to be involved in the autoamplification of insulin signaling, maintaining the active conformation of insulin receptors and promoting greater glucose uptake (Vincent, 2001). Yet, Cr-propionate supplementation may also impact insulin resistance parameters in adipose and other body tissues through immunological signals such as proinflammatory cytokine response (Wellen and Hotamisligil, 2005). Therefore, additional research is still warranted to further comprehend the physiological mechanisms responsible for the outcomes observed herein, particularly why Cr-propionate supplementation enhanced insulin sensitivity parameters in CRN cows, but not PLP cows during the GTT.

Milk production

No main treatment effects were detected ($P \geq 0.51$; Table 5) for milk yield, milk fat, 3.5% fat-corrected milk yield, milk total solids, and 12% solids-corrected milk yield. No Cr-propionate supplementation effect was detected ($P = 0.48$) for milk protein concentration (Table 5). Insulin resistance may negatively impact milk yield and mammary synthesis of milk constituents in lactating dairy cattle (McGuire *et al.*, 1995; LeBlanc, 2010). Perhaps Cr-propionate supplementation effects detected within CRN cows during the GTT were not sufficient to impact milk production and concentration of constituents. Leiva *et al.* (2015) also failed to detect milk yield differences in cows supplemented or not with Cr-propionate and consuming excessive energy. Nevertheless, milk production by the mammary gland is regulated by lactose synthesis

from glucose, whereas glucose uptake by the mammary gland is relatively insulin-independent (Zhao *et al.,* 1996). Hence, insulin resistance may not be a critical factor influencing milk yield in lactating dairy cows, corroborating with the outcomes reported herein and Leiva *et al.* (2015).

Research evaluating milk production in dairy cows offered corn-based vs. citrus pulp-based concentrate has yielded variable results, such as similar (Belibasakis and Tsirgogianni 1996; Leiva *et al.,* 2000) or greater milk production when corn-based concentrate is fed (Leiva *et al.,* 2000; Cabrita et el., 2007). Variables results were also detected when evaluating milk fat and total solids (Belibasakis and Tsirgogianni 1996; Leiva *et al.,* 2000; Cabrita et el., 2007). It is important to note that these research studies evaluated cows receiving diets to meet their NE_L requirements. In this experiment, all diets were formulated to provide excessive NE_L, which likely allowed cows from all treatment combinations to produce their maximum milk yield while the additional energy supplied was converted into BCS (Table 1).

A concentrate type effect was detected for milk protein, which was greater ($P < 0.01$) in CRN cows compared with PLP cows (Table 5). Others have also reported greater milk protein content when concentrate is based on corn instead of citrus pulp (Leiva *et al.*, 2000; Cabrita *et al.*, 2007). This outcome can be attributed a two different mechanisms: 1) greater availability of glucogenic precursors in the CRN diet such as starch, reducing the utilization of aminoacids for gluconeogenesis and increasing the supply and of these aminoacids to the mammary gland (Lemosquet *et al.*, 2004); 2) lower ruminal bacteria concentrations in PLP cows, decreasing the flow of microbial protein to the intestine that can be used to support the protein requirement in the mammary gland (NRC, 2001; Hristov e Ropp, 2003). Still, differences in milk protein were not sufficient to impact 12% solids-corrected milk in CRN vs. PLP cows.

Reproductive variables

No main treatment effects were detected ($P \geq 0.35$) for number of viable oocytes collected, embryos produced per collection, or proportion of embryo produced per oocyte collected (Table 6). Insulin resistance has been shown to impair oocyte fertility (Adamiak *et al.*, 2005; Leiva *et al.*, 2015), and such outcome can be attributed to reduced mRNA concentrations of IGF-I binding proteins as well as insulin receptors within small follicles (Baruselli *et al.*, 2016). The lack of treatment differences for reproductive variables herein indicate that both concentrate type and Cr-propionate supplementation did not impact oocyte production and fertility in lactating dairy cows consuming excessive energy. Further, Cr-supplementation effects on insulin sensitivity parameters within CRN cows during the GTT were also not sufficient to impact these reproductive variables, although others have reported reproductive benefits of organic Cr supplementation to dairy cows consuming corn-based concentrate (Bryan *et al.*, 2004; Soltan *et al.*, 2010). Therefore, research is still warranted to develop strategies that mitigate potential reproductive losses caused by excessive energy intake and subsequent increase in insulin resistance in lactating dairy cattle (Leiva *et al.*, 2015).

Overall conclusions

This experiment evaluated if feeding a concentrate based on citrus pulp instead of corn and providing Cr-propionate supplementation to lactating dairy cows consuming excessive energy would benefit insulin sensitivity parameters, milk production, and reproductive outcomes. Feeding the citrus pulp-based concentrate did not improve any of the aforementioned variables, whereas Cr-propionate supplementation only enhanced insulin sensitivity parameters in cows receiving a corn-based concentrate during a GTT. Given the negative relationship among excessive energy intake, insulin sensitivity parameters, and productive responses in lactating cows (LeBlanc, 2010;

Baruselli *et al.*, 2016), research is still warranted to develop nutritional strategies that mitigate insulin resistance and optimize performance and welfare in dairy cattle

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Table 1 *Composition and nutritional profile of concentrate based on ground corn (**CRN**) or citrus pulp (**PLP**).*

Item	CRN	PLP
Composition (% as-fed basis)		
Ground corn	57	25
Citrus pulp	0	31
Soybean meal	40	41
Mineral mix[1]	3	3
Nutritional profile (DM basis)		
NDF, %	9.3	14.9
Starch, %	38.1	18.1
Net energy for maintenance, MJ/kg	8.1	8.0
Net energy for lactation, MJ/kg	8.1	8.0
Crude protein, %	22.9	23.1

[1] Containing 22% Ca, 7.5% P, 6.5% Na, 1.0% K, 3.6% Mg, 2.0% S, 0.003% Co, 0.115% Cu, 0.004% I, 0.220% Mn, 0.003% Se, 0.400% Zn, 400,000 IU/kg of vitamin A, 100,000 IU/kg of vitamin D3, and 0.150% of vitamin E (Milk MAC, M. Cassab Tecnologia Animal, São Paulo, Brazil).

Table 2 *Body weight (**BW**), body condition score (**BCS**), and milk yield of lactating dairy cows consuming excessive energy, and receiving in a 2 × 2 factorial arrangement design: 1) concentrate based on ground corn (**CRN**; n = 13) or citrus pulp (**PLP**; n = 13), and 2) supplemented (n = 14) or not (n = 12) with 2.5 g/day of Cr-propionate.*

	Concentrate type				**Cr supplementation**			
Item	**CRN**	**PLP**	**SEM**	***P*=**	**Yes**	**No**	**SEM**	***P*=**
Body weight, kg								
Initial BW (day 0), kg	581	569	15	0.54	557	592	15	0.25
Final BW (day 182), kg	617	595	15	0.29	598	615	15	0.44
BW change, kg	35	25	7	0.35	39	21	8	0.20
BCS [1]								
Initial BCS (day 0)	2.75	2.84	0.08	0.43	2.78	2.81	0.08	0.98
Final BCS (day 182)	3.14	3.14	0.08	0.80	3.12	3.14	0.08	0.85
BCS change	0.37	0.30	0.07	0.38	0.34	0.33	0.07	0.95

[1] According to Wildman et al. (1982).

Table 3 *Serum parameters and revised quantitative insulin sensitivity check index (**RQUICKI**) of lactating dairy cows consuming excessive energy, and receiving in a 2 × 2 factorial arrangement design: 1) concentrate based on ground corn (**CRN**; n = 13) or citrus pulp (**PLP**; n = 13), and 2) supplemented (n = 14) or not (n = 12) with 2.5 g/day of Cr-propionate.*[1]

	Concentrate type				Cr supplementation			
Item	**CRN**	**PLP**	**SEM**	***P*=**	**Yes**	**No**	**SEM**	***P*=**
Weekly collections								
Serum glucose, mmol/L	3.02	2.97	0.06	0.57	2.97	3.02	0.06	0.60
Serum insulin, µIU/mL	6.74	7.72	0.97	0.48	6.84	7.62	0.98	0.58
Insulin:glucose ratio	0.124	0.147	0.019	0.40	0.130	0.141	0.019	0.70
Serum NEFA, mmol/L	0.177	0.215	0.009	< 0.01	0.198	0.195	0.009	0.79
RQUICKI	0.631	0.626	0.045	0.93	0.671	0.586	0.045	0.19
Glucose tolerance test[1]								
Serum glucose, mmol/L	7.76	8.43	0.33	0.17	8.21	7.99	0.33	0.69
Glucose - area under the curve, mmol/L ·min	959	1052	45	0.16	1,011	997	46	0.80
Glucose clearance rate, %/min	0.99	0.95	0.05	0.51	0.98	0.96	0.05	0.81
Glucose half-life, min	79.1	81.3	8.1	0.84	80.1	80.3	8.2	0.98
Serum insulin, µIU/mL	10.8	9.8	1.2	0.52	8.7	11.9	1.2	0.07
Insulin - area under the curve, µIU/mL ·min	1,398	1,300	200	0.73	1,108	1,590	200	0.10
Insulin:glucose ratio	1.57	1.40	0.20	0.56	1.33	1.66	0.20	0.27

[1] Glucose tolerance tests were performed on days -3, 60, 120, and 180 as described by Leiva *et al.* (2015). Values obtained on day -3 served as covariate; therefore, values reported are covariately-adjusted means.

Table 4 *Serum parameters during a glucose tolerance test of lactating dairy cows consuming excessive energy, and receiving in a 2 × 2 factorial arrangement design: 1) concentrate based on ground corn (**CRN**; n = 13) or citrus pulp (**PLP**; n = 13), and 2) supplemented (n = 14) or not (n = 12) with 2.5 g/day of Cr-propionate.*[1,2]

	CRN				PLP			
Item	**Cr**	**No Cr**	**SEM**	***P*=**	**Cr**	**No Cr**	**SEM**	***P*=**
Serum insulin, µIU/mL	8.2	13.5	1.7	0.04	9.3	10.3	1.7	0.70
Insulin - area under the curve, µIU/mL·min	975	1821	288	0.05	1241	1360	280	0.77
Insulin:glucose ratio	1.28	1.89	0.27	0.09	1.40	1.42	0.27	0.96

[1] Glucose tolerance tests were performed on days -3, 60, 120, and 180 as described by Leiva *et al.* (2015). Values obtained on day -3 served as covariate; therefore, values reported are covariately-adjusted means.

[2] Concentrate type × Cr-propionate supplementation interactions were detected ($P \leq 0.04$) for serum insulin concentrations, serum insulin AUC, and serum I:G. Hence, values are being reported within concentrate type.

Table 5 *Milk yield of lactating dairy cows consuming excessive energy, and receiving in a 2 × 2 factorial arrangement design: 1) concentrate based on ground corn (**CRN**; n = 13) or citrus pulp (**PLP**; n = 13), and 2) supplemented (n = 14) or not (n = 12) with 2.5 g/day of Cr-propionate.*[1]

	Concentrate type				Cr supplementation			
Item	**CRN**	**PLP**	**SEM**	***P*=**	**Yes**	**No**	**SEM**	***P*=**
Milk yield, kg/day	22.7	21.3	1.5	0.51	21.5	22.5	1.5	0.63
Milk fat, %	4.25	4.37	0.33	0.80	4.37	4.25	0.33	0.80
3.5% fat-corrected milk, kg/day	26.5	25.7	1.6	0.73	25.9	26.2	1.6	0.88
Milk protein, %	3.54	3.14	0.08	< 0.01	3.38	3.30	0.08	0.48
Milk total solids, %	12.9	13.2	0.4	0.62	13.1	13.1	0.4	0.96
12% solids-corrected milk, kg/day	24.7	22.5	1.4	0.24	23.1	24.0	1.3	0.64

[1] Milk production was recorded daily, and milk samples were collected weekly from each cow, which was analyzed using infrared spectrometry (method 972.16; AOAC, 1999) by a commercial laboratory (Clínica do Leite; Universidade de São Paulo, Piracicaba, Brazil).

