FERNANDA FERREIRA DOS SANTOS

Nutrição energética de ovelhas e implicações na reprodução e programação fetal

Pirassununga

2021

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Tese apresentada ao Programa de Pós-Graduação em Nutrição e Produção Animal da Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo para a obtenção do título de Doutor em Ciências.

Departamento:

Nutrição e Produção Animal

Área de concentração:

Nutrição e Produção Animal

Orientador:

Profa. Dra. Sarita Bonagurio Gallo

Coorientador:

Prof. Dr. Augusto Hauber Gameiro

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CERTIFICADO

Certificamos que a proposta intitulada "Nutrição Energetica de Ovelhas e Implicaçoes na Reprodução, Programação Fetal e Genética.", protocolada sob o CEUA nº 2700201218 (ID 006443), sob a responsabilidade de **Sarita Bonagurio Gallo** e *equipe; FERNANDA FERREIRA DOS SANTOS* - que envolve a produção, manutenção e/ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto o homem), para fins de pesquisa científica ou ensino - está de acordo com os preceitos da Lei 11.794 de 8 de outubro de 2008, com o Decreto 6.899 de 15 de julho de 2009, bem como com as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), e foi **aprovada** pela Comissão de Ética no Uso de Animais da Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo (CEUA/FMVZ) na reunião de 17/04/2019.

We certify that the proposal "Energetic Nutrition of Sheep and Implications in Reproduction, Fetal Programming and Genetics.", utilizing 111 Ovines (111 females), protocol number CEUA 2700201218 (ID 006443), under the responsibility of **Sarita Bonagurio Gallo** *and team; FERNANDA FERREIRA DOS SANTOS* - which involves the production, maintenance and/or use of animals belonging to the phylum Chordata, subphylum Vertebrata (except human beings), for scientific research purposes or teaching - is in accordance with Law 11.794 of October 8, 2008, Decree 6899 of July 15, 2009, as well as with the rules issued by the National Council for Control of Animal Experimentation (CONCEA), and was **approved** by the Ethic Committee on Animal Use of the School of Veterinary Medicine and Animal Science (University of São Paulo) (CEUA/FMVZ) in the meeting of 04/17/2019.

Finalidade da Proposta: Pesquisa

Vigência da Proposta: de 03/2017 a 03/2020 Área: Nutrição E Produção Animal

Origem:	Animais provenientes de outros projetos						
Espécie:	Ovinos	sexo:	Fêmeas	idade:	2 a 8 anos	N:	71
Linhagem:	mestiças			Peso:	33 a 90 kg		
Origem:	Animais provenientes de outros projetos						
Espécie:	Ovinos	sexo:	Fêmeas	idade:	2 a 14 meses	N:	40
Linhagem:	mestiças			Peso:	15 a 80 kg		

Local do experimento: Prefeitura Fernando Costa, Universidade de São Paulo, Campus de Pirassununga.

Comentário da CEUA: Foi apresentado a certificado de aprovação pela CEUA da FZEA. Gostaríamos de lembrar que todos os projetos que venham a ser desenvolvidos como orientadora da FMVZ, deverão ser submetidos a esta CEUA, independente de sua aprovação por outro orgão de ensino e pesquisa

São Paulo, 02 de fevereiro de 2021

Prof. Dr. Marcelo Bahia Labruna
Coordenador da Comissão de Ética no Uso de Animais
Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo

Camilla Mota Mendes
Vice-Coordenadora da Comissão de Ética no Uso de Animais
Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo

FOLHA DE AVALIAÇÃO

Autor: Santos, Fernanda Ferreira dos

Título: **Nutrição energética de ovelhas e implicações na reprodução e programação fetal**

Tese apresentada ao Programa de Pós-Graduação em Nutrição e produção Animal da Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo para obtenção do título de Doutor em Ciências.

Data: _____/_____/_____

Banca Examinadora

Prof. Dr.____

Instituição:___________________________Julgamento:____________________

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Prof. Dr.____

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Prof. Dr.____

Instituição:___________________________Julgamento:____________________

DEDICATÓRIA

À minha família, meu porto seguro.

AGRADECIMENTOS

Não há palavras suficientes para agradecer à minha família, meus pais (Cidinha e Wagner), meus irmãos (Carla, Paulo, Júnior e Jess) e minhas sobrinhas perfeitas, Lina e Luna. Obrigada por todo amor incondicional, todo apoio, aprendizados, risadas, broncas.

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“Nada vale mais do que sorrir. É uma força sorrir e abandonar a si mesmo, para ser leve.”

Frida Kahlo

“Faça o seu melhor em cada momento.”

Monja Coen

RESUMO

SANTOS, F.F. **Nutrição energética de ovelhas e implicações na reprodução e programação fetal.** 2021. 240 f. Tese (Doutorado em Ciências) – Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, Pirassununga, 2021.

A nutrição corresponde à grande parte do custo total de produção de ovinos, sendo a energia da dieta fator determinante para um bom desempenho zootécnico. O objetivo deste trabalho foi verificar os efeitos de diferentes níveis nutricionais (90%, 100% e 110% do recomendado), e diferentes fontes de energia (amido, amido com propionato de cromo e gordura protegida) na nutrição de ovinos. Para isso, dois experimentos foram realizados. No primeiro, 72 ovelhas mestiças foram divididas em cinco tratamentos, de acordo com as exigências do NRC: CTL (n=14) 100% dos requerimentos para energia metabolizável, LOW (n=14) 90% do recomendado, ST (n=15) 110% do recomendado sendo o amido a fonte energética; ST+Cr (n=15) dieta ST com suplementação de cromo propionato e ST+RPF (n=14) dieta ST com suplementação de sais de cálcio de óleo de palma. As dietas foram fornecidas durante a estação de monta, início de gestação, final de gestação e lactação. A progênie foi desmamada com 80 dias e as cordeiras ficaram em mesmo piquete recebendo dieta para fase de crescimento, de acordo com o NRC. Análises reprodutivas e de desempenho tanto das ovelhas quanto das filhas foram avaliadas. Os dados de índices reprodutivos e de análises comportamentais foram considerados não paramétricos e analisados pelo Teste exato de Fisher a 5% de significância e Kruskall-Wallis a 10% de significância. Os dados de desempenho foram analisados pelo teste de Tukey a 5% de significância. Análise economica de curto prazo e um fluxo de caixa de 10 anos foram elaborados e analisados No experimento dois, 32 borregas desmamadas foram confinadas e divididas nos tratamentos: CTL, ST, ST+Cr e ST+RPF. Análises de desempenho e econômicas foram realizadas e analisadas pelo teste de Tukey a 5% de significância. Dietas com baixa energia têm alta mortalidade e baixo desempenho de ovelhas e borregas ($P<0.05$) e, apesar de baixo custo variável, não é viável economicamente (valor presente líquido negativo). Dieta ST melhora o desempenho reprodutivo e

produtivo de ovelhas e sua prole ($p<0.05$), sendo viável economicamente (valor presente líquido positivo e taxa interna de retorno >9%). A suplementação com cromo na gestação proporciona o melhor desempenho econômico (maior valor presente líquido e taxa interna de retorno >13%). A suplementação com gordura protegida tem efeitos negativos na reprodução da ovelha e de suas filhas ($P<0.05$), não sendo viável sua utilização durante a gestação, mas para fêmeas em confinamento proporciona um melhor rendimento de carcaça ($P<0.05$).
Palavras-chave: Energia. Desenvolvimento fetal. Subnutrição. Gordura Protegida. Cromo propionato

ABSTRACT

SANTOS, F.F. **Ewes energy nutrition and implications for reproduction and fetal programming.** 2021. 240 f. Tese (Doutorado em Ciências) – Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, Pirassununga, 2021.

Nutrition accounts for a large part of the total cost of sheep production, with dietary energy being a determining factor for good zootechnical performance. The objective of this work was to verify the effects of different nutritional levels (90%, 100% and 110% of the recommended), and different sources of energy (starch, starch with chromium propionate and protected fat) on sheep nutrition. For this, two experiments were carried out. In the first 72 crossbred ewes were divided into five treatments, according to the requirements of the NRC: CTL (n = 14) 100% of the requirements for metabolizable energy, LOW (n = 14) 90% of the recommended, ST (n = 15) 110% of the recommended, with starch being the energy source; ST + Cr (n = 15) ST diet with supplementation of chromium propionate and ST + RPF (n = 14) ST diet with supplementation of calcium salts from palm oil. Diets were provided during the breeding season, beginning of pregnancy, end of pregnancy and lactation. The progeny were weaned at 80 days and the lambs were in the same paddock receiving a diet for the growth phase, according to the NRC. Reproductive and performance analyzes of both sheep and daughters were evaluated. The data of reproductive indices and behavioral analyzes were considered non-parametric and analyzed by the Exact fisher test at 5% significance and Kruskall-wallis at 10% significance, respectively. Performance data were analyzed using the Tukey´s test at 5% significance level. Short-term economic analysis and a 10-year cash flow were prepared. In experiment two, 32 weaned lambs were confined and divided into treatments: CTL, ST, ST + Cr and ST + RPF. Performance and economic analyzes were performed and analyzed by Tukey's test at 5% significance. Low energy diets have high mortality and low performance of sheep and lambs ($P < 0.05$) and, despite low variable cost, it is not economically viable (negative net present value). ST diet improves the reproductive and productive performance of sheep and their offspring ($p < 0.05$), being economically viable (positive net present value and internal rate of return> 9%). Chromium supplementation during pregnancy provides the best economic performance (highest net present value and internal rate of return> 13%). Supplementation with protected fat has negative effects on the reproduction of sheep and their daughters (P <0.05), not being viable during pregnancy, but for confined females it provides a better carcass yield (P <0.05).

Keywords: Energy. Fetal development. Malnutrition. Protected fat. Chromium propionate.

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ADF	Acid detergent fiber
ADG	Average daily gain
AFRC	Agricultural and Food Research Council
ALT	Alanine Aminotransferase
ANVISA	Agência nacional de vigilância sanitária
AST	Aspartate Aminotransferase
BCS ou ECC	Body condition score
BHB	Beta-hidroxibutirato
BW ou PV	Body weight
BWG	Body weight gain
Ca	Calcium
CaOFA	Calcium salts of olive oil
CaSSO	Calcium salts of soy oil
CAT	Catalase
CCW	Cold carcass weight
CF	Crude fiber
CHOL	Total cholesterol
CLA	Conjugated Linoleic Acid
CM	Contribution margin
CMU	Contribution margen per unit
CP ou PB	Crude protein
CRBC	Chickens red blood cells
CrCl3	Chromium chloride
Cr^{III}	Trivalent chromium
CrLys	Chromium lysine--
CrMet	Chromium metionine
CrNic	Chromium nicotinate
CrPic	Chromium picolinate
CrPro	Chromium propionate
Cr^{VI}	Hexavalent chromium
CrYst	Chromium Yeast
CS	Calcium salts

CSFA	Calcium salts of fatty acids
CSIRO	Commonwealth Scientific and Industrial Research Organisation
CWC	Cell wall constituents
DHA	Docosahexaenoic acid (C22:6)
DM	Dry matter
DMI	Dry matter intake
EE	Ethereal extract
EL ou NE	Net energy
eNos	Endothelial nitric acid
EPA	Eicosapentaenoic acid (C20:5)
EPG	Eggs per gram
ESO	Emulsified soy oil
FA ou AG	Fatty acid
FBW	Final body weight
FC	Cash flow
FCR	Feed conversion rate
FDA	Food and Drug Administration
FFA	Free fatty acid
GGT	Gamma-glutamyl transferase
GHR	Growth hormone receptor
GnRH	Gonadotropin-releasing hormone
GUCY1b3	Guanylate Cyclase 1 Beta 3
HCW	Hot carcass weight
HDL	High density lipoprotein
HPO	Hydrogenated palm oil
IGF1	Insulin-like growth factor type 1
IGF2	Insulin-like growth factor type 2
IgG	Immunoglobulin G
IL-6	Interleukin-6
INRA	Institut National de la Recherche Agronomique
IRR	Internal rate of return
IVGTT	Intravenous glucose tolerance test
IVICT	Insulin challenge test

LDL	Low density lipoprotein
LH	Luteinizing hormone
LT	*Longissimus Thoracis*
MDA	Malondialdehyde
ME ou EM	Metabolizable energy
MFD	Milk fat depression
MUFA	Monounsaturated fatty acid
MUN	Milk urea nitrogen
N	Nitrogen
nCrPic	Nano chromium picolinate
NDF	Neutral detergent fiber
NEFA	non-esterified fatty acids
NOS3	Nitric Oxide Synthase 3
NPV	Net presente value
NRC	National Research Council
OM	Organic matter
OT	Oxytocin
P	Profit
PHA	Phytohaemagglutinin
PS	Period for heard stabilization
PU	Profit per unit
PUFA	Polyunsaturated fatty acids
ROS	Reactive oxygen species
SFA	Saturated fatty acid
SO	Soy oil
SOD	Superoxide dismutase
T3	Triiodothyronine
T4	Thyroxine
TDN	Total digestive nutrients
TG	Triglycerides
TNF-α	Tumor necrosis fator α
VLDL	Very low density lipoprotein
YE	Yucca extract

SUMÁRIO

INTRODUÇÃO GERAL E OBJETIVOS

O número estimado de ovinos no mundo é de 1.238.719.591, com um aumentou de 4,63% no rebanho mundial entre os anos de 2015 a 2019, e um crescimento de 5,13% na produção de carne (FAOSTAT, 2021). No Brasil, neste mesmo período e pela estimativa da mesma agência, o aumento no número de cabeças foi de 7,08% e na produção de carne de 7,97%, com valor atual de 19.715.587 cabeças. Observa-se um aumento na produtividade animal, a qual é possível devido às melhorias de manejos e, principalmente, às melhorias na nutrição animal. De fato, para garantir a rentabilidade com a produção de carne de cordeiro é necessário ter escala de produção e para isso são necessários planejamento e investimento em tecnologias (RAINERI; NUNES; GAMEIRO, 2015).

A ingestão de energia é o principal fator determinante na criação de ruminantes, sendo fundamental para sobrevivência e produtividade animal (GUIMARÃES et al., 2012). Os nutrientes absorvidos seguem uma ordem preferencial, sendo que as atividades relacionadas à reprodução, como ciclo estral e início de gestação, são de menor prioridade (MAGGIONE et al., 2008).

Assim, erros nutricionais afetam a reprodução, diminuindo a fertilidade e o retorno ao cio, atrasando a puberdade e aumentando a mortalidade embrionária e de recém-nascidos (CHILLIARD; BOCQUIER; DOREAU, 1998; BINDARI et al., 2013). Contudo a manipulação da dieta, com aumento do aporte de energia fornecida, pode ser benéfica para aumentar as taxas de concepção, como feito no caso do flushing alimentar na estação de monta (HABIBIZAD et al., 2015a, 2015b). O aumento da energia na dieta também melhora o escore de condição corporal (ECC) e o peso vivo próximo ao parto e aumenta a produção de leite e colostro (MCGOVERN et al., 2015a; DONNEM et al., 2020).

Além dos efeitos diretos na reprodução e desempenho da fêmea, a nutrição durante a gestação também interfere no desenvolvimento fetal, a chamada “programação fetal” (DU et al., 2015). Erros nutricionais podem levar a produção de cordeiros com menor capacidade reprodutiva, menor crescimento, menor ganho de peso, diminuindo a produtividade do rebanho. Em contrapartida, sabendo-se os efeitos da nutrição no feto, é possível manipular as dietas maternas a fim de aumentar a produtividade da prole (KENYON, 2013; ASMAD et al., 2015; WILSON; FAULKNER; SHIKE, 2016; PIAGGIO et al., 2018; CASTRO-RODRÍGUEZ et al., 2020; LOPES et al., 2020; RAMÍREZ et al., 2020).

A principal forma de aumentar o fornecimento de energia na dieta, principalmente no final de gestação, quando o espaço para o enchimento ruminal é diminuído, é aumentando o fornecimento de amido ou de gordura. A partir do amido fermentado no rúmen, maiores quantidades de propionato são produzidos, aumentando a glicose disponível no sangue, por meio da gliconeogênese hepática (GÓMEZ; POSADA; OLIVERA, 2016; FERREIRA et al., 2020).

Já as fontes de gordura podem fornecer duas vezes mais energia do que os grãos (HESS; MOSS; RULE, 2008), sendo que os efeitos negativos na digestibilidade da fibra e fermentação ruminal podem ser evitados com o uso de gorduras na forma protegida como, por exemplo, o uso de sais de cálcio de ácidos graxos (BIONAZ; VARGAS-BELLO-PÉREZ; BUSATO, 2020). Além disso, podem ser utilizados aditivos alimentares que auxiliem na melhor utilização da energia fornecida na dieta, como é o caso do cromo propionato. O cromo intensifica a sinalização da insulina, tendo efeitos positivos no metabolismo da glicose, além de auxiliar no metabolismo lipídico e proteico. Contudo, seu uso na alimentação animal é ainda recente e com poucos resultados conclusivos (VINCENT, 2000, 2014; LINDEMANN, 2007; LASHKARI; HABIBIAN; JENSEN, 2018).

Para que as tecnologias e novos manejos sejam implementados a campo, é necessário mostrar suas consequências econômicas, para que os produtores percebam o quanto poderiam agregar na propriedade. É importante ressaltar que o principal obstáculo para viabilidade econômica na produção de ovinos é a baixa tecnificação da atividade (RAINERI; STIVARI; GAMEIRO, 2015). Avaliações econômicas, incluindo análises de curto e longo prazo, são estruturas para a tomada de decisões (UDEH, 2020). Estudos nesse sentido, com ovinos, são bem menos realizados e publicados (RAINERI; STIVARI; GAMEIRO, 2015).

O objetivo deste trabalho é verificar os efeitos da nutrição energética para ovinos, tanto de diferentes níveis (90%, 100% e 110% do recomendado), quanto fontes de energia (amido, propionato de cromo e gordura protegida), com ênfase na reprodução e desempenho das ovelhas, consequências na progênie fêmea e na viabilidade econômica.

As hipóteses são:

- A subnutrição tem efeitos negativos na reprodução da fêmea e no desempenho da progênie, não sendo viável economicamente apesar do menor investimento na alimentação.

- O excesso de energia fornecida na dieta tem efeitos positivos na reprodução da fêmea e no desempenho da progênie, sendo viável economicamente, apesar do maior investimento na alimentação.
- Tanto o cromo quanto a gordura protegida melhoram o desempenho da ovelha e da prole, sendo economicamente viáveis.

Esta tese foi escrita em formato de capítulos, sendo cada capítulo referente a um artigo científico. Além disso, ela foi dividida em dois módulos: (1) Revisões de literatura e (2) Artigos completos. O módulo (1) apresenta quatro artigos de revisão que serão submetidos a revistas científicas: (a) “Nutrição energética de ovelhas: níveis de energia e consequências reprodutivas”; (b) “Protected fat supplementation in sheep diet: a review”; (c) “Chromium upplementation and ewe production: a systematic review” e (d) “Effects of ewe nutrition on the development of their offspring: the role of fetal programming”.

Para o módulo (2), mais quatro artigos completos foram escritos. O artigo 1 “The addition of chromium in a high energy diet for pregnant ewes has a beneficial effect on reproduction traits for both ewes and their offspring” será submetido para a revista Scientia Agricola (JCR 1,625). O artigo 2 “Performance and behavior of the progeny of ewes fed with different sources and energy feeds” foi submetido para a revista Livestock Science (JCR 1,7) e encontra-se em “under review” na data de fevereiro de 2021. O artigo 3 “Economic performance of high energy diets and supplementation with chromium or protected fat in ewes production” será submetido para a Revista Brasileira de Zootecnia (JCR 0,853). O artigo 4 “Feedlot of lambs supplemented with rumen protected fat or chromium propionate: performance and economic evaluation” está em “under review” na revista Tropical Animal Health and Production (JCR 1,333) na data de fevereiro de 2021.

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MÓDULO 1. REVISÕES DE LITERATURA

CAPÍTULO 1.

Nutrição energética de ovelhas: níveis de energia e consequências reprodutivas

Resumo Em ruminantes, a principal fonte de energia são os ácidos graxos voláteis, produzidos por meio da fermentação de carboidratos no rúmen. Os requerimentos de energia são descritos em sistemas de alimentação diversos como NRC, AFRC, INRA e CSIRO, mas há trabalhos determinando a exigência nutricional de determinadas raças e regiões, o que os tornam mais acurados. A restrição alimentar da ovelha causa prejuízos tanto reprodutivos quanto no desempenho de cordeiros. Fêmeas subnutridas tem menor taxa de ovulação, menor taxa de prenhez e demoram mais para retornar ao estro no pós parto. Fornecer dietas acima das exigências pode ser benéfico para animais em crescimento e em estação de monta (flushing alimentar). Contudo, é necessário controlar o escore de condição corporal de animais em reprodução para que fiquem próximo ao ideal para cada fase reprodutiva.

Palavras-chave: níveis energia; nutrição; ovinos; reprodução.

Introdução

A maior parte da energia consumida pelos ruminantes é derivada de polissacarídeos presentes na parede de células vegetais (celulose, hemicelulose e pectina) ou de carboidratos não-fibrosos, como o amido (KOZLOSKI, 2012). O alimento ingerido sofre fermentação no rúmen, resultando na produção de ácidos graxos voláteis (acetato, butirato e propionato), os quais são responsáveis por 50-85% do fornecimento de energia metabolizável (HARMON; SWANSON, 2020). Nesses animais, grande parte da glicose é proveniente da gliconeogênese hepática cujo principal percursor é o propionato (CALDEIRA et al., 2007b). As proporções molares de acetato:propionato:butirato são variáveis e dependem da dieta. Dietas ricas em carboidratos fibrosos produzem maior proporção de acetato, enquanto que o maior fornecimento de amido aumenta a proporção do propionato (GOULARTE et al., 2011).

Os requerimentos de energia geralmente são descritos, nas tabelas de exigência nutricional, como "energia metabolizável (EM)" ou "energia líquida (EL)". EM é definida como o calor de combustão da dieta consumida menos o calor de combustão do metano, urina e fezes decorrentes de sua digestão e a EL é a energia efetivamente obtida pelo animal com sua dieta e usada para manutenção, crescimento, gestação ou lactação (EM menos o incremento calórico) (NRC, 2007; DOVE, 2010).

O requerimento nutricional é calculado para mantença ou produção. A exigência de mantença é a energia necessária para que os processos vitais do seu corpo permaneçam normais, em outras palavras, é a energia utilizada pelo animal quando não está sofrendo alterações na sua composição corporal. No caso da energia, essa exigência de mantença é referente ao consumo de oxigênio pelo corpo, principalmente pelo trato gastrointestinal e fígado para absorção e metabolismo dos alimentos (NRC, 2007; RESENDE et al., 2008). Condições como raça, sexo, idade, conforto térmico e nível de atividade influenciam a exigência energética de mantença.

A exigência em energia ultrapassa o requerimento de mantença quando o animal está em crescimento (aumento do tecido ósseo, muscular e adiposo), gestação (crescimento do útero, feto, placenta, fluidos fetais e glândula mamária) e lactação (GUIMARÃES et al., 2012).

Os nutrientes absorvidos tendem a seguir uma ordem de prioridade, sendo ela: metabolismo basal, atividades ou trabalho, crescimento, reserva de energia, gestação, lactação, ciclo estral, início de gestação e por último reserva de energia em excesso (SHORT; ADAMS, 1988; BINDARI et al., 2013). No caso de uma falha no plano nutricional, a reprodução é uma das primeiras e principais funções afetadas.

As exigências nutricionais de ovinos são descritas e tabuladas em diversos sistemas de alimentação e servem de base para os cálculos de formulação de ração. As normas mais utilizadas são: National Research Council (NRC, 2007) dos Estados Unidos, Institut National de la Recherche Agronomique (INRA, 2007) da França, Commonwealth Scientific and Industrial Research Organisation (CSIRO, 2007) da Austrália, Agricultural and Food Research Council (AFRC, 1993) do Reino Unido.

Por essas tabelas conterem informações mais generalizadas, alguns estudos com raças e condições de alimentação específicas foram realizados para determinar as exigências desses animais. Por exemplo, Zhang et al. (2018) determinaram os requerimentos de energia de mantença durante a gestação, para ovinos da raça Hu, na China; Pereira et al. (2018a) determinaram as exigências de mantença e crescimento para de cordeiros Morada Nova. Oliveira et al. (2018) reuniram em uma meta-análise, as exigências de energia para ovinos deslanados em região tropical e concluíram ser diferente de ovelhas lanadas de regiões temperadas. Salah et al. (2014) demonstraram que as exigências energéticas de animais em climas quentes são superiores as propostas pelas tabelas tradicionais (NRC, INRA, AFCR). E para Deng et al. (2014) e Ma et al. (2016), as tabelas de exigência disponíveis superestimam os requerimentos energéticos em cordeiros mestiços de Dorper. Para ovinos Santa Inês, os valores de exigência disponíveis do NRC (2007) são semelhantes às exigências de energia para cordeiros encontrado por Regadas Filho (2013), mas superiores a estimada por Pereira et al. (2017).

Outros estudos também indicam que o fornecimento de dietas acima dos requerimentos pode ser benéfico para melhorar o desempenho do animal. Fornecimentos de energia acima das recomendações de mantença é uma ferramenta importante para mitigar emissão de metano entérico e perda de eficiência energética (CHAOKAUR et al., 2015; ZHANG et al., 2018). Altos níveis de energia na dieta de ovelhas em crescimento (10.41MJ/ kg de EM) altera a proporção de ácidos graxos voláteis produzidos no rúmen, aumentando butirato, valerato e isso-valerato, além de otimizar a funcionalidade do rúmen (WANG et al., 2020a).

O aumento da energia na dieta pode ser atingido com o aumento de grãos ou o uso de fontes de lipídios. Contudo, há um limite de capacidade de digestão do amido pelos ruminantes pela atividade de carboidrase limitada, tempo insuficiente para hidrólise completa do amido, acesso inadequado de enzimas aos grânulos de amido e absorção limitada de glicose, além de que o escape de grande quantidade de amido para o intestino grosso pode provocar diarreia e acidose (HARMON; SWANSON, 2020). No caso de lipídios, o excesso pode diminuir o consumo do animal, a digestibilidade da fibra e a gordura no leite, além de ter efeito toxico para as bactérias ruminais, afetando a fermentação ruminal (GRUMMER, 1988; BIONAZ; VARGAS-BELLO-PÉREZ;

BUSATO, 2020). Assim, esta revisão tem por objetivo esclarecer os efeitos de diferentes níveis de energia na dieta de ovinos, com ênfase em aspectos reprodutivos.

Nutrição e Reprodução

Ovelhas são animais poliéstricos estacionais de dias curtos (SENGER, 2003), contudo em regiões tropicais, elas podem apresentam estro o ano todo, dependendo da qualidade e disponibilidade de alimento (ROSA; BRYANT, 2003).

A nutrição afeta a reprodução em todos os processos da cadeia reprodutiva, desde a gametogênese até puberdade e gestação (SCARAMUZZI et al., 2006a). Dentre os fatores nutricionais, o balanço energético é o principal fator relacionado à eficiência reprodutiva (BINDARI et al., 2013). Quando a ingestão de energia é menor do que o requerimento, o animal utiliza a energia armazenada no organismo (glicogênio, triglicerídeos ou proteína) e entra em balanço energético negativo. De forma semelhante, quando a ingestão é maior do que a exigência, o animal deposita o excesso de energia em forma de glicogênio ou triglicerídeos ou dispersa a energia em forma de calor metabólico, entrando em balanço energético positivo (SCARAMUZZI et al., 2006b). A tabela 1 relaciona os efeitos dos balanços energéticos com a reprodução da fêmea ovina, adaptado de Scaramuzzi et al. (2006b).

Tabela 1 *Alterações metabólicas e reprodutivas do balanço energético negativo e positivo*

	Balanço Energético Negativo	Neutro	Balanço Energético Positivo
Alterações Metabólicas	• Perda de peso • Diminuição no armazenamento de gordura • Debilidade muscular • Hipoinsulinemia • Baixa Leptina • Supressão do sistema IGF • Ureia aumentada	• Insulina normal • Glicose Normal • Leptina Normal • IGF normal • Ureia normal	• Hiperinsulinemia • Leptina aumentada • Estímulo do sistema IGF • Ureia normal (se a dieta for com alta proteína, a ureia pode estar elevada)
Alterações Reprodutivas	• Inibição da secreção do GnRH pelo hipotálamo • Ausência de pulsos de LH • Diminuição da concentração de FSH • Inibição da foliculogênese • Diminuição da concentração de estradiol • Anovulação • Anestro	• Funcionamento normal do eixo hipotálamo-hipófise-gônadas • Ovulação • Estro	• Funcionamento normal do eixo hipotálamo-hipófise-gônadas • Concentração de FSH aumentada • Aumento da foliculogênese • Diminuição do estradiol e da progesterona (com alta ingestão de MS) • Ovulação • Estro

Adaptado de Scaramuzzi et al. (2006b)

As reservas de gordura corpórea tem um papel importante para garantir a fertilidade de animais que passam por períodos de restrição energética e também tem benefícios em animais com nível de energia na dieta dentro das recomendações (GERNAND et al., 2008). Vatankhah et al. (2012) correlacionaram o escore de condição corporal (ECC) com eficiência reprodutiva utilizando 1099 dados gerados por 442 ovelhas durante três anos.

O ECC, considerado pelos autores, como ideal para a fase de estação de monta foi de 3 a 3,5, com correlação positiva para taxa de concepção, prolificidade e taxa de desmame.

De forma semelhante, Fernández-Foren et al. (2019) também comprovaram que o ECC influencia no desempenho reprodutivo de ovelhas subnutridas. Trinta e seis ovelhas com diferentes ECC (>2.75 ou <2,25) foram suplementadas com dois níveis de energia: controle e restrita (50% controle) no início da gestação. Como resultado observaram uma relação do ECC com resposta endócrina e expressão gênica uterina, sendo que ovelhas com maior ECC apresentam maior concentração de hormônios, número de embriões recuperados e maior taxa de viabilidade embrionária. Além disso, os animais subnutridos, mas com alto ECC inicial apresentaram aumento na expressão gênica uterina de GHR (receptor do hormônio do crescimento), sugerindo mecanismo compensatório contra fatores ambientais adversos.

O aumento de energia na dieta antes da estação de monta, chamado de “flushing”, já é uma prática consolidada e utilizada para melhorar os índices reprodutivos, como prolificidade, em ovinos (GUTIERREZ et al., 2011; HABIBIZAD et al., 2015a). Isso porque o aumento do fornecimento de energia gera um aumento no aporte de glicose, sendo este o maior substrato metabólico para o ovário, para o desenvolvimento folicular e para o sistema nervoso central, o qual regula a produção de hormônios envolvidos na reprodução (RABIEE; LEAN, 2000; VIÑOLES; MEIKLE; MARTIN, 2009; SCARAMUZZI; BROWN; DUPONT, 2010; SUTTON-MCDOWALL; GILCHRIST; THOMPSON, 2010; HABIBIZAD et al., 2015a).

Habibizad et al. (2015a) utilizou a estratégia de flushing alimentar (3.0 Mcal/kg MS) por 16 ou seis dias e comparou com animais controle (2.4Mcal/kg MS). O excesso de energia por 16 dias aumentou o número de folículos pequenos e grandes e, independente do tempo de flushing, a suplementação aumentou a porcentagem de ovulações duplas de 25% no controle, para 50% nos tratamentos com flushing e aumentou a concentração de glicose, insulina e colesterol plasmático.

Para confirmar os efeitos do flushing por curto período de tempo, Habibizad et al. (2015b) suplementou 45 ovelhas com uma dieta de alta energia ou dieta controle, quatro dias antes até um dia após a retirada do dispositivo de progesterona (totalizando 6 dias). A dieta com alta energia obteve maior número de folículos grandes, maior concentração de glicose, colesterol e insulina plasmática, além de maior concentração de estradiol, após a retirada do dispositivo de progesterona.

Farrag (2019) utilizou o flushing em pastagens áridas na zona sudeste do Egito e melhorou os índices reprodutivos de ovelhas, principalmente intensificando o estro, aumentando a prolificidade e a taxa de parição. Para Koyuncu e Canbolat (2009) ovelhas com 11.6 ou 12.2 MJ EM na dieta 21 dias antes da monta apresentaram estro em até 96 horas após a sincronização, diferente dos tratamentos de menor energia. Além disso, a prolificidade, fecundidade e peso do cordeiro ao nascer também foram maiores para essas ovelhas. Para Naqvi (2012), a utilização do flushing teve influência significativa no peso corporal, ganho de peso médio, porcentagem de estro, duração do estro, início do estro, resposta da ovulação, glicose plasmática, proteína total e ureia.

Mori et al. (2006) utilizaram 112 ovelhas para verificar o efeito de flushing apenas com suplementação de milho ou milho e soja, além de um tratamento controle (sem flushing). Ovelhas suplementadas tiveram maior ganho de peso e ECC até o final da estação de monta, contudo a dieta não alterou as taxas de concepção ou aumento de partos gemelares. A suplementação apenas com milho melhorou a taxa de natalidade.

El-Hag et al. (2007) suplementaram ovelhas durante o período de monta, 45 dias antes do parto ou uma combinação desses dois tratamentos, além do tratamento controle. Tratamento controle obteve os menores PV e maior perda de peso durante a gestação, menor taxa de concepção e de nascimento e maior taxa de aborto. A suplementação somente no período de monta melhorou a taxa de concepção e de nascimento, mas não afetou o peso ao nascer. Animais suplementados tanto na monta quanto no final de gestação tiveram os melhores resultados reprodutivos. Portanto, maior nível de suplementação energética antes da monta trás benefícios reprodutivos (KOYUNCU; CANBOLAT, 2009; NAQVI; SEJIAN; KARIM, 2012; FARRAG, 2019).

Debus et al. (2012) restringiram a alimentação (50% do requerido) de 64 ovelhas desde -15 até +30 dias após a concepção e mantiveram 52 ovelhas no grupo controle (100% do requerimento). O peso, ECC e a concentração de leptina diminuíram nos animais com restrição, enquanto que o cortisol e o NEFA aumentaram. Contudo, não houve diferenças para taxa de prenhez, prolificidade, peso do cordeiro ao nascer e mortalidade do cordeiro.

Macías-Cruz et al. (2017) verificaram os efeitos da restrição alimentar (60% dos requerimentos energéticos) por 30 dias antes da monta, por 50 dias após a monta ou restrição pré e pós monta (80 dias). O comportamento de estro, taxa de prenhez, taxa de aborto, período de gestação e prolificidade não foram afetados. A concentração de progesterona até oito dias após a monta também não foi afetada. Contudo, o escore, a condição corporal e o peso vivo (PV) foram alterados. A restrição antes da monta gerou animais de menor ECC e menor peso durante a monta, mas, no parto, apenas as fêmeas subnutridas durante os 80 dias tiveram menor PV e ECC.

Maurya et al (2004) dividiu 16 ovelhas em dois grupos, sendo um controle e outro com restrição alimentar, recebendo 30% da ingestão do grupo controle durante o verão e depois todas passaram a receber dieta ad libitum. As ovelhas subnutridas tem um ganho compensatório após a adequação nutricional, contudo a performance reprodutiva continua em déficit em comparação com ovelhas do tratamento controle. A restrição alimentar reduziu a duração do estro, aumentou o intervalo entre estros, precisaram de um maior número de montas para concepção e o peso ao nascer dos cordeiros foi menor.

Em ovelhas não prenhes, Grazul-Bilska et al. (2019) verificaram o efeito de três níveis energéticos na dieta (controle, 200% controle ou 60% controle) sobre a função ovariana por oito semanas. Como resultado observaram maior número de folículos presentes no tratamento controle e com excesso de energia. A proliferação celular das células da granulosa também foi maior nas ovelhas com excesso de energia comparado com as da subnutrição e maior das células da teca comparado ao controle. Ácido nítrico endotelial (eNOS), expressão RNA de receptores NOS3 (síntese endotelial de oxido nítrico) e GUCY1b3 (Guanilato Ciclase 1 Beta 3) foram maiores nos animais subnutridos.

Grazul-Bilska et al. (2012) alimentaram 48 ovelhas com dieta controle (100% recomendações do NRC 2007), excesso de energia (dieta *ad libitum* com >200% MS da dieta controle) e restrição alimentar com 60% da dieta controle por dois meses. Enquanto os tratamentos controle e com restrição perderam peso (0.02 e 0.240kg/d, respectivamente), as ovelhas com excesso de energia ganharam 0.200 kg/d. Ao final do experimento. O ECC de ovelhas subalimentadas foi menor que o controle e este, menor que as ovelhas com excesso de energia. Apesar do número de folículos e a porcentagem de oócitos saudáveis não diferirem entre tratamentos, a proporção de oócitos clivados após a fertilização in vitro e a formação de mórula e blastocisto foi reduzida com a redução ou excesso de energia. E os autores recomendaram a dieta controle para ovelhas doadoras de oócitos.

Brink (1990) forneceu dietas com 50, 100 ou 150% das recomendações de energia, de acordo com NRC (1985), para ovelhas nos primeiros 109 dias de gestação e depois, forneceu alimentação ad libitum até o parto. Ovelhas com alimentação restritiva tiveram um menor peso, menor ganho de peso e menor ECC aos 109 dias. Contudo, após a alimentação ad libitum, tiveram um ganho de peso superior aos outros tratamentos e o peso ao nascer e de desmame dos cordeiros não foi influenciado.

Tanto a restrição (60% dos requerimentos) quanto o excesso (140%) de energia durante o terço médio e final de gestação diminuiu o peso e volume do colostro (SWANSON et al., 2008; MEYER et al., 2011), além de diminuir o IgG total (SWANSON et al., 2008). Além disso, a composição do colostro também sofre alteração, sendo o tratamento controle com maior quantidade de gordura, sólidos-não-gordurosos, lactose e proteína do que tratamentos com 60 ou

140% do requerimento de energia, fornecido a partir de 40 dias de gestação (MEYER et al., 2011).

Em contrapartida, para McGovern et al. (2015a), forneceu dieta com 80%, 100% e 120% do recomendado em energia no final da gestação, e a dieta com 120% aumentou a produção de colostro após uma e 18 horas do parto e a produção de leite na terceira semana, além de aumentar os ácidos graxos do leite.

Para Donnem et al. (2020), a suplementação acima das recomendações (120%) para ovelhas no final de gestação com fetos múltiplos, aumentou o ganho de peso e ECC das ovelhas pré-parto, mas também teve uma maior perda de ECC durante a lactação, em comparação com tratamento controle. A concentração de NEFA antes do parto foi maior em ovelhas subnutridas (85% controle) em comparação ao controle. Ovelhas com excesso de energia desmamaram maior número de cordeiros (21/27) em comparação com controle (15/27) e subnutridas (9/27).

Keomanivong et al. (2015) restringiram a alimentação de ovelhas prenhes (60% do requerimento de energia) dos 50 a 130 dias de gestação. A restrição energética diminuiu a massa pancreática, atividade da α-amilase e a concentração de insulina plasmática, proveniente das veias uterinas e artéria e veia umbilical. Trotta et al. (2020) restringiram o aporte energético (60%) em ovelhas prenhes de 50 até 90 dias de gestação (meio da gestação) e depois do dia 90 ao dia 130 (final da gestação). A restrição diminuiu a massa pancreática e o peso do intestino delgado no meio e no final de gestação. A produção de α-amilase foi menor em ovelhas subnutridas no meio da gestação. No final de gestação, a subnutrição aumentou a concentração de maltase e lactase intestinal.

Nutrição e desempenho de borregas

Alimentação energética influencia o perfil endócrino e metabólico de borregas durante o terço médio e final de gestação, sendo que o excesso de energia fornecido (140% do requerimento energético) diminui concentrações de NEFA, progesterona e estradiol e aumenta nitrogênio ureico no sangue, insulina, T3, T4 e cortisol (VONNAHME et al., 2013).

Junqueira et al. (2019) também trabalharam com aspectos reprodutivos em borregas, mas com restrição energética. De 95 dias de idade até sete meses (ou primeiro estro), 35 borregas foram divididas entre os tratamentos: (i) controle (dieta *ad libitum*), (ii) restrita (85% dieta controle), (iii) controle + suplementação com lipídios e (iv) restrita + suplementação com lipídios. Comparando os animais do controle e com restrição, não houve diferença para conversão alimentar, concentração de glicose ou insulina, concentração de progesterona na puberdade e número de dias até atingir a puberdade, apesar do ganho de peso diário ter sido menor no tratamento com restrição energética.

Ferreira et al. (2020) avaliaram a puberdade de borregas recebendo dietas formuladas para ganho de peso de 200g/d com inclusão de milho ou polpa cítrica ou 100g/d com apenas feno, por 119 dias. O peso vivo, ganho de peso e eficiência alimentar foram melhores em animais com maior fornecimento de energia, mas essas diferenças não foram suficientes para alterar a idade a puberdade.

El-Hag et al. (2007) suplementaram borregas de quatro meses por aproximadamente seis meses. As fêmeas suplementadas tiveram um maior crescimento corpóreo e melhores índices reprodutivos comparados a borregas sem suplementação (controle).

Nutrição e desempenho de cordeiros

A nutrição materna principalmente durante o terço final de gestação e durante a lactação afetam a sobrevivência dos cordeiros e seu crescimento e desenvolvimento até o abate (GAO et al., 2008; VAN DER LINDEN et al., 2010; DU et al., 2015; GREENWOOD; BELL, 2019a; KALYAN DE et al., 2019). Contudo, a nutrição do cordeiro pós-parto é fator determinante para seu desempenho e, consequentemente, para produtividade e lucratividade do rebanho.

Trinta cordeiros de região tropical foram divididos em três níveis de alimentação: restrita I (300g/kg PV), restrita II (600g/kg PV) e ad libitum por 105 dias. Animais com dieta restrita tiveram maior concentração de beta-hidroxibutirato e de insulina, menor concentração de glicose e nitrogênio ureico no sangue. Além disso, animais com alimentação *ad libitum* apresentaram melhor ECC, PV, altura de cernelha, altura da garupa, comprimento do corpo, largura da garupa, comprimento da garupa e largura do tórax, evidenciando que a restrição alimentar prejudica o crescimento de cordeiros (PEREIRA et al., 2018b).

Cui et al. (2019) avaliaram o efeito da subnutrição em cordeiros após o desmame. Animais com menor aporte energético e/ou proteico têm menor ganho de peso e menor produção de ácidos graxos voláteis, menor peso de rúmen e espessura da mucosa ruminal, mostrando um menor desenvolvimento deste órgão.

Quarenta e oito cordeiros castrados e 48 cordeiras foram confinados com dieta *ad libitum* (controle) ou 85% do controle. O ganho de peso médio nos animais controle foi maior, levando um menor tempo até atingir o peso estipulado. Além disso, animais com maior aporte energético tiveram uma melhor eficiência alimentar, maior peso de carcaça quente e rendimento de carcaça, sendo mais vantajosos economicamente (JABOREK et al., 2018).

Ríos-Rincón et al. (2014) forneceram dietas com diferentes níveis de energia e proteína para cordeiros confinados. Os animais com alta energia (3.05 Mcal/kg energia metabolizável - EM) tiveram uma melhor eficiência alimentar em comparação com animais de baixa energia (2.83 Mcal/kg EM). Outros autores também afirmam a melhora na eficiência alimentar com o aumento do nível de energia na dieta (CRADDOCK; FIELD; RILEY, 1974; SHERIDAN; FERREIRA; HOFFMAN, 2003; HADDAD; HUSEIN, 2004; EBRAHIMI et al., 2007).

Majdoub-Mathlouthi et al. (2013) forneceram dietas com 300 ou 600g de concentrado para cordeiros desmamados até atingirem 35 ou 42kg. Os cordeiros com maior nível de suplementação obtiveram melhor ganho de peso, melhor características de carcaça, maior espessura de gordura, mas não afetou o pH da carne, coloração da gordura e composição de ácidos graxos. Para Sheridan et al. (2003) maior nível de suplementação de cordeiros aumentou o ganho de peso diário, o peso vivo final, eficiência alimentar, peso de carcaça fria e o rendimento de carcaça. O desempenho e características da carcaça de cordeiros alimentados com alta energia em confinamento também apresentaram melhores resultados do que cordeiros com menor aporte energético (HADDAD; HUSEIN, 2004; EBRAHIMI et al., 2007).

Considerações finais

A nutrição afeta o desempenho reprodutivo e produtivo de ovinos, sendo a energia fator determinante. Para animais em crescimento, o aumento da energia da dieta melhora a eficiência alimentar e o desempenho do animal, podendo ser formuladas dietas com energia acima das recomendações do NRC. Para animais em estação de monta, o flushing alimentar também é recomendado. Quando há uma falha na nutrição, a reprodução é a primeira função a ser afetada, diminuindo taxas de concepção, prolificidade, produção hormonal, diminuindo o peso das ovelhas e aumentando o balanço energético negativo. Experimentos com fornecimento de energia em excesso durante diferentes fases da gestação têm resultados contraditórios. De forma geral, tanto o excesso quanto a restrição alimentar podem trazer prejuízos à reprodução da fêmea, sendo que a melhor abordagem é manter os animais dentro do ECC ideal para cada fase produtiva, utilizando as recomendações do NRC.

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CAPÍTULO 2.

Protected fat supplementation in sheep diet: a review

Abstract: Supplementation with fat in the sheep diet is mainly accomplished by increasing energy intake. In addition, the use of fat has already been shown to be beneficial in sheep and other ruminant species, for improving postpartum performance, milk production and milk quality, reproductive aspects and growing performance. However, fat has a toxic effect on rumen bacteria, in addition to decreasing fiber digestibility. Thus, studies on different ways of protecting the fat against biohidrogenation have been carried out. Protected fat, in theory, is not metabolized in rumen, passing inert to the intestine. The positive effects of supplementation with protected fat depend on its source and amount of inclusion. But in general, calcium salts of fatty acids improve the quality of oocytes, ovulatory follicles, ovulation rate, return to estrus after lambing, anticipate lamb puberty and increase total cholesterol. Supplementation with protected palm oil increases milk production and fat in milk but decreases protein, while supplementation with CLA decreases fat in milk. Fat supplementation is recommended in sheep production, but in its protected form to avoid the negative effects on rumen fermentation.

Keywords: calcium salts of fatty acids, lipids, nutrition, energy, palm oil, PUFA

Introduction

The primary function of supplementing fat to ruminant diet is to increase energy intake. In fact, fat sources can provide two times more energy than cereal grains (NRC, 2007; HESS; MOSS; RULE, 2008). Many other beneficial results may outcome from fat supplementation, such as: improving milk production and content (HUSVÉTH et al., 2010; PALMQUIST; JENKINS, 2017; SAVOINI et al., 2019; ANGELES-HERNANDEZ et al., 2020), reproductive efficiency of the male (VAN TRAN et al., 2017), reproductive efficiency of the female (HESS; MOSS; RULE, 2008; SILVESTRE et al., 2011; HILLER, 2014; HOSSEIN; SAFDAR; HOSSEIN, 2015; ABD EL-HAMID et al., 2016a), alleviates negatives effects of heat stress (VIR SINGH et al., 2018), improve growth performance (LADEIRA et al., 2012; BHATT et al., 2013a; WANG et al., 2020b) and carcass quality (LADEIRA et al., 2012; URRUTIA et al., 2020). Also, depending on the type and level of medium-chain fatty acid supplementation, the production of methane can be reduced (YANZA et al., 2020).

The fat ingested by the sheep goes through the lipolysis process, performed by lipases released from rumen bacteria, mostly by *Anaerovibrio lipolytica* and *Butyrivibrio spp* (BIONAZ; VARGAS-BELLO-PÉREZ; BUSATO, 2020). The fatty acids (FAs) released by lipolysis are hydrogenated by bacteria isomesares followed by the activity of reductases. Biohydrogenation is a protection mechanism made by rumen bacteria, that involves the saturation of FA molecules, so dietary polyunsaturated fatty acids (PUFA) are convert into more saturated products, losing their health features (GADEYNE et al., 2017).

The inclusion of fat in ruminant diet, especially PUFAs, may bring negatives consequences. For instance, depending on the amount of fat and source, a reduction on fiber digestion in the rumen and milk fat percentage may happen (GRUMMER, 1988). Inhibition of fermentation may be due to a toxic effect of FA in the bacteria membranes and a coating effect of FA to feed particles, blocking the actions of the bacteria (BIONAZ; VARGAS-BELLO-PÉREZ; BUSATO, 2020).

Because of the biohydrogenation process, manipulating fat contents of ruminant products is not an easy task, considering that the FAs content in these products, such as milk and meat, are not a reflection of dietary FA composition (JENKINS; BRIDGES, 2007; GADEYNE et al., 2017; BIONAZ; VARGAS-BELLO-PÉREZ; BUSATO, 2020). Increasing PUFA absorption is not only beneficial for human health, but is also involved with increase fertility and a better reproductive performance of ruminants. Therefore, it is interesting to use rumen protect forms of fatty acids, as an alternative to protect the rumen bacteria population, increase energy intake, increase the absorption of PUFA (GADEYNE et al., 2017; BIONAZ; VARGAS-BELLO-PÉREZ; BUSATO, 2020), and minimize the negatives effects of fat on ruminal fermentation (GRUMMER, 1988).

To protect the fat from biohydrogenation, a few technologies have been developed: production of calcium soap or calcium salts of fatty acids (CSFA), encapsulating FA in a matrix of protein treated with formaldehyde, heat treatment, conversion of FA to fatty acyl amide (GADEYNE et al., 2017; BIONAZ; VARGAS-BELLO-PÉREZ; BUSATO, 2020), non-enzymatic browning, lipid composite gels, encapsulation within lipid and tyrosinase cross-linking (GADEYNE et al., 2017). This protections are only partial (JENKINS; BRIDGES, 2007; BIONAZ; VARGAS-BELLO-PÉREZ; BUSATO, 2020) but it brings greater benefits than the use of unprotected fats, by increasing the flow of unsaturated fatty acids to the intestine and decreasing the negatives effects on feed intake, ruminal fermentation and digestion, and milk fat percentage (JENKINS;

BRIDGES, 2007). This paper aims to review the effects of supplementing fatty acids in a protected form to ewe's diet.

Reproductive Parameters

In order for sheep production to be efficient, reproductive performance (increase lambing rate and decrease mortality) and lamb performance (greater number of lambs with greater slaughter weight) must be improved (HOSSEIN; SAFDAR, 2015). The most effective way to improve reproduction index is by manipulating the animal's nutrition (D'OCCHIO; BARUSELLI; CAMPANILE, 2019).

Fat supplementation improves establishment and maintenance of pregnancy, improves oocyte and embryo quality, increases progesterone levels and improve follicular development (SANTOS et al., 2008). Lipids are precursors of sex steroids and integral parts of the membranes of oocytes and spermatocytes; oocytes store lipids for supplying energy to embryonic development and adipose tissue–derived adipokines such as leptin significantly affects fertility (HILLER, 2014).

For ewes, calcium soap of fish oil supplemented during 13 weeks can improve oocytes quality. Fish oil is rich in polyunsaturated fatty acid, especially C20:5 (eicosapentaenoic acid; EPA) and C22:6 (docosahexaenoic acid; DHA). Number of oocytes per ovary, oocytes with grade I and PUFA in the plasma and cumulus cells were higher in supplemented ewes. Also, after have been exposed to chilling, oocytes from ewes fed the PUFA diet had more intact membranes (ZERON; SKLAN; ARAV, 2002). Cheng et al. (2005) collected ovine uterine epithelial cells, injected with PUFA and showed that it can influence prostaglandin production. Linoleic acid reduced responsiveness to oxytocin (OT) challenge; γ-linolenic acid and arachidonic acids increases overall prostaglandins and prevented the cells to respond to OT.

El-Shahat and Abo-El Maaty (2010) investigated the effect of supplementing calcium salts of fatty acids (CSFA) (3% in the DM) on ovarian activity and found an improvement in the number and size of preovulatory follicles and in the ovulation rate, in addition to improving body condition score (BCS) and body weight. For El-Nour et al. (2012), supplementing 50g of CSFA after synchronization to ewes out of breeding season improved ovarian activity with the presence of a greater number of follicles and larger follicles, in addition to increasing the conception rate.

Production of corpus luteum per ewe five weeks after weaning was higher for ewes supplemented with calcium soap of olive fatty acids (CaOFA), probably because of the increase in energy intake. This increase would probably not be possible just by adding more grains to the diet as this would trigger ruminal acidosis (PEREZ ALBA et al., 1997). Protected palm oil (50g/head/d) was supplemented to 30 ewes during early to mid-postpartum, reducing body weight loss and produced more ovulatory follicles and corpora lutea (HASHEM; EL-ZARKOUNY, 2017).

Titi and Kridli (2008) used 25 females receiving 0, 3 or 5% of CSFA, rich in C16:0, C18:2 and C18:1, 45 days before mating. Gestation length was increase with fat supplementation. Although 3% fat did not affect reproductive parameters, 5% inclusion negative effect litter size, twinning rate and lamb´s birth weight. Control group had the highest pregnancy rate and conception rate at the first cycle. This same diets were used by Titi et al. (2008) during postpartum period and the 3% of fat inclusion was beneficial to recovery ewes cyclicity in breeding season.

The supplementation with CSFA during the flushing period is beneficial especially when the source of fat is the flaxseed oil (rich in omega-3). CSFA supplementation from flaxseed oil or sunflower oil improves fertility, lambing rate and lambing birth weight (HOSSEIN; SAFDAR; HOSSEIN, 2015). When the CSFA supplementation was 50g/head/day from a source rich in palmitic and oleic, there was no effect for twinning, prolificacy, pregnancy, lamb survival and lambing rates (ABD EL-HAMID et al., 2016b).

Progesterone increases on days 9 and 11 of the estrus cycle with the supplementation of 2.5 % of CSFA (Megalac ®). Serum LH concentration before GnRH injection and GnRH-induced LH release also increases with CSFA supplementation (ESPINOZA et al., 1997). After synchronization, 3% of CSFA supplementation improved progesterone concentration from 1.1 to 2.5 ng/ml, result of a higher ovulation rate (EL-SHAHAT; ABO-EL MAATY, 2010). Similarly, for ewes out of breeding season and supplemented with 50g CSFA after synchronization, progesterone levels increase (EL-NOUR; NASR; HASSAN, 2012). Supplementation of protected omega 6 (n-6) or omega 3 (n-3) during the flushing period also increases progesterone and estrogen (HOSSEIN; SAFDAR; HOSSEIN, 2015).

In contrast, Titi and Kridli (2008) found no differences for progesterone levels between ewes supplemented with 0, 3 or 5% CSFA 45 days before mating but higher progesterone levels when supplemented post-partum (TITI et al., 2008). Abd El-Hamid et al.(2016b) also found no differences for progesterone during the luteal phase with CSFA rich in palmitic acid.

CSFA, rich in long-chain FA, were able to anticipated ewe lambs puberty by five weeks. The supplementation also improved final body weight (FBW), body weight gain (BWG), dry matter intake (DMI), BCS, withers height and heart girth (EL-SHAHAT; KHALED; EL-FAR, 2010). In male reproduction, inert fat supplementation after weaning increases body weight (BW), testicular length, testicular volume, semen volume, sperm concentration, mass motility, % motility, % rapid and medium or slow motile spermatozoa (KUMAR et al., 2014).

Overall, calcium salts of fatty acids improved ewe's reproductive parameters, mainly with the supplementation with PUFAs. Oocytes quality, preovulatory follicles, corpus luteum, progesterone, conception rate, puberty and estrus return after lambing may be improved by CSFA supplementation. Calcium salts rich in palmitic acid (C116:0) are not recommended over 5% supplementation.

Lipids Metabolites

Supplementation of CSFA increased total cholesterol (CHOL) and high density lipoprotein (HDL) but decrease serum insulin and does not affect very low-density lipoprotein cholesterol (VLDL), low-density lipoprotein cholesterol (LDL) and triglycerides (TG) (ESPINOZA et al., 1997). For El- Shahat et al. (2010), CSFA also increase CHOL, glucose and total lipids in ewe lambs with 12 weeks old. For Obeidat et al. (2012) CSFA supplementation did not affect TG, LDL and calcium, but increased glucose, CHOL and HDL in ewe lambs with 50 or 100g/d of CSFA and for cycling ewes with 50g/d of CSFA (ABD EL-HAMID et al., 2016b).

Cholesterol, TG, LDL and glucose also increase in ewes supplemented out of the breeding season with 50g CSFA (EL-NOUR; NASR; HASSAN, 2012).

There was no effect for lactate and insulin when the dietary carbohydrate was replaced by CSFA at 4, 7 or 11% of the DM. But glucose was lower for lambs with 11% CSFA compared with the control group (SEABROOK; PEEL; ENGLE, 2011a).

Calcium salts of fish oil only increase cholesterol levels in ewes during peripartum period. Total protein, albumin, TG, urea, glucose and VLDL were not affected (SHEIBANI et al., 2017). For Hashem and El-Zarkouny (2017) calcium salts of palm oil decrease glucose and increase TG, but did not affect T_3, insulin, total protein and urea.

For lambs supplemented between 12 and 92 days old with calcium salts of linseed oil, the fat inclusion increased glucose, cholesterol. HDL and insulin, but did not affected beta-hydroxybutyrate (BHB), LDL, total protein, albumin, blood urea nitrogen, AST and ALT. Also, linseed oil improved nitrogen utilization efficiency in lambs feed high protein diets (KANDI et al., 2020).

Improvements on lipids metabolites depend on the levels of fat supplementation and source. However, higher cholesterol levels are expected with the addition of fat in the diet.

Ewes Performance and Milk production

Table 2 shows the effects of protected fat supplementation on milk production and composition.