Table 6 *Oocyte collection and in vitro embryo production from lactating dairy cows consuming excessive energy, and receiving in a 2 × 2 factorial arrangement design: 1) concentrate based on ground corn (**CRN**; n = 13) or citrus pulp (**PLP**; n = 13), and 2) supplemented (n = 14) or not (n = 12) with 2.5 g/day of Cr-propionate.*[1]

	Concentrate type				Cr supplementation			
Item	**CRN**	**PLP**	**SEM**	***P*=**	**Yes**	**No**	**SEM**	***P*=**
Oocytes per collection, n	7.3	9.5	1.5	0.35	8.6	8.2	1.5	0.87
Embryos produced per collections, n	0.76	1.03	0.34	0.59	0.81	0.98	0.34	0.72
Embryo produced/oocyte collected	0.07	0.10	0.03	0.39	0.09	0.08	0.03	0.87

[1] Follicle aspiration was performed on days -1, 62, and 162 via transvaginal ovum pick-up, processed and maturated for in vitro fertilization and fertilized with semen from the same sire (Bilby *et al.*, 2006). Values obtained on day -1 served as covariate; therefore, values reported are covariately-adjusted means.

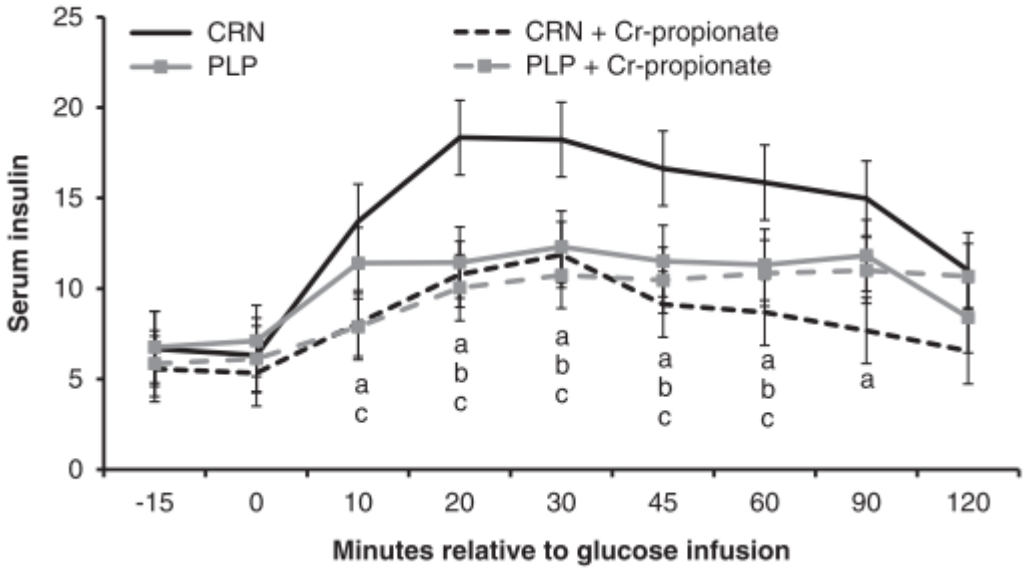

Figure 1 Serum insulin concentrations (μIU/mL) following a glucose tolerance test (intravenous infusion of 0.5 g of glucose/kg of body weight at 0 min) of lactating dairy cows consuming excessive energy, and receiving in a 2 × 2 factorial arrangement design: 1) concentrate based on ground corn (**CRN**; n = 13) or citrus pulp (**PLP**; n = 13), and 2) supplemented (n = 14) or not (n = 12) with 2.5 g/day of Cr-propionate. Although the concentrate type × Cr-propionate supplementation × time interaction was not detected (P = 0.34), differences among treatment combinations were only detected from 10 to 120 min relative to glucose infusion. Within min, letters indicate the following treatment differences ($P \leq 0.05$); a = CRN vs. CRN + Cr-propionate, b = CRN vs. PLP, c = CRN vs. PLP + Cr-propionate.

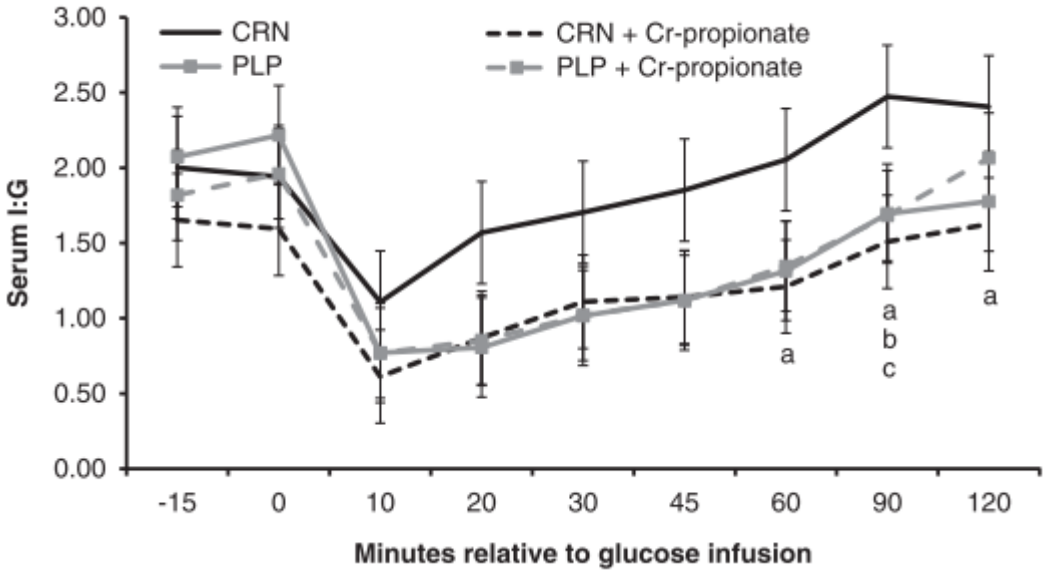

Figure 2 Serum insulin:glucose (**I:G**) ratio following a glucose tolerance test (intravenous infusion of 0.5 g of glucose/kg of body weight at 0 min) of lactating dairy cows consuming excessive energy, and receiving in a 2 × 2 factorial arrangement design: 1) concentrate based on ground corn (**CRN**; n = 13) or citrus pulp (**PLP**; n = 13), and 2) supplemented (n = 14) or not (n = 12) with 2.5 g/day of Cr-propionate. Although the concentrate type × Cr-propionate supplementation × time interaction was not detected (P = 0.41), differences among treatment combinations were only detected from 60 to 120 min relative to glucose infusion. Within min, letters indicate the following treatment differences ($P \leq 0.05$); a = CRN vs. CRN + Cr-propionate, b = CRN vs. PLP, c = CRN vs. PLP + Cr-propionate.

CAPÍTULO 3

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Interpretative summary: Effects of supplemental Ca salts of palm oil and Cr-propionate on insulin sensitivity, productive, and reproductive parameters of lactating dairy cows consuming excessive energy. Leiva. Excessive energy intake and hyperinsulinemia reduce insulin sensitivity in lactating dairy cows, whereas Cr-propionate alleviates this outcome. In this experiment, replacing dietary corn by Ca salts of palm oil, with the intent of reducing insulinogenic feed ingredients, was detrimental to basal insulin sensitivity parameters and prevented the benefits of Cr-supplementation. Hence, substituting dietary starch by fat is not an effective alternative to enhance insulin sensitivity in dairy cows consuming excessive energy.

Running Head: Concentrate energy source and Cr-propionate to dairy cows

Effects of supplemental Ca salts of palm oil and Cr-propionate on insulin sensitivity, productive, and reproductive parameters of lactating dairy cows consuming excessive energy [1,2]

T. Leiva,* R. F. Cooke,† A. P. Brandão,† R. D. Bertin,* E. A. Colombo,* V. F. B. Miranda,* L. A. C. Lourenço,* S. M. B. Rodrigues,* and J. L. M. Vasconcelos*

* São Paulo State University - Department of Animal Production, Botucatu 18168-000, Brazil
† Oregon State University - Eastern Oregon Agricultural Research Center, Burns, OR 97720
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[2] Corresponding authors: vasconcelos@fmvz.unesp.br and reinaldo.cooke@oregonstate.edu. Dr. Reinaldo Cooke is also affiliated as graduate professor to the Programa de Pós-Graduação em Zootecnia / FMVZ - UNESP, Botucatu, SP, Brazil, 18618-970.

ABSTRACT: This experiment compared insulin sensitivity, milk production, and reproductive outcomes in lactating dairy cows consuming excessive energy, and receiving in a 2 × 2 factorial arrangement design: 1) concentrate based on ground corn (**CRN**; n = 20) or including 8% (DM basis) of Ca salts of palm oil (**CSPO**; n = 20), and 2) supplemented (n = 20) or not (n = 20) with 2.5 g/d of Cr-propionate. During the experiment (d 0 to 203), 40 multiparous, non-pregnant, lactating Gir × Holstein cows (initial DIM = 81 ± 2) were offered corn silage for ad libitum consumption, and individually received concentrate formulated to allow diets to provide 160% of their daily NE_L requirements. From d -15 to 203, cow milk production was recorded daily, blood samples collected weekly, while cow BW and BCS were recorded on d 0 and 203. For DMI evaluation, cows from both treatments were randomly divided in 5 groups of 8 cows each, and allocated to 8 individual feeding stations for 3 d. Intake was evaluated 6 times/group. Glucose tolerance tests (**GTT;** 0.5 g of glucose/kg of BW) were performed on d -3, 100, and 200. Follicle aspiration for in vitro embryo production was performed via transvaginal ovum pick-up on d -1, 98, and 198. Mean DMI, NE_L intake, as well as BW and BCS change were similar across treatments. On average, cows gained 40 kg of BW and 0.49 of BCS during the experiment. Within weekly blood samples, CRN cows had lower serum concentrations of glucose, insulin, fatty acids, and insulin-to-glucose ratio compared with CSPO cows, suggesting increased insulin sensitivity in CRN cows. During the GTT, insulin-sensitivity traits were also greater in CRN versus CSPO cows. Supplemental Cr-propionate resulted in lower serum insulin concentrations and insulin-to-glucose ratio within CRN cows only, indicating that Cr-propionate improved basal insulin sensitivity in CRN but not in CSPO cows. During the GTT, however, Cr-propionate supplementation reduced hyperinsulinemia and insulin-to-glucose ratio across CSPO and CRN

cows. Milk production, as well as number of viable oocytes collected and embryos produced within each aspiration, were not affected by treatments. Hence, replacing corn by Ca salts of palm oil in the concentrate did not improve insulin sensitivity in Holstein × Gir dairy cows consuming excessive energy during mid to late lactation, whereas Cr-supplementation was effective in improving basal insulin sensitivity in cows not receiving Ca salts of palm oil.

Key Words: Chromium, dairy cows, energy intake, fat, insulin sensitivity, starch

INTRODUCTION

Excessive energy intake reduces insulin sensitivity and leads to insulin resistance in non-lactating and lactating dairy cows (Leiva et al., 2014; Leiva et al., 2015). This syndrome, characterized by persistent hyperglycemia despite increased insulin secretion, has been shown to negatively impact welfare and reproductive responses of dairy cattle (Adamiak et al., 2005; Leiva et al., 2015; Baruselli et al., 2016). Excessive energy intake is common and often inevitable among late-lactating and non-lactating cows in commercial dairies (Van Saun and Sniffen, 1996). Hence, nutritional and management strategies that mitigate insulin resistance are warranted to optimize productivity and welfare of dairy cattle.