Table 2 *Milk production and composition affected by protected fat supplementation*

Author	Source	Milk	Fat	Protein	Total solids	FA profile
(HORTON et al., 1992a)	Palm oil	Unaffected	Increase	Decrease		
(PEREZ et al., 1986)	Palm oil		Increase	Decrease		
(CASALS et al., 2006)	Palm oil	Unaffected	Increase	Unaffected	Increase	Decrease short and medium chain FA and SFA; Increased long-chain FA
(CASTRO et al., 2009)	Palm Oil	Increase	Increase	Increase		Decrease C18:0 and C18:1; Increase C14:1, C16:1, C16:0
(LURUEÑA-MARTÍNEZ et al., 2010)	Palm Oil					Increase C16:0; Decrease C4:0, C6:0 C18:2 CLA
(HASHEM; EL-ZARKOUNY, 2017)	Palm oil	Increase	Unaffected	Unaffected	Unaffected	
(BIANCHI et al., 2018a, 2018b)	Palm oil	Increase	Increase	Decrease	Decrease	Increase SFA (6% inclusion) or decrease SFA (2 or 4% inclusion); Decrease PUFA
(PEREZ ALBA et al., 1997)	Olive oil	Unaffected	Increase	Decrease	Increase	Decrease short-chain FA; Increase C18:0 and C18:1;
(DOBARGANES GARCÍA et al., 2005)	Olive oil	Unaffected	Increase	Unaffected		Increase C18:1
(SHEIBANI et al., 2017)	Fish oil		Decrease			Increase PUFA
(LOCK et al., 2006)	CLA	Increase	Decrease	Increase		
(SINCLAIR et al., 2007)	CLA		Decrease			

Table 2. *Milk production and composition affected by protected fat supplementation (continuation)*

Author	Source	Milk	Fat	Protein	Total solids	FA profile
(HUSVÉTH et al., 2010)	CLA	Increase	Decrease	Increase		
(WEERASINGHE et al., 2012)	CLA	Increase	Decrease	Increase		
(BALDIN et al., 2017)			Decrease			
(NGUYEN et al., 2018)	DHA+EPA	Increase		Increase		
(NICKLES et al., 2019)	DHA+EPA	Unaffected	Unaffected	Unaffected	Increase	

CLA: conjugated linoleic acid; EPA: eicosapentaenoic; DHA: docosahexaenoic; PUFA: polyunsaturated fatty acid; SFA: saturated fatty acids; FA: fatty acids

Horton et al. (1992b) studied the inclusion of 0, 75, 150 or 300 g of Ca soap of palm oil to 32 ewes nursing two lambs each. No differences were found for ewes or lambs BW, but fat supplementation decrease lambs feed consumption. Milk yield was also unaffected, milk fat content increased, especially with 150g of Ca soap supplementation, but milk protein decrease. The supplementation did not improved energy balance of lactating ewes. Different from Horton et al (1992b?), Perez et al. (1986) found that supplementation with Ca soap of palm oil to lactating ewes, increase lambs daily gain and milk yield. Increase in fat percentage and decrease protein percentage in milk was similar between these researches.

Casals et al. (2006) supplemented 50 ewes with 0, 50, 100, 150 or 200g/kg of CSFA from palm oil (Megalac ®). During total lactation, milk and protein yields were similar between treatments, but fat content and total solids increase linearly with fat supplementation. Ewes BW and BCS and lambs growth, weaning weight and milk conversion index (kg milk/ kg lamb gain) were also not affected. For a second trial, 94 ewes in mid lactation were divided into two groups: control or 42g CSFA/kg DM. DMI, milk production and protein content in the milk were not affected by diet, but body weigh variation, fat (g/kg milk or g/day), lactose (g/kg milk) and milk solids (g/kg or g/day) were improved by fat supplementation. Milk FA profile was also influenced by fat in the diet, decreasing short and medium chain FA, saturated FA (SFA) and SFA/(MUFA+PUFA) and increasing long chain FA and MUFA (monounsaturated FA). PUFA was not affected.

Castro et al. (2009) supplemented dairy ewes with no oil, sunflower oil (SO) (12g/kgDM) or hydrogenated palm oil (HPO - Hidropalm®) (12g/kgDM).

Sunflower oil is rich in C18:2 (60.3%) and palm oil is rich in C16:0 (65.5%). HPO increased milk yield, energy-corrected milk, milk fat and protein (g/day) and alters fatty acid profile in the milk, with lower C18:0 and C18:1 cis-9 but higher C14:1, C16:1 and C16:0. Compared with control, HPO produced milk fat with 15% more cis-9 trans-11 CLA.

Fatty composition of ewe´s milk was affected by 4% calcium soap supplementation (Magnapac®, rich in C16:0), with increasing C16:0 and decreasing C4:0, C6:0 and C18:2 CLA, but more significant changes in the FA profile of the milk was duo to different breeds (LURUEÑA-MARTÍNEZ et al., 2010).

For Hashem and El-Zarkouny et al. (2017), 50 g/head/ day of protected palm oil improved milk yield and energy corrected milk yield, but did not affected milk composition and fat, protein and lactose yield. Bianchi et al. (2018a) used 0, 20, 40 and 60g/ kg DM of protected palm oil and found a linear improvement on milk fat content and negative linear effect for milk protein, lactose, and non-fat solids. Ewes with 60g supplementation had higher milk production but lower BCS. Also, 6% of protected palm oil increases SFA levels in the milk and decreases PUFA, especially alpha-linolenic (C18:3 n-3) and arachdonic acid (C20:4 n-6). The addition of 2 or 4% palm oil decrease SFA (BIANCHI et al., 2018b).

Total solids in milk were improved by CaOFA, but percentage of protein was decrease and milk yield was not affected. CaOFA supplementation also decrease short-chain fat acids (C6:0-C12:0) and C18:2 in the milk and increase C18:0 and C18:1, including trans-isomers of C18:1, showing that at least, part of the supplemented fat undergoes the process of biohydrogenation (PEREZ

ALBA et al., 1997). Similar results were found by Dobarganes-García et al (2005): the inclusion of protected olive fatty acids with calcium salts or as emulsified oil increases fat yield and milk fat content but no effects was found for milk, protein or lactose yield and CaOFA also increases C18:1 in the milk.

The effect of milk fat depression (MFD) caused by the trans-10, cis-12 isomer of conjugated linoleic acid (CLA) is well documented in cows. Lock at al. (2006) used 20 ewes to test with the same occurs with this specie. They used 25g/d of the CLA-encapsulated to ewe’s diet for 10 days and found a similar response with reduction in milk fat synthesis and increase in milk and milk protein yield. Sinclair et al. (2007) also proved that milk fat percentage and milk fat yield are decreased with the inclusion of 25g/d of encapsulated CLA but beneficially altered the flavor characteristics of the cheese. Husvéth et al. (2010) also with 25g/d of protected CLA founded higher milk production and protein yield but lower fat content. However this supplementation decreases fat accumulation in the ewes’ liver, comparing with protected palm oil. Weerasinghe et al. (2012) with 2.4 g/d of trans-10,cis-12 CLA (encapsulated CLA) found a decrease in fat percentage by 33% and 24% decrease for fat yield but increase protein, lactose and milk yields. Baldin et al. (2017) confirmed that CLA supplementation decrease fat yield and percentage in the milk but when associated with CS of palm oil, this decrease is less prominent. Toral et al. (2015, 2020) affirm that although CLA may decrease milk fat content, it is not the main cause of MFD in lactating ewes.

Sheibani et al. (2017) supplemented ewes from four weeks before delivery to three weeks after with calcium salts of fish oil and found a lower feed intake prepartum but no differences postpartum or for body weight. Milk composition showed lover fat percentage with fat supplementation but the profile of FA was improved with increasing PUFA (EPA, DHA, CLA, C20:3, C22:2, C24:1, C22:5 n-3).

Sixty lactating ewes were supplemented with rumen-protect DHA+EPA and milk yield, protein yield, BCS and ether extract (EE) intake were increased. However, feed intake, DMI and intake of organic matter (OM), acid detergent fiber (ADF), neutral detergent fiber (NDF), crude protein (CP) were decreased (NGUYEN et al., 2018). For Nickles et al. (2019) rumen protected DHA+EPA during late pregnancy did not affect BW and BCS, milk yield, fat (%), protein (%), milk urea nitrogen (MUN) (%) and milk energy but linearly increase lactose and total solids with increasing inclusion of 1 and 2% DHA+EPA in the DM.

The best dose of Ca soap of palm oil seems to be 100-200g/d and it is recommended to improve milk composition and fatty acids profile. This recommendation was based by reviewing 11 experimental trials (MELE; BUCCIONI; SERRA, 2005). Another review made by Gargouri et al. (2006) showed that CSFA supplementation has a positive effect in increasing fat yield, mainly at the beginning of lactation and the negative effects in decreasing protein content are mainly in late lactation. Also, CSFA improves milk quality by decreasing short and medium chain fatty acids and increasing long fatty chains fatty acids but with no better responses from lamb’s performance. In agreement with Mele, Buccioni e Serra (2005), the addiction up to 150-200g/d is recommended (GARGOURI et al., 2006). Angeles-Hernandez et al. (2020) published a meta-analysis evaluating the effects of fat supplementation on milk composition. They evaluated several papers with fat different sources of fat and in a protected or unprotected form. Overall, the conclusion was that the optimal fat inclusion to increase milk yield is between 150-200 g/day and the supplementation also increases fat and CLA content but decreases protein.

The effects of fat supplementation on milk production and composition depend on the fat source. Palm oil increases milk production and fat yield but decreases protein yield. On the other hand, fish oil and CLA supplementation decreases fat percentage in the milk, but increases protein and milk production.

Neonatal behavior, colostrum and immune responses

Capper et al. (2006a) tested the possibility of supplementing PUFA (fish oil) to ewes at late pregnancy as a manner to improve neonatal behavior and therefore, decrease neonatal mortality, comparing with the use of calcium salts of palm oil. Fish oil supplementation increases length of gestation, decrease the latency to suckle, and increased 22:6(n-3): 20:4(n-6) ratio in the neonatal brain. However negatives effects were found in the colostrum, reducing fat and protein yield. Annett et al. (2008; 2009) also founded that supplementation with fish oil decrease colostrum production and IgG yields. Percentage of fat in the colostrum decrease with calcium salts of fish oil from 10.3% to 8.5% (SHEIBANI et al., 2017).

Thirty ewes were supplemented with 0, 4 or 6% calcium salt of palm oil to assess their immune response. Palm oil decrease eggs per gram (EPG) on the feces, and increased immunoglobulin levels and cytokine level (BIANCHI et al., 2014).

Fetal programming

Mother's diet can influence fatty acid profile in the lamb's meat, more than the breed and other fators. FA content in the adipose tissue of lambs born and fed by ewes supplemented with 4% of CSFA (Magnapac®, rich in C16:0) shows higher oleic acid (C18:1 n-9), palmitic acid (C16:0), stearic acid (C18:0) and SFA and lower MUFA and PUFA (LURUEÑA-MARTÍNEZ et al., 2010). Manso et al. (2011) also stated that fat supplementation to the ewes diet can influence FA profile in the lambs meat and subcutaneous fat. For example, hydrogenated palm oil produced lambs with more C16:0 and less C18:0 than other sources of unprotected fat (olive, soybean or linseed oil).

For Gallardo (2014), ewes supplementation with calcium soaps of fish oil produces a better FA profile in lambs than CS of palm oil or olive because of the increased n-3 PUFAs and lower SFA and trans-FAs. However, even if it produces a better fatty acid profile in lamb, supplementation with calcium soap of fish oil has a shorter shelf life, being rejected for poor sensory quality after just 5 days of storage (VIEIRA et al., 2019).

Also, dam's nutrition affected offspring performance: increased BW, DMI and glucose but not ADG, gain to feed ratio, NEFA and insulin. The supplementation with rumen protected DHA+EPA during late pregnancy had a tendency (P=0,07) to decrease lamb's ghrelin (36.7pM control x 27.2pM 2% DHA+EPA) (NICKLES et al., 2019).

Lamb Performance and carcass

Haddad and Younis (2004) worked with 21 lambs for 72 days with 0, 2.5 or 5% of protected fat from a 100% saturated form (Ultralac^{TM}), but there was no differences for BW changes, final BW, average weight gain and feed to ratio gain, explained by the not affected metabolizable energy (ME) intake. Similar was found (not affected BW, days until achieve 25kg, ADG and DMI) by Manso et al. (2006) using 30 lambs with a high (41g fatty acids/kg) or low (25g fatty acids/kg) level of fat from palm oil, with calcium salts or not. However, inclusion of fat improved feed to ratio gain. Slaughter weight, empty BW, hot carcass weight (HCW), cold carcass weight (CCW), carcass yield, non-carcass weight and body chemical composition was not affect by fat inclusion or by type of fat (protected or unprotected).

Salinas et al. (2006) supplemented 20 lambs with different levels of calcium soap of tallow (0, 1.5, 3.0 or 4.5%) in a diet with 14%CP and 2.6 Mcal ME/kgDM. Fat supplementation did not affect lamb performance: initial or final weight, DMI, ADG, feed efficiency *longissimus dorsi* area and subcutaneous fat (cm).

Seabrook et al. (2011a) used 60 lambs with 0, 4, 7 or 11% of CSFA on a DM basis, replacing the dietary carbohydrate with the fat and the diets were isoenergetics. CSFA supplementation decrease daily DMI, 7 or 11% inclusion decrease BW and gain to feed ratio and 11% inclusion decrease ADG, HCW, *longissimus* muscle area, back fat and yield grade. Although lambs were fed ad-libitum, lambs with 11%CSFA consumed 15% less than control but total digestive nutrients (TDN) and protein consumption was similar.

Kandi et al.(2020) worked with 36 lambs from 12 to 92 days old with calcium salt of linseed oil and two levels of protein 18 and 21%. Fat addition, alone, only improved feed efficiency post weaning, but the association between fat supplementation and 21% CP improved average daily gain and final body weight, without affecting ruminal parameters.

Bhatt et al. (2020) supplemented 36 lambs with three months old with no fat, CSFA or linseed for 90 days. No differences were found for empty body weight, HCW, carcass length, back fat thickness, total fat, lean:fat, lean:bone, water holding capacity and cooking loss. Also, chemical composition (DM, OM, total ash, CP, EE) of *longissimus thoracis* (LT) and adipose tissue were similar between groups. However, fatty acid profile from LT and adipose tissue were improved with CSFA supplementation and linseed, respectively.

Protected fat supplementation has only a small effect on lambs performance and carcass, improving feed efficiency. High levels of supplementation (11% CSFA of DM) decrease intake and have negatives effect on BW, feed efficiency, ADG, HCW, and carcass. However, the inclusion of fat in the mother diet changes fatty acid profile in the lamb meat, with the supplementation with long chain fatty acids producing a better fatty acid profile.

The use of protected fat may be beneficial for culling ewes. Thirty ewes, over six years old, were supplemented with 0, 20 or 40g/kg rumen bypass (made from ice bran oil) for three months. Final weight, ADG, percent increase, final BCS, hot carcass weight, dressing percentage, loin eye area and meat:bone ratio were higher for supplemented ewes (BHATT et al., 2013b).

Digestibility and rumen fermentation

Enjalbert et al. (1994) using isoenergetic diets, supplemented ewes with soy oil (SO), emulsifified soy oil (ESO), calcium salts of soy oil (CaSSO) or no supplementation was used (control). SO and ESO decrease apparent digestibility of organic matter, ADF and NDF, which did not occur with CaSSO, proving to be an effective energy source for ruminant rations. Likewise, apparent digestibility of crude protein and crude fiber was not affected by the inclusion of Ca soap of palm acid, but digestibility of fatty acids was decrease with fat supplementation (Horton et al., 1992).

However, Perez Alba et al. (1997) supplemented dairy ewes with Ca soap of olive fatty acids at 10% of the basal diet and found a decrease in the digestibility of dietary dry matter. Digestibility of crude fat was improved while other components were not affected (OM, nitrogen, NDF, ADF, gross energy).

Reddy et al. (2003) evaluated the inclusion of 0, 0.05, 0.10 and 0.15g/kg DM of calcium salts of red palm oil for a month in adult rams. DMI from sorghum straw was decrease but only with a 0.15g inclusion and total DMI was not affected. Digestibility of DM, OM, CP, and cell wall constituents (CWC) was also not affected but the digestibility of EE was higher with inert fat supplementation. This same supplementation levels were added to 16 lambs kept in metabolic cages. Average body weight and digestibility of DM, OM, CP, crude fiber (CF), NDF, ADF, hemicellulose was similar between treatments. Control diet had lower EE digestibility and only the 15% inclusion of fat decreased DMI and cellulose digestibility (KUMAR et al., 2006). Hydrogenated palm oil seems to increase apparent digestibility of OM but not of DM, CP, EE, NDF and ADF (CASTRO et al., 2009).

Fat supplementation (0, 2.5 or 5% Ultralac ™) improved the digestibility of DM, CP, OM, EE, NDF, ADF and energy for fattening lambs, but energy intake was not affected. The increase of NDF and ADF digestibility may suggest a better protection of the fat, alleviating negatives effects of oils in the rumen (HADDAD; YOUNIS, 2004). In contrast, Manso et al. (2006) found a decrease in ADF apparent digestibility and increase EE digestibility with fat supplementation (palm oil, protected with calcium soap or unprotected). However, only the unprotected form, in high levels of palm oil (41g fatty acids/kg) decrease OM and NDF digestibility, showing that the use of calcium soap to protected the palm oil is beneficial for rumen fermentation.

Obeidat et al. (2012) supplemented ewe lambs with 0, 50 or 100g of CS long-chain FA. Nutrient intake of DM, OM, CP, NDF and ADF was similar between groups and the fat inclusion increase EE and EM intake. The higher inclusion of CSFA (100g CSFA/ewe/day) increased the digestibility of OM, NDF, ADF and EE.

Supplementation with 20 or 40g/kg of rumen bypass fat (from rice bran oil) improved apparent digestibility of DM, OM, CP, EE, ADF, NDF, hemicellulose and cellulose (BHATT et al., 2013a).

Overall, when the inclusion of fat is made with a protected form, no negative effects are found for nutrient digestibility. In fact, calcium salts of fatty acids may increase several nutrients apparent digestibility.

Considerations

Calcium salts of fatty acids are recommended for ewe’s production, to improve reproductive performance, milk production and fatty acid profile in the lamb’s meat. Besides the positive effects of fat supplementation, its protected form does not seem to effect rumen fermentation.

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CAPÍTULO 3.

Chromium supplementation and ewe production: a systematic review

Abstract: Chromium is a heavy metal found naturally in nature. In its trivalent form, it is considered an essential micronutrient for glucose and lipid metabolism. Since the 90s, it has been allowed to be used in farm animals and since then, a range of studies on each species has been carried out. Chromium enhances the effects of insulin, improves antioxidant status, stimulates the use of amino acids by cells and is related to lipid metabolism. Little evidence has suggested that chromium supplementation can improve lamb performance, carcass and meat quality, especially by reducing carcass fat. In animals under stress or in physiological conditions with high nutritional demands, such as pregnancy and lactation, chromium supplementation looks promising. It is not yet possible to recommend the better dose of chromium, but it is recommended that further studies be carried out, mainly in animals under stress, pregnancy or lactation. In addition, further studies are needed to elucidate and quantify the damage caused by bioaccumulation and environmental contamination with the use of chromium in the sheep diet.

Keywords: organic chromium; insulin; glucose; energy; sheep

Introduction

In 1959, Schwarz and Mertz (1959) conclude that chromium is an active constituent of a “glucose tolerance factor” and from there the interests and research on chromium as a essential trace element started (SAMSELL; SPEARS, 1989).

Chromium is a naturally occurring heavy metal (AMATA, 2013) that can exist as Cr^{III} (non-toxic) and Cr^{VI} (toxic), depending on human activities or environmental conditions such as pH, organic matter and metal oxide concentration; Trivalente Cr^{III} is an essential micronutrient for lipid and glucose metabolism, acting on the amplification of insulin signaling (AMATA, 2013; HAMILTON et al., 2020) and it is the most stable form in the food supply (STOECKER, 1999).

In 2002, Food and Nutrition Board of the Institute of Medicine of the National Academies of Science (USA) stated that the estimated safe and adequate daily dietary intake recommendation of chromium was 30-35µg/day for adults and 25 µg/day for young people (Vincent, 2014, 2004), less than before (1980), which was 50-200 µg/day of Cr (Stoecker, 1999). Because it is involved with lipid and glucose metabolism, its use for weight loss was widespread (Hamilton et al., 2020). Although for healthy people Cr^{III} supplementation is unlikely to have beneficial effects, patients with type 2 diabetics or pregnant women, might benefit from pharmacological quantities of Cr^{III} (VINCENT, 2004).

The use of chromium as a feed supplement for animal production has begun only in the 1990s and the utilization of minerals on animals diet in under the purview of a regulatory agency responsible for public health and food safety, such as Food and Drug Administration (FDA) in the USA, Canadian Food Inspection Agency in Canada (LINDEMANN, 2007) and National Health Surveillance Agency (ANVISA) in Brazil .

Historically, the first permission for the use of chromium in the animal diet was by the FDA, in 1996, with the release of the use of chromium picolinate

(CrPic) in the diet of pigs with the addition of up to 200ppb in the diet. In Canada, the first release was in 1999 for lactating cows, and up to 400ppb of chromium yeast (Cr Yst). The FDA also released for use in swines, chromium propionate in 2000 at up to 200ppb in the diet and chromium methionine in 2003 at up to 400 ppb (LINDEMANN, 2007). Until 2020, chromium propionate obtained from the Kemin Industries Inc. USA was released by the FDA for use in swine, broiler chickens, cattle and horses (FDA, 2020).

Chromium metabolism

Organic sources of chromium (chromium propionate, chromium picolinate, chromium nicotinate and chromium yeast) are over ten times more bio-avaiable than inorganic sources (LINDEMANN, 2007; AMATA, 2013; LASHKARI; HABIBIAN; JENSEN, 2018). However, trivalent chromium as chromium picolinate has been shown evidence of toxicity with more oxidative stress and DNA damages (SPEETJENS et al., 1999; VINCENT, 2004; BAILEY et al., 2006; LEVINA; LAY, 2008; AMATA, 2013).

Chromium is absorbed mostly in the small intestine and it is related inversely to dietary intake (Lashkari et al., 2018; NRC, 1997). In ruminants, mechanisms of Cr absorption are not completely understood but it has been evidence that it also occurs in the jejunum and the Cr absorption in the rumen is negligible (LASHKARI; HABIBIAN; JENSEN, 2018). It is suggested that the major physiologic chromium transport agent is transferrin (STOECKER, 1999; VINCENT, 2000), competing with iron (STOECKER, 1999).

Trivalent chromium acts on energy metabolism by binding four ions of Cr^{III} to an oligopeptide with four types of aminoacidic residues (glycine, cysteine, glutamate and aspartate), called apochromodulin and located in the intracellular environment. Together, they form the chromodulin whose main ability is to potentiate the effects of insulin on the conversion of glucose into carbon dioxide or into lipid (Amata, 2013; Bin-Jumah et al., 2020; NRC, 1997; Vincent, 2004, 2000; Yamamoto et al., 1989).

Chromodulin promotes a small activation of a membrane phosphotyrosine phosphatase and a higher insulin sensitivity by stimulate the tyrosine kinase activity from insulin receptor in the plasma membrane (VINCENT, 2000; LASHKARI; HABIBIAN; JENSEN, 2018).

Also, by improving insulin activity, Cr^{III} may also increase the numbers of insulin receptor glucose transporter type 4 by upregulates mRNA levels, increase glycogen synthase, uncoupling protein-3 in skeletal muscle cells (DAVIS; HEATHER SUMRALL; VINCENT, 1996; LASHKARI; HABIBIAN; JENSEN, 2018), boost antioxidant status (EVANS; BOWMAN, 1992; LASHKARI; HABIBIAN; JENSEN, 2018) and has a positive effect on protein metabolism by stimulating the uptake of aminoacids by cells (NRC, 1997; Rezende Gomes et al., 2005).

Chromium is also related to lipid metabolism and may decrease total cholesterol, low density lipoprotein (LDL) cholesterol and triacylglycerols, in addition to increasing high-density lipoprotein (HDL) cholesterol (NATIONAL RESEARCH COUNCIL, 1997; BROADHURST; DOMENICO, 2006) by inhibiting the liver enzyme hydroxymethylglutaryl-CoA reductase (REZENDE; MACEDO; TIRAPEGUI, 2005), circulating levels of TNF-α and IL-6 (JAIN; RAINS; CROAD, 2007).

Although there is plenty of evidence of the benefices of chromium on energy metabolism, there is a lack of consistent results about doses and beneficial effects of supplementing chromium in farm livestock. Some review papers have been published about chromium supplementation in farm animal, but none of them focus on sheep. For example, White and Vicent (2019) wrote a systematic review analyzing the effects of trivalent chromium on chickens. Lashkari et al. (2018) review the role of chromium in ruminants nutrition but there was only nine papers about sheep. Bin-Jumah et al. (2020) studied the effects of chromium for animals under heat stress, but there was no papers about sheep. Therefore, this reviews aims to elucidate and summarize the effects of chromium in sheep production so it is possible to recommend or not this use.

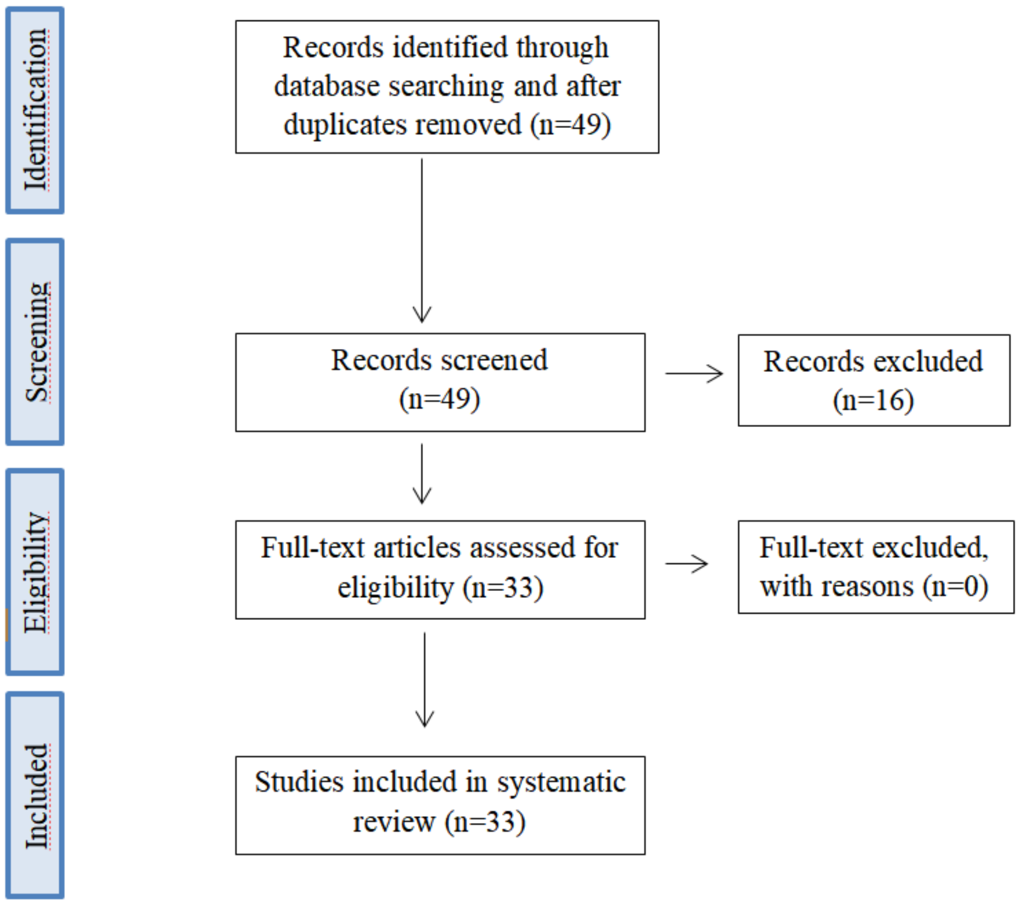
Methods

The scope of this review is experimental trials of chromium supplementation on ewes published until 2020. A systematic review protocol was used, according to Salameh et al. (2020) (Figure 1).

The research was made on the fallowing databases: Web of Science, Scopus, Google Scolar, Scielo and Science Direct and the keywords were chromium, ewe, sheep and lambs, in various combination. Duplicates were removed and titles and abstract were screened to identify studies that were potentially relevant. Reviews paper and studies that did not used chromium or sheep were excluded. Papers written in English, Portuguese or Spanish and published before November 2020 were selected.

Elegible studies were identifies after reading the full text and articles that did not used chromium as a treatment and did not have a control group were excluded and the following data were extracted: first author, publication year, animal category, number of animals, study design and duration, treatments and period of treatments, type and dose of chromium, main parameters evaluated, main results and general conclusion. Regardless of the level of statistical significance used by the authors, differences between means were deemed at the level of $P < 0.05$.

Figure 1 *Flow diagram for selection of studies for the systematic review.*



Adapted from Salameh et al. (2020)

Results and Discussion

Lamb performance, carcass and meat quality

The first published article about the supplementation of chromium in lambs was in 1989 by Samsell and Spears (1989) at the journal “Nutrition Research”.

They performed two trials with sixteen lambs each and two doses of chromium (0 and 10µg/g DM as $CrCl_3$) and analyzed blood samples for glucose, insulin and cholesterol (table 3). On the first trial, lambs were divided into four treatments: Low fiber (18.6% NDF) with or without chromium and High fiber (55.6%NDF) with or without chromium and fed for 28 days followed by ad libitum feeding for 14 days. On the second trial, there were only two treatments, high fiber diets with or without chromium. Overall, chromium seems to cause an effect on reducing plasma glucose only when receiving low fiber diets and after a 48-hour fast. Glucose, insulin and cholesterol were not affected but chromium decrease free fatty acids form lambs on a high fiber diet, as confirmed by trial two.

Fatma Uyaniki (2001a) supplemented 17 lambs with 0, 200 or 400ppb of Cr as CrCl3 during 55 days. Subcutaneous fat thickness was lower for lambs supplemented with 400ppb, followed by lambs with 200ppb and higher for lambs with no chromium. However, live weight (table 4), serum protein and enzymes (ALT, GGT and AST) were similar during all trial. Serum triacylglycerol was lower for treatments with chromium but no differences were found for total cholesterol and LDL.

Yulistiani, Puastuti and Mathius (2013) studied replacing 10% of the protein concentrate with feather meal and supplementing it with CrCl3 or inorganic chromium (1.5mg/kg DM), but found that chromium organic or inorganic did not affect lamb performance (table 4).