Chromium is a critical component of the glucose tolerance factor and facilitates the action of insulin on body cells (Mertz, 1992) by enhancing auto-amplification of insulin signaling, maintaining the active conformation of insulin receptors, and promoting greater glucose uptake (Vincent, 2001). Accordingly, supplementing Cr-propionate to lactating and non-lactating dairy cows prevented the decrease in insulin sensitivity caused by excessive energy intake (Leiva et al., 2014; Leiva et al., 2015). Insulin resistance can also be caused by chronic hyperinsulinemia, which down-regulates insulin receptors and decreases cellular sensitivity to insulin (Moller and Flier, 1991). Thus, reducing dietary content of insulinogenic ingredients, such as starch, may also mitigate the incidence of this syndrome in cattle. Garnsworthy et al. (2008) reported that reducing starch intake by substituting wheat for Ca salts of palm oil reduced mean plasma insulin concentrations in lactating dairy cows, although the effects of this dietary strategy on insulin sensitivity parameters still needs investigation.

Based on this information, we hypothesized that replacing corn by Ca salts of palm oil in the dietary concentrate mitigates the decline in insulin sensitivity in lactating dairy cows

consuming excessive energy, whereas Cr-propionate supplementation is an alternative to further alleviate this outcome. Therefore, this experiment compared insulin sensitivity parameters, milk production, and reproductive outcomes in lactating dairy cows consuming excessive energy, receiving a corn-based concentrate including or not Ca salts of palm oil, and supplemented or not with Cr-propionate.

MATERIALS AND METHODS

This experiment was conducted at the São Paulo State University – Lageado Experimental Station, located in Botucatu, São Paulo, Brazil. The animals utilized were cared for in accordance with acceptable practices and experimental protocols reviewed and approved by the São Paulo State University - Animal Ethics Committee (#17/2015).

Animals and diets

Forty lactating, multiparous, pregnant Holstein × Gir cows (initial mean ± SE; parity = 2.3 ± 0.12 parities, BW = 590 ± 9.4 kg, BCS = 3.18 ± 0.06, milk yield = 26.9 ± 0.95 kg and DIM = 81 ± 2 d) were assigned to this experiment (d 0 to 203). On d 0, cows were ranked by DIM, milk yield, BW and BCS (Wildman et al., 1982), and assigned to 2 × 2 factorial arrangement design containing the following treatments: 1) concentrate based only on ground corn CRN; n = 20) or including 8% (DM basis) of Ca salts of palm oil (EnerFAT^{TM} Kemin Agrifoods South America, Indaiatuba, São Paulo, Brazil**; CSPO**; n = 20), and 2) supplemented (n = 20) or not (n = 20) with 2.5 g/day of Cr-propionate (10 mg of Cr/cow daily; KemTrace 0.4% Cr; Kemin Agrifoods South America). All treatment combinations had equivalent initial average DIM, milk yield, BW and BCS.

Beginning on day -15 and until day 203, cows were maintained in a single drylot pen with ad libitum access to corn silage, water and a commercial mineral mix without the inclusion of Cr (described in Table 1). Corn silage was provided in feed bunks that allowed 1.5 m of linear bunk space/cow and offered at daily rates to result in ≥ 15% (DM basis) of non-consumed silage. Cows were milked twice daily in a side-by-side milking system (0600 and 1700 h), and individually received their concentrate through self-locking head gates immediately after each milking.

From day -15 to -1 (adaptation period), cows received a concentrate containing (as-fed basis) 40% of soybean meal, 57% of ground corn, and 3.0% of a commercial mineral mix (described in Table 1). From day 0 to 203, cows received concentrate treatments described in Table 1. Concentrate intake was formulated to each individual cow so the diet (concentrate + corn silage) provided 100% (day -15 to -1) or 160% (day 0 to 203) of their daily NE_L requirements, as previously described and accomplished by Leiva et al. (2015). All dietary treatments were formulated to similarly meet CP, mineral, and vitamin requirements (NRC, 2001). Concentrate intake was adjusted weekly (day -15 to 203) using the Spartan Dairy Ration Evaluator/Balancer (version 3.0; Michigan State University, East Lansing, MI), according to DIM, milk yield, BW, and BCS, treatment, and corn silage intake estimated by the software.

Chromium-propionate was offered in the amount recommended by the manufacturer (2.5 g/cow daily of KemTrace; Kemin Agrifoods South America), mixed with 97.5 g of finely ground corn and top-dressed daily into the morning concentrate feeding of each supplemented cow. Finely ground corn (97.5 g/cow) was also top-dressed into the morning concentrate feeding of cows not assigned to Cr-proprionate supplementation, but without the addition of the Cr-propionate. The Ca salts of palm oil was added to the CSPO concentrate to result in less than 6% of total dietary fat

(NRC, 2001) according to the expected corn silage and concentrate DMI based on Leiva et al. (2015) and Leiva et al. (2016).

Sampling

Intake parameters. Twice monthly, samples of the offered TMR were collected weekly, pooled into one sample, and analyzed for nutrient content (Table 1) via wet chemistry procedures by a bromatology laboratory (3rlab, Belo Horizonte, Brazil). Calculations of NE_L and NE_M used the equation proposed by the NRC (2001). Nutritive value of corn silage was 37.8% DM, 1.44 Mcal/kg of NE_L, 1.57 Mcal/kg of NE_M, 20.1% starch, 3.1% ether extract, and 7.1% CP (DM basis). Nutritive values of experimental concentrates are described in Table 1. Nutritive value of concentrate offered from day −15 to −1 was 90.1% DM, 1.96 Mcal/kg of NE_L, 1.96 Mcal/kg of NE_M, 49.1% starch, 3.2% ether extract and 23.1% CP (DM basis).

To determine treatment effects on voluntary DMI, cows from both treatments were randomly divided in 5 groups of 8 cows each (2 cows/treatment combination in each group). Cows from each group were allocated to 8 individual feeding stations (15 m^2; 2.0 m of linear bunk space) with soft rubber flooring for 3 d as in Leiva et al. (2017). During this period, cows from the selected group continued to have ad libitum access to water and corn silage, and received treatments after milking as previously described. Corn silage DMI was evaluated daily from each feeding station by collecting and weighing refusals daily. Samples of the offered and non-consumed corn silage were collected daily from each feeding station and dried for 96 h at 50°C in forced-air ovens for DM calculation. At the end of the 3-d period, cows returned to the drylot pen and another group was assigned to the individual feeding stations, in a manner that corn silage DMI was evaluated 6 times/group during the experimental period.

Cow productive parameters. Cow BW and BCS were recorded weekly before (d −15 to −1) and during the experimental period (d 0 to 203). Cow milk production was recorded daily from day −15 to 203. These parameters were used to adjust concentrate intake of each cow on a weekly basis. Further, BCS was evaluated (Wildman et al., 1982) by the same two evaluators throughout the experiment, and evaluators were blinded to which treatment the assessed cow was assigned to. Milk samples were collected weekly (d -15 to 203) from each cow during both milkings of the day, combined into one daily sample (50 mL from each milking). Milk samples were analyzed for SCC via flow cytometry (AOAC, 1990) with a Somacount 300 instrument (Bentley Instruments Inc.; Chaska, MN), and concentrations of fat, protein, and total solids via infrared spectrometry (method 972.16; AOAC, 1999), by a commercial laboratory (Clínica do Leite; Universidade de São Paulo, Piracicaba, Brazil). Daily milk yield was adjusted to FCM or TS-corrected milk based on milk concentrations of fat and total solids, respectively, of the concurrent week. Starch, fat, and NE_L intake within each treatment were estimated according to the NRC (2001) model, using dietary nutrient profile, average milk yield, BW, BCS, and DMI values observed during the experimental period.

Blood samples were collected weekly (d 0 to 203), before the morning concentrate feeding during the experiment for determination of serum glucose, insulin, and non-esterified fatty acids (**NEFA**) concentrations. Serum insulin and glucose concentrations within each sampling time were used to calculate insulin to glucose ratio (I:G; Bernhard et al., 2012) and homeostasis model of insulin resistance (HOMA-IR; Mann et al., 2016). Glucose tolerance tests (GTT) were performed on d −3, 100, and 200 by intravenously infusing cows with 0.5 g of glucose/kg of BW. More specifically, cows were weighed the day before each GTT and had access to water but were not offered corn silage, Cr-propionate, or concentrate 12 h before and during the GTT. Cows were

fitted with indwelling jugular catheters according to the procedures described by Curley et al. (2008) immediately before infusion and received a 50% saline:dextrose solution via gravity intravenous infusion (Glicocalbos 50%; Calbos Saúde Animal, Curitiba, Brazil) according to their BW (1 g of saline:dextrose solution/kg of BW). Across all GTT, total glucose infusion time was 9.9 ± 0.5 min. Catheters were removed after infusion was complete. Blood samples were collected at −15, 0, 10, 20, 30, 45, 60, 90, and 120 min relative to infusion and analyzed for serum concentrations of glucose and insulin. During each GTT, area under the curve (AUC) for glucose and insulin were calculated with the trapezoidal method (Shiang, 2004), whereas I:G and HOMA-IR were also determined within each sampling time (Bernhard et al., 2012). Glucose clearance rate and half-life were calculated with the equations described by Bernhard et al. (2012), using incremental serum glucose concentrations between 30 and 120 min postinfusion during the GTT.

Laboratorial analysis

During the weekly or GTT blood collections, samples were obtained from coccygeal vessels into commercial blood collection tubes (Vacutainer, 10 mL; Becton Dickinson, Franklin Lakes, NJ), placed immediately on ice, centrifuged at 3000 × g at 4 °C for 30 min for serum collection, and stored at -20°C on the same day of collection. Glucose, insulin, and NEFA concentrations were analyzed as in Leiva et al. (2015). The intra- and interassay CV were, respectively, 3.6 and 6.2% for glucose, 2.1 and 3.0% for insulin, and 6.7 and 9.3% for NEFA. Assay sensitivity was 0.0005 mmol/L for glucose, 0.01 mmol/L for NEFA, and 0.1 µIU/mL for insulin.

Reproductive Management

Follicle aspiration was performed on d -1, 98, and 198 to evaluate treatment effects on production of viable oocytes, as well as subsequent in vitro embryo production. Cows were at

random stages of the estrous cycle when assigned to follicle aspiration, which was performed via transvaginal ovum pick-up according to the procedures described by Bilby et al. (2006). Oocytes were collected, processed and maturated for IVF as described by Leiva et al. (2015), and fertilized with semen from the same sire according to the procedures described by Bilby et al. (2006). Presumptive zygotes were incubated at 38.5°C in 5% O_2, 5% CO_2, in 100% humidified air for 7 d (Bilby et al., 2006). After incubation, number of cleaved and viable embryos was recorded with a dissecting microscope. Variables that were utilized for the present experiment were; number of oocytes collected that were viable to IVF (Grades I, II, and III), number of embryos produced, and ratio of embryos produced/viable oocytes collected within each sampling day.

Statistical analysis

Cow was considered the experimental unit given that concentrate energy source (CRN or CSPO) and choice of Cr supplementation were individually applied to cows. All data were analyzed using the MIXED procedure of SAS (SAS Inst., Inc., Cary, NC; version 9.3) and Satterthwaite approximation to determine the denominator df for the tests of fixed effects. Cow(concentrate energy source × Cr-propionate supplementation) was included as random variable into all analyses, but for DMI that used cow(concentrate energy source × Cr-propionate supplementation × group).