Mostafa-Tehrani et al. (2006a) evaluated two forms of chromium - chromium nicotinate (CrNic) and chromium chloride ($CrCl_3$) - and three doses – 200, 600 and 1000 µg/kg of DM, besides a control group, with no chromium supplementation. Seventy ram lambs were used and their non-carcass components were analyzed after a 15-week period of trial. Final BW, BW gain and ADG were similar between treatments. Statistical analysis was performed using contrasts and little effect was found between control treatment x supplemented with chromium treatments. Control produced lighter proximal thoracic and pelvic limbs. Chromium nicotinate produce lambs with heavier skin and lighter heart. The 200µg dose produced heavier head for both forms of chromium and 200 µg from CrNic produced heavier skin and kidneys. Chromium supplementation may be beneficial when considering non carcass components sells and wholesale cuts.

In the 90s, Kitchalong et al. (1995) worked with 25 Suffolk lambs for 85 days feeding a diet formulated to exceed the nutritional requirement proposed by NRC (1985) and 250 µg/DM as chromium picolinate (CrPic). Although the majority of the results showed no statistical difference, some evidence suggests a positive effect of chromium supplementation for growing lambs: lower yield grade, 17% less plasma cholesterol on the second week of trial, overall less plasma NEFA (table 3) and positives effects on week 2 during the IVGTT (intravenous glucose tolerance test) and IVICT (insulin challenge test).

Gentry et al. (1999) studied the influence of chromium picolinate and dietary protein in lambs. Thirty-two lambs were fed for 84 days in a 2x2 factorial trial with two levels of protein (high 12.8-14.4% CP or low 9-12.1%), with or without 400ppb Cr. There were only some effects for carcass with a decrease of 19% on liver weight, an increase of 12% in kidney weight, and a decrease in NEFA on lambs receiving a high protein diet. Chromium also increased total cholesterol on lambs with high protein diets and decrease cholesterol on lambs with the low protein diet (table 3). Regardless of the protein, Cr increased platelets and heat precipitable protein in the blood. No effects were found for; growth, DMI, gain:feed ratio, carcass weight, dressing percentage, heart weight, pelvic fat, yield grad, loin-eye area, midrib fat, and any parameters evaluated in the IVGTT (table 4 and table 5).

Similar to Gentry et al. (1999), Yan et al. (2008, 2010) used 48 weaned male lambs to study the interaction between chromium supplementation and two levels of crude protein (Low: 157-171g/d or High: 189-209g/d). Chromium yeast was chosen at three levels: no supplementation, 400 or 800ppb. Chromium supplementation had no effect on ADG, final weight (table 4) and carcass measurements except for reducing intramuscular fat rate (table 5) and triglycerides (table 3) (YAN et al., 2008). Chromium didn’t affect insulin, glucagon, insulin/glucagon, leptin, TNF-α (tumor necrosis factor α), glycogen on muscle and enzyme activities in the liver or kidney (Glucose-6-phosphatase, hexokinase) and in adipose tissue (fatty acid synthetase and hormone-sensitive lipase). However liver glycogen was increased with chromium, reinforcing the conclusion that chromium regulates glycogen metabolism.

In 2009, Domínguez-Vara et al. (2009a) supplemented 54 lambs with chromium yeast (0, 0.25 or 0.35mg/head/d) during 95 days and the carcass performance (table 5) and characteristics were evaluated. Chromium decrease daily intake and the fat in the carcass and increase plasma glucose (mg/dL) and area 12th rib (cm^2) but did not influence final BW, ADG, feed conversion, slaughter BW, hot carcass weight and cold carcass weight. The supplementation of 0.25mg CrYeast reduced triglycerides (mg/dL) and increased insulin (µIU/ml).

A completely random trial was design with a 2x2x2 factorial arrangement of treatments (genotype x nutritional supplementation x organic chromium) using 32 male lambs. The daily chromium yeast dose was 2.5mg per lamb. Interaction effect was not found. Chromium had no effect for hot carcass weight, cold carcass weight, carcass yield, carcass length, leg length, leg perimeter, major and minor width of thorax and rib eye area at 12^{th} rib. However, chromium was able to reduce dorsal fat content in meat and carcass (table 5) (ARVIZU et al., 2011a).

Zhou et al. (2013) studied the effects of chromium yeast supplementation (300µg/kg DM) during 56 days for lambs. Chromium decreases abdominal fat mass and abdominal fat percentage (table 5), decrease serum insulin, triglycerides and total cholesterol and increases the ratio of glucose/insulin, serum FFA (free fatty acid) and high-density lipoproteincholesterol (HDL-C) (table 3). Therefore, chromium regulates insulin and lipid metabolism, improving meat quality.

Da Rocha et al. (2013) evaluated the metabolism of organic chromium (1mg of Cr Yeast) using four lambs kept in metabolic cages for 20 days and discovery that organic chromium derived from S. cerevisiae yeast is not absorbed and was totally eliminated in the feces. Also, does not affect the intake and digestibility of lambs.

Estrada-Ângulo et al. (2013) worked with 0.4, 0.8 and 1.2 mg/Cr from Chromium yeast/ lamb/ day and found differences for performance and carcass traits: ADG, gain for feed, final BW, empty BW, HCW, longissimus muscle area and dietary energetic efficiency. Other parameters were similar between treatments and control: DMI and organs mass (tables 4 and 5).

To assess the performance, carcass and meat quality of 18 lambs, Moreno-Camarena et al. (2015) used three doses of chromium (0, 0.2 and 0.4mg / kg DM) from Cr yeast. Chromium supplementation linearly decrease perirenal fat and increase leg perimeter and muscle conformation, but no other performance was effect (tables 4 and 5). For *longissimus dorsi*, chromium increase shear force and decrease ash content, but did not affect moisture, crude protein and fat contents.

Rodríguez-Gaxiola et al. (2020a) evaluated the performance, carcass and *longissimus* muscle of 14 finishing lambs receiving a control or 0.3 mg Cr/kg DM from Chromium yeast for 74 days (tables 4 and 5). There was no effect for performance, carcass chemical composition of *longissimus* muscle and muscle fatty acid profile except for increasing palmitic acid.

Sánchez-Mendoza et al. (2015) studying chromium methionine, used 24 males lambs for 56 days and a high energy finishing diet. Lambs receive 0, 0.6, 1.2 or 1.8 mg Cr/ lamb/ day. Although there was no effect for performance (DMI, feed to gain ratio, FBW, slaughter weight, hot and cold carcass weight, dressing percentage) and carcass measurements (carcass length, leg length, leg circumference, chest perimeter, chest depth, and longissimus muscle area), chromium supplementation was able to linearly decrease the fat thickness and improve yield grade on the carcass and decrease fat concentration and improve protein/ fat ratio on longissimus muscle (tables 4 and 5).

Griss et al. (2020) studied the effects of yucca extract (YE) and chromium lysine (2mg Cr-lys/kg concentrate) on 64 lactating lambs for 40 days. When analyzing only the influence of chromium, not associated with YE, there was no effect for BW, weight gain, ADG, some hematological variables such as erythrocytes, hematocrit, hemoglobin and eosinophil, for serum albumin, glucose, urea, cholesterol and globulin (tables 3 and 4). However, chromium reduced leucocytes, lymphocytes and monocytes, increase total protein and triglycerides. Chromium also reduces ROS (reactive oxygen species), a free radical responsible for causing oxidative damages, and increases antioxidant enzymes: CAT (catalase) and SOD (superoxide dismutase) on day 40.

In 1997, Sano et al. (1997) published an article about the effects of supplemental chromium on kinetics of plasma glucose, lactate and propionate in rams fed a high grain diet (tables 3 and 4). Using a commercial chelated chromium (chromium chevalite ® - chromium dinicotinate glycine chelate) and six rams fed 2% DM/kg BW for 14 days and 2,5% DM/kg BW for more 9 days, the researchers found no effect for body weight and dry matter intake, but weight gain was higher for supplemented rams. Plasma concentration of glucose, lactate and propionate was similar between treatments, but percent of glucose derived from propionate was higher for supplemented rams.

Most of the works analyzing lamb performance, carcass and/or meat quality have shown, for the most part, no effects or, when present, positive effects. Chromium seems to produce a leaner carcass, which may be economical beneficial for producers and more appreciated by consumers.

Table 3 *Effects of chromium supplementation on serum glucose, insulin, cholesterol and others metabolites*

Authors	**Chromium Source and level**	**Glucose**	**Insulin**	**Cholesterol**	**Others**
(SAMSELL; SPEARS, 1989)	CrCl3 (0, 10µg/g DM)	Not affected; decrease after 48h fasting and with low fiber diets.	Not affected	Not affected	Decrease free fatty acids in a high fiber diet
(UYANIK, 2001b)	CrCl3 (0, 200 or 400ppb)	Decrease with 200ppm		Not affected (total, HDL or LDL)	Decrease triacylglycerol Not affected: Serum protein, ALT, AST and GGT
(SANO et al., 1997)	Cr chevilate ® (0.5ppm)	Not affected			%glucose from propionate was higher
(KITCHALONG et al., 1995)	CrPic (0, 250 µg/g DM)	Not affected	Not affected	Decrease	Decrease NEFA Not affected: plasma albumin, T3, T4, urea N, glucagon
(GENTRY et al., 1999)	CrPic (0, 400ppb)	Not affected		Increase (diet with high protein) and decrease diet with low protein)	Decrease NEFA and cortisol
(YAN et al., 2008, 2010)	CrYeast (0, 400, 800ppb)	Not affected	Not affected	Not affected (total, HDL or LDL)	
(DOMÍNGUEZ-VARA et al., 2009b)	CrYeast (0, 0.25, 0.35mg/head/d)	Increase	Increase (0.250mg level)		Decrease triglycerides (0.250mg level)
(ZHOU et al., 2013)	CrYeast (0, 300µg/kg DM)	Not affected	Decrease	Decrease total cholesterol and increase HDL and free fatty acids	Decrease triglycerides

Table 3 Effects of chromium supplementation on serum glucose, insulin, cholesterol and others metabolites (cont.)

Authors	Chromium Source and level	Glucose	Insulin	Cholesterol	Others
(GRISS et al., 2020a)	CrLys (2.0mg/kg concentrade)	Not affected		Not affected	Increase total protein, triglycerides; CAT and SOD; decrease ROS

CrCl3: chromium chloride; CrPic: chromium picolinate; CrYeast: chromium yeast; CrLys: chromium lysine; CAT: catalase; SOD: superoxide dismutase; ROS: reactive oxygen species.

Table 4 *Effect of chromium on lamb performance*

Authors	Chromium Source and level	DMI	ADG	BW	FCR
(UYANIK, 2001b)	CrCl3 (0, 200 or 400ppb)			No effect	
(MOSTAFA-TEHRANI et al., 2006b)	CrCl3 and CrNic (0, 200, 600, 1000 µg/ kg DM)		No effect	No effect	
(KITCHALONG et al., 1995)	CrPic (0, 250 µg/g DM)	No effect	No effect	No effect	
(SANO et al., 1997)	Cr chevilate ® (0.5ppm)	No effect	Improved		
(GENTRY et al., 1999)	CrPic (0, 400ppb)	No effect	No effect	No effect	No effect
(YAN et al., 2008, 2010)	CrYeast (0, 400, 800ppb)		No effect	No effect	
(DOMÍNGUEZ-VARA et al., 2009b)	CrYeast (0, 0.25, 0.35mg/head/d)	Decrease	No effect	No effect	No effect
(YULISTIANI; PUASTUTI; MATHIUS, 2013)	CrCl3 or Inorganic Cr(1.5mg/kg DM)	No effect	No effect	No effect	No effect
(ESTRADA-ANGULO et al., 2013)	CrYeast (0, 0.4, 0.8, 1.2mg Cr/head/d)		Improved	Improved	Improved
(MORENO-CAMARENA et al., 2015)	CrYeast (0, 0.2, 0.4 mg/ kgDM)			No effect	
(SÁNCHEZ-MENDOZA et al., 2015)	Cr-Met (0, 0.6, 1.2, 1.8 mg Cr/head/d)	No effect	No effect	No effect	No effect
(GRISS et al., 2020a)	CrLys (2.0mg/kg concentrade)		No effect	No effect	
(RODRÍGUEZ-GAXIOLA et al., 2020b)	CrYeast (0, 0.3mg Cr/kg DM)	No effect	No effect	No effect	No effect

CrCl3: chromium chloride; CrPic: chromium picolinate; CrYeast: chromium yeast; CrLys: chromium lysine; Cr-Met: chromium methionine.

Table 5 *Effects of chromium supplementation on carcass characteristics*

Authors	Chromium Source and level	HCW	Yield grad	Dressing %	Fat at 10th rib	Other fats	LMA
(KITCHALONG et al., 1995)	CrPic (0, 250 µg/g DM)	No effect	Decrease		Decrease	No effect for pelvic fat	No effect
(GENTRY et al., 1999)	CrPic (0, 400ppb)	No effect	No effect	No effect		No effect for midrib fat and pelvic fat	No effect
(YAN et al., 2008)	CrYeast (0, 400, 800ppb)	No effect		No effect	Decrease	No effect: pelvic fat, wall fat thickness Decrease: intramuscular fat rate	No effect
(DOMÍNGUEZ-VARA et al., 2009b)	CrYeast (0, 0.25, 0.35mg/head/d)	No effect					
(ARVIZU et al., 2011b)	CrYeast (2.5mg/head/d)	No effect	No effect			Reduce fat in meat and carcass	No effect
(ZHOU et al., 2013)	CrYeast (0, 300µg/kg DM)					Reduce abdominal fat mass and percentage	
(ESTRADA-ANGULO et al., 2013)	CrYeast (0, 0.4, 0.8, 1.2mg Cr/head/d)	Improved		No effect			Improved
(SÁNCHEZ-MENDOZA et al., 2015)	Cr-Met (0, 0.6, 1.2, 1.8 mg Cr/head/d)	No effect	Improved	No effect	Decrease	Decrease fat concentration on LM	No effect
(MORENO-CAMARENA et al., 2015)	CrYeast (0, 0.2, 0.4 mg/ kgDM)	No effect		No effect		Decrease perineal fat	

Table 5 Effects of chromium supplementation on carcass characteristics (cont.)

Authors	**Chromium Source and level**	**HCW**	**Yield grad**	**Dressing %**	**Fat at 10^{th} rib**	**Other fats**	**LMA**
(RODRÍGUEZ-GAXIOLA et al., 2020b)	CrYeast (0, 0.3mg Cr/kg DM)	No effect		No effect			No effect

CrPic: chromium picolinate; CrYeast: chromium yeast; Cr-Met: chromium methionine;

Ewes

Forbes et al. (1998) working with two different breeds (Suffolk and Gulf Coast Native) of yearling ewes, a concentrated-based diet and 370 ppm of supplemented chromium picolinate found no effects for ADG, DMI, gain-to-feed ratio, glucose and insulin kinetics on IVGTT test, plasma glucose, triacylglycerol, urea N, albumin, insulin, cortisol, T3 and T4. However a reduction in the mobilization of adipose tissue is suggestive by the lower concentration of NEFA influenced by chromium supplementation.

Célia et al. (2012) evaluated chromium supplementation on reproductive parameters of the ewe. Twenty-six ewes were divided into two groups: with 1.5 mg CrYeast or no chromium supplementation. There was no effect for BW and body condition score during the whole pregnancy, for pregnancy rate and prolificacy, and also for birth weight.

Mousaie et al. (2017) studied the effects of 3 mg CrMet/kg DM from five weeks prior to partum to 5 weeks post-partum supplemented to 40 ewes in a restricted diet (60% of NRC energy requirements in pre-partum). Chromium increase dry matter intake, decrease cholesterol and MDA pre-partum and alleviates BW changes. However, no effect was found for glucose and total protein and for offspring performance.

In 2020, two research groups evaluated colostrum profile of ewe supplemented with chromium during breeding season, pregnancy and lactation. Bompadre et al. (2020) worked with 32 ewes and four doses of chromium from Cr picolinate: 0, 0.15, 0.30 and 0.45mg CrPic per ewe. Chemical composition was not affected, but the chromium decreased lactoperoxidase activity, an important component of an innate defense mechanism in the body. Gallo et al.

(2020), on the other hand, found that chromium increase the concentration of glutathione peroxidade witch may improve the quality of colostrum, but others immune and oxidative evaluations were the same between treatments. For this trial, 32 ewes were allocated to one of three treatments: control diet (2.24Mcal of ME/kg DM), high energy diet (2.46Mcal of ME/kg DM) and a high energy diet with chromium propionate.

There is only a few works evaluating chromium supplementation in the female diet, but chromium may be an important tool for ewes in late pregnancy and beginning of lactation once this is a crucial phase for ewes and their progeny and chromium seems to alleviate BW changes and changes colostrum quality. Therefore, more studies with chromium supplementation for ewes at late pregnancy are strongly recommended.

Animals under stress

Sano et al. (1999) aimed to evaluate the effects of chromium supplementation on sheep under stress conditions (isolation). They used 0.5 mg/kg of chrome yeast. During the isolation period, no effect was found for glucose, NEFA, lactate, insulin and cortisol. However, in this case, the isolation itself did not affect these parameters either, showing that it did not cause much stress.

In 2000, Sano et al. (2000) worked with eight sheep in cold exposure to evaluated the effects of chromium on blood glucose turnover rate and sensitivity to insulin. The exposure last for nine days and ewes were supplemented or not with 1mg of Cr/kg from Cr yeast, but this supplementation had little or no effect on glucose metabolism.

Al-Mufarrej et al. (2008) worked with 48 stressed by transport lambs. After transport, lambs were allocated to four treatments groups: 0, 0.3, 0.6 or 0.9ppm Cr from Cr Yeast and stayed for 84 days. There was no effect for plasma glucose, total protein, albumin, total cholesterol, urea-N and cortisol, but chromium reduce rectal temperatures. Lambs were repeated immunized with CRBC (chicken red blood cells) and chromium supplementation was able to improve both humoral and cell-mediated immune responses. Performance, digestibility and carcass characteristics of these stressed lambs were published by the same researched group in 2009, showing that chromium yeast supplementation improves DMI and ADG, the best dose being 0.3ppm. The 0.3ppm doses also increased final life weight, slaughter weight, carcass, and kidneys weights. No effects were found for digestibility (KRAIDEES et al., 2009).

In 2014, Mousaie et al. (2014) supplemented 24 ewe lambs during nine weeks with 0.8mg Cr from chromium-methionine/kg DM. There was no effect for concentration of urea, insulin, cholesterol, total protein, globulin and T4. Chromium supplementation had no adverse effect on liver as there were no changes in the ALT and AST enzymes and had a positive effect on performance and stress mitigation. Feed intake, final BW, ADG, total gain, T3 and T3:T4 were increase and glucose concentration, feed conversation rate and malondialdehyde (MDA - indicator of lipid peroxidation) were decrease. The ewe lambs were transported for 30 minutes at week 8. After transportation, lambs that receive chromium had less glucose concentration than control, but no other parameter changed.

Sharma et al. (2020) evaluated the effect of nano chromium picolinate (nCrPic) during heat stress in 36 cross-bred sheep. The animals were allocated to treatments 0, 400 or 800 μg/kg nCrPic for eight weeks and then moved to metabolic cages and divided into two groups: neutral temperatures and heat stress for three weeks. Dietary nCrPic alleviates heat stress symptoms such as rectal temperatures and respiration rate and maintain average daily feed intake.

For stressed animals, chromium supplementation seems to mitigate the negatives effects of the stress and improve performance and immunity. Despite the promising preliminary results, more studies are necessary to recommend the best dose of chromium supplementation for stressed ewes. Chromium supplementation for heat-stressed poultry is already recommended for altering nutrient partitioning, improve immune and metabolic functions (BIN-JUMAH et al., 2020).

Toxicity and negatives effects

Dallago et al. (2011, 2013, 2015, 2016) worked with 24 lambs allocated to one of four Cr doses from chromium picolinate: 0, 0.250, 0.375 and 0.500 mg/CrPic/animal/day for 84 days and published four papers with the results. They were kept in individual pens and fed with Panicum maximum cv Massai hay and concentrate (85% cassava flour, 11.5% mineral salt and 3.5% urea). They showed negative effects of chromium supplementation: decrease protozoa count, lower reaction for skin thickness after intradermal injection of PHA (phytohaemagglutinin), findings of hystopathological damage and bioaccumulation.

Ruminal protozoa population was evaluated in supplemented sheep by Dallago et al. (2011). Chromium did not affect animal performance (DMI, initial

BW, daily gain, total body weight gain) and ruminal pH but decrease protozoa count, showing a possible toxic effect of chromium. On the other hand, Rocha et al (2016) found that Cr Yeast does not affect the concentration of protozoa in lambs. They used four lambs, in a cross over design, in individual metabolic cages and one gram of organic chromium diluted in 10mL of water was administered by intra ruminal infusion in two lambs while the other two, only water was infused during 20 days.

Dallago et al. (2013) also found that CrPic may be harmfull in terms of immunity when working with lambs that are not in a stressful situation. Although chromium supplementation did not affect leukocyte counts and anti-ovalbumin IgG production after, lambs on the control group had the highest reaction for skin thickness after intradermal injection of PHA.

Histopathological analysis in liver, kidney, heart, lung and testis was made but no significant differences were found. For blood parameters, during the role trial, the averages were within the references values and similar to control. No statistical proof of Cr toxicity was found (DALLAGO et al., 2015). Linearly increase in Cr concentration was observed in the heart, lungs and tests, but not for liver, kidney, spleen, lymph nodes, muscle and bone. Also, higher chromium in the diet equals higher elimination in the urine, in a quantity-dependent way (DALLAGO et al., 2016). However, although the histopathological exams were not statistically different, it still exists a minimal damage observed and, associated with the bioaccumulation capacity of some tissue, the authors recommend caution and to avoid the use of chromium picolinate as a dietary supplement.

White and Vincent (2019) also found the bioaccumulation capacity of chromium by reviewing papers about the Cr supplementation on chickens. But Spears at al. (2019) support the safety of chromium propionate in broilers diet, indicating that Cr supplementation at 2 or 10 times the approved feeding level does not present a human health concern. Andersson et al. (2007) gave an intraperitoneal injection of CrPic in mices to test the genotoxicity of chromium, but found that a high concentration of CrPic may damage the DNA but only under non-physiological conditions. Asad et al. (2019) demonstrated that inorganic chromium may be toxic for fish but organic chromium is not and should be recommended for fish produces to improve growth performance. Berner et al. (2004) also determinate that is safe the supplementation of chromium tripicolinate for humans at a maximum level of 2.4mg of Chromax® and CrPic have not been shown to be mutagenic in vivo.

Final considerations

Most chromium supplementation experiments have no effect on sheep or small evidence of a positive effect. Due to the lack of clarification on the levels of chromium requirement for ruminants, in addition to the presence of chromium in the different foods normally used, it is possible that this lack of response is related to the initial concentration of chromium in these animals. However, in under stress or more demanding phases such as late pregnancy and lactation, chromium supplementation seems to have more promising results. In addition, chromium supplementation appears to decrease fat in lamb carcasses, demonstrating its action in shifting energy partitioning. If this characteristic is economically viable for producers, it is recommended that more studies be done to suggest the best dose.

In general, with the data provided today, it is not possible to recommend the use of chromium in the diet of sheep; however it is recommended that further studies be carried out, especially in animals under stress conditions and in the most demanding production phases (final gestation and lactation).

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CAPÍTULO 4.

Effects of ewe nutrition on the development of their offspring: the role of fetal programming

Abstract: Fetal programming is the term used to refer to the consequences on the formation and development of fetuses, caused by different stimuli during pregnancy, especially maternal nutrition. These effects need not always be negative, but knowing the effects of maternal nutrition on fetal development, it is possible to manipulate diets to increase the productivity of the herd. Maternal nutrition, both malnutrition and excess energy in diets, can affect the offspring's muscle, fat and bone development, in addition to the organs. It also influences birth weight, especially nutrition at the end of pregnancy, and postnatal growth. Evidence also suggests that maternal nutrition may affect the reproductive performance of their offspring. Sheep's nutrition during pregnancy must be controlled to ensure maximum productivity and survival of its offspring.

Keywords: maternal nutrition, energy, lamb production, malnutrition.

Introduction

Fetal programming or developmental programming refers to the short- and long-term consequences on the formation and development of the fetus, caused by maternal insults during pregnancy (GAO et al., 2008; KENYON, 2008; IGWEBUIKE, 2010; ROCA FRAGA et al., 2018; GREENWOOD; BELL, 2019a; REYNOLDS et al., 2019; HUBER et al., 2020; TROTTA et al., 2021).

This concept was first described by Barker et al. (1992), in an epidemiological study in humans in which it was possible to associate low birth weight with insults that occurred during pregnancy (for example exposure to stress-related hormones) and the risks of developing pathological conditions as adults or adolescents.

Controlled studies have shown that virtually every organ system and metabolic function are affected by developmental programming in livestock and other food animals (REYNOLDS et al., 2019). It is important to understand that fetal programming is not always bad (GREENWOOD; BELL, 2019b; REYNOLDS et al., 2019). Knowing the effects of maternal nutrition on fetal development is useful to prevent its negative effects and also to be able to manipulate the maternal diet to obtain more lambs and of better quality. Therefore, this review aims to summarize the latest research on the consequences of maternal nutrition on progeny development.

Development of muscle, adipose tissue and bone

The fetal stage is critical for skeletal muscle, adipose tissue and connective tissue development (YAN et al., 2013). Multipotent stem-cells are converted into muscle cells in a process called myogenesis and in livestock, all muscle fibers are formed during prenatal stages (MARKHAM et al., 2009; DU et al., 2010, 2015; YAN et al., 2013). The fetal stage is critical for skeletal muscle development because is the phase that secondary myogenesis is ongoing (from about day 38 of pregnancy to day 110) and forming the majority of muscle fibers (Du et al., 2010; Greenwood and Bell, 2019; Markham et al., 2009; Yan et al., 2013). Skeletal muscle matures at late gestation in sheep (105 of gestation) and nutrient restriction after this stage has no major impact on muscle fiber number but can influence the size (DU et al., 2010, 2015; YAN et al., 2013). After birth, there is no more increase in muscle fiber number (DU et al., 2010, 2015).

Therefore, maternal nutrition is important to ensure the good performance and development of offspring.

Compared to vital organs such as the brain, heart and liver, the skeletal muscle has a lower priority in nutrient partitioning during fetal development, becoming more vulnerable to maternal malnutrition (ZHU et al., 2004; DU et al., 2010; REYNOLDS et al., 2019). Maternal nutrient restriction around days 30 to 70 of gestation indicates a reduction in the numbers of fast fibers (predominantly secondary fibers) formed in young lambs (FAHEY et al., 2005) and produce lambs with lighter muscle (DANIEL et al., 2007), but that did not happen when the restriction was made during days 55 to 95 or 85 to 115 of pregnancy (FAHEY et al., 2005).

Ithurralde et al. (2019) studied the effects of maternal undernutrition on days 30 to 143 of pregnancy and found that male lambs born from ewes on low pasture allowance showed the reduced area and tended to show reduced perimeter of the *Longissimus* muscle and poorer quality carcasses and that the effects of maternal nutrition on growth performance, carcass and meat quality of lambs are sex-dependent.

Sen et al. (2016a) investigated the effects of maternal nutrition (50%, 100% and 150% of daily requirements) during mid-gestation (days 30-80) and found lighter muscles (semitendinosus, semimembranosus and gastrocnemius) on lambs born to the undernutrition ewes and lambs born to the overnutrition ewes had higher number of Type IIA and IIB muscle fiber and had an increase in the number of fibers/mm2 muscle area, but treatments did not affect meat quality.

Maternal restriction during periconception period (six days before and 7 days after mating) may alter muscle fiber diameter without affecting fiber types (SEN et al., 2016b) and maternal overnutrition from 18 days before and six days after ovulation increased total muscle fiber numbers and secondary fiber numbers (QUIGLEY et al., 2005). Prenatal nutrition restriction produces six months old lambs with heavier semitendinous muscle weight, larger muscle fiber diameters and shorter sarcomere length but does not affect carcass characteristics (NODBY et al., 1987).

In addition to muscle tissue, adipose tissue and fibroblasts are also derived from the mesoderm (DU et al., 2015). Intramuscular fat is important to add flavor and juiciness to the beef meat and it is determined by the number and size of intramuscular adipocytes (Du et al., 2015) and fibrogenesis refers to the formation of connective tissue (YAN et al., 2013). Myogenic, adipogenic or fibrogenic lineages can be considered a competitive process and are "shaped" by numerous inductive regulators. Switching of stem cells from myogenesis to adipogenesis may increase intramuscular fat and promote insulin resistance and switching to fibrogenesis leads to muscle malfunction (DU et al., 2010; YAN et al., 2013).

Exposure to widely different types of nutrition (either overnutrition or undernutrition) in late gestation can depress expandability of the subcutaneous adipose tissue in postnatal life. Maternal poor nutrition interferes with subcutaneous adipose tissue function resulting in decreased ability to accumulate fat in the subcutaneous area (KHANAL et al., 2014). However neither maternal under or overnutrition seems to have an impact on body size, weights of major organs, muscle, adipose tissue mass in adult offspring (KHANAL; NIELSEN, 2017).

Consequences on bone development and mineralization caused by maternal nutrition are less common to be observed (SARTORI et al., 2020). Bone development precedes the formation of muscles and adipose tissue and therefore, has priority use of nutrients, compared to soft tissues (GREENWOOD; BELL, 2019b). As a result, bone development becomes less sensitive to the mother's nutritional stimuli, requiring severe restriction to be affected (PILLAI et al., 2016).

Studies have shown that maternal nutrient restriction can result in smaller bones and a higher proportion of bone relative to muscle and fat within the fetus and newborn (BELL; GREENWOOD, 2016; PILLAI et al., 2016; HOFFMAN et al., 2017; GREENWOOD; BELL, 2019b) but overnutrition of pregnant ewes did not affect bone characteristics at birth (HOFFMAN et al., 2017; GREENWOOD; BELL, 2019b). Bone weight can be lighter on prenatal undernutrition lambs but with higher cortical density and higher wall thickness (TYGESEN et al., 2007). Osteoclast metabolism can be reduced and osteoblast metabolism not affected by nutritional constraint, overall, resulting in the relative increase in osteogenic activity (LI et al., 2020).

Fetal organ development

During sensitive periods of cellular proliferation, differentiation, and maturation, the developing embryo may respond to environmental stimuli, such as maternal nutrition. In response, there may be changes in the formation, metabolism or functionality of cells, tissues or organs in the long term (MOSSA et al., 2015).

Nutritional restriction may negatively affect the gastrointestinal tract because of its relatively large consumption of total energy expenditure (CATON; HESS, 2010; TROTTA et al., 2020). Nutrient restriction during late gestation may also influence fetal digestive enzymes as total fetal pancreatic trypsin content and α-amylase activity decrease with 60% of maternal nutrient restriction but there is no effect on fetal carbohydrase, glucoamylase, maltase, or isomaltase activities (TROTTA et al., 2020, 2021). Keomanivong et al. (2017) also found a decrease in α-amylase activity but no effects on pancreatic digestive enzymes.

Maternal undernutrition (50% maintenance requirements) during periconception period (SEN et al., 2016b) or mid-gestation (SEN et al., 2016a) did not influenced organ development (heart, liver, lungs, spleen, kidneys, brain and testes). However, overnutrition at 150% of NRC recommendation during periconception period can increase fetal heart, pancreas, and liver weights, as well as lipid content of fetal liver (GEORGE et al., 2010).

To verify at what time maternal nutritional restriction would have the greatest impact on organ development, Blair et al. (2011) allocated the ewes to *ad-libitum* (A) or maintenance (M) nutritional regiments from day 21 to 140 of pregnancy and collected foetal organs at days 65 (P65), 100 (P100) and 140 (P140) of pregnancy. No differences were found at P65. At P100, M-lambs had heavier livers and at P140 M-lambs had lighter thyroid glands but heavier livers and kidneys. However, overall, there were only a few effects of maternal undernutrition on organ development. Kenyon et al. (2011a) allocated ewes to ad libitum (A) or maintenance (M) nutritional regimens from day 21 to day 140 of pregnancy and collected fetal organ on day 140 of gestation found no effect on foetal weight or dimensions, the weight of kidney, perirenal fat, heart, thymus, adrenal glands or gastrointestinal tract, mammary gland, ovaries or scrotum.

Kleemann et al. (2015) worked with 466 Merino ewes allocated to treatments with different levels of energy during periconception period (70, 100 and 150% of requirements). At five days old, fetal organs were collected. Neck thymus, ovaries and peri-renal fat were heavier for the high energy treatment and liver mass were heavier for low energy lambs.

Posnatal performance

Mistakes in the nutritional management of pregnant ewes can lead to a deficient uteroplacental blood flow and supply of nutrients compromising fetal development (GREENWOOD; BELL, 2003, 2019b; REDMER; WALLACE; REYNOLDS, 2004; REYNOLDS et al., 2019; CASTRO-RODRÍGUEZ et al., 2020; SARTORI et al., 2020), consequently decreasing the efficiency in lamb production (SARTORI et al., 2020). The main factor that influences fetal growth and postnatal performance is maternal nutrition (CATON; HESS, 2010; KENYON; BLAIR, 2014; KHANAL; NIELSEN, 2017).

Major fetal growth occurs in the final third of pregnancy, so the fetus becomes more susceptible to changes in the environment at this time. However, the formation of the placenta occurs earlier and maternal malnutrition can lead to a failure in placental development, consequently preventing the full growth potential of the fetus at the end of pregnancy (GREENWOOD; BELL, 2019b).

Sartori et al. (2020) published a review identifying studies with maternal under and overnutrition during pregnancy and its consequences on weight morphometric measurements of the progeny. This meta-analysis showed that effects of overnutrition are heterogeneous but those of undernutrition are consistent and influenced by litter size, time and duration of the intervention and overall reducing nutrient intake during pregnancy, mainly in the last third or during more than 90 days, produces lighter lambs, but does not influence their size. Mean lamb birth weights can be 18% lower in undernutrition than control ewes (BORWICK et al., 2003).

Kenyon and Blair (2014) also reviewed the effects of maternal nutrition on fetal growth, birth weight and growth until weaning. They suggested that overnutrition has a limited impact on birth and weaning weight and undernutrition has to be severe at the beginning of pregnancy to cause any effects on birth weight or occurs in the last third of pregnancy.

For Greenwood and Thompson (2007) and Igwebuike (2010), when the negative stimulus occurs at the beginning of pregnancy, the adequacy of nutrition in the middle and end of gestation can overcome the negative effects, producing lambs with normal weight but this recovery depends on the female's body condition score during the preconception period.

Just like the authors above, Gardner et al. (2007) state that the maternal diet at the first and second third of gestation has little influence on the lamb's birth weight, but energy intake at the final third has a positive association. Also, the body condition score of the female at late gestation has a positive correlation with the birth weight of lambs (KALYAN DE et al., 2019).

Daniel at al. (2007) restricted maternal nutrition between day 30 and 70 of pregnancy and found that maternal nutrition had a negative impact on growth and slaughter weight but had no influence on birth weight. Ithurralde et al.

(2019) worked with maternal undernutrition (low pasture allowance x high pasture allowance) from day 30 of pregnancy until day 143 and found no differences for lambs birth weight or body measurements at birth, probably because ewes were supplemented from day 143 and that could have mitigated any undernutrition negative effects. Lambs born from ewes with nutritional treatments during mid-pregnancy (50, 100 and 150% daily requirements) had no differences for birth weight and weaning weight but those born from undernutrition ewes were lighter at slaughter (SEN et al., 2016a).

Lambs born to nutrient restricted (60% of nutrients requirement) ewes at the end of pregnancy weighed 19.5% less at birth and had a 25% lower growth rate than those born to non-restricted ewes, but showed higher compensatory growth after weaning (TYGESEN et al., 2007).

Overnutrition at late gestation may also be negative for the offspring, producing lighter placental weight and lighter lambs, but with normal nutrition, after birth, the negatives effects can be ameliorated with rapid catch-up growth of the lambs from birth to six months (WALLACE et al., 2012). In this same study, Wallace et al. (2012) found that different from overnutrition, lambs born from undernutrition ewes didn’t have a rapid catch-up growth, keeping with the relatively modest degree of prenatal growth restriction. On the other hand, overnutrition (125% requirements) during periconception period may produce bigger lambs with higher fetal crown-rump length, thoracic and abdominal girths, and fetal perirenal fat (GEORGE et al., 2010).

Maternal nutrition during the preconception period can also affect fetal development (CASTRO-RODRÍGUEZ et al., 2020; ROSALES-NIETO et al., 2020). Ewes receiving 50% of nutrients required for maintenance produced heavier female lambs at birth and weaning and with faster growth, comparing with ewes receiving 100% or 200%, suggestive of reprogramming of pathways regulating growth before conception (ROSALES-NIETO et al., 2020). Body conditional score during the breeding season and the maternal nutrition in the final third of pregnancy are the most important factors to determine the lamb's birth weight (GARDNER et al., 2007; CASTRO-RODRÍGUEZ et al., 2020; ROSALES-NIETO et al., 2020).

Igwebuike (2010) review the impact of maternal nutrition on fetal development and suggested that alteration of the insulin-like factors cascade suck as IGF-1 and IGF-2 may play an important role in intrauterine nutrition-associated compromised fetal growth. Insulin-like factors mediate maternal and placental metabolism and transplacental nutrient transfer and the synthesis of these proteins or their receptors may be affected by undernutrition.

Extremely important for lamb production, neonatal mortality is also influenced by fetal programming because many factors associated with an increased risk of neonatal mortality and morbidity are reflexes of maternal malnutrition during pregnancy (PERRY et al., 2019). Among the factors associated with higher lamb mortality, the most important are: premature birth, birth weight, dystocia, poor adaptation to the postnatal environment, maternal-lamb behavior and colostrum intake by the lamb (KHALAF et al., 1979; PERRY et al., 2019).

Consequences on reproduction

A good performance of a female depends on her ovulation rate, ability to conceive and to generate lambs with high weight at weaning (VAN DER LINDEN et al., 2010). Maternal nutrition may reduce the ovulation rate of the offspring and affect ovarian development (RAE et al., 2001, 2002a; BORWICK et al., 2003; RHIND, 2004). Mossa et al. (2015) affirm that long-term effects caused by maternal nutrition may impair reproductive efficiency in the offspring and therefore, special attention should be given to nutrition throughout pregnancy. However, there are not many studies following the long-term effects on the reproductive functions of female offspring and the results are still divergent.

Unlike productive performance, such as birth weight and weight gain after birth, in which maternal nutrition at the end of pregnancy has a fundamental role as it is the period of greatest fetal development, changes in the reproductive functions of the offspring can occur during all periods. This is because the development of the reproductive system happens throughout the gestational period: in the early third of pregnancy, there is sexual differentiation and the onset of meiosis in females. In the second third, there are primordial follicles and primary follicles visible in the ovaries of the offspring and aglandular nodules in the uterine endometrium. In the final third, there is the glandular development of the endometrium and antral follicles are visualized (MOSSA et al., 2015).

Borwick et al. (2003), Rae et al (2002a) and Kotsampasi (2009) studied the effects maternal undernutrition on the hypothalamic–pituitary–gonadal axis function in the offspring and founded different results, depended on the timing of the nutritional restriction. Borwick et al. (2003) limited maternal nutrition by 70% at the end of pregnancy and found no consequences on reproductive parameters of the female offspring. Rae et al. (2002a, 2002b) showed that maternal undernutrition throughout the first 3 months of gestation decreased male fetal pituitary sensitivity to exogenous GnRH but female fetal pituitary sensitivity was unaffected. In contrast, Kotsampasi (2009) founded that maternal undernutrition during the first month of pregnancy resulted in increased pituitary sensitivity to GnRH on female lambs at 10 months of age and decrease number of corpora lutea. It has been suggested that effects on pituitary sensitivity to GnRH may be related to reduced thyroid hormone concentrations in the fetus (RAE et al., 2002a).

Rae et al. (2002b) limited ewe nutrition by 50% during early and middle pregnancy and conclude that pre-natal undernutrition reduced ovulation rate in female progeny but had no effect on male reproductive development and adult function. Undernutrition during periconception period may increase oocyte population of the 30-days old ewe lambs (ABECIA et al., 2014) and 60-days-old ewe lambs (ABECIA et al., 2015), which is likely to reflect a delay in ovarian development (MOSSA et al., 2015). Overnutrition can also cause a negative effect on ovulation rate since high maternal nutrient intakes alter foetal ovarian follicular development before the final third of pregnancy (DA SILVA et al., 2003).

Van Der Linden et al. (2010) followed 207 ewe lambs from birth to first pregnancy at two years old born from ewes with ad libitum or maintenance nutritional regiments during pregnancy and found no differences in reproductive performance but maintenance regiment increased lamb birth and weaning weight and lamb growth rates of the grand-offspring.

Asmad et al. (2015) allocated 450 heavy ewes and 450 light ewes to receive maintenance or ad libitum diets from day 21 to 140 of pregnancy and then follow reproductive parameters of the female offspring during five years. At

2050 days of age, ewe lambs born from ewes receiving ad libitum diets had higher ovulation rate and gave birth to more lambs, but results had minimal impact.

Da Silva et al (2001) shown that if female lambs were fed ad libitum after birth, prenatal growth restriction is not detrimental to the onset of puberty, but that does not mean that undernutrition won't affect reproductive parameters later in life.

Further studies are needed to verify the extent of the problems generated by maternal malnutrition, but there is evidence that, depending on the period of pregnancy and the degree of insult, negative effects may occur on the reproductive development of the offspring.

Metabolics Consequences

The glucose-insulin regulatory axis is an important target for fetal programming and under/overnutrition can cause permanent changes in its function (KHANAL; NIELSEN, 2017). Kongstedet al. (2014) fed 50 (low) or 100% (ctl) energy and protein requirements during late pregnancy and low lambs had reduced insulin sensitivity at six months old. Husted et al. (2007) using this same diet and period found that low lambs at 19 weeks old had less insulin-secretory capacity. Overall, maternal undernutrition during late pregnancy have a negative impact on the offspring energetic efficiency (HUSTED et al., 2007; KONGSTED et al., 2014; KHANAL; NIELSEN, 2017; ADHIKARI; KHANAL; NIELSEN, 2018).

Maternal overnutrition also has long terms implications and reduces hepatic glucagon signaling of 6 months old lambs (ADHIKARI; KHANAL; NIELSEN, 2018) and can also predisposed for hyperglycaemia and hyperlactataemia, possibly due to increased hepatic gluconeogenetic capacity (KHANAL et al., 2015).

The use of starch as a source of maternal energy during pregnancy can increase basal insulin levels and lower insulin sensitivity during the first pregnancy of the daughter, comparing with the use of high digestible fiber as the energy source, therefore, the use of starch diets during intrauterine life could be beneficial to favor nutrient portioning towards insulin-independent sites such as the fetus and mammary gland (LUNESU et al., 2020).

However, it's necessary to pay attention to the energy level of maternal diets. Overnutrition can produce lambs with higher total and LDL-cholesterol at birth and during the 11-week suckling period and with mildly impaired glucose tolerance at 6 months of age, but by 6 months of age, the fasting lipid profile was back to normal and fat metabolism unaffected by prenatal diet (WALLACE et al., 2012).

Late gestation undernutrition can also negatively impact postnatal cholesterol metabolism in sheep offspring, but how bad the consequences depend on postnatal nutrition (KHANAL; NIELSEN, 2017). Prenatal undernutrition was also associated with increased triglyceride, ceramide and free fatty acid contents in livers of adult sheep (HOU et al., 2014).

Consequences on lamb behavior at birth

Maternal nutritional may affect lamb behavior at birth, but data are inconsistent. Maternal under or overnutrition during late gestation did not affect birth assistance, lamb vigor, suckling assistance, length of time from birth until stood successfully, and rectal temperature of the lamb (DONNEM et al., 2020).

In agreement with these findings, McGovern et al. (2015b) supplying 80, 100 or 120% of nutrients required for late gestation found no effect for the length of time both lambs spent attempting to stand after birth or the length of time from birth until they stood successfully. Freitas-de-Melo et al. (FREITAS-DE-MELO et al., 2015, 2017, 2018) found no effect for ewe-lamb attachment and lamb behavior when ewes were in low pasture allowance during pregnancy and Gronqvist et al. (GRONQVIST et al., 2016, 2018), Rocha et al. (2018) and Dwyer et al. (2003) also found no effect of maternal diet on lamb behavior.

On the other hand, Corner et al. (2010) showed that ewes with less pasture available during pregnancy produce lambs weaker and less likely to vocalize. Dwyer (2003) found that ewes that did not lose body condition score during their pregnancy produce lambs that stood, sought the udder, and sucked more quickly than those that needed to mobilized body reserves. Although some adverse consequences may occur due to poor maternal nutrition during pregnancy, this effect does not seem to interfere with the lambs' survival.

Supplementation with fish oil for ewe at late gestation can decrease the latency to suckle, increases gestation length, and may improve lamb survival rate (CAPPER et al., 2006b).

Abecia et al.(2014) studied the effects of maternal undernutrition during periconceptional period and concluded that this insult leads to a significant decrease in the voluntary locomotor activity of one months old lambs but the duration of the insult wasn´t enough to produce chances in the cognitive development of the offspring at 60 days old, no more difference were found for voluntary locomotor activity or cognitive responses (ABECIA et al., 2015).

Kleemann et al. (2015) also working during the periconceptional period, used three different diets (70, 100, and 150% maintenance requirements) and found that lambs from undernutrition ewes took less time to make contact with their mothers and contacted the udder quicker than other lambs too, but there were no differences for lamb vigor score, time to bleat and stand after release at tagging. Lambs born from overnutrition mothers had no differences from those in the control group.

Conclusion

There is evidence that maternal nutrition throughout pregnancy can affect offspring development with long terms consequences. Under or overnutrition may negatively affect the number of fiber muscles and that cannot be overcome with postnatal nutrition. Undernutrition at the end of pregnancy can produce lighter lambs and affect mother-lamb behavior at birth, reducing the lamb's survival rate. A few consequences can also be reflected on the ewe lamb reproduction later in life. Therefore, maternal nutrition has to be closely looked at during the role pregnancy to avoid negatives effects on the development of the offspring.

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MÓDULO 2. ARTIGOS COMPLETOS

CAPÍTULO 5.

Artigo n°1

Artigo que será submetido à revista Scientia Agricola

The addition of chromium in a high energy diet for pregnant ewes has a beneficial effect on reproduction traits for both ewes and their offspring

Abstract: The amount and source of energy are important factors that influence an ewe's reproduction ability, but the effects of the diet on the reproductive performance of its offspring are not clear. The aim of this study was to investigate the effects of maternal nutrition (energy level and source) on the reproductive performance of the ewe and its offspring. Seventy-two Dorper x Santa Inês ewes were allocated to five treatments in a completely randomized block design; Treatments were based on NRC recommendations: LOW (*n*=14) 90% of predicted ME requirement, CTL (*n*=14) 100% of predicted ME requirement; ST (*n*=15) 110% of predicted ME requirement and the energy source was starch; ST+Cr (*n*=15) ST more chromium propionate and ST+RPF (*n*=14) ST more palm oil rumen protected fat. Non-parametric data was evaluated by the Fisher exact test and parametric data by the Tukey test, at 5% significance. The treatments did not differ in terms of pregnancy rate (*P*=0.1944) or prolificacy (*P*=0.5729), but the ST+Cr diet anticipated the return to estrus after lambing (*P*<0.001). The LOW diet decreased lamb survival (*P*<0.005) and decreased the offspring's reproductive potential. The use of chromium had a positive effect on the reproductive parameters of the offspring and ST+RPF had a negative effect. This work shows evidence that the source

and the energy level fed to the ewe affect the fetal programming of the offspring.

Keywords: Fetal programming; Lamb survival; Nutrition; Protect Fat, Puberty, Sheep.

Introduction

The nutrition of ewes affects age at puberty, ovulation rate, birth interval, embryo survival, fetus development and neonatal metabolism. Mainly, dietary energy alters different reproductive parameters. Scaramuzzi et al. (2006), state that a negative energy balance diet has as a consequence: inhibition of GnRH secretion by the hypothalamus, absence of LH pulses, low FSH concentrations, inhibition of folliculogenesis, low estradiol, high negative feedback sensitivity, anovulation, anoestrus and delayed puberty.

The main source of energy supplementation for ewes is corn starch. When starch is fermented in the rumen, greater amounts of propionate are produced in relation to acetate, increasing the glucose available in the blood, through hepatic gluconeogenesis. However, it can decrease the fat in the milk and generate a greater production of lactic acid, decreasing the ruminal pH (KLEVENHUSEN, 2019). Another source of energy is the calcium salts of fatty acids (protected fat), which is less affect by biohydrogenation and release more energy than starch (SILVESTRE et al., 2011). Chromium supplementation focuses on making a better use of the energy available in the diet, as it facilitates the action of insulin on body cells (VINCENT, 2000, 2004; LEIVA et al., 2017).

The maternal diet not only affect the reproductive life of the ewe but also can change the reproductive parameters of its offspring. Since the 1970s, researchers have recognized that metabolic changes during prenatal nutrition can impact the productivity of offspring after birth. But few have tried to manipulate the maternal diet in order to "program" the fetus' performance during its life (BELL, 2006).

Maternal under nutrition during the mating season and early gestation slows the development of the ovaries in the fetus (RAE et al., 2001; BORWICK et al., 2003) and reduces the rate of ovulation in the adult offspring (RAE et al., 2002a) . Under nutrition in the early and middle gestation period promotes a decrease in the number of ovulations in the offsprings (KOTSAMPASI et al., 2009). The excess of nutrients at the beginning and middle of pregnancy can impair the establishment of the ovarian follicular reserve and, consequently, the reproductive potential in female fetuses (DA SILVA et al., 2003).

The hypotheses are that a low energy diet has a negative effect on the ewes' reproduction and on their offspring and that the addiction of chromium propionate and rumen protected fat (RPF) can have a positive effect on the offspring reproduction life.

The aim of the study was to investigate the effects of maternal nutrition on the reproductive performance of the ewe and its offspring studying the energy levels (low, control and high), different source of energy (starch and RPF) and addiction of chromium propionate.

Material and methods

All procedures were approved by the Ethics Committee for the Use of Animals, Faculty of Veterinary Medicine and Animal Science, University of São

Paulo (protocol no CEUA 2700201218). This study was conducted at University of São Paulo, Faculty of Veterinary Medicine and Animal Science, Pirassununga, São Paulo, Brazil.

<u>Animals and nutritional treatments</u>

Seventy-two Dorper x Santa Inês sheep (59.65 ± 10 kg, 2 to 4 age) were allocated in a completely randomized block design and distributed in five treatments: The treatment were based on the National Research Council (2007) as follows: **CTL** (*n*=14) 100% of predicted ME requirement; **LOW** (*n*=14) 90% of predicted ME requirement, **ST** (*n*=15) 110% of predicted ME requirement and the energy source was starch; **ST+Cr** (*n*=15) ST more chromium propionate and **ST+RPF** (*n*=14) ST more palm oil rumen protected fat (Table 6).

The requirement was calculated used this equation (NRC, 2007):

$$MEpreg\ (Mcal/d)\ = (36.9644\ x\ e^{(-11.465\ x\ e^{(-0.00643\ xT)-0.00643\ x\ T)}}x\ \left(\frac{LBW}{4}\right)/0.13]$$

[equation 1]

In which ME is metabolizable energy during pregnancy, T is the gestation length in days, LBW is the lamb's birth weight in kg.

For lactating ewes the determination of the metabolizable energy according to NRC (2007), is represented in equation 2 and 3:

NE_{LR} = MY x (0.25173 + 0.08964 x MkF + 0.03785 x MkTP / 0.93) [equation 2]

ME_{LR} = NE_{LR} / 0.644 [equation 3]

In which: MkF is the milk fat content, g/100g; MkTP is the milk total protein, g/100g; MY is the total milk production, kg/d.

Total milk production value of 1 L / d was considered, protein content of 5 grams and 7 grams of fat. The lambing date was estimated by the date of the mating and the pregnancy was evaluated by ultrasound. The requirement for crude protein, calcium and phosphorus was as recommended by the NRC (2007).

Ewes received the experimental feed from 30 days before the start of mating up to 50 days of pregnancy and then from 100 days of gestation until weaning. From day 51 until 99 days in gestation, all animals received the CTL feeding.

Sheep management

Ewes were treated with a controlled intra-vaginal drug release device (Eazi-breed CIDR Sheep and Goat Device, 0.33mg progesterone in inert silicone elastomer - ZOETIS Veterinary Products Industries Ltda) for 12 days. At CIDR® removal, the ewe were injected with 1.25ml (250ui) Pregnant Mare Serum Gonadotropin (PMSG; Novormon® ZOETIS Veterinary Products Industries Ltda) and 2,0 ml prostaglandin (Cloprostenol sodium 25mg; Sincrocio® Ouro Fino Animal Health) for better estrus synchronization (URIOL et al., 2019). At 24h and 36h after CIDR® removal, ewes were put together with a male for controlled breeding: one male with 5-6 females for an hour to observe the mating. Rectal ultrasound on day 30 after breeding was performed to determine pregnancy rate (number of ewe pregnant per ewe in mating) and prolificacy was calculated on number of lambs born per sheep.

Offspring management

At birth season, all paddocks were inspected during day (600 to 1900) to record ewe and lamb identity, litter size, birth-weight, sex, mortality and to dip the navel of each lamb in a 10% iodine solution. In all paddocks there was a creep feeding available for lambs, with ration ad libitum. The creep feeding concentrate had 22% of crude protein and 3.2 Mcal of ME.

Lamb survival was defined and analyzed in ways (a) proportion of lambs born alive of all the lambs born (b) proportion of lambs alive on day 7 of lambs alive at birth, (c) proportion of lambs alive on day 7 of all lambs born (d) proportion of lambs alive on weaning of lambs alive at birth and (e) proportion of lambs alive on weaning of all lambs born.

After weaning, ewe lambs (n=46) stayed together and were fed with maize silage, corn and soybean meal, meeting their growth requirements (NRC, 2007; Table 6). The total diet had values of 14% crude protein and 2.6 Mcal of ME. Ewe lamb survival was defined as the proportion of ewe lambs alive at breeding season on ewe lambs alive at weaning.

Table 6 *Chemical composition of feed used in experimental diets of ewe during gestation and lactation.*

Nutrient	Maize silage	Corn grain	Soybean meal	Rumen Protected Fat
Dry Matter, %	26	88	91	98
Crude Protein, % of DM	7,0	9,0	40	
NDF, % of DM	70	9	15	
ADF, % of DM	44	3	10	
ME, Mcal/kg	1,98	3,2	3,0	5,41
EE, % of DM	1,3	4,3	1,6	84,5
Mineral material, % of DM	7	2	7	15
Calcium, % of DM	0,35	0,02	0,38	12
Phosphor, % of DM	0,19	0,3	0,71	

EE = ether extract; ME = metabolizable energy; ADF = acid detergent fiber; NDF = neutral detergent fiber.

Ewe lambs reproduction management

At seven months old, thirty-one lambs were selected for the breeding season and considered as lambs for replacement. The criteria used was minimum body weight of 40 kg, thus ensuring that the animals were in puberty, therefore, able to reproduce. Several criteria can be used to define puberty in the female. According to Senger (2003), it can be considered that the animal achieves its puberty at age witch a female can support pregnancy without deleterious effects. The average age in puberty in female sheep is seven months (SENGER, 2003). The onset of puberty is closely linked to the female's weight, and in sheep it can be considered when reaching 60-70% of adult weight (KENYON, 2013; KENYON; THOMPSON; MORRIS, 2014)

For the breeding season, two White Dorper rams were introduced into the paddock of 31 ewes for 34 days. Each ram was fitted with a crayon harness (different colors), and on Day 17 the crayon color was changed to allow detection of ewes that did not conceive, expressed a second estrus and re-mated. In that way it was possible to determinate the mating day.

Transrectal ultrasound evaluations were performed on day 28 after mating in order to diagnose pregnancy (MORAES et al., 2009). On days 40 and 60 of pregnancy, ultrasound was carried out to determine embryonic and fetal death, respectively. Late embryonic death occurs between 30-40th days of gestation and after that is considered fetal death (YOTOV, 2012).

At birth, it was recorded the lamb identity, litter size, birth-weight, sex, mortality and dipped the navel of each lamb in a 10% iodine solution. It was calculated for each treatment: pregnancy rate and prolificacy.

<u>Blood samples and hormonal assays</u>

Blood samples were collected from jugular vein of all ewe during the estrus synchronization (n=72) and for estrus return (n=71), and for puberty of all female lambs (=37) into 9 ml vacutainers with no anticoagulant. Samples were centrifuged at 1500xg for 15 min and serum separated and stored at -20°C until analysis for estradiol (E2) and progesterone (P4).

With the use of synchronization protocols with P4 and eCG, the onset of estrus behavior occurs at 34 hours after the CIDR removal (URIOL et al., 2019). Therefore, for E2 analyses during estrus synchronization, blood was collected at -12h, 0h, 12h, 24h and 36h, considering 0h as ovulation time. For progesterone analysis during this period, blood was collected at days 1, 4 and 7 after ovulation, to verify the formation and competence of the corpus luteum.

To verify return of estrus (moment when the progesterone dosage exceeds 1ng / ml), blood was collected twice a week from day 30 to day 60 after lambing. In the ewe lambs, progesterone measurement was performed twice a week from the fifth to the eighth month of life in order to verify the onset of puberty. To measure serum progesterone, the radioimmunoassay technique (RIE) in solid phase was used, using a commercial diagnostic kit from Siemens® (COAT - A - COUNT, Diagnostic Products Corporation, Los Angeles, CA, USA) developed for quantitative evaluation of progesterone in human serum, and previously validated for use in sheep serum. The quantification of serum estrogen was performed by the RIE technique, using the diagnostic set of double-stradiol 3rd generation estradiol from DSL®-39100 (Diagnostic System Laboratories, Webster, Texas, USA) due to its ultra-sensitivity.

<u>Statistical analyses</u>

The experimental design was completely randomized blocks based on parturition (nulliparous and pluriparous). Data were analyzed using the statistical package SAS 9.1.2 for Windows (SAS Institute Cary, NC, USA). The homogeneity of the variances and the normality of the residues were verified by the Shapiro-wilk and Levene's tests, respectively. Reproductive indices (pregnancy rate, estrus return, survival, puberty, suitable for reproduction and abortion) were analyzed using non-parametric frequency statistics (PROC FREQ), using the Fisher exact test at 5% significance. Hormonal analyzes and prolificacy were analyzed by the Tukey test, at 5% significance.

Results

Ewes

The percentage of pregnant sheep was not different between treatments (P =0.1944) as well as the prolificacy (P=0.5729). The percentage of sheep that returned to estrus between 30 and 60 days after partum was different among the diet groups (P<0.001). Half of the ewes on ST+Cr (53,33%) were able to return to estrus before weaning, while on LOW and CTL, only 7,7% and 14,3% did, respectively (Table 7).