The model statement used for analysis of BW and BCS change, as well as initial and final BCS and BW during the experiment contained the effect of concentrate energy source, Cr-propionate supplementation, and the resultant interaction. The model statement used for analysis of weekly BW, BCS, milk yield and serum variables contained the effects of concentrate energy source, Cr-propionate supplementation, time (day or week), and the resultant interactions. Average milk production and milk component concentrations from d -15 to -1, as well as results from serum

samples collected on d 0, were included as covariate within each respective analysis. The model statement used for analysis of DMI and nutrient intake contained the effects of concentrate energy source, Cr-propionate supplementation, day, group, and all resultant interactions. The model statement used for serum glucose, serum insulin, I:G, and HOMA-IR obtained during the GTT contained the effects of concentrate energy source, Cr-propionate supplementation, day of GTT, min of sampling, all resultant interactions, and mean values obtained from the GTT on day -3 as independent covariate. The model statement used for follicle aspiration and IVF outcomes, as well as glucose and insulin AUC, glucose clearance rate, and glucose half-life during the GTT contained the effects of concentrate energy source, Cr-propionate supplementation, day of follicle collection or GTT, all resultant interactions, and values obtained from collection on day -1 (reproductive variables) or -3 (GTT) as independent covariate. The specified term for the repeated statement was week for the weekly collections, day for reproductive variables, day(group) for NE_L intake and DMI, and hour for the GTT. Cow(concentrate energy source × Cr-propionate supplementation) was included as subject for all repeated statements, but for NE_L intake and DMI that included cow(concentrate energy source × Cr-propionate supplementation × group) as subject. The covariance structure utilized for all repeated statements was autoregressive, which provided the best fit for these analyses according to the Akaike information criterion.

Results are reported as least square means, or covariately adjusted means for GTT, milk yield, weekly serum variables, and reproductive responses, and separated using PDIFF. Significance was set at $P \leq 0.05$, and tendencies were determined if $P > 0.05$ and ≤ 0.10. Results are reported according to main treatment effects (concentrate energy source and Cr supplementation) if no interactions were significant, or according to the highest-order significant ($P \leq 0.05$) interaction containing one of both main treatment effects.

RESULTS

Intake, BW, and BCS parameters

Mean DMI during the experiment, as kg/d or % of BW, were similar ($P \geq 0.25$) within concentrate energy source or Cr supplementation effects (Table 2). No differences among mean NE_L intake, % of NE_L intake according to requirements (NRC, 2001), and mean starch intake were detected ($P \geq 0.53$) within concentrate energy source or Cr supplementation effects (Table 2). However, mean dietary starch content was greater ($P < 0.01$) in CRN vs. CSPO cows, and similar ($P = 0.59$) between Cr supplementation treatments (Table 2). Mean fat intake and dietary fat content were greater ($P < 0.01$) in CSPO vs. CRN cows, and similar ($P = 0.38$) between Cr supplementation treatments (Table 2).

No concentrate energy source or Cr supplementation effects were detected ($P \geq 0.22$) for final BW and BW change, as well as final BCS and BCS change (Table 3). Moreover, all cows gained (day effect, $P < 0.01$) BW (560 vs. 600 kg on day 0 and 203, respectively; SEM = 11) and BCS (3.18 vs. 3.67 of BCS on day 0 and 203, respectively; SEM = 0.07) during the experiment.

Serum variables evaluated weekly

A concentrate energy source × day interaction was detected ($P = 0.01$) for serum glucose; CSPO cows often had greater serum glucose concentrations compared with CRN cows (Figure 1). Accordingly, mean serum glucose concentrations during the experimental period were greater in CSPO vs. CRN cows (Table 4). A tendency for Cr supplementation effect was detected ($P = 0.09$) for serum glucose, as Cr-supplemented cows had less mean serum glucose concentrations compared with non-supplemented cohorts during the experimental period (Table 4).

Concentrate energy source × Cr supplementation × day interactions were detected (P = 0.02) for serum insulin, I:G, and HOMA-IR. Cows receiving CRN + Cr-propionate often had lower ($P \leq 0.05$) serum insulin concentrations (Figure 2), I:G (Figure 3), and HOMAIR (data not shown) compared with all other treatment combinations. Accordingly, mean serum insulin concentrations, I:G, and HOMA-IR were lower ($P \leq 0.05$) in Cr-supplemented versus non-supplemented CRN cows, but similar ($P \geq 0.93$) within CSPO cows (Table 5; concentrate energy source × Cr supplementation interaction, $P \leq 0.10$). Moreover, mean serum insulin concentrations, I:G, and HOMA-IR during the experimental period were ($P \leq 0.02$) greater in CSPO versus CRN cows (Table 4). A concentrate energy source × Cr supplementation interaction was detected ($P < 0.01$) for serum fatty acids (Table 5). During the experimental period, mean serum fatty acid concentrations were lower ($P < 0.01$) in Cr-supplemented versus nonsupplemented CSPO cows, but similar ($P = 0.91$) within CRN cows (Table 5). In addition, CSPO cows has greater ($P < 0.01$) mean serum fatty acid concentrations compared CRN cows during the experiment (Table 4).

Serum variables evaluated during the GTT

A concentrate energy source × min interaction was detected ($P < 0.01$) for serum glucose, given that peak serum glucose concentration was greater ($P < 0.01$) in CRN vs. CSPO cows (Figure 4). However, glucose AUC, half-life, and clearance rate were similar (P = 0.40) between CSPO and CRN cows (Table 4). No Cr supplementation effects were detected ($P \geq 0.39$) for serum glucose concentrations (172 vs. 177 mg/dL for Cr supplemented and non-supplemented cows, respectively; SEM = 4.5), as well as glucose AUC, half-life, and clearance rate during the GTT (Table 4).

Concentrate energy source × min interactions were detected ($P \leq 0.02$) for serum insulin, I:G, and HOMAIR during the GTT. Serum insulin concentrations were greater ($P \leq 0.05$) in CSPO

versus CRN cows beginning at 20 min (Figure 5a), whereas I:G (Figure 6a) and HOMA-IR (data not shown) were often greater ($P \leq 0.05$) in CSPO versus CRN cows before and after glucose infusion. Accordingly, serum insulin AUC during the GTT were lower ($P = 0.03$) in CRN versus CSPO cows (Table 4). A Cr supplementation × minute interaction was detected ($P = 0.03$) for serum insulin during the GTT, which was greater ($P = 0.05$) at 30 min and tended to be greater ($P = 0.09$) at 45 min relative to glucose infusion in nonsupplemented versus supplemented cows (Figure 5b). No differences were detected ($P = 0.57$) for serum insulin AUC between cows supplemented or not with Cr-propionate (Table 4). Although a Cr supplementation × minute interaction was also detected ($P < 0.01$) for I:G during the GTT, only numerical differences were detected ($P \geq 0.16$) between nonsupplemented versus supplemented cows (Figure 6b).

Milk production

No concentrate energy source effects were detected ($P \geq 0.24$; Table 6) for milk yield, milk fat, milk protein, SCC, milk total solids, as well as 3.5% FCM yield and 12% SCM. Cows supplemented with Cr-propionate had ($P = 0.05$) less milk fat compared with non-supplemented cows, whereas no further Cr supplementation effects were detected ($P \geq 0.21$) for milk production parameters (Table 6).

Reproductive variables

No concentrate energy source or Cr supplementation effects were detected ($P \geq 0.35$) for number of viable oocytes collected, embryos produced per collection, or proportion of embryo produced per oocyte collected (Table 7).

DISCUSSION

Intake, BW, and BCS parameters

The similar DMI, BCS, and BW parameters among CSPO and CRN cows, as well as cows supplemented or not with Cr-propionate (Tables 2 and 3), corroborate that cows from all treatment similarly consumed excessive energy, which was designed to impair insulin sensitivity as previous accomplished by our research group (Leiva et al., 2015; Leiva et al., 2016). Although estimated NE_L intake was below the targeted 160% (Table 2), BW and BCS gain during the experimental period was equivalent to Leiva et al. (2015); hence, adequate to test our main hypotheses. Moreover, starch and fat intake, as % of DMI, differed between CRN and CSPO cows as designed (Table 2), while such differences were not sufficient to alter daily (kg/d) starch intake between concentrate energy treatments (Table 2). This latter outcome can be associated with the starch content of the corn silage, which contributed to nearly 50% of the dietary DM and likely hindered major differences in starch intake between CRN and CSPO cows. Hence, concentrate energy source or Cr supplementation effects on all physiological and productive responses evaluated herein should not be associated with differences in energy intake among treatment groups (Vizcarra et al., 1998; Butler et al., 2003; Grummer, 1995), but with designed differences on Ca salts of palm oil and Cr-propionate intake.

Serum variables evaluated weekly and during the GTT

Starch is the major dietary precursor for glucose in ruminants (Huntington, 1997) and is classified as an insulinogenic nutrient (Cabrita et al., 2007); hence, it would be expected that CRN cows had greater serum glucose and insulin concentration compared with CSPO cows (Garnsworthy et al., 2008). In turn, reduced hyperinsulinemia would lessen the incidence of insulin resistance in CSPO versus CRN cows (Moller and Flier, 1991). However, concentrate energy source effects on serum

glucose, insulin, I:G, and HOMAIR during weekly sampling (Table 4) are suggestive of increased insulin sensitivity in CRN versus CSPO cows (Hayirli et al., 2001; Leiva et al., 2015, 2017b; Mann et al., 2016), which should be largely attributed to Cr-supplementation effects on CRN cows, as discussed in the next paragraph. Nevertheless, results from the GTT further suggest that CSPO cows experienced hyperinsulinemia and reduced insulin sensitivity, based on I:G and HOMA-IR results (Muniyappa et al., 2008; Bernhard et al., 2012), compared with CRN cows (Table 4; Figures 5 and 6). Perhaps differences in starch intake between CRN and CSPO cows were not sufficient to modulate hyperinsulinemia in CSPO cows. These outcomes should also be associated with greater serum fatty acid concentrations in CSPO versus CRN cows (Table 4). Inclusion of supplemental fat in diets to lactating dairy cows results in increased circulating fatty acid concentrations due to incomplete dietary lipid oxidation (Hayirli, 2006). In turn, fatty acid metabolism interferes with the intracellular insulin receptor-signaling cascade and reduces cell sensitivity to insulin (Lewis et al., 2002; Pires et al., 2007). Garnsworthy et al. (2008) also reported increased serum fatty acids when substituting wheat for Ca salts of palm oil, although plasma insulin concentrations decreased and plasma glucose concentrations did not change in fat-enriched diets. However, those authors formulated their experimental diets to meet NEL requirements of early-lactating cows. Cows from the present experiment were fed excessive energy during mid to late lactation, which is known to result in different fatty acid metabolism compared with that of early-lactating dairy cows (Bell, 1995). Moreover, insulin resistance increases mobilization of body fat reserves and subsequent fatty acid release into the circulation to compensate for the reduced uptake of glucose by body tissues (Barbour et al., 2002), which may have further contributed to concentrate energy source effects on serum fatty acid concentrations. Hence, replacing concentrate corn by Ca salts of palm oil failed to improve insulin sensitivity in dairy

cows consuming excessive energy during mid to late lactation, likely due to their altered fatty acid metabolism (Bell, 1995) or insufficient decrease in daily starch intake. Previous research reported that Cr-propionate supplementation to lactating and nonlactating Holstein × Gir dairy cows affected serum concentrations of glucose, insulin, and fatty acids, and prevented the decrease in insulin sensitivity caused by excessive energy intake (Leiva et al., 2014, 2015). Chromium is a critical component of the glucose tolerance factor that facilitates the action of insulin on body cells (Mertz, 1992) via chromodulin—an oligopeptide that binds with high affinity to 4 chromic ions and enables Cr to be involved in the autoamplification of insulin signaling—maintaining the active conformation of insulin receptors and promoting greater glucose uptake (Vincent, 2001). Supplemental Cr-propionate may affect insulin-sensitivity traits in adipose and other body tissues through immunological signals such as proinflammatory cytokine response (Wellen and Hotamisligil, 2005). In our experiment, Cr-propionate reduced serum glucose concentrations independently of concentrate energy source in weekly samples, suggesting a potential increase in glucose uptake by body tissues across CSPO and CRN cows (Table 4). Conversely, Cr-propionate supplementation also reduced serum fatty acid concentrations in CSPO but not in CRN cows within weekly samples (Table 5), denoting enhanced dietary fat oxidation or increased body fat deposition due to increased glucose uptake in CSPO cows only (Barbour et al., 2002; Hayirli, 2006). Nonetheless, Cr-propionate supplementation reduced serum insulin, I:G, and HOMA-IR in CRN cows only within weekly samples (Table 5), which indicates that Cr supplementation improved basal insulin-sensitivity traits in CRN but not in CSPO cows (Muniyappa et al., 2008; Leiva et al., 2015, 2017b). Perhaps the elevated serum fatty acid concentrations in CSPO cows prevented the benefits of Cr-propionate supplementation on insulin-sensitivity traits, such as enhanced insulin activity in target cells (Lewis et al., 2002; Pires et al., 2007). Conversely, Cr-

propionate supplementation reduced hyperinsulinemia and I:G, although numerically, independently of concentrate energy source during the GTT (Figures 5 and 6). Hence, Cr-propionate supplementation was effective in improving insulin-sensitivity traits in both CRN and CSPO cows when cows were exposed to supraphysiological circulating glucose concentrations as previously reported (Hayirli et al., 2001; Leiva et al., 2015, 2017b).