Table 7 *Ewes reproductive traits and Lamb Survival rate from birth to weaning, from ewes with different levels and source of energy in the diet.*

	Pregnancy (%)	Prolificy	Estrus (%)	Survival A	Survival B	Survival C	Survival D	Survival E
Treatments	n=72	n=72	n=72	n=100	n=98	n=100	n=98	n=100
LOW	100	1.43±0.06	7.69b	90b	88.8b	80b	88.8b	80b
CTL	100	1.36±0.06	14.28b	100a	100a	100a	100a	100a
ST	100	1.53±0.06	35.71ab	100a	100a	100a	100a	100a
ST+CR	100	1.53±0.06	53.33a	100a	100a	100a	100a	100a
ST+RPF	92.85	1.23±0.06	38.46ab	100a	100a	100a	100a	100a
Litter size								
Single				97.7	95.2	95.3	95.2	95.3
Multiple				96.5	98.2	98.2	98.2	98.2
Treat*Litter size[£]								
LOW*Single				100a	90a	90a	90a	90a
LOW*multiple				80b	87b	70b	87b	70b

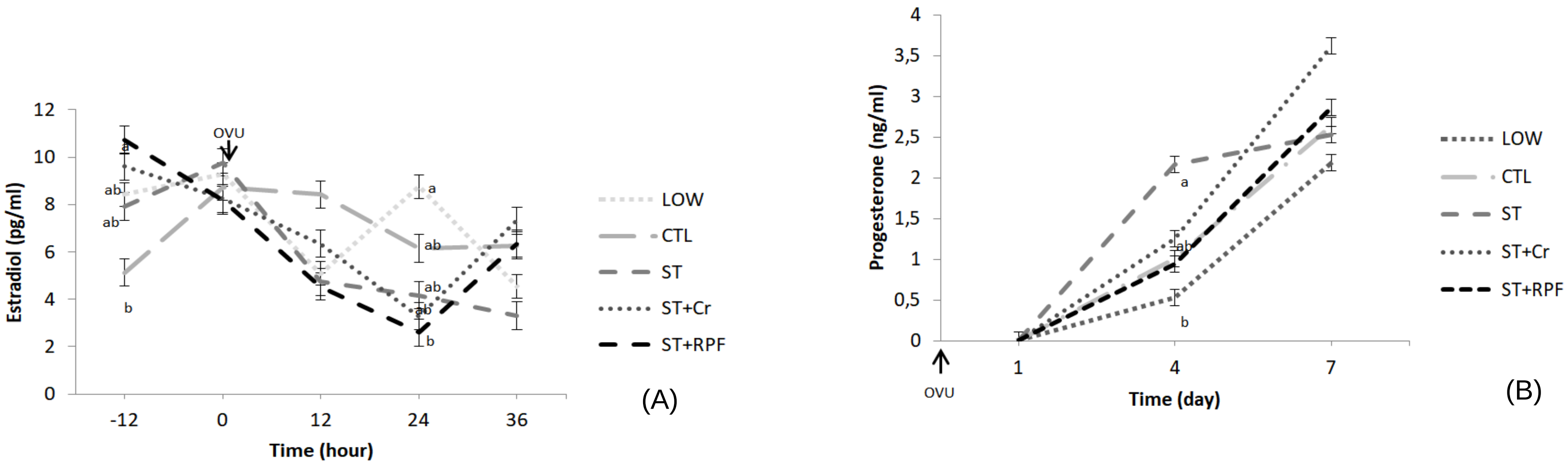
LOW (*n*=14) - 90% of predicted ME requirement, CTL (*n*=14) - 100% of predicted ME requirement, ST (*n*=15) - 110% of predicted ME requirement and the energy source was starch, ST+Cr (*n*=15) - ST more chromium propionate and ST+RPF (*n*=14) - ST more palm oil rumen protected fat. Prolificy - lambs born/ewe; Estrus – estrus return after lambing; Survival A - % alive d0/total born; Survival B - % alive d7/ born alive; Survavil C - % alive d7/ total born; Survival D - % alive at weaning/born alive; Survival E - % alive at weaning/total born; Letters on the column differ from the averages by the Fisher test at 5% probability for pregnancy, estrus and survival and by Tukey test at 5% probability for prolificacy.

£: the interaction between treatments CTL, ST, ST+CR, ST+RPF and litter size (single or multiple) had 100% of survival and letter a for Fisher test.

After the estrus synchronization, serum E2 differs between treatments at -12h (P=0.0234), when ewes on ST+RPF diet had the highest concentration and CTL the lowest, and 24h (P=0.0273) after ovulation, with LOW ewes having the highest and ST+RPF the lowest concentration (Figure 2A). Progesterone only differs at day 4 (P=0.0534), being ST treatment the highest and LOW, the lowest (Figure 2B). There is no difference for E2 at ovulation time (P=0.8711), 12h (P=0.1663) and 36h (P=0.2005) and for P4 at day 1 (P=0.5686) and day 7 (P=0.3386).

Lambs born from LOW ewes had lower survival rates (Table 7) for alive d0/total born (*P*=0.0027), alive d7/ born alive (*P*=0.0038), alive d7/total born (*P*=0.001), alive at weaning/born alive (*P*=0.0038) and alive at weaning/total born (*P*=0.0001). Litter size (single or multiple) had no influence on lamb survival alone, but the interaction between LOW group and multiple litter showed higher mortality on d0 (20%), d7 (30%) and weaning (30%) among all lambs born (*P*<0.001).

Figure 2 *Hormonal concentration after estrus synchronization from ewes with different levels and source of energy in the diet.*



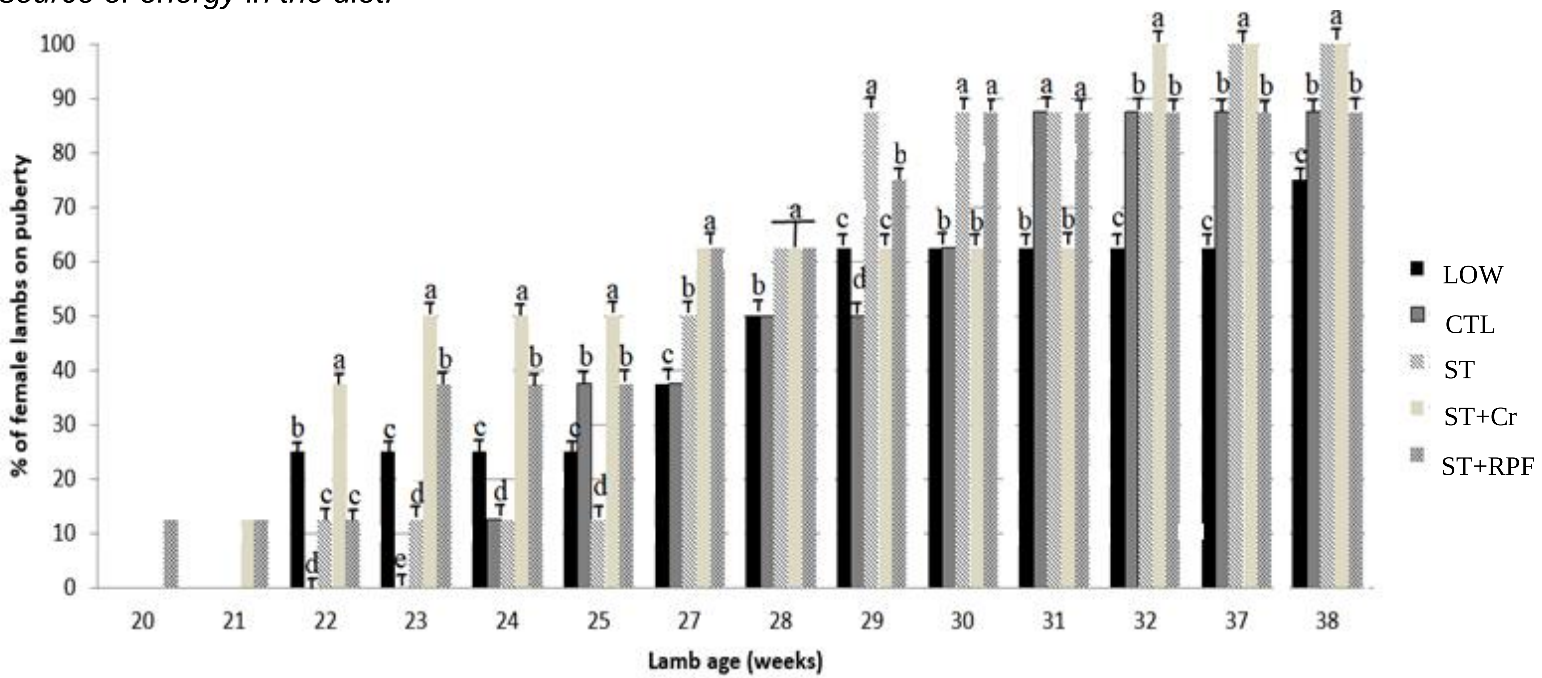
(A) Estradiol concentration; (B) Progesterone concentration; LOW (*n*=14) - 90% of predicted ME requirement, CTL (*n*=14) - 100% of predicted ME requirement, ST (*n*=15) - 110% of predicted ME requirement and the energy source was starch, ST+Cr (*n*=15) - ST more chromium propionate and ST+RPF (*n*=14) - ST more palm oil rumen protected fat. OVU=Ovulation time. Letters on the line differ from the averages by the Tukey test at 5% probability.

Ewe lambs

From 20 weeks of age, concentration of serum progesterone on ewe lambs was evaluated every week, to determine when they achieve puberty (progesterone >1.0ng/ml). Figure 3 shows the sum of the percentage of females who reached puberty between weeks 20 to 38, the dams diets influenced the beginning of puberty of their ewe lambs (P<0.005).

Between weeks 22 and 25, there were more ewe lambs that reached puberty on ST+Cr groups than any other (Figure 3), with 37.5% of ST+Cr ewe lambs in puberty on week 22, against 25% LOW, 12.5% ST+RPF and ST and 0% CTL (P=0.0054) and 50% on weeks 23 to 25, against 37.5% ST+RPF, 25% LOW, 12.5% STA and 0, 12.5 and 37.5% CTL on weeks 23, 24 and 25, respectively (P=0,001). On week 32 (P=0.0026), 37 (P=0.0013) and 38 (P=0.0033), LOW group had the lowest percentage of ewe lambs in puberty (62.5, 62.5 and 75%, respectively). All lambs from ST+Cr achieve puberty on week 32 and, from ST, on week 37. At the last week of measurements, CTL and ST+RPF had 87.5% of the lambs on puberty and from LOW group, only 70% which was significantly different (P=0.0003). Therefore, treatments ST+Cr and ST had a positive effect on anticipating puberty, especially ST+Cr. Treatments CTL, ST+RPF and LOW had negative effects.

Figure 3 *Sum of percentage of ewe lambs on puberty between weeks 20 and 38 of life, born from ewes with different levels and source of energy in the diet.*



LOW (*n*=14) - 90% of predicted ME requirement, CTL (*n*=14) - 100% of predicted ME requirement, ST (*n*=15) - 110% of predicted ME requirement and the energy source was starch, ST+Cr (*n*=15) - ST more chromium propionate and ST+RPF (*n*=14) - ST more palm oil rumen protected fat. OVU=Ovulation time. Letters on the line differ from the averages by the fisher test at 5% probability.

From all the lambs alive at weaning, survival rate was calculated on day 240 (Table 8) and the differences was significant (*P*=0.0095). ST and ST+RPF had 100% of survival while LOW and ST+Cr 75%. During this period, the animals were affected by worms (*Haemonchus contortus* and *Moniezia sp*.) that can result in mortality. On day 240, females over 40 kg were selected as suitable for reproduction (Table 8). All lambs from mothers on CTL, ST and ST+Cr were ready for reproduction, while 75% from ST+RPF and only 66.7% from the LOW diet (*P*=0.0080). There were significant different for pregnancy rates (*P*=0.0214) and abortion rates (*P*=0.005) but not for prolificacy (*P*=0.452) and lamb survival (*P*=0.006) (Table 8). CTL and ST+RPF had 100% of pregnancy rate, different from LOW and ST with 75% but not different as ST+Cr (83%). There was one case of abortion on treatment LOW.

Table 8 *Reproductive traits for ewe lambs, born from ewes with different levels and source of energy in the diet.*

	Survival (% d240/total alive on weaning)	Suitable for repro †	Pregnancy rate	Abortion	Prolificacy	Lamb survival (% d7/total born)	Total lamb born alive (F3 generation)/total female lamb (F2 generation)
Treatments							
LOW	75 (6/8)b	66.7 (4/6)c	75 (3/4)b	33.3 (1/3)a	1.0 (3/3)	100 (3/3)	0.375 (3/8)
CTL	87.5 (7/8)ab	100 (7/7)a	100 (7/7)a	0 (0/7)	1.2 (6/5)	83.33 (5/6)	0.625 (5/8)
ST	100 (8/8)a	100 (8/8)a	75 (6/8)b	0 (0/6)	1.17 (7/6)	100 (7/7)	0.875 (7/8)
ST+Cr	75 (6/8)b	100 (6/6)a	83 (5/6)ab	0 (0/5)	1.2 (6/5)	83.33 (5/6)	0.625 (5/8)
ST+RPF	100 (8/8)a	75 (6/8)b	100 (6/6)a	0 (0/6)	1.0 (6/6)	83.33 (5/6)	0.625 (5/8)

LOW (*n*=8) - 90% of predicted ME requirement, CTL (*n*=8) - 100% of predicted ME requirement, ST (*n*=8) - 110% of predicted ME requirement and the energy source was starch, ST+Cr (*n*=8) - ST more chromium propionate and ST+RPF (*n*=8) - ST more palm oil rumen protected fat. Letters on thecolumn differ from the averages by the fisher test at 5% probability. Letters on the line differ from the averages by the fisher test at 5% probability.

†ewe lambs at 8 months and more than 40kg.

Discussion

This study indicates that diets with more energy available can positively affect reproductive parameters of the ewe and the offspring. Although there were no significant differences on pregnancy rate and prolificacy, the diet can accelerate the return to estrus after lambing, especially when supplemented with chromium. Similar, the replacement of part of the starch with protected palm oil and Cr-propionate supplementation failed to affect fertility in cows (LEIVA et al., 2018). Supplementation with fat had no differences in days postpartum to the first progesterone rise and days postpartum to ovulation comparing with a control diet (OQLA; KRIDLI; HADDAD, 2004; TITI et al., 2008).

In this study there was a difference between the treatments for return to estrus after lambing (7.69 up to 53.33% of ewes ovulated within 60 days after lambing). For non-seasonal sheep breeds, for exemple Santa Inês breed, to enable the production of more lambs throughout their life, it is necessary to reduce lambing interval and obtain three births in two years. The duration of postpartum anestrous is one important economic factor, given that a minimal period between birth and the onset of ovarian cyclicity enables a new conception within a shorter period after lambing. Nutrition plays an important role in follicular development and consequently influences the period from lambing until the first estrus and ovulation (ASCARI et al., 2016). Adequate nutritional support during the early postpartum period is necessary for the animals to resume early ovarian activity. The nutritional supplementation with high energy diets stimulates folliculogenesis decreasing the negative feedback to induce compensatory increases in folliculogenesis (CROWE, 2008; ASCARI et al., 2016).

Delayed resumption of ovulation is invariably because of a lack of LH pulse frequency that can be a result of metabolic-related stressors (CROWE, 2008; CROWE; DISKIN; WILLIAMS, 2014) and in this study diets with less energy were not able to resume ovarian activity before 60 days as expected. The effects of low energy diets on reproduction are primarily at the hypothalamo-pituitary level of reproductive control and are characterized by suppressed plasma IGF-1 and elevated plasma GH, which are associated with anovulation and anoestrus in the female (WADE; JONES, 2004; SCARAMUZZI et al., 2006b).

Among the interactions of nutrition with reproduction in mammalian species, increased DMI in sheep was found to reduce circulating progesterone (P4) concentrations and reduce embryo survival. High energy intake decreased circulating P4 concentrations, probably through a primary effect on increased liver blood flow (MAHMOUD et al., 2012; VONNAHME et al., 2013; JING et al., 2017). Vonnahme et al. (2013) found that ewes with higher feed intake had lower circulating cortisol, with can argue against a generalized effect on hepatic steroid metabolism. The use of a diet with 30% more energy showed no effect on the concentration of E2 and P4 (MAHMOUD et al., 2012) despite that, supplementation with more energy level raised P4 and E2 concentration (JING et al., 2017). However, our study does not show a reduction on plasma E2 and P4 on ST, ST+Cr or ST+RPF treatments, maybe because there wasn´t a difference between DMI and only on the amount of energy available in each diet.

Ewes that received the lowest energy diet had lower percentage of survival lambs until weaning with a greater impact on multiple births. The ewe´s diet also influenced lamb survival from weaning to puberty in the same way, with the exception on the ST+Cr treatment that had a problem with worms. The successful rearing of lambs for slaughter and replacement of breeding stock is a key factor in profitable sheep enterprises (PERRY et al., 2019). The greatest loss in ruminant production systems occurs during the neonatal period (PERRY et al., 2019). Maternal malnutrition causes restrictions on intrauterine growth and is a major cause of fetal mortality and morbidity (GAO et al., 2014). Studies shows that diets with more energy given only near the insemination day does not influence lamb survival, probably because it's a short period of supplementation, but multiple birth promote less survival lambs (KLEEMANN et al., 2015) and lamb mortality was reduced when ewes were supplemented with safflower (rich in omega-6), probably associated with a higher total energy intake (GULLIVER et al., 2012). Nutrient restriction may affect neonate survival by influencing a) dystocia via placental dysfunction, b) thermoregulation after birth, c) modification of the developing immune system and d) modification of maternal and neonatal behavior (PERRY et al., 2019).

The term "programming" has been adopted to describe the process whereby a stimulus (good or bad) at a period of fetal life has permanent effects on the physiology, structure and metabolism of different systems (MOSSA et al., 2015; PERRY et al., 2019; REYNOLDS et al., 2019). However, only few studies have investigated the impact of maternal environment on the reproductive potential of offspring and even less studies have put this potential to the test. Mossa et al. (2015) in their review speculated that in utero undernutrition of female ovine fetuses during the first and second third of gestation causes a delay in ovarian development and reduces ovulation rate in adulthood and, similar to undernutrition, overnutrition may impair the establishment of the ovarian follicular reserve and consequently reproductive potential in female fetuses.

In this study, low-energy diets (LOW) also had a negative effect on the reproduction of the offspring, producing fewer replacement ewes, lower pregnancy rates, higher abortion rates, delaying puberty, showing that indeed malnutrition can decrease the reproductive potential of offspring.

Of particular interest it is evidence that the supplementation with rumen protected fat can have a negative effect on fetal programming. The protected palm oil delayed female puberty and by the age of eight months less than 90% of the female lambs were in puberty and only 75% of them were suitable for reproduction based on their weight. It has been well documented that increasing the precursor of fatty acids leads to an increase on steroid synthesis, especially high level of eicosapentaenoic acid (C20:5; EPA) and docosahexaenoic acid (C22:6; DHA) that are two components crucial of steroidogenesis and feeding supplemental fatty acids provides a higher level of energy and have positive impacts on reproductive performance by controlling ovarian function and follicular development (MIRZAEI-ALAMOUTI et al., 2018). However studies comparing palm oil (protected or not) with others oils or with no supplementation at all have shown that palm oil does not have the benefits of supplementation with protect fat (ABDEL-HAKIM et al., 2016; ASGARI SAFDAR; SADEGHI; CHAMANI, 2017; MIRZAEI-ALAMOUTI et al., 2018).

Supplementation with chromium may have positive effects on ewe reproduction and their offspring. In this study, more than half of the ewes receiving chromium propionate were able for mating 60 days after lambing and 100% of the ewe lambs achieve puberty before week 32, accelerating the cyclicality of both mothers and daughters.

On lactating cows, chromium supplementation tended to reduce the number of days to first estrus (63 vs 75d) and to first artificial insemination (71 vs 90d) and seemed to reduce the numbers of cows that did not conceive (YANG et al., 1996). Beef cows receiving supplemental Cr tended to have higher pregnancy rates and chromium may improve fertility. (STAHLHUT; WHISNANT; SPEARS, 2006). When given to periparturient cows under heat stress conditions, chromium supplementation increased the percentage of cows that were pregnant within the first 28 d of the breeding period (SOLTAN, 2010). Numbers of days to first ovulation after calving tended to be less for cows fed the diet supplemented with chromium (KAFILZADEH et al., 2012).

Conclusion

This study shows that it is possible to program the fetus's reproductive performance during its life by manipulating the mother diet. Providing diets with energy below the recommended is not indicated by its negative effects on the reproduction of the ewe, on the survival of the lambs and on the reproduction of the offspring. The use of rumen protected fat from palm oil had a negative effect on fetal programming, delaying puberty and decreasing the number of ewes for replacement, suggesting that other sources of oils should be used in a protected manner. As far as we know, this is the first work suggesting that chromium propionate supplementation may have a positive effect on the ewe reproduction by anticipating the return to estrus after lambing and on fetal programming, anticipating female puberty.

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CAPÍTULO 6.

Artigo N°2

Artigo Submetido na revista Livestock Science

Performance and behavior of the progeny of ewes fed with different sources and energy feeds

Abstract: The aim of this study was to investigate the effects of increased dietary energy intake, different sources of energy (starch and rumen protected fat) and chromium propionate addition to the diet during pregnancy on ewe and offspring performance as well as maternal-neonatal behavior. Seventy-two Dorper x Santa Inês ewes were allocated in a completely randomized block design to 1 of 5 treatments based on the National Research Council (2007) as follows: CTL (n=14) = 100% of predicted ME requirement; LOW (n=14) = 90% of predicted ME requirement, ST (n=15) = 110% of predicted ME requirement and the energy source was starch; ST+Cr (n=15) = ST more chromium propionate and ST+PF (n=14) = ST more palm oil rumen protected fat. Parametric data were evaluated by Tukey test, at 5% significance and behavior analyses by Kruskal-Wallis at 10% significance. Energy levels influenced ewes live weight, body condition score (BCS) and Famacha® score before and after lambing and during lactation, being that the use of palm oil protected fat had greater benefits. Although the dam's diet did not influence the offspring weight gain, body measurements and BCS and Famacha® at 260 days old, the addiction of chromium propionate produced heavier offspring. Lower energy diet negatively influenced the degree of Famacha® on mothers and offspring and mother-lamb behavior. This work shows evidence that the source and the

energy level fed to the ewe influences the performance of the ewe, fetal programming of the offspring and postpartum behavior.

Keywords: Chromium propionate; Fetal programming; Nutrition; Palm oil protected fat; Postpartum behavior.

Introduction

Lamb meat is the main product sold in the sheep meat production system (REINTKE et al., 2020) but to be profitable it is necessary to use technologies that improve animal performance. New knowledge about the effect of maternal diet on progeny aims to improve animal performance, and the energy diet may be a possibility (KENYON, 2013; KENYON; BLAIR, 2014; D'OCCHIO; BARUSELLI; CAMPANILE, 2019).

Maternal under and overnutrition have the potential to influence fetal growth, lamb birth weight, lamb growth from birth to weaning, live weight at weaning and also, maternal nutrition will have a great influence on when a lamb reaches target slaughter weight or whether a ewe can be bred in her first year of live (KENYON; BLAIR, 2014; MCGREGOR, 2017). Also, optimum ewe and lamb behavior at parturition is crucial to the well-being and performance of the neonate (RAMÍREZ-VERA et al., 2012; MCGOVERN et al., 2015b) and studies have shown that maternal nutrient restriction impairs the duration of ewe grooming behavior, weakening the ewe-lamb bond (MCGOVERN et al., 2015b) and ewes were more aggressive towards their lambs at birth than adequately fed ewes (RAMÍREZ-VERA et al., 2012).

The increase in energy available in the diet can be accomplished with supplementation of starch, fat or chromium propionate (LEIVA et al., 2018).

Diets with high starch content promote an increase in propionate, increasing glucose production and insulin release, which is favorable to the development of the fetus in the middle and final third of gestation (RADUNZ et al., 2011). Inclusion of fat in ruminant diets improve energy efficiency due to the direct use of long chain fattyacids in the metabolic pathways of fat synthesis and the inclusion of calcium is used to form a insoluble soaps that protects the fatty acids (FA) from rumen degradation (BHATT et al., 2013a). Chromium increases tissue sensitivity to insulin, making better use of all the energy provided by the diet (DAVIS; VINCENT, 1997a; SUMNER; VALDEZ; MCNAMARA, 2007a)

We hypothesized that greater maternal dietary energy mainly with the inclusion of chromium propionate and rumen protected fat (RPF) would increase ewe body weight (BW) and body condition score (BCS), progeny BW from birth to puberty, and strengthen mother-lamb bond. Objectives were to evaluate the effects of increased dietary energy intake, different sources of energy (starch and RPF) and chromium propionate addition to the diet during pregnancy on ewe and subsequent progeny performance as well as maternal-neonatal behavior.

Material and methods

All experimental procedures followed were approved by the Ethics Committee for the Use of Animals, Faculty of Veterinary Medicine and Animal Science, University of São Paulo (protocol no CEUA 2700201218). This study was conducted at University of São Paulo, Faculty of Veterinary Medicine and Animal Science, Pirassununga, São Paulo, Brazil.

Animals and Nutritional Management

Seventy-two Santa Inês x Dorper sheep (59.65 ± 10 kg, 2 to 4 age, parity block) and their progeny were used to evaluate the effects of energy levels and different ingredients (starch, chromium propionate or palm oil protected fat) during gestation on sheep performance as well as subsequent progeny growth and maternal-lamb behavior.

Ewes were allocated to 1 of 5 treatments based on the National Research Council (2007) as follows: **CTL** (n=14) = 100% of predicted ME requirement; **LOW** (n=14) = 90% of predicted ME requirement , **ST** (n=15)= 110% of predicted ME requirement and the energy source was starch; **ST+Cr** (n=15) = ST more chromium propionate and **ST+PF** (n=14) = ST more palm oil protected fat (Table 9).

Table 9 *Chemical composition of feed used in experimental diets of sheep during gestation and lactation.*

Nutrient	Corn silage	Corn grain	Soybean meal	Protect Fat
Dry Matter, %	26	88	91	98
Crude Protein, % of DM	7.0	9.0	40	
NDF, % of DM	70	9	15	
ADF, % of DM	44	3	10	
ME, Mcal/kg	1.98	3.2	3.0	5.41
EE, % of DM	1.3	4.3	1.6	84.5
Mineral material, % of DM	7	2	7	15
Calcium, % of DM	0.35	0.02	0.38	12
Phosphor, % of DM	0.19	0.3	0.71	

EE = ether extract; ME = metabolizable energy; ADF = acid detergent fiber; NDF = neutral detergent fiber.

In the control treatment, the value of metabolizable energy at the beginning of pregnancy was 2 Mcal/kg/d, at the end of pregnancy 2.24 Mcal/kg/d and in lactation 2.4 Mcal/kg/d (NRC 2017).

The feed was offered twice daily (800 and 1600 h) during early and late gestation and lactation (-30 until 50 days of gestation and later, 100 days of gestation until 60 days *pos partum*). Ewes had the oestrus synchronized and natural and controlled mating, using one male for five females, put together at 24h and 36 hours after synchronization. After 30 days, pregnancy was confirm via rectal ultrasonography.

Ewes measurements

Ewe body weight (BW), body condition score (BCS) and Famacha® were evaluated on days 50 and 135 of pregnancy and days 15 and 80 after lambing. Body weight was measured on electronic scale, with a minimum capacity of 10 kg and a maximum of 300 kg. Body conditional score (BCS) was done by the same person, on a 1-5 scale, with 0.25 intervals and one being emaciated and five being obese (RUSSEL; DONEY; GUNN, 1969). The Famacha® Chart method was used for color score of the ocular mucosa by the same experienced person on 1-5 scale, and each ewe was classified into five categories: 1=red, non-anemic; 2=red-pink, non-anemic; 3=pink, middle anemic; 4=pink-white, anemic; 5=white, severely anemic (SOTO-BARRIENTOS et al., 2018)

Offspring management

Fifty-two ewe lambs were born and kept with the mothers until weaning at 80 days. At birth season, all paddocks were inspected during day (600 to 1900) to record ewe and lamb identity, litter size, birth weight, sex and to dip the navel of each lamb in a 10% iodine solution. In all paddocks there was a creep feeding available for lambs, with ration ad libitum (Table 9). The creep feeding concentrate had 22% crude protein and 3.2 Mcal of ME. The feed with corn silage and the concentrate was *ad libitum*. BW was evaluated on birth, 15, 30, 45, 60 days and weaning. Weight gain was calculated for this period.

After weaning, eight ewe lambs from each treatment remained, discarding animals that died or were born with any type of disability that could compromise growth. They stayed together and fed with a corn silage, corn and soybean meal, with diet to growth requirements (NRC, 2007; Table 9). The total diet had values of 14% crude protein and 2.6 Mcal of ME. From weaning to 264 days, BW was record approximately every 15 days, totaling 11 measurements. Weight gain was calculated for this period. BCS and Famacha® were evaluated at 160, 180, 200, 220, 240 and 260 days, date that they were considered able to reproduction.

Biometric measures were performed at day 260 and taken using a tape measure and a hypometer with the animal standing in a proper vertical position. All measurements were taken from the left side of the animal, for uniformity purposes. The following body measurements were performed: HG (heart girth) - external circumference of the thoracic cavity, under the armpits; BL (body length) - distance between the cervicothoracic junction and the tail base at the first intercoccygeal joint; WH (withers height) - distance between the withers and the distal end of the forelimb; RH (rump height) - distance between the sacral tuberosity and the distal end of the hindlimb; RW (rump width) - distance between the greater trochanters of the femurs (SOUZA et al., 2019).

Behavior analysis

The effect of dietary treatment on maternal behavior during the first hour postpartum, was determined only 26 mothers. The observations started when a doe displayed signs of imminent parturition and mother–kid interactions were recorded every minute until the first successful suckling by a trained person using the individual focal method with continuous recording (HILD et al., 2011; COULON et al., 2014). These data were transformed into frequency for statistical analysis.