Milk production

Reduced insulin sensitivity may negatively impact milk yield and mammary synthesis of milk constituents in lactating dairy cattle (McGuire et al., 1995; LeBlanc, 2010). However, milk parameters were not impacted by concentrate energy source (Table 6) despite differences detected for insulin sensitivity between CSPO and CRN cows; perhaps the magnitude of such differences among CSPO and CRN cows were not sufficient to affect milk yield and constituents. Garnsworthy et al. (2008) and Garnsworthy et al. (2009) also reported that reducing starch intake by substituting wheat for Ca salts of palm oil did not impact milk yield, although milk fat was increased in cows receiving Ca salts of palm oil in both studies. Likewise, Cr-propionate supplementation also failed to increase milk yield despite improved insulin sensitivity parameters during weekly sampling and GTT compared with non-supplemented cows, corroborating previous research from our group evaluating dairy cows consuming excessive energy (Leiva et al., 2015; Leiva et al., 2016). Yet, Cr-propionate supplementation reduced milk fat, although such difference was not sufficient to impact 3.5% FCM yield (Table 6). Greater milk fat concentration in non-supplemented cows may be associated with their greater serum NEFA concentrations compared with cows supplemented with Cr-propionate, given that preformed fatty acids such as NEFA taken up by the mammary gland and directly used for milk fat synthesis (Bauman and Griinari, 2003). It is important, however, that diets in the present experiment were formulated to provide excessive NE_L. Hence,

such diets allowed cows from all treatment combinations to produce their maximum milk, which may have hindered potential effects of concentrate energy source or Cr supplementation treatments on milk production parameters.

Reproductive variables

Insulin resistance has been shown to impair oocyte fertility (Adamiak et al., 2005; Leiva et al., 2015), which can be attributed to reduced mRNA concentrations of IGF-I binding proteins as well as insulin receptors within small follicles (Baruselli et al., 2016). The lack of treatment differences for reproductive variables herein indicate that both concentrate energy sources and Cr-propionate supplementation failed to impact oocyte production and fertility in lactating dairy cows consuming excessive energy. As in milk production parameters, differences in insulin sensitivity parameters among treatment combinations were not sufficient to affect reproductive variables, although others have reported reproductive benefits of organic Cr supplementation to dairy cows consuming corn-based concentrate (Bryan et al., 2004; Soltan et al., 2010). Therefore, research is still warranted to develop strategies that mitigate potential reproductive losses caused by excessive energy intake and subsequent increase in insulin resistance in lactating dairy cattle (Leiva et al., 2015; Leiva et al., 2016).

Overall conclusions

This experiment evaluated if replacing corn by Ca salts of palm oil in the dietary concentrate while providing Cr-propionate supplementation to lactating dairy cows consuming excessive energy would benefit insulin sensitivity parameters, milk production, and reproductive outcomes. Inclusion of Ca salts of palm oil was in fact detrimental to insulin sensitivity parameters, whereas Cr-supplementation was effective in improving basal insulin sensitivity in cows not receiving Ca salts of palm oil. Nonetheless, milk yield and reproductive outcomes were not

impacted concentrate energy source or Cr supplementation. Given the negative relationship among excessive energy intake, insulin sensitivity parameters, and productive responses in lactating cows (LeBlanc, 2010; Baruselli et al., 2016), research is still warranted to develop nutritional strategies that mitigate insulin resistance and optimize performance and welfare in dairy cattle.

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Table 1. Composition and nutritional profile of concentrate based on ground corn (**CRN**; n = 20) or with the inclusion of Ca salts of palm fatty oil (**CSPO**; n = 20).

Item	**CRN**	**CSPO**
Composition (% as-fed basis)		
Ground corn	69.0	59.5
Soybean meal	28.0	29.5
Ca salts of palm fatty acids[1]	-	8.0
Mineral mix[2]	3.0	3.0
Nutritional profile (DM basis)		
NDF, %	11.8	11.1
Starch, %	50.2	42.8
Ether extract, %	3.3	10.3
NE_M, Mcal/kg	2.13	2.36
NE_L, Mcal/kg	1.96	2.24
CP, %	20.4	20.3

[1] EnerFAT™ (Kemin Agrifoods South America, Indaiatuba, São Paulo, Brazil).
[2] Containing 22% Ca, 7.5% P, 6.5% Na, 1.0% K, 3.6% Mg, 2.0% S, 0.003% Co, 0.115% Cu, 0.004% I, 0.220% Mn, 0.003% Se, 0.400% Zn, 400 000 IU/kg of vitamin A, 100 000 IU/kg of vitamin D3 and 0.150% of vitamin E (Milk MAC; M. Cassab Tecnologia Animal, São Paulo, Brazil).

Table 2. Feed and nutrient intake of lactating dairy cows consuming excessive energy, and receiving in a 2 × 2 factorial arrangement design: 1) concentrate based only on ground corn CRN; n = 20) or including 8% (DM basis) of Ca salts of palm oil (EnerFAT™ Kemin Agrifoods South America, Indaiatuba, São Paulo, Brazil**; CSPO**; n = 20), and 2) supplemented (n = 20) or not (n = 20) with 2.5 g/d of Cr-propionate (10 mg of Cr/cow daily; KemTrace 0.4% Cr; Kemin Agrifoods South America).[1,2]

	Concentrate energy source				Cr supplementation			
Item	**CRN**	**CSPO**	**SEM**	***P* =**	**Yes**	**No**	**SEM**	***P* =**
DMI as kg/d								
Silage	14.6	15.4	1.0	0.57	15.7	14.3	1.0	0.33
Concentrate	7.39	7.25	0.84	0.90	7.46	7.18	0.84	0.81
Total	22.0	22.7	1.6	0.76	23.2	21.5	1.6	0.46
Total DMI as % of BW	3.96	3.75	0.20	0.47	3.97	3.75	0.20	0.25
NE_L intake								
Total, Mcal/d	36.8	39.4	2.9	0.53	39.5	36.7	2.9	0.49
NE_L required, Mcal/d	25.9	26.9	-	-	26.1	26.4	-	-
% of NE_L requirement	142	146	11	0.77	149	139	11	0.50
Fat intake								
Total intake, kg/d	0.71	1.24	0.07	< 0.01	1.02	0.93	0.07	0.38
% of DMI	3.23	5.48	0.09	<0.01	4.35	4.36	0.09	0.96
Starch intake								
Total intake, kg/d	6.63	6.19	0.53	0.57	6.61	6.21	0.53	0.60
% of DMI	30.1	27.3	0.6	< 0.01	28.5	28.9	0.6	0.59

[1] Cows from both treatments were randomly divided in 5 groups of 8 cows each, and allocated to 8 individual feeding stations (15 m^2; 2.0 m of linear bunk space) for 3 d as in Leiva et al. (2017). Corn silage and concentrate DMI were evaluated daily from each cow by collecting and weighing refusals daily. At the end of the 3-d period, cows returned to the drylot pen and another group was assigned to the individual feeding stations, in a manner that DMI was evaluated 6 times/group during the experimental period.

[2] Starch, fat, and NE_L intake and requirements within each treatment were estimated according to the NRC (2001) model, using dietary nutrient profile, average milk yield, BW, BCS, and DMI values observed during the experimental period.

Table 3. Body weight and BCS of lactating dairy cows consuming excessive energy, and receiving in a 2 × 2 factorial arrangement design: 1) concentrate based only on ground corn CRN; n = 20) or including 8% (DM basis) of Ca salts of palm oil (EnerFAT™ Kemin Agrifoods South America, Indaiatuba, São Paulo, Brazil**; CSPO**; n = 20), and 2) supplemented (n = 20) or not (n = 20) with 2.5 g/d of Cr-propionate (10 mg of Cr/cow daily; KemTrace 0.4% Cr; Kemin Agrifoods South America).[1]

	Concentrate energy source				Cr supplementation			
Item	**CRN**	**CSPO**	**SEM**	***P* =**	**Yes**	**No**	**SEM**	***P* =**
BW, kg								
Initial BW (d 0), kg	552	568	13	0.39	558	562	13	0.80
Final BW (d 210), kg	593	605	18	0.64	592	607	19	0.57
BW change, kg	33	48	12	0.39	33	48	12	0.36
BCS								
Initial BCS (d 0)	3.17	3.20	0.08	0.83	3.14	3.23	0.08	0.47
Final BCS (d 210)	3.64	3.76	0.12	0.49	3.60	3.81	0.12	0.22
BCS change	0.44	0.50	0.08	0.58	0.43	0.52	0.08	0.43

[1]According to Wildman et al., (1982).

Table 4. Serum parameters of lactating dairy cows consuming excessive energy, and receiving in a 2 × 2 factorial arrangement design: 1) concentrate based only on ground corn CRN; n = 20) or including 8% (DM basis) of Ca salts of palm oil (EnerFAT™ Kemin Agrifoods South America, Indaiatuba, São Paulo, Brazil**;** **CSPO**; n = 20), and 2) supplemented (n = 20) or not (n = 20) with 2.5 g/d of Cr-propionate (10 mg of Cr/cow daily; KemTrace 0.4% Cr; Kemin Agrifoods South America).

	Concentrate type				**Cr supplementation**			
Item	**CRN**	**CSPO**	**SEM**	***P* =**	**Yes**	**No**	**SEM**	***P* =**
Weekly collections[1]								
Serum glucose, mg/dL	61.7	64.4	0.8	0.03	62.1	64.0	0.8	0.09
Serum insulin, µIU/mL	8.6	16.6	2.1	0.01	10.5	14.7	2.2	0.20
Insulin:glucose ratio	0.145	0.261	0.033	0.02	0.168	0.238	0.035	0.18
Homeostasis model of insulin resistance[2]	0.182	0.382	0.049	<0.01	0.239	0.325	0.05	0.24
Serum non-esterified fatty acids, µEq/L	0.146	0.244	0.007	< 0.01	0.181	0.209	0.007	0.01
Glucose tolerance test[3]								
Glucose - area under the curve, mg/dL ·min	21,575	22,131	607	0.52	21,474	22,232	613	0.39
Glucose clearance rate, %/min	0.887	0.862	0.043	0.69	0.870	0.879	0.043	0.89
Glucose half-life, min	82.8	89.7	5.6	0.40	84.3	88.2	5.7	0.64
Insulin - area under the curve, µIU/mL ·min	1,010	2,417	440	0.03	1,528	1,900	449	0.57

[1] Blood samples were collected weekly (d 0 to 203), before the morning concentrate feeding during the experiment. Values obtained on d 0 were included as covariate within each respective analysis; therefore, values reported are covariately-adjusted means

[2]According to Mann et al. (2016).

[3]Glucose tolerance tests were performed on days -3, 100, and 200 as described by Leiva et al. (2015). Values obtained on day -3 served as covariate; therefore, values reported are covariately-adjusted means.