The following maternal behaviors at parturition were measured: (1) posture standing (with the four supports on the floor) or lying down, (2) grooming (when the mother cleans the lamb by licking and consuming the amniotic membrane tissue adhered to the lambs body) (3) amniotic membrane consumption (when the mother consumes amniotic membrane tissue adhered to her body or on the ground), (4) facilitating the suckling (when the mother allows her kid to begin suckling), (5) disturbing the suckling (when the mother does not allowed access to her udder), (6) moving away from the kid and (7) others activities.

The following lamb behaviors at parturition were measured: (1) posture (standing or lying), (2) trying to stand up (action to stay upright), (3) looking for udder (when the lamb moves towards the udder and try to find it), (4) trying to suckling (when the lamb makes suction movements with the lips but it’s not in the right place or doesn’t swallow the milk), (5) successfully suckling (when the lamb swallow the milk), (6) no activity (moment when the lamb only receives the sheep's action) and (7) other activity (when the lamb does anything but the activities describe above).

Statistical analysis

Data were evaluated using the statistical package SAS 9.1.2 for Windows (SAS Institute Cary, NC, USA). The homogeneity of the variances and the normality of the residues were verified by the Shapiro-wilk and Levene's tests, respectively and then, the variables analyzed using Tukey test at 5% significance. The treatments and the type of birth (single or multiple) were analyzed in the model as fixed effects. Behavior analyses were transformed into frequency and considered non-parametric. The frequency of mothers and lamb behaviors at parturition were analyzed using Kruskal-wallis test at 10% of significance.

Results

Ewe performance

At the beginning of this study, there was no difference for ewes body weight (BW), body condition score (BCS) and Famacha® between treatments. Before lambing, at 135 days of pregnancy (DP), ST ewes were heavier and similar to treatments ST+Cr and ST+PF (P=0.0442) (Table 10). Ewes receiving palm oil protected fat reach the end of lactation (day 80) heavier (P=0.0145).

LOW treatment had lower BCS before lambing (P<0.001) and during lactation (P<0.001). ST+PF ewes had higher BCS than CTL and similar than ST and ST+Cr on days 15 and 80 of lactation (DL) (Table 10). During pregnancy, LOW ewes lost BCS, while ST, ST+Cr and ST+PF gain BCS, and CTL were statistically similar to all treatments (P=0.0489) (Figure 4). After lambing and during lactation, LOW ewes lost more BCS than ST+PF (P=0.0799) and numerically they lost more condition score than all the others treatments. ST+PF did not lose BCS after lambing and lost only 0.02 points during lactation.

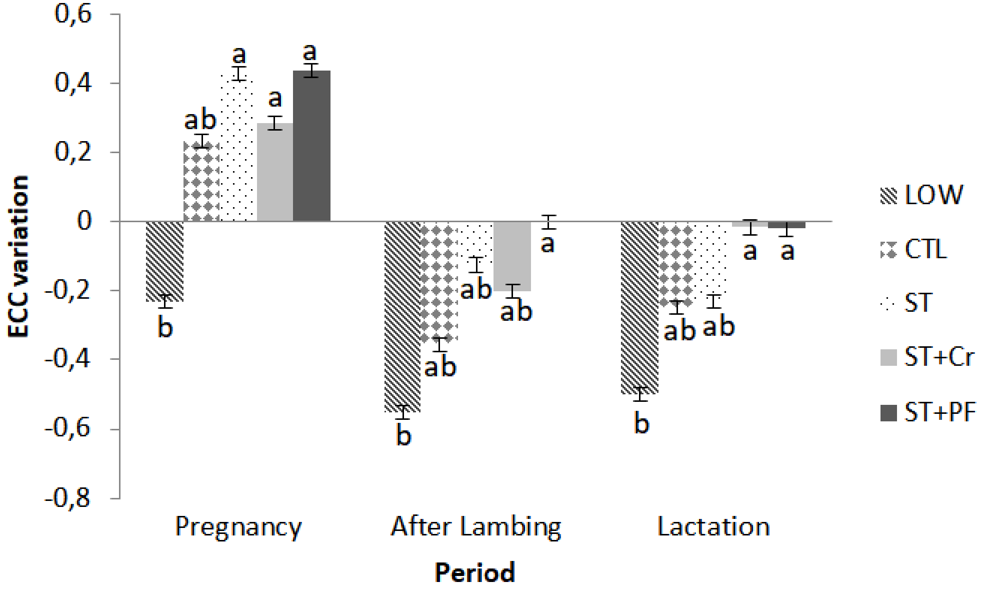
Famacha® score was different before lambing (P=0.0019) and in the end of lactation (*P*<0.0001). LOW ewes had the highest score on both periods. At 80 DL, ST ewes and ST+PF had lower Famacha® score and similar to ST+Cr.

Table 10 *Body weight, body condition score and Famacha® during pregnancy and lactation, from ewes receiving diets with different energy levels and starch, chromium propionate and/or palm oil protected fat*

	TREATMENTS							
	LOW	CTL	ST	ST+Cr	ST+PF	MEAN	SEM	*P*-value
	(n=14)	(n=14)	(n=15)	(n=15)	(n=14)			
Body Weight								
Pregnancy								
50 d	56.88	53.16	59.30	59.10	59.11	57.51	1.114	0.3828
135 d	68.54bc	67.75c	77.50a	77.09ab	74.10ab	73.02	1.359	0.0442
Lactation								
15 d	59.02b	60.13ab	68.62ab	65.58ab	70.42a	64.57	1.301	0.0156
80 d	57.67b	57.45b	65.18ab	62.91ab	68.75a	62.22	1.237	0.0145
Body condition score								
Pregnancy								
50 d	2.89	3.14	3.31	3.63	3.38	3.27	0.079	0.0699
135 d	2.66b	3.37a	3.82a	3.91a	3.75a	3.50	0.090	<0.001
Lactation								
15 d	2.10c	3.01b	3.71ab	3.71ab	3.75a	3.24	0.111	<0.001
80 d	1.82c	2.92b	3.30ab	3.55ab	3.70a	3.05	0.111	<0.001
Famacha®								
Pregnancy								
50 d	3.00	3.21	3.13	2.92	2.84	3.02	0.090	0.6463
135 d	2.84[a]	2.07b	2.2ab	1.93b	2.00b	2.20	0.814	0.0019
Lactation								
15 d	3.16	3.07	2.93	2.73	2.53	2.88	0.124	0.5449
80 d	3.58a	3.14ab	2.46c	2.86bc	2.46c	2.88	0.088	<0.0001

LOW = 90% of predicted ME requirement; CTL = 100% of predicted ME requirement; ST = 110% of predicted ME requirement and the energy source was starch; ST+Cr = ST more chromium propionate; ST+PF = ST more palm oil protected fat. Letters on the line differ from the averages by the tukey test at 5% probability.

Figure 4 *BCS variation of ewes with different levels of energy and supplemented with chromium propionate or palm oil protected fat during pregnancy (Day 135-50 of pregnancy), after lambing (day 15 in lactation – day 135 of pregnancy) and during lactation (day 80-15 in lactation)*

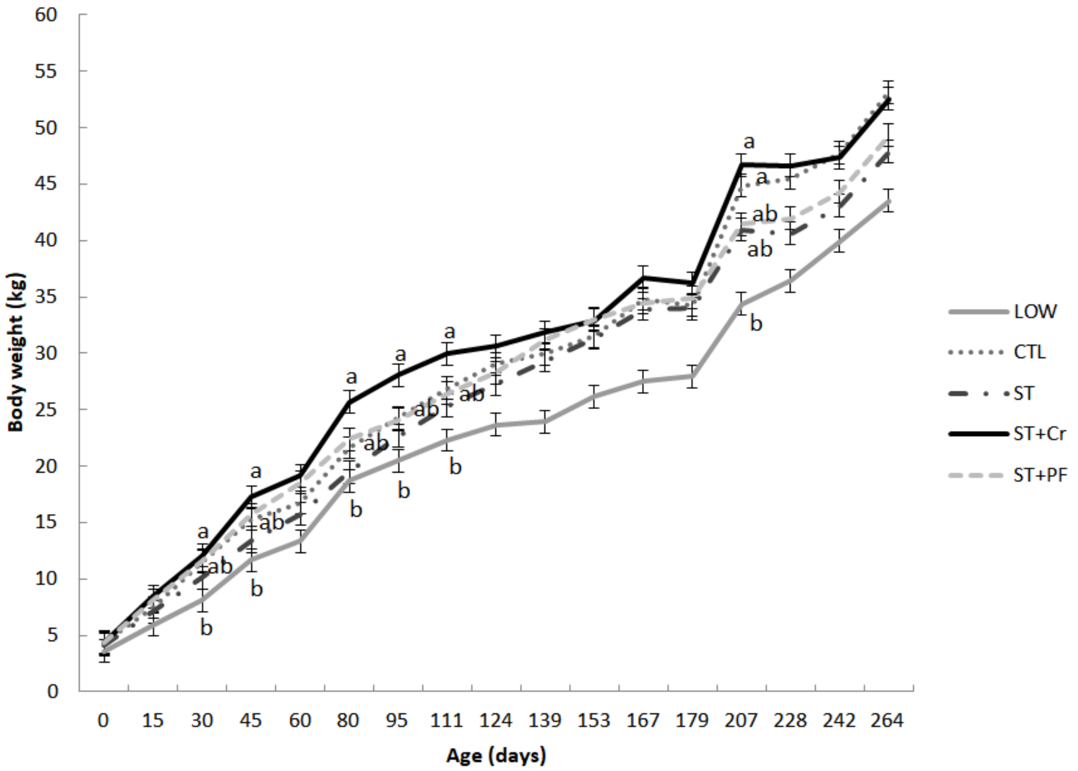


LOW = 90% of predicted ME requirement; CTL = 100% of predicted ME requirement; ST = 110% of predicted ME requirement and the energy source was starch; ST+Cr = ST more chromium propionate; ST+PF = ST more palm oil protected fat. Letters on the line differ from the averages by the Tukey test at 5% probability

Progeny performance

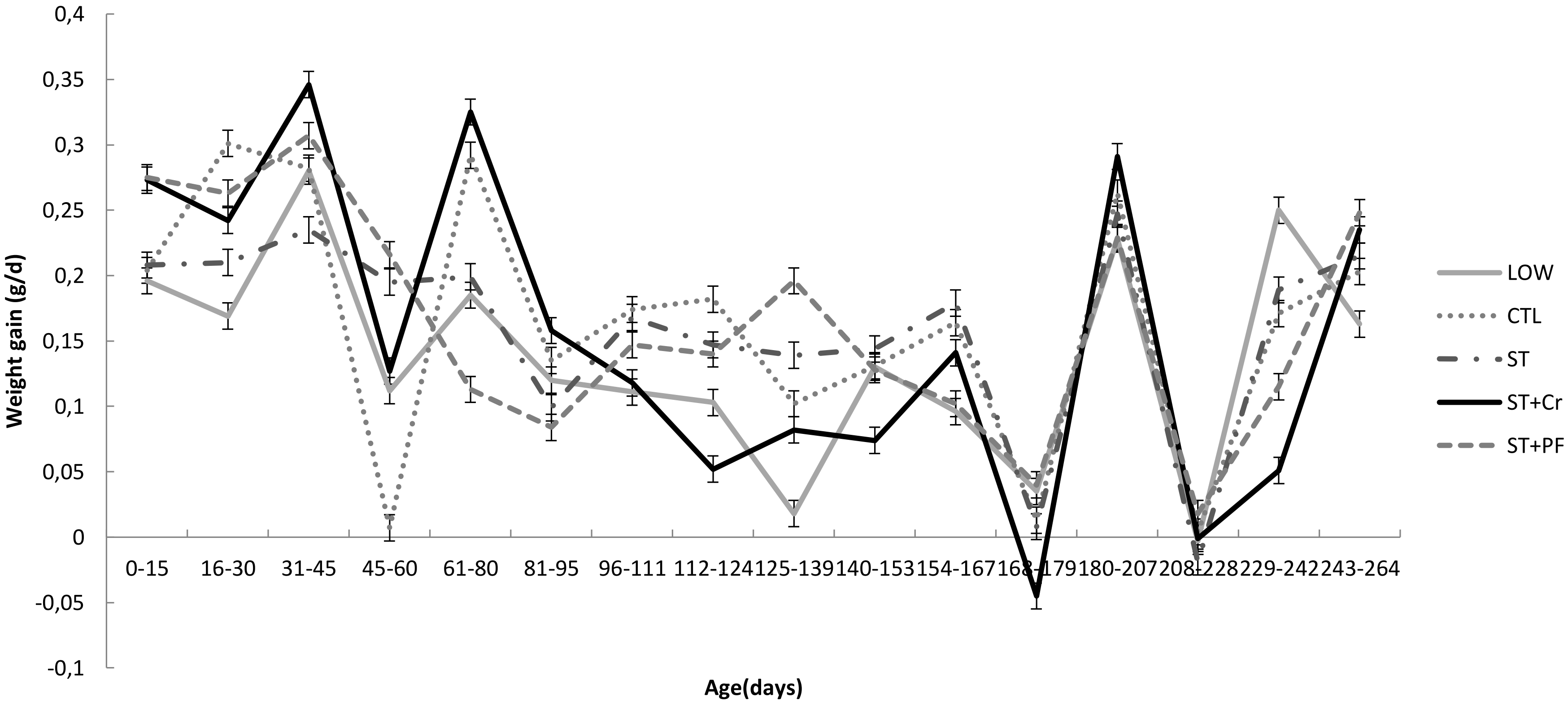
Birth BW was not different (*P*=0.0933) (Figure 5). However, BW was different on days 30 (*P*=0.0243), 45(*P*=0.0376), 80 (*P*=0.0304), 95 (*P*=0.0288), 111(*P*=0.0494) and 207 (*P*=0.0140). For all those periods, ewe lambs born from ST+Cr ewes were heavier than LOW ewes. On day 207, LOW lambs were lighter than CTL too. There was no difference for weight gain in any of the analyzed periods (Figure 6).

Figure 5 *Body weight from ewe lambs born from ewes with different levels and source of energy in the diet*



LOW = 90% of predicted ME requirement; CTL = 100% of predicted ME requirement; ST = 110% of predicted ME requirement and the energy source was starch; ST+Cr = ST more chromium propionate; ST+PF = ST more palm oil protected fat. Letters differ from the averages by the Tukey test at 5% probability.

Figure 6 *Weight gain from ewe lambs born from ewes with different levels and source of energy in the diet*



LOW = 90% of predicted ME requirement; CTL = 100% of predicted ME requirement; ST = 110% of predicted ME requirement and the energy source was starch; ST+Cr = ST more chromium propionate; ST+PF = ST more palm oil protected fat. Letters differ from the averages by the Tukey test at 5% probability.

Body condition score and Famacha® (Table 11) were evaluated between 160 days and 260 days old. LOW ewe lambs had lower BCS on days 160 (*P*=0.0296) and 180 (*P*=0.0108) than ST, ST+Cr and ST+PF but similar to CTL. At 200 days old there was a tendency (*P*=0.0798) for LOW ewes lamb to have lower BCS than ST+Cr, but CTL, ST and ST+PF were similar. At 220 (*P*=0.1756), 240 (*P*=0.5117) and 260 days old (*P*=0.9737), BCS were not different between treatments. By the time they achieved puberty (260 days old), all ewe lambs had BCS scores higher than 3.5 and therefore, suitable for reproduction according to this parameter (KENYON; MALONEY; BLACHE, 2014; MOREL et al., 2016).

Famacha® was different on days 160 (*P*=0.0107), 200 (*P*=0.0464) and 220 (*P*=0.0007), but not on days 180 (*P*=0.0623), 240 (*P*=0.1952) and 260 (*P*=0.3190). On day 160, LOW lambs had the highest anemia, with a Famacha® score of 4, but it was similar to ST+Cr (3.28) and ST+PF (3.14) and higher than CTL (2.71) and ST (2.75). On day 100, LOW lambs had higher Famacha® score (3.28) than ST+PF (2.14) and on day 220, ST+Cr lambs had lower Famacha® score (1.0) comparing with LOW lambs (2.0) and ST (1.87), but no group had anemia.

Table 11 *Body conditional score and Famacha® score from ewe lambs between 160 and 260 days of live, born from ewes with different levels and source of energy in the diet*

	Treatments							
	LOW	CTL	ST	ST+Cr	ST+PF	Mean	SEM	*P*-value
BCS								
160d	2.62b	3.50ab	3.50a	3.66a	3.39a	3.35	0.133	0.0296
180d	2.91b	3.71ab	4.00a	3.91a	3.92a	3.72	0.114	0.0108
200d	3.29b	3.70ab	3.96ab	4.25a	3.92ab	3.84	0.108	0.0798
220d	2.83	3.00	3.46	3.54	3.35	3.26	0.105	0.1756
240d	3.16	3.60	3.68	3.41	3.32	3.45	0.095	0.5117
260d	3.58	3.64	3.59	3.62	3.78	3.64	0.096	0.9737
Mean	3.06	3.53	3.70	3.73	3.61			
SEM	0.106	0.110	0.097	0.089	0.087			
P-value	0.1020	0.4866	0.4682	0.0753	0.0851			
Famacha®								
160d	3.83a A	2.42b A	2.75b AB	3.00ab A	3.14ab AB	3.00	0.133	0.0107
180d	3.33AB	2.42A	2.87 AB	2.83A	3.85ABC	2.85	0.095	0.0623
200d	3.29a AB	2.66ab A	2.37ab AB	2.50ab A	2.14b C	2.56	0.128	0.0464
220d	2.33a B	1.33b B	1.87a B	1.00b B	1.28b D	1.57	0.115	0.0007
240d	3.66A	3.00A	3.62A	3.33A	3.57A	3.44	0.109	0.1952
260d	3.04AB	2.57A	2.37AB	2.50A	2.57BC	2.59	0.107	0.3190
Mean	3.25	2.42	2.64	2.52	2.59			
SEM	0.128	0.112	0.124	0.146	0.136			
P-value	0.0061	0.0002	0.0004	<0.0001	<0.0001			

LOW = 90% of predicted ME requirement; CTL = 100% of predicted ME requirement; ST = 110% of predicted ME requirement and the energy source was starch; ST+Cr = ST more chromium propionate; ST+PF = ST more palm oil protected fat. Uppercase letters on the collumn and lowercase letters on the line differ from the averages by the Tukey test at 5% probability.

At the age of eight months, body measurements were made, but no differences was found between treatments (Table 12) for heart girth (*P*=0.3401), body length (*P*=0.7693), withers high (*P*=0.5807), rump high (*P*=0.4625) and rump width (*P*=0.2451).

Table 12 *Body measurements of ewe lambs at puberty (8 months), born from ewes with different levels and source of energy in the diet*

	Treatments							
	LOW	CTR	ST	ST+Cr	ST+PF	MEAN	SEM	P value
Heart girth	87.00	91.71	90.25	92.16	89.28	90.11	0.822	0.3401
Body length	64.50	64.28	66.75	67.50	64.42	65.50	0.960	0.7693
Withers height	54.83	56.57	57.62	56.00	56.42	56.38	0.522	0.5807
Rump height	57.00	60.57	58.00	59.00	59.28	58.79	0.617	0.4625
Rump width	32.66	37.42	34.12	36.00	35.00	35.05	0.679	0.2451

LOW = 90% of predicted ME requirement; CTL = 100% of predicted ME requirement; ST = 110% of predicted ME requirement and the energy source was starch; ST+Cr = ST more chromium propionate; ST+PF = ST more palm oil protected fat. Letters on the line differ from the averages by tukey test at 5% probability.

Behavior analysis

After lambing, ewes on CTL, ST+Cr, and ST+PF spent more time standing up than lying down (*P*=0.0692) (Table 13). There was no other effect of dietary treatment on maternal behavior at lambing, the frequency that the ewes were grooming (*P*=0.7189), consuming amniotic membranes from her own body or the ground (*P*=0.2758), facilitating (*P*=0.8680) or disturbing the suckling (*P*=0.3611), moving away (*P*=0.5714) and doing other activities (*P*=0.7429) was the same between treatments. No ewe showed any aggressive behavior towards the lambs.

Table 13 *Maternal and lamb behavior after lambing*

	Treatments							
	LOW	CTL	ST	ST+Cr	ST+PF	MEAN	SEM	P-value
Mother Behavior	(n=6)	(n=6)	(n=6)	(n=5)	(n=3)			
Standing	95.074b	99.663a	97.116ab	100a	100a	98.120	0.840	0.0692
Lying	4.925a	0.336b	2.884ab	0b	0b	1.483	0.824	0.0692
Grooming	73.865	64.822	86.156	71.270	75.026	74.249	4.331	0.7189
Amniotic membranas comsumption	2.073	0	1.100	6.053	6.349	2.629	1.237	0.2758
Facilitating the suckling	5.439	12.011	9.094	6.217	13.333	8.860	2.112	0.8680
Disturbing the suckling	1.555	2.564	0	0	0	0.950	0.629	0.3611
Moving away	0.862	0	0	8.666	0	1.865	1.670	0.5714
Other activity	10.105	10.374	3.648	7.458	5.291	7.612	1.984	0.7429
Lamb Behavior								
Standing	50.239	62.382	47.751	52.607	59.172	53.953	3.872	0.7127
Lying	49.760	37.617	52.248	47.392	40.827	46.046	3.872	0.7127
Trying to stand	27.920	12.162	14.208	13.309	12.225	16.499	2.735	0.8980
Looking for udder	24.436ab	54.726a	39.567ab	14.878b	35.941ab	34.407	4.206	0.0261
Trying to suckle	4.544ab	0b	1.190b	12.669a	0.952b	3.869	1.653	0.1022
Suckling	4.062	4.830	4.550	3.852	3.971	4.301	0.440	0.8484
No activity	37.430	28.281	40.482	55.289	45.851	40.429	3.324	0.1538
Other activity	1.606	0	0	0	1.058	0.492	0.385	0.3406

LOW = 90% of predicted ME requirement; CTL = 100% of predicted ME requirement; ST = 110% of predicted ME requirement and the energy source was starch; ST+Cr = ST more chromium propionate; ST+PF = ST more palm oil protected fat; Letters on the line differ from the averages by Kruskal-Wallis test at 10% probability

The diet affect the time that lambs spending looking for udder (*P*=0.0261), in which CTL lambs spend 54.72% of the period analyzed trying to find the udder, while the ST+Cr, only 14.87%. On the other hand, CTL lambs didn't spend any time trying to suckle, while ST+Cr lambs spend 12.66% of the period (*P*=0.1022). However, there was no difference for the percentage of time that the lambs spend suckling (*P*=0.8484), tryind to stand (*P*=0.8980), doing no activity (*P*=0.1538) or doing other activities (*P*=0.3406). The diet did not affect the lamb posture (*P*=0.7127).

Discussion

Ewe performance

Energy level on a diet can influence ewes BW. A study using different energy levels and pregnant ewes showed that ewes that were fed low energy sheep had lower body weight than ewe in the control diets (DONNEM et al., 2020). After eight weeks of diets with different energy levels, overfeed ewes had greater BW and BCS than control and both were greater than underfeed ewes (GRAZUL-BILSKA et al., 2012). On another study, after 21 days of different energy diets, ewes in the low energy treatment (10.3ME/kg DM) gained 2.3% of initial body weight and treatments with 11.0, 11.6 and 12.2 ME/kg DM gained on average 4.5, 7.4 and 10.9%, respectively, during the same period (KOYUNCU; CANBOLAT, 2009).

In this experiment, after lambing, the addition of palm oil protected fat had positive effect on body weight. ST+PF ewes were heavier than LOW ewes after 15 DL and heavier than LOW and CTL after 80 DL. As shown by Abdel-Hakim et al. (2016), Eknæs et al. (2017), Leiva et al. (2018) and Bianchi et al. (2018) fat supplementation decrease the loss in body weight after lambing.

Feeding fat sources to lactating dairy cows may increase energy intake without reduce diet fiber content, providing higher energy intake (ARAÚJO et al., 2018) and supplemental fat can reduce, at least in part, cereal grains that may decreased ruminal pH, neutral detergent fiber digestibility, total DMI and milk production (SOUZA; BATISTEL; SANTOS, 2017).

Body condition score (BCS) is the best practical indicator to evaluate and control mechanisms of body reserves management and to monitor the general metabolic status of the ewe (CALDEIRA et al., 2007a). It is established that the quantity of body reserves has a definite effect on production efficiency of animals (CALDEIRA et al., 2007b). The BCS of cows provides a good index of subcutaneous fat which is an important source of readily available energy in postpartum cows (D'OCCHIO; BARUSELLI; CAMPANILE, 2019).

Implementing target BCS at specific points in the production cycle is considered to be a key management tool for the performance of breeding ewes (MOREL et al., 2016). In early pregnancy, BCS is suggested to be in the range of 2.5-3.5 (KENYON; MALONEY; BLACHE, 2014) and all treatments were in agreement with the proposed. Ideally, ewes should be of BCS 2.5-3.0 at lambing, with an absolute minimum of 2.0, because of the likely further loss of body condition during lactation (KENYON; MALONEY; BLACHE, 2014). In this study, near lambing date (135d of pregnancy), LOW treatment had 2.6 of BCS, lower than others treatments, but still within the expected. However, ST, ST+Cr and ST+PF ewes had BCS over 3.5 and increasing BCS above 3.5 would be inefficient for the utilization of energy (MOREL et al., 2016). For cows, BCS at calving is arguably the single most important factor linked to the timely resumption of fertile ovulations postpartum (D’OCCHIO; BARUSELLI; CAMPANILE, 2019).

At weaning, ewes should not have a BCS below 2.0 and should not have lost more than 1.0 unit of BCS in lactation (KENYON; MALONEY; BLACHE, 2014). LOW ewes had BCS of 1.8 at weaning, below expected. Overall, this long-lasting effect due to long malnutrition on body status of LOW treatments ewes may occur because the feed consumed after the d50 of pregnancy was used directly for fetal nutrition and to meet nutritional needs for maintenance of mothers and therefore, maintain body condition was not a priority in LOW ewes.

Progeny performance

Long term effects on body composition and performance of the offspring may occur due to the nutritional environment in which the fetus grow and develop (SYMONDS; SEBERT; BUDGE, 2010). However this and others studies did not found any differences on lambs birth weight, born from ewes with different energy levels and source diets (RAE et al., 2002b; FORD et al., 2007; BLAIR et al., 2011; KENYON et al., 2011a; DEBUS et al., 2012; KLEEMANN et al., 2015; MCGOVERN et al., 2015a, 2015b; SEN et al., 2016a; WERNER et al., 2019; ITHURRALDE et al., 2019; DONNEM et al., 2020). It seems to be only cases of severe malnutrition during late gestation that significantly reduces birth weight, for example feeding ewes 60% of NRC requirement (1995) can reduce newborn weight by 0.4kg (MEYER et al., 2010) or when the reduction is 50-60% of AFRC (1993) ME requirement at late gestation, the lamb's birth weight decreases 0.2 kg by MJ decrease in energy allowance (GARDNER et al., 2007) and reducing the energy intake to 65% can cause a reduction in mean lamb birth-weight of 9% when compared with well-fed ewes (DWYER et al., 2003). In the present experiment, the ewes were less undernourished than in both studies and therefore, was not able to reduce lamb birth weight.

Although energy on the diet was able to change BCS of the ewes, in the majority of studies, BCS has had no effect on lamb birth weight, but there is large variation between studies (KENYON; MALONEY; BLACHE, 2014). Lamb birth weight have not been related to BCS in late pregnancy with Scottish Halfbred and Polypay ewes (KENYON; MALONEY; BLACHE, 2014) and on Welsh Mountain ewes even under conditions where were fed less than maintenance (OSGERBY; GADD; WATHES, 2003). For not compromising fetal growth, maternal plasma glucose concentration is maintained by a kind of adaptation, for example, it may occur the reduction in plasma concentration of catabolic hormones including cortisol, thyroid hormones and insulin when there is maternal nutrient restriction before or during pregnancy (SYMONDS; SEBERT; BUDGE, 2010). Even if high energy diets were able to increase BCS, BCS greater than 3 in late pregnancy has a little benefit in terms of lamb live weight (GRONQVIST et al., 2018) and apparent energy levels above pregnancy maintenance has little additional impact on the offspring's early life weight (GREENWOOD; THOMPSON, 2007; KENYON, 2013)

Ewe BCS has been reported to have either no influence on lamb growth to weaning (LITHERLAND; LAMBERT; MCLAREN, 1999) or weaning weight (LITHERLAND; LAMBERT; MCLAREN, 1999; ALIYARI et al., 2012; VERBEEK et al., 2012; PASTURES et al., 2018), or a positive effect on lamb growth (KENYON; MOREL; MORRIS, 2004; KENYON et al., 2011b; MATHIAS-DAVIS et al., 2013) and weaning weight (KENYON; MALONEY; BLACHE, 2014).

Nutrition levels also had no influence on lamb growth (RAE et al., 2002b; TYGESEN et al., 2007; MUÑOZ et al., 2008; DEBUS et al., 2012; SEN et al., 2016a; ITHURRALDE et al., 2019) or low energy diets produced lambs with lower potential growth (EVERITT, 1960; BORWICK et al., 2003).

Although there was no differences on weight gain, ST+Cr ewes had heavier ewe lambs than LOW group on days 30, 45, 80 (weaning), 95, 111 and 207. As we also found, the addition of palm oil protected fat on the dams diet did not change ewe lambs body weight from birth to weaning, even in high energy diets, reflecting that fat supplementation may not have a significant effect on milk production (ABDEL-HAKIM et al., 2016).