Table 5. Serum parameters of lactating dairy cows consuming excessive energy, and receiving in a 2 × 2 factorial arrangement design: 1) concentrate based only on ground corn CRN; n = 20) or including 8% (DM basis) of Ca salts of palm oil (EnerFAT^{TM} Kemin Agrifoods South America, Indaiatuba, São Paulo, Brazil**;** **CSPO**; n = 20), and 2) supplemented (n = 20) or not (n = 20) with 2.5 g/d of Cr-propionate (10 mg of Cr/cow daily; KemTrace 0.4% Cr; Kemin Agrifoods South America).[1,2]

	CRN		**CSPO**			
Item	**Cr**	**No Cr**	**Cr**	**No Cr**	**SEM**	***P* =**
Serum insulin, μIU/mL	4.3[a]	12.8[b]	16.7[b]	16.5[b]	3.1	0.08
Insulin:glucose ratio	0.076[a]	0.214[b]	0.262[b]	0.261[b]	0.048	0.09
Homeostasis model of insulin resistance[3]	0.091[a]	0.273[b]	0.387[b]	0.378[b]	0.070	0.10
Serum Serum non-esterified fatty acids, μEq/L	0.147[a]	0.145[a]	0.216[b]	0.272[c]	0.010	< 0.01

[1] Blood samples were collected weekly (d 0 to 203), before the morning concentrate feeding during the experiment. Values obtained on d 0 were included as covariate within each respective analysis; therefore, values reported are covariately-adjusted means.
[2] P-value represents the concentrate energy source × Cr supplementation interaction. Within rows, means with different superscripts (a,b,c) differ ($P < 0.05$).
[3]According to Mann et al. (2016).

Table 6. Milk yield of lactating dairy cows consuming excessive energy, and receiving in a 2 × 2 factorial arrangement design: 1) concentrate based only on ground corn CRN; n = 20) or including 8% (DM basis) of Ca salts of palm oil (EnerFAT™ Kemin Agrifoods South America, Indaiatuba, São Paulo, Brazil**; CSPO**; n = 20), and 2) supplemented (n = 20) or not (n = 20) with 2.5 g/d of Cr-propionate (10 mg of Cr/cow daily; KemTrace 0.4% Cr; Kemin Agrifoods South America).[1]

	Concentrate type				Cr supplementation			
Item	**CRN**	**CSPO**	**SEM**	***P* =**	**Yes**	**No**	**SEM**	***P* =**
Milk yield, kg/d	19.6	20.7	0.7	0.27	20.5	19.7	0.7	0.47
Milk fat, %	5.04	4.97	0.14	0.71	4.81	5.20	0.14	0.05
3.5% fat-corrected milk, kg/d	27.2	28.9	1.4	0.41	27.9	28.2	1.4	0.89
Milk protein, %	3.46	3.28	0.05	0.24	3.33	3.40	0.05	0.32
Milk total solids, %	13.9	13.7	0.2	0.58	13.6	13.9	0.2	0.21
12% solids-corrected milk, kg/d	22.4	23.3	0.9	0.63	23.1	22.6	0.9	0.79
SCC, cells/ml	785	823	140	0.85	723	885	141	0.42

[1] Milk production was recorded daily (d -15 to 203). Milk samples were collected weekly (d -15 to 203) from each cow, and analyzed for SCC via flow cytometry (AOAC, 1990) with a Somacount 300 instrument (Bentley Instruments Inc.; Chaska, MN), and concentrations of fat, protein, and total solids via infrared spectrometry (method 972.16; AOAC, 1999), by a commercial laboratory (Clínica do Leite; Universidade de São Paulo, Piracicaba, Brazil). Daily milk yield was adjusted to FCM or TS-corrected milk based on milk concentrations of fat and total solids, respectively, of the concurrent week. Values obtained from d-15 to -1 were averaged and included as covariate within each respective analysis; therefore, values reported are covariately-adjusted means.

Table 7. Oocyte collection and in vitro embryo production from lactating dairy cows consuming excessive energy, and receiving in a 2 × 2 factorial arrangement design: 1) concentrate based only on ground corn CRN; n = 20) or including 8% (DM basis) of Ca salts of palm oil (EnerFAT™ Kemin Agrifoods South America, Indaiatuba, São Paulo, Brazil**; CSPO**; n = 20), and 2) supplemented (n = 20) or not (n = 20) with 2.5 g/d of Cr-propionate (10 mg of Cr/cow daily; KemTrace 0.4% Cr; Kemin Agrifoods South America).

	Concentrate type				**Cr supplementation**			
Item	**CRN**	**CSPO**	**SEM**	***P* =**	**Yes**	**No**	**SEM**	***P* =**
Oocytes per collection, n	6.1	7.7	1.4	0.43	7.6	6.2	1.4	0.50
Embryos produced per collections, n	0.83	0.95	0.40	0.84	0.62	1.16	0.40	0.35
Embryo produced/oocyte collected, %	9.5	7.1	3.2	0.60	7.7	9.0	3.2	0.77

[1] Follicle aspiration was performed on days -1, 98, and 200 via transvaginal ovum pick-up, processed and maturated for in vitro fertilization and fertilized with semen from the same sire (Bilby et al., 2006). Values obtained on day -1 served as covariate; therefore, values reported are covariately-adjusted means.

Figure 1. Serum glucose concentrations of lactating dairy cows consuming excessive energy, and receiving in a 2 × 2 factorial arrangement design: 1) concentrate based only on ground corn CRN; n = 20) or including 8% (DM basis) of Ca salts of palm oil (EnerFAT™ Kemin Agrifoods South America, Indaiatuba, São Paulo, Brazil**; CSPO**; n = 20), and 2) supplemented (n = 20) or not (n = 20) with 2.5 g/d of Cr-propionate (10 mg of Cr/cow daily; KemTrace 0.4% Cr; Kemin Agrifoods South America). Values obtained on d 0 were included as covariate; therefore, values reported are covariately-adjusted means. A concentrate type × day interaction was detected ($P = 0.01$). Within days, ** $P \leq 0.01$, * = $P \leq 0.05$, and † = $P \leq 0.10$.

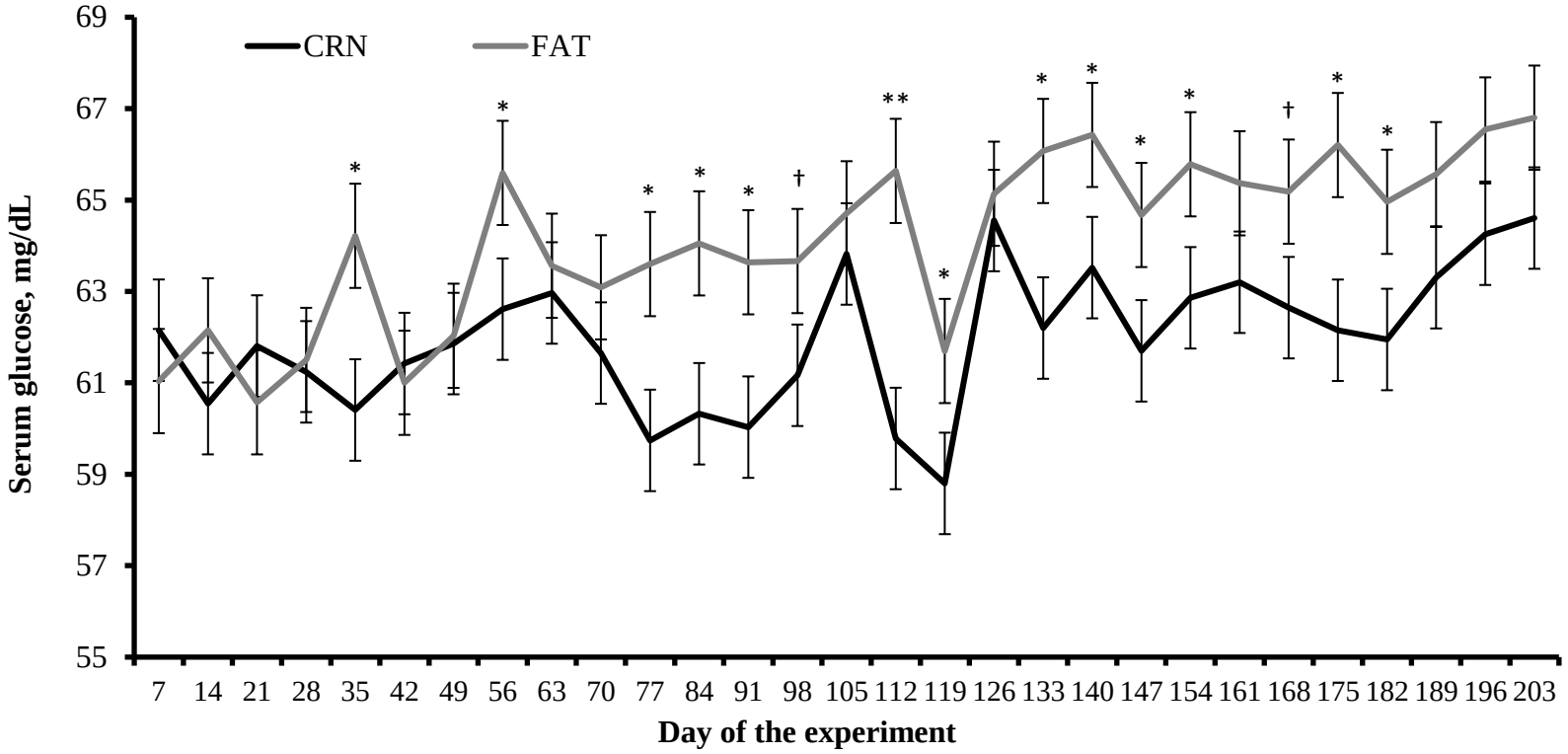


Figure 2. Serum insulin concentrations of lactating dairy cows consuming excessive energy, and receiving in a 2 × 2 factorial arrangement design: 1) concentrate based only on ground corn CRN; n = 20) or including 8% (DM basis) of Ca salts of palm oil (EnerFAT^{TM} Kemin Agrifoods South America, Indaiatuba, São Paulo, Brazil**; CSPO**; n = 20), and 2) supplemented (n = 20) or not (n = 20) with 2.5 g/d of Cr-propionate (10 mg of Cr/cow daily; KemTrace 0.4% Cr; Kemin Agrifoods South America). Values obtained on d 0 were included as covariate; therefore, values reported are covariately-adjusted means. A concentrate type × Cr supplementation × day interaction was detected ($P = 0.02$). Within days ($P \leq 0.05$); a = CRN vs. CSPO, b = CRN + Cr-propionate vs. CSPO + Cr-propionate, c = CRN + Cr-propionate vs. CSPO, d = CRN vs. CRN + Cr-propionate.

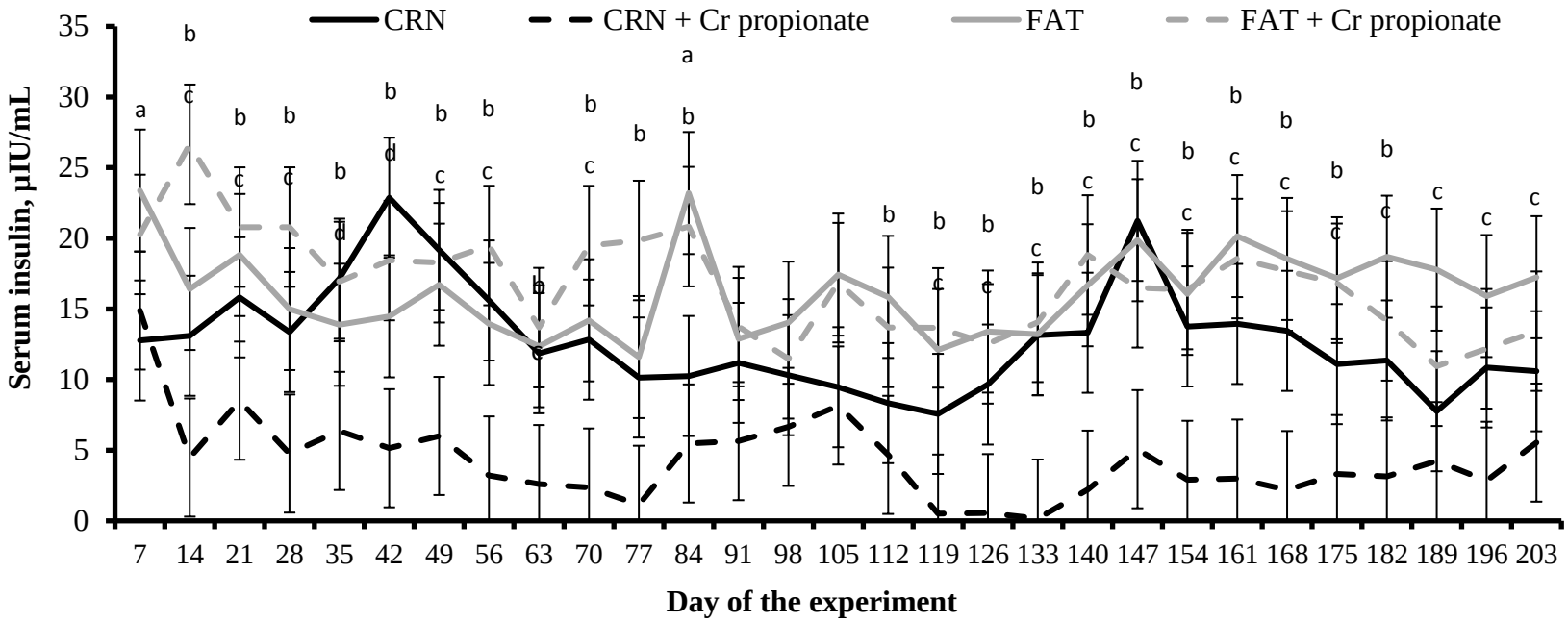

CRN
CRN + Cr propionate
FAT
FAT + Cr propionate
Serum insulin, µIU/mL
0
5
10
15
20
25
30
35
7 14 21 28 35 42 49 56 63 70 77 84 91 98 105 112 119 126 133 140 147 154 161 168 175 182 189 196 203
Day of the experiment

Figure 3. Serum insulin:glucose ratio of lactating dairy cows consuming excessive energy, and receiving in a 2 × 2 factorial arrangement design: 1) concentrate based only on ground corn CRN; n = 20) or including 8% (DM basis) of Ca salts of palm oil (EnerFAT^{TM} Kemin Agrifoods South America, Indaiatuba, São Paulo, Brazil**; CSPO**; n = 20), and 2) supplemented (n = 20) or not (n = 20) with 2.5 g/d of Cr-propionate (10 mg of Cr/cow daily; KemTrace 0.4% Cr; Kemin Agrifoods South America). Values obtained on d 0 were included as covariate; therefore, values reported are covariately-adjusted means. A concentrate type × Cr supplementation × day interaction was detected ($P = 0.02$). Within days ($P \leq 0.05$); a = CRN + Cr-propionate vs. CSPO + Cr-propionate, b = CRN + Cr-propionate vs. CSPO, c = CSPO vs. CSPO + Cr-propionate, d = CRN vs. CRN + Cr-propionate.

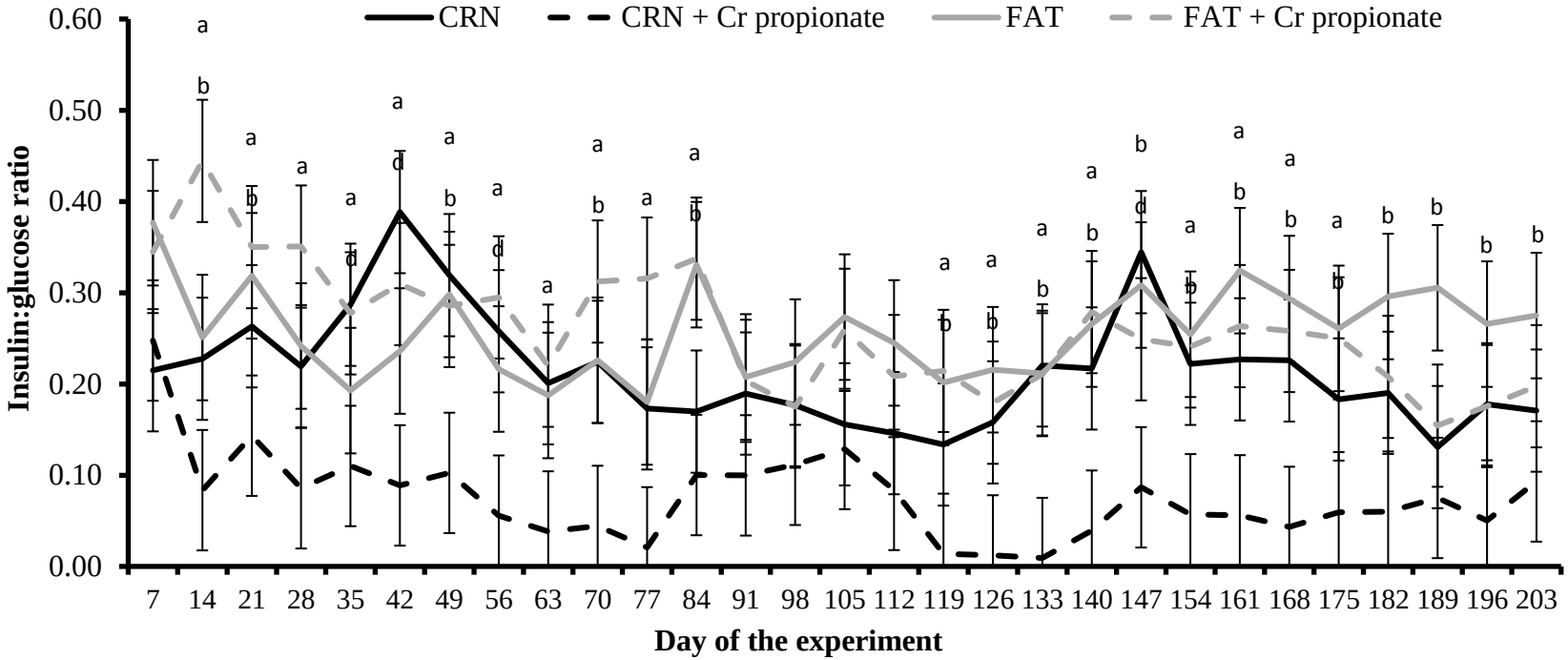


Figure 4. Serum glucose concentrations following a glucose tolerance test (intravenous infusion of 0.5 g of glucose/kg of body weight at 0 min) of lactating dairy cows consuming excessive energy, and receiving in a 2 × 2 factorial arrangement design: 1) concentrate based only on ground corn CRN; n = 20) or including 8% (DM basis) of Ca salts of palm oil (EnerFAT™ Kemin Agrifoods South America, Indaiatuba, São Paulo, Brazil**; CSPO**; n = 20), and 2) supplemented (n = 20) or not (n = 20) with 2.5 g/d of Cr-propionate (10 mg of Cr/cow daily; KemTrace 0.4% Cr; Kemin Agrifoods South America). Values obtained on d 0 were included as covariate; therefore, values reported are covariately-adjusted means. A concentrate type × min interaction was detected ($P < 0.01$). Within min, ** $P \leq 0.01$.

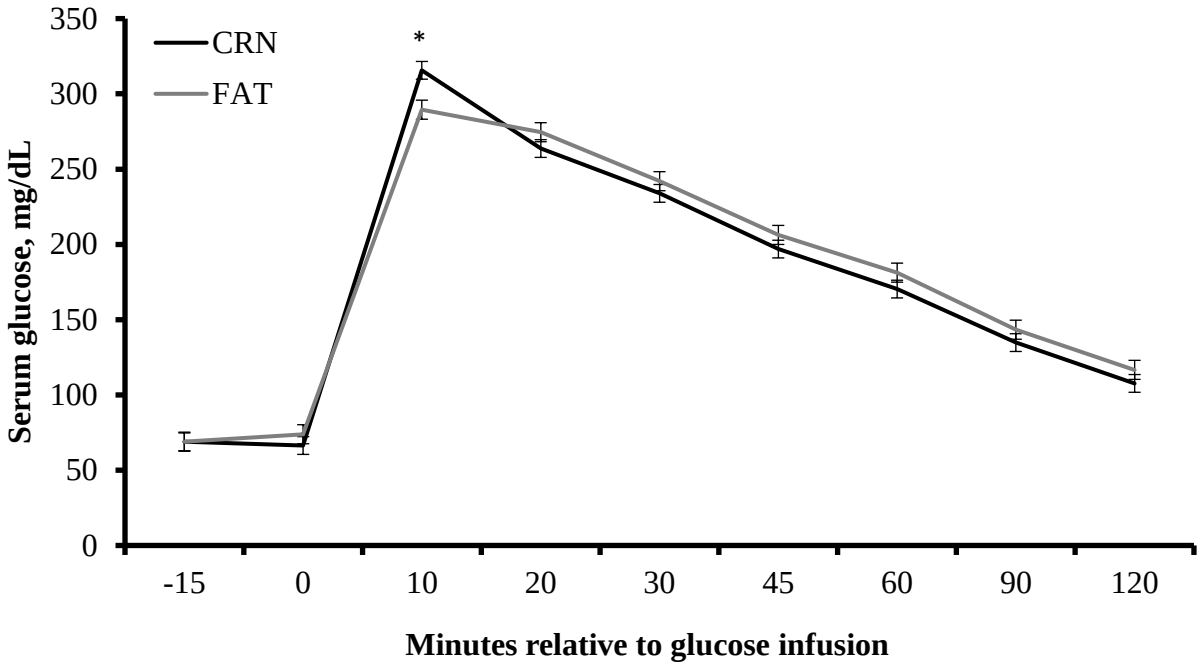


Figure 5. Serum insulin concentrations following a glucose tolerance test (intravenous infusion of 0.5 g of glucose/kg of body weight at 0 min) of lactating dairy cows consuming excessive energy, and receiving in a 2 × 2 factorial arrangement design: 1) concentrate based only on ground corn CRN; n = 20) or including 8% (DM basis) of Ca salts of palm oil (EnerFAT™ Kemin Agrifoods South America, Indaiatuba, São Paulo, Brazil**; CSPO**; n = 20), and 2) supplemented (n = 20) or not (n = 20) with 2.5 g/d of Cr-propionate (10 mg of Cr/cow daily; KemTrace 0.4% Cr; Kemin Agrifoods South America). Values obtained on d 0 were included as covariate; therefore, values reported are covariately-adjusted means. Concentrate type × min (Panel A, $P = 0.02$) and Cr supplementation × min (Panel B, $P = 0.03$) interactions were detected. Within min, ** $P \leq 0.01$, * $P \leq 0.05$, and † = $P \leq 0.10$

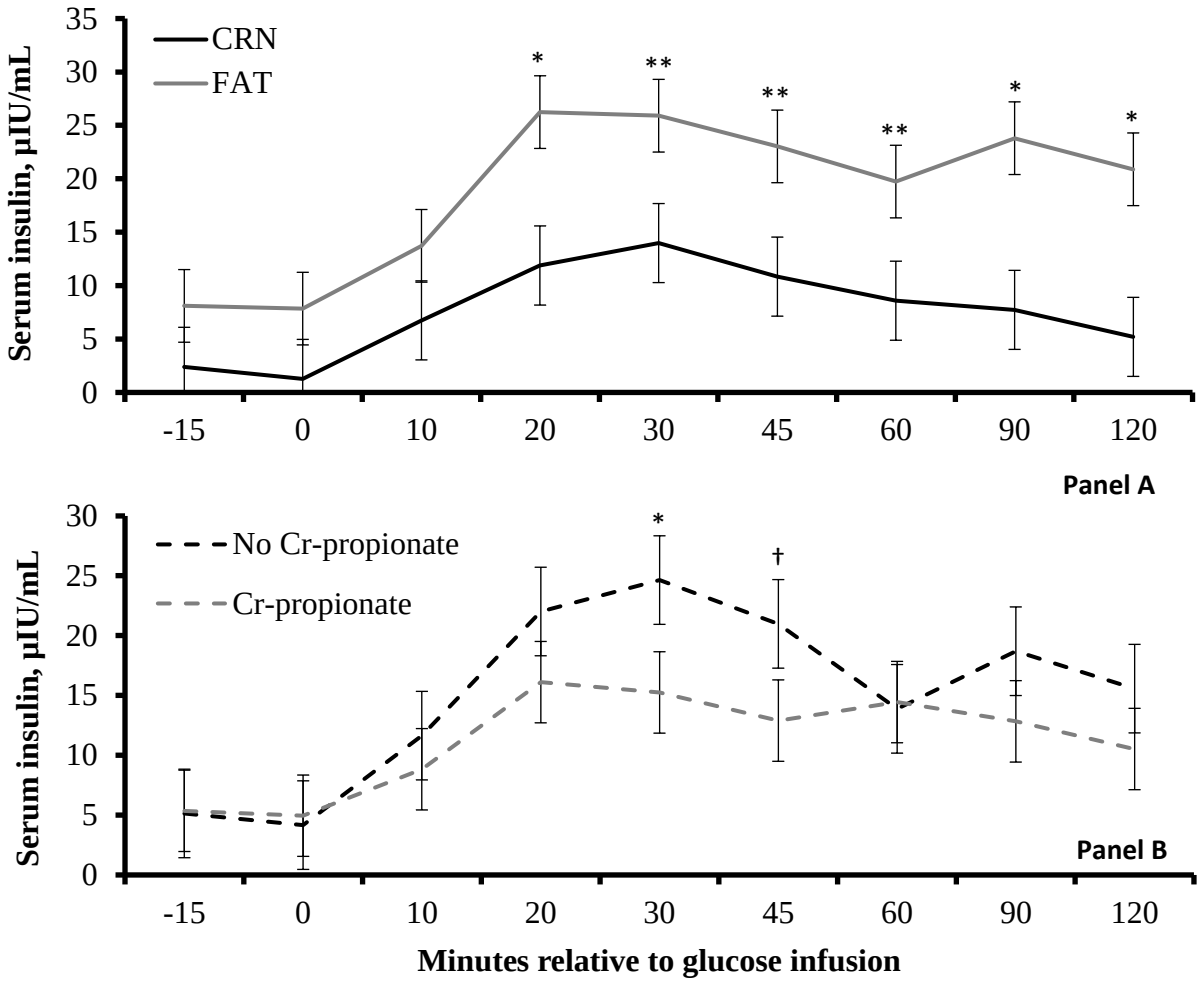
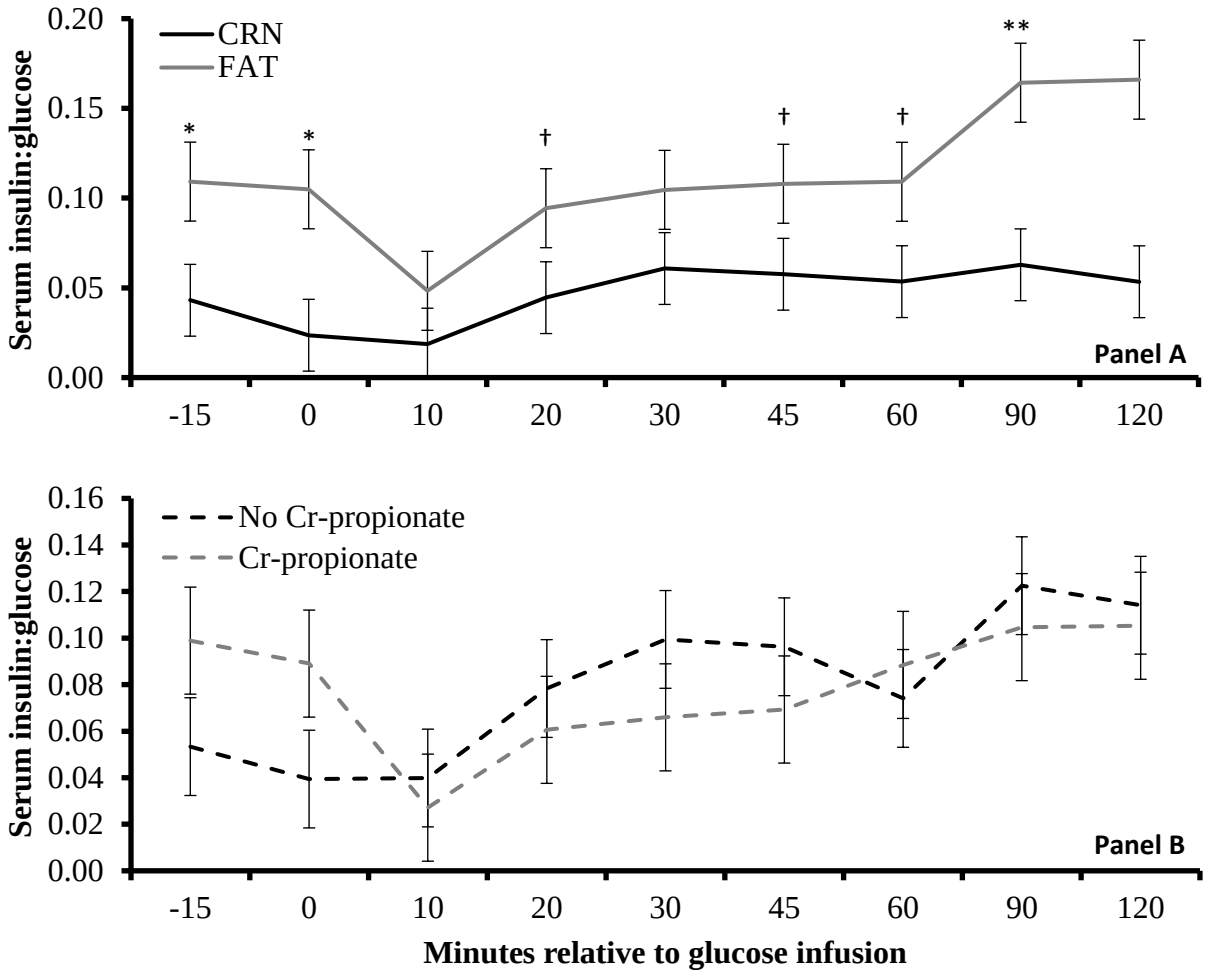


Figure 6. Serum insulin:glucose ratio following a glucose tolerance test (intravenous infusion of 0.5 g of glucose/kg of body weight at 0 min) of lactating dairy cows consuming excessive energy, and receiving in a 2 × 2 factorial arrangement design: 1) concentrate based only on ground corn CRN; n = 20) or including 8% (DM basis) of Ca salts of palm oil (EnerFAT™ Kemin Agrifoods South America, Indaiatuba, São Paulo, Brazil**; CSPO**; n = 20), and 2) supplemented (n = 20) or not (n = 20) with 2.5 g/d of Cr-propionate (10 mg of Cr/cow daily; KemTrace 0.4% Cr; Kemin Agrifoods South America). Values obtained on d 0 were included as covariate; therefore, values reported are covariately-adjusted means. Concentrate type × min (Panel A, $P < 0.01$) and Cr supplementation × min (Panel B, $P < 0.01$) interactions were detected. Within min, ** $P \leq 0.01$, * $P \leq 0.05$, and † = $P \leq 0.10$


0.20
0.15
0.10
0.05
0.00
Serum insulin:glucose
CRN
FAT
*
*
†
†
†
**
Panel A
-15
0
10
20
30
45
60
90
120
0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
Serum insulin:glucose
No Cr-propionate
Cr-propionate
Panel B
-15
0
10
20
30
45
60
90
120
Minutes relative to glucose infusion

Implicações

Estados de resistência à insulina pode estar envolvido com consumo excessivo de energia em vacas leiteiras lactantes ou não. Em 2014, nosso grupo de pesquisa começou a estudar a influência do consumo excessivo de energia em vacas leiteiras não lactantes consumindo dietas a base de milho que visavam a atender 160% dos requerimentos de mantença dos animais. Em 2015, similar estudo foi realizado substituindo vacas não lactantes por vacas em meio de lactação. Ambos os estudos tinham por objetivo investigar se o alto consumo de energia aliado a dietas que continham milho como base energética, favoreciam o aparecimento de resistência à insulina. Com esses estudos fomos capazes de verificar que o consumo excessivo de energia utilizando dietas a base de milho foi efetivo em causar resistência à insulina.

Em estados de balanço energético positivo, resistência à insulina é descrita como uma síndrome metabólica marcada pela insensibilização dos tecidos periféricos a insulina levando a um aumento nas concentrações circulantes deste hormônio (diminuição da ligação insulina e receptores de membrana) podendo acarretar em queda do desempenho produtivo, reprodutivo e aumento na incidência de doenças, tornando-se um fator de grande relevância ao sistema produtivo de gado leiteiro. Assim, estratégias que visam mitigar o aparecimento desta síndrome é de fundamental importância para melhoria do sistema produtivo.

Nos dois trabalhos acima mencionado, nosso grupo de pesquisa observou que a suplementação de propionato de cromo para animais consumindo excesso de energia recebendo dietas a base de milho era eficaz em diminuir a resistência à insulina, provavelmente devido ao cromo ser um facilitador da interação insulina-receptor aumentando a captação de glicose pelas células insulinodependentes.

Sabendo que aumento nas concentrações de insulina está relacionado com surgimento de resistência à insulina e que a suplementação com propionato de cromo pode diminuir essa síndrome em vacas leiteiras, essa tese teve como objetivo buscar estratégias que poderiam ajudar na diminuição da resistência à insulina proporcionando melhoria nos índices produtivos dos animais. Conduzimos dois estudos utilizando diferentes fontes de energia e suplementação com propionato de cromo em dietas que favoreciam o alto consumo energético em vacas em meio de lactação. Já que as concentrações de insulina são aumentadas em resposta ao aumento das concentrações de glicose e que dietas a base que de milho favorecem essa resposta, passamos a substituir parte do milho do concentrado por polpa cítrica peletizada ou gordura de palma protegida, ingredientes que não visam o aumento glicêmico. Ainda, avaliamos se a suplementação com propionato de cromo nessas dietas seria eficiente em diminuir resistência à insulina.

No primeiro estudo onde comparamos substituição de milho por polpa cítrica peletizada observamos que adição de polpa cítrica no concentrado minimizou resistência à insulina independente do fornecimento de propionato de cromo, porém em dietas a base de milho a adição de propionato de cromo se fez necessária para que a resistência à insulina fosse minimizada.

No segundo estudo gordura protegida aumentou a resistência à insulina e mais uma vez propionato de cromo foi eficiente em diminuir resistência à insulina quando os animais eram alimentados com dietas a base de milho.

Em ambos os estudos foram avaliados parâmetros produtivos e reprodutivos dos animais, os quais foram semelhantes entre os grupos. No entanto, esse estudo foi desenhado com o intuito de avaliar os efeitos das dietas nas variáveis metabólicas, tornando assim necessário a realização de outros estudos para um melhor entendimento do real impacto dessa síndrome nas variáveis produtivas e reprodutivas dos animais.

De modo geral, esses estudos mostraram que a utilização de polpa cítrica no concentrado pode ser uma ferramenta interessante para promover diminuição na resistência à insulina, enquanto que a adição de gordura protegida saturada pode ser um fator agravante à essa síndrome, porém a adição de cromo em dietas que utilizam milho como principal ingrediente energético é efetiva em mitigar resistência à insulina.