More studies are required before conclusions can be made on whether the in utero environment can influence an animal's susceptibility to internal parasites (KENYON; BLAIR, 2014). Asmad et al. (2015) reported no effect on male offspring fecal egg count when the mothers were treat with low energy diets, maintenance and high energy while Paten et al. (2011) found no effect on female offprinsg and Rooke et al. (2010) did not found any effect on egg fecal count for both female and male offspring for Blackface and Suffolk sheep. On this trial, LOW group had the highest Famacha® score during all periods analyzed and, except for day 220, the Famacha® remained above 3, which indicates that the animals were a little anemic. On the other hand, treatments ST and ST + Cr had Famacha® above 3 in only one period.

Dam's nutrition during pregnancy did not influence body measurement of the female offspring. Similar results were found by Ithurralde et al. (2019) where ewe's nutrition (high and low energy) treatment did not affect lamb thoracic perimeter, cranium-caudal length and hind limb perimeter. Not finding any differences can be due the fact that we worked only with females and ewe lambs born to undernourished ewes seemed to be less affected in terms of growth performance (ITHURRALDE et al., 2019).

Behavior analysis

A small effect of nutritional treatments on ewe and lamb behavior was found in this trial. Adding chromium propionate and palm oil protected fat to the diet influence the ewes to stay 100% of the time standing up and not lying down for any minute while LOW ewes spend more time resting (4.92%). Also, LOW lambs spend more time trying to stand and, despite statistically finding no difference, they took twice as long trying to stand than others treatments, showing that they were born weaker. ST+Cr and ST+PF lambs spend more time doing nothing and less time sucking, showing that the diets may have influenced milk production. Studies shows that supplementation with calcium salts of palm FA increased milk production and yield of milk components (LOFTEN et al., 2014; BOERMAN et al., 2015; SOUZA; BATISTEL; SANTOS, 2017; BIANCHI et al., 2018a). The lack of differences between treatments may be due to the fact that only a few animals were used. No other behavior was change because of the treatments.

Few studies have considered the effects of nutrition on the behavior of ewe or lamb (DWYER et al., 2003) and the results do not reach consensus. For example, Gronqvist et al. (2018, 2016) shows that increasing feeding during late pregnancy, above the level of pregnancy maintenance would not be effective as a management tool to improve behaviors of the ewe and her lambs soon after birth and Donnem et al. (2020) found no effect of ewe nutritional treatment (100%ME, 85%ME and 120%ME) on the lambs behavioral scores. On the other hand, McGovern et al. (2015b) say that lambs born to ewes offered the excess energy treatment (120% ME) were quickest to stand and attempt to suckle after birth, in addition to having a greater live weight at weaning (P = 0.01) and Dwyer et al. (2003) said that ewes in high energy diets during pregnancy spend more of the first 30 min after lamb birth grooming the lamb than ewes on low energy diets, but had no effect of nutritional treatment on the time to start grooming the lamb after delivery, no outright maternal rejection of lambs by any ewe and no differences for lamb behavior.

Conclusion

Energy levels as recommended by the NRC and above those recommendation were positive for maintaining live weight and body condition score, but levels above were better for resistance to worms in the end of lactation and the use of rumen protected fat promoted lower variation in BCS. Dam’s diet does not interfere with the growth and performance of the progeny but the use of chromium can improve body weight. Levels above recommendation can improve the degree of anemia caused by worms. Energy level under NRC recommendation may compromise maternal-lamb behavior by generating weaker mothers and offspring after lambing.

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CAPÍTULO 7.

Artigo n°3

Artigo que será submetido na Revista Brasileira de Zootecnia

Economic performance of high energy diets and supplementation with chromium or protected fat in ewes production

Abstract: The experiment to evaluate the economic viability of diets with different levels of energy and feed (supplementation of chromium and protected fat) the diet of ewes during pregnancy and lactation. Seventy-two crossbred sheep were allocated in five treatments: CTL (n=14) with 100% of the NRC recommendation for metabolizable energy per kg of dry matter intake ($ME.kg^{-1}$ DMI), LOW (n=14) with 90% $ME.kg^{-1}$ DMI, ST (n=15) with 110% $ME.kg^{-1}$ DMI and the energy feed was corn, ST+Cr (n=15) diet ST more chromium propionate, and ST+RPF (n=14) diet ST more palm oil protected fat. Based on performance data for each treatment, a short-term analysis was carried out for a module of 1000 ewes, with cost, revenues, and profitability assessment and long-term analysis, through a 10-year cash flow. CTL and ST+RPF treatments were not viable due to the higher cost and lower revenue, in addition to presenting negative NPV and IRR. LOW treatment, despite presenting low costs and positive profitability, NPV was negative and IRR lower than the discount rate, not being viable in the long run. ST and ST+Cr treatments were considered feasible because they presented lower unit cost and higher profitability, in addition to high NPV and higher IRR than the discount rate. The use of high energy diets, with chromium supplementation, promotes better performance and, consequently, better economic return.

Keywords: economic viability, cash flow, profitability, chromium, protect fat.

Introduction

Brazilian sheep farming must leave the condition of subsistence exploitation and gain space as a specialized, planned, organized, and quality-oriented business activity. To become economically viable, it needs to overcome several challenges, mainly technical (RAINERI; STIVARI; GAMEIRO, 2015) .

Strategic use of feed can increase productivity and maximize the economic return on lamb production (ERMIAS; SOLOMON; MENGISTU, 2013). For grazing animals, it is harder to estimate nutrient supply because diet composition and forage intake are difficult to quantify and continually change as pasture conditions change. Therefore, even the most accurate system of nutrient requirement, such as NRC or CSIRO, may underestimate the real nutritional requirement of ewes, especially during pregnancy and lactation (DOVE, 2010). In this context, high energy diets are an interesting management tool for pregnant and lactating ewes.

The increase of energy level in the diet brings reproductive benefits, due to the higher ovulation rate, a shorter interval between births, anticipates age to puberty, lower mortality and embryonic loss (RAE et al., 2002b; SCARAMUZZI et al., 2006b; DONNEM et al., 2020), and performance benefits, due to better fetal growth and greater birth, weaning and slaughter weight (KENYON; BLAIR, 2014; MCGREGOR, 2017). It is possible to increase the energy level in the diet with supplementation with corn (starch), protected fat, and chromium propionate (LEIVA et al., 2018).

Starch fermentation increases the proportion of propionate produced in the rumen, increasing the availability of glucose in the blood, through hepatic gluconeogenesis (KLEVENHUSEN, 2019). The inclusion of fat in the ruminant diet improves energy efficiency by the direct supply of long-chain fatty acids for fatty acid synthesis metabolism (BHATT et al., 2013b). In turn, chromium increases the sensitivity of tissues to insulin, improving energy efficiency (DAVIS; VINCENT, 1997b; SUMNER; VALDEZ; MCNAMARA, 2007b).

Nutritional management is a major influencing variable in the success of a production system (SANTOS et al., 2018). To assess whether the implementation of a feed strategy is economically viable, it is necessary to carry out the economic viability analysis, so it is possible to determine if the project can meet the expectations and demands of investors so that the decision to invest can be made (ROJAS, 2015).

This paper hypothesizes that, although investment in high energy diets can increase production costs, at the end of a productive cycle this value is offset by higher productivity. The aim was to analyze the economic viability of using high-energy diets and the supplementation of chromium or protected fat in the diet of ewes from the breeding season until the end of lactation.

Materials and Methods

Data collection

To carry out the economic analysis, data from the experiment carried out in the Goats and Sheep Production Center at the University of São Paulo, in Pirassununga (SP), between March 2017 and January 2019, were used.

Seventy-two Santa Inês x Dorper sheep, 59.65 ± 10 kg body weight, 2 to 4 years old, allocated five treatments: CTL (n=14) with 100% of the NRC recommendation for metabolizable energy per kg of dry matter intake ($ME.kg^{-1}$ DMI), LOW (n=14) with 90% $ME.kg^{-1}$ DMI, ST (n=15) with 110% $ME.kg^{-1}$ DMI and the energy feed was corn, ST+Cr (n=15) diet ST more chromium propionate, and ST+RPF (n=14) diet ST more palm oil protected fat (Table 14).

Diets were provided twice a day during the breeding season, the initial and final third of pregnancy and lactation. After birth, the lambs were weighed and identified. Creep-feeding, containing 22% crude protein (CP) and 3.2 Mcal of metabolizable energy (ME), was available in all paddocks.

***Table 14** Chemical composition of feed used in experimental diets of sheep during gestation and lactation.*

Nutrient	Corn silage	Corn grain	Soybean meal	Protect Fat
Dry Matter, %	26	88	91	98
Crude Protein, % of DM	7.0	9.0	40	
NDF, % of DM	70	9	15	
ADF, % of DM	44	3	10	
ME, Mcal/kg	1.98	3.2	3.0	5.41
EE, % of DM	1.3	4.3	1.6	84.5
Mineral material, % of DM	7	2	7	15
Calcium, % of DM	0.35	0.02	0.38	12
Phosphor, % of DM	0.19	0.3	0.71	

EE = ether extract; ME = metabolizable energy; ADF = acid detergent fiber; NDF = neutral detergent fiber.

Weaning was performed at 80 ± 10 days. Males lambs were fattened up in feedlot, receiving the same diet formulated for a daily weight gain of 300 g, according to NRC (2007) for 56 days, and slaughtered. Ewe lambs stayed in collective stalls with a diet formulated to meet the growth requirements according to NRC (2007), with 14% CP and 2.6 Mcal ME until they reached puberty (eight months). The body condition score, Famacha® score and health condition of the herd were monitored monthly and the use of medications was performed according to the need for each treatment. Vaccination against clostridiosis was performed once in lambs and mothers (Table 15).

Table 15 *Productive data of ewes in diets with different levels and sources of energy.*

	Treatments				
Productive data	LOW	CTL	STA	STA+Cr	STA+RPF
Birth Weight (kg)	3.8	4.4	4.2	4.3	4.5
Weaning weight (kg)	16.2	15.2	16	18.1	20.1
Lamb slaughter weight (kg)	42.38	37.13	42.90	46.81	44.75
Lamb weight at 8 months (kg)	43.5	52.2	47.85	52.57	48.89
Pregnancy rate (%)	100	100	100	100	92.85
Prolificity	1.43	1.36	1.53	1.53	1.23
Mortality until weaning (%)	20	0	0	4.4	0
Ewe lambs suitable for breeding (%)	66.7	100	100	100	75

LOW = 90% of predicted ME requirement; CTL = 100% of predicted ME requirement; ST = 110% of predicted ME requirement and the energy source was starch; ST+Cr = ST more chromium propionate; ST+RPF = ST more palm oil protected fat.

Economic-financial analysis

Based on performance data, the economic-financial analysis was performed for each treatment, with a short and long-term focus. The short-term analyzes included the calculation of contribution margin (CM = total revenue - variable cost), contribution margin per unit (CMU = CM / total kg produced) (PADOVEZE, 2013), profit (P = total revenue - total cost), profit per unit (PU = profit / total kg produced) for a one-year period (MANKIW, 2014), representative of a production cycle. For such analyzes, a representative commercial property of 1000 matrixes was designed, pre-fixed, with the surplus considered as sold (replacement ewes).

For long-term analysis, an incremental monthly cash flow (FC) was prepared for the 10 year forward. The herd began with 100 sheeps and the number of 1000 ewes was considered for a stabilized herd. The replacement was performed only with ewe lambs born in the herd and after stabilizing it, the excess was considered as sold. After the first five years, the replacement rate of 20% per year was considered. To represent the dynamics of the commercial herd, it was established the use of one breeder for 50 ewes (STIVARI et al., 2013).

From the cash flow, the indicators of net present value (NPV), internal return rate (IRR) and simple payback were calculated according to Gitman et al. (2015), considering the discount annual rate of 2% (Selic) and the period for herd stabilization (PS), considered as the time, in months, to reach 1000 breeding stock. The initial investment in month zero was considered to be R$ 150,000.00 for all treatments, taking into account the purchase of the 100 initial ewes and the necessary equipment (tractor, fence and other equipment). At the end of the 120th month, all animals that remained in the herd were sold at R$ 5.00 / kg animal.

Production costs were allocated according to Raineri et al. (2015) in variable costs (feed and veterinary costs) and fixed costs (labor, depreciation, maintenance and conservation or equipment, taxes and the capital and land rent). For calculation details see Raineri et al. (2015).

To obtain the prices of feed ingredients, the database of the Institute of Agricultural Economics (IEA) was used. Prices for protected fat and chromium propionate were provided by the supplier (Kemin Industries Inc). Prices of other inputs such as vaccination and medicines, in general, were obtained from agricultural input stores in the São Paulo region. The price of milk supplied to newborn lambs was obtained using the database of the Center for Advanced

Studies in Applied Economics (CEPEA) for the São Paulo region. The values not found in the databases were obtained from agricultural input stores in the São Paulo region. All values were deflated for September 2020, using the National Consumer Price Index (IGP - DI / FGV) as a reference.

Revenue was assessed by selling live lambs (R$ 9.50 / kg live weight) (CEPEA), selling animals for disposal (R$ 4.00 / kg live weight) and selling a young female aged eight months of life (R$ 8.00 / kg of live weight), considering the sale value in the Pirassununga / SP region and that US$ 1.00 is equivalent to R$ 5.32 (Brazil). The results were obtained by calculations in spreadsheets prepared in Excel® (Microsoft Corporation, 2010) and compared using descriptive statistics.

Results

LOW presented the lowest variable cost (R$ 276.621,61), total cost (R$ 294.661,93), and cost per kg/animal produced (R$ 7.11). ST and ST+Cr had similar variable costs (R$ 563.590,77 and R$ 579.617,73, respectively), total cost (R$ 581.631,09 and R$ 597.658,05, respectively), and average cost (R$ 8.86 and R$ 8.34, respectively). The CTL and ST+RPF were the most expensive, with the average cost to produce a kg of the animal from the CTL treatment of R$ 11.40 and R$ 10.67 for ST + RPF (Table 16).

The total revenue generated by the LOW was the lowest (R$ 373.387,48), followed by the ST+RPF (R$ 468.888,69), CTL (R$ 594.357,50), ST (R$ 633.716,14), and ST+Cr with the highest revenues (R$ 696.316,09).

For the short-term analysis, for a module of 1000 ewes, the CTL and ST+RPF were negative and with values close to each other, with the profit per unit (profit/total kg produced) of R$ -1.26) and R$ -1.45, respectively.

Table 16 *Annual economic-financial analysis (R$ / year) and long-term (10 years) of ewes fed different levels and energy sources, for modules of 1000 ewes.*

	Treatments				
Expenses (R$)	LOW	CTL	STA	STA+Cr	STA+RPF
A - Variable costs	276.621,61	653.395,97	563.590,77	579.617,73	527.911,35
B - Fixed Costs	18.040,32	18.040,32	18.040,32	18.040,32	18.040,32
C - Total Cost (A + B)	294.661,93	671.436,29	581.631,09	597.658,05	545.951,67
D - Average cost (R $) / Kg live	7.11	11.40	8.86	8.34	10.67
Revenue (R $)					
Sale of live finished lambs	140.543,76	235.522,56	296.929,29	421.789,69	243.150,24
Sale of replacements ewes	166.392,10	328.834,94	306.786,86	245.126,40	167.775,81
Sale of discard animals	66.451,62	30.000	30.000	30.000	57.962,64
E - Total revenue	373.387,48	594.357,50	633.716,14	696.316,09	468.888,69
F - Average revenue (R $) / live kg	9.01	10.09	9.65	9.72	9.16
G - Total produced (kg)	41.441,45	58.905,60	65.670,07	71.637,46	51.188,72
Short-term viability (annual)					
H - Contribution margin (E-A) (R $)	96.765,87	-59.038,47	70.125,37	116.698,36	-59.022,66
I - Contribution margin per unit (H / G) (R $ / kg)	2.34	- 1.00	1.07	1.63	1.15
J - Profit (E-C) (R $)	78.725,55	-77.078,79	52.085,05	98.658,04	-77.062,98
Profit per unit (J / G) (R $ / kg)	1.96	-1.26	0.83	1.41	-1.45
Long-term viability (10 years)					
NPV (R $)	R$ -4.713,69	R$ -1.176.932,31	R$ 350.428,24	R$ 566.471,07	R$ -1.455.968,15
IRR (per year) (%)	1.85%	-24.27%	9.03%	13.65%	-38.06%
Simple payback (months)	120	-	113	101	-
PS (months)	85	45	45	53	69

NPV: Net present value; IRR: internal rate of return; PS: herd stabilization period, considering the time, in months, to reach 1000 ewes. LOW = 90% of predicted ME requirement; CTL = 100% of predicted ME requirement; ST = 110% of predicted ME requirement and the energy source was starch; ST+Cr = ST more chromium propionate; ST+RPF = ST more palm oil protected fat.

The contribution margin (CM = total revenue - variable cost) and profit (P = total revenue - total cost) of the LOW were intermediate (CM = R$ 96.765,87 and P = R$ 78.725,55) between the ST (CM = R$70.125,37 and P = R$ 52.085,05) and ST+Cr (CM = R$ 116.698,36 and P = R$ 98.658,04). Values per unit of kg / produced of the LOW were higher (CMU = R$ 2.34 and PU = R$ 1.96), followed by ST+Cr (CMU = R$ 1.63 and PU = R$ 1.41) and ST (CMU = R$ 1.07 and PU = R$ 0.83).

By analyzing the cash flow, the NPV and IRR of the CTL and ST+RPF were negative and there was no simple payback for the stipulated period of 10 years. The LOW had a negative NPV and IRR lower than the rate of return (2%), in addition to the payback only appearing at the end of the project, with the sale of all animals. Therefore, they are not considered economically viable.

The ST+Cr had the best NPV value (R$ 566.471,07), IRR (13.65% per year), and simple payback (101st month), followed by ST treatment with NPV of R$ 350.428,24, IRR of 9.03% per year and simple payback in the 113th month.

The period for herd stabilization (PS) was 45 months for CTL and ST treatments, 53 months for ST+Cr, 73 months for ST+RPF and 81 months for the lowest energy treatment.

Discussion

As expected, the LOW showed a lower variable cost, total cost, and cost per kg of animal produced, since there is no investment in feed, which is equivalent to 63.17% of the total costs of animal production (Raineri et al., 2015b). The similarity of costs between treatments ST and ST+Cr was also expected since the difference was only in the inclusion of chromium in 0.1% of the concentrate.

A higher cost with food wasn´t expected for CTL treatment. However, the higher consumption of corn silage by the control treatment was the main reason for increasing the variable cost and consequently the total and average costs. The silage cost for the CTL treatment was R$ 454.958,05, while for the ST, ST+Cr and ST+RPF treatments were R$ 303.505,13, R$ 307.469,96 and R$ 319.829,69, respectively. For ST+RPF treatment, the highest cost was determined both by the inclusion of protected fat, which increased the total cost of the diet by R$ 12.960 per year and the higher consumption of corn silage. The inclusion of chromium may also decrease DMI (DOMÍNGUEZ-VARA et al., 2009b) and therefore, a lower cost with silage intake.

According to the Production Cost Index for Lamb Production in Sao Paulo State, updated monthly by the Laboratory of Socioeconomic Analysis and Animal Sciences (LAE) of the University of São Paulo, in September 2020, the average cost of lamb production in the state of São Paulo was R$ 12.65 / kg live, ranging from R$ 9.63 (Araçatuba region) to R$ 21.28 (Campinas region). In this experiment, all treatments had an average production cost per kg of live animal lower than the average for the state of São Paulo, probably due to differences in nutritional management. Food cost is the main variable responsible for the total cost, not only in Brazilian production (BARROS et al., 2009; VIANA; SILVEIRA, 2009; LÔBO et al., 2011; PAIM et al., 2011; ZIGUER et al., 2011; RAINERI; STIVARI; GAMEIRO, 2015; ROMANZINI et al., 2018;

SANTOS et al., 2018; REIJERS et al., 2019) but also in other countries (STOTT et al., 2005; HILALI et al., 2011; BOHAN et al., 2018; DEMIRHAN, 2019).

Few studies analyzed the profitability in sheep production and, the majority choose to disregard some production costs (opportunity cost of land, pasture, others) by option or ignorance (Raineri et al., 2015a). Viana et al. (2009) carried out an economic analysis of sheep farming (cut and wool) in the region of Rio Grande do Sul, Brazil, and concluded that it was a profitable activity due to the positive balance of operating income, but does not compensate for the opportunity costs of the main factors of production (land and capital). Toro-Mujica et al. (2011) assessed the economic viability of dairy sheep properties in Spain and concluded that 45% of the properties were non-viable and needed a better balance between the use of feeding supplements and the sheep productive capacity. Barros et al. (2009) analyzed the profitability of sheep production in pasture and feedlot, with or without weaning, which showed a positive gross margin only when there is no use of feedlot, but did not obtain an economic return, NPV was negative and IRR lower than the discount rate, concluding that none of the scenarios were viable.

Lamb meat is a commodity and therefore, producers cannot control the price of the product they sell, so they need to manage the variables that are under their control (RAINERI; STIVARI; GAMEIRO, 2015). The implementation of new technologies, mainly nutritional, capable of reducing costs and increasing revenue are necessary (SANTOS et al., 2018). ST and ST+Cr diets demonstrated that the supply of diets with a higher energy level and with chromium supplementation, not only decrease the total cost but also improves the economic return, due to higher productivity. These findings are in agreement with Santos et al. (2018) that found that supplementation of both ewes and lambs promoted an increase in animal stocking rate and had a positive economic return. Supplementation nutrition also provides a high level of ewe reproductive performance, which brings a significant economic return (ATSAN et al., 2007) and profitability is mainly affect by revenues witch, in turn, is mainly affected by productivity (GAZZARIN; BENNI, 2020). The increase in any performance indicator has a greater impact on production profitability than any increase in the prices of inputs used in the diet (RAINERI; STIVARI; GAMEIRO, 2015). The traits with higher economics importance are carcass yield, number of lambing per year and lambing percentage (KOSGEY; VAN ARENDONK; BAKER, 2003; LÔBO et al., 2011).

For the investment to be considered viable, NPV has to be greater than zero, that is, the expected flow of revenues must be higher than the investment that generated it and the internal rate of return should be higher than the savings account, for example (PINHEIRO ROGÉRIO et al., 2019). In the short-term analysis, in a module with 1000 ewes, the LOW treatment seems viable, due to the higher unit profit. However, in the long-term analysis, the NPV is negative and the IRR is lower than the discount rate. The payback occurs only in the last month, with the sale of the entire remaining herd. Throughout the analyzed period (months 0-119), the accumulated cash flow is negative, therefore it is not a promising project. Although the LOW treatment seems to be profitable, the accumulated losses until reaching 1000 ewes (PS = 85 months) can only be recovered with the sale of all animals in the last month, confirming that this treatment is not promising.

CTL and ST+RPF treatments were not economically viable in the short or long term. In the short term, both have no contribution margin, that is, the revenues cannot pay the costs with food and medicine (variable costs). The variable costs of these treatments were higher, while the total production of kg produced was not big enough as the other treatments, so they did not reach the expected return.

In contrast, both ST and ST+Cr treatments were economically viable. The ST+Cr treatment proved to be the most economically profitable, with a high NPV (1.6 higher than the ST treatment) and an IRR seven times higher than the Selic rate (2%), while the ST treatment showed an IRR of almost five times the Selic. Production systems with more efficient lambs seem to promote a higher net present value (NPV) and internal rate of return (IRR), while low-efficiency treatments may have negative NPV and IRR (LIMA et al., 2017)

Regarding the simple payback, all treatments showed a high value (minimum eight years for recovery the amount invested, in the ST+Cr treatment), and the CTL and ST+RPF treatments did not present simple payback. However, for agricultural projects, with an emphasis on sheep farming, it is expected to find higher simple payback values, such as Pinheiro Rogério et al. (2019), that also found no payback value for finishing system for Morada Nova sheep fed a standard diet including traditional feedstuff and when lambs were supplemented with bovine cheese sérum, the payback was 10 years. For França et al. (2016), sheep production in an agrosilvpastoral system also takes 10 years to return the investment and more than 10 years in traditional systems.

The herd stabilization period (PS), considered as the time in months required to reach 1000 ewes, considers the performance indexes (Table 15). As the LOW treatment showed high mortality (20%) and a low percentage of lambs suitable for breeding (66.7%), it took longer to reach 1000 ewes, almost twice the time of the CTL and ST. Neonatal mortality has a major impact on herd profitability (BYRNE et al., 2010; REIJERS et al., 2019). The ST+RPF treatment showed lower reproductive rates such as pregnancy rate (92.85%), prolificacy (1.23 lambs/sheep), and lambs able to reproduce (70%) and therefore, also showed a higher PS.

Conclusion

CTL and ST + RPF treatments are not economically viable. The LOW treatment, despite presenting short-term profit, is not recommended, since it is not viable in the long term and the financial return only occurs with the sale of the entire herd and the completion of the activity. The treatments ST and ST+Cr are economically viable, and the inclusion of chromium in the diet of sheep promoted a higher NPV and higher IRR, therefore the treatment with the best economic viability, among those studied. The increase of energy in the diet and supplementation of chromium promoted better performance, which led to the best economic return.

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CAPÍTULO 8.

Artigo n°4

Artigo submetido na revista Tropical Animal Health Production

Feedlot of lambs supplemented with rumen protected fat or chromium propionate: performance and economic evaluation

Abstract: Our purpose was to analyze the performance and economic viability of lambs finished in a feedlot system with high energy diets and different sources of energy. Thirty-two lambs were divided into four treatments group according to the energy level and source: CTL = 100% of predicted ME requirement; ST = 110% of predicted ME requirement and the energy source was starch; ST+Cr = ST more chromium propionate and ST+PF = ST more palm oil protected fat. There was no differences on dry matter intake, initial or final body weight, feed conversion, total weight gain and carcass weight ($P > 0.05$), but there was significant differences for average daily gain (P=0.0011) and dressing percentage (P=0.0465). ST+Cr and ST+RPF had the smallest weight gain, but CTL had the lower dressing percentage (45.04%) and ST+RPF had the highest (47.42%). Considering selling live animal for slaughter, ST and ST+Cr had the highest profitability, with US$0.62 and US$0.64 more gain per animal, respectively, comparing with CTL. Considering selling slaughter lambs, ST+RPF had the highest gain (US$2.57). It is economically beneficial to use diets with more energy for feedlot lambs, however the addition of chromium has no effect. The addition of rumen protected fat of palm oil had a positive effect on dressing percentage and economic gain when selling slaughter lambs.

Keywords: Partial budgets; Energy; Nutrition; finishing lambs

Introduction

The implementation of feedlot of lambs provides a method of fattening lambs in a more efficient manner and alleviates grazing pressure, improving the production per hectare (VAN DER MERWE; BRAND; HOFFMAN, 2020), in addition to shortening the production cycle and improve the quality of slaughtered lamb (SILVA et al., 2019).

Feeding high energy diets improve the feedlot performance of growing animals (SULTAN; JAVAID; ASLAM, 2010) and since energy provide by forage is narrowed by bulk volume limitation of the rumen, finishing rations lean on the inclusion of concentrates to obtain optimal rates of gain (SEABROOK; PEEL; ENGLE, 2011b).

The most common component in feedlot rations is the starch (GAO; OBA, 2016). Ruminants have the ability to convert starch into propionate via microbial fermentation, which is the primary substrate for gluconeogenesis in ruminants (WARD; SCOTT; DAWSON, 1964; SEABROOK; PEEL; ENGLE, 2011b), but feeding too much starch may increase the risk of ruminal acidosis (GAO; OBA, 2016).

Chromium (Cr) is an essential micro mineral, metabolic modifier that influences insulin activity and modify carbohydrate metabolism, increasing the performance of ruminants (RODRÍGUEZ-GAXIOLA et al., 2020b). The use of fat in the diet has become interesting due to its high energy density and low heat increment (KUMAR et al., 2006) and therefore, improve animal performance by optimizing energy intake (OBEIDAT et al., 2011).

Profit margins in a feedlotting enterprise is relatively narrow and dietary costs are the most relevant, so a careful management and planning are necessary to maintain profitability (SANTOS et al., 2016; VAN DER MERWE; BRAND; HOFFMAN, 2020).

Therefore, the aim of this study was to evaluate the performance and economic viability of lambs finished in a feedlot system with high energy diets and different sources of energy. The hypothesis is that the use of high energy diets supplemented with chromium propionate or rumen-protected fat is economically viable as they improve feedlot performance of lambs.

Materials and Methods

The experimental was developed at the Faculty of Veterinary Medicine and Animal Science – USP (Campus Pirassununga, Brazil). Thirty-two ewe lambs, crossbred, with initial BW of 23.40kg ±3.5kg and the average of 90 days were allocated to individuals pens (1.5m²) for 45 days with slatted floor, individual feeders and waterers. The experimental design was completely randomized with four treatments and eight repetitions each based on the energy level and source: CTL (n=8) = 100% of predicted ME requirement; ST (n=8) = 110% of predicted ME requirement and the energy source was starch; ST+Cr (n=8) = ST more chromium propionate and ST+PF (n=8) = ST more palm oil protected fat (Table 17).

The feed was chemically characterized according to the AOAC (1995) by determination of dry matter (DM) (ID 930.15), mineral matter (MM) (ID 942.05), crude protein (CP) (ID 954.01) and acid detergent fiber (ADF) (ID 973.18). Neutral detergent fiber (NDF) was determinate according to Mertens (1997).

The value of metabolizable energy of the diets was calculated using SRNS program (Cornell University).

During the first three days, they were all fed with the same diet for adaptation to the feedlot. The animals were then fed the treatments diets twice a day, with feed amount adjusted to allow approximately 5% of refusal. Throughout the experimental period (45 days), lambs were weighed four times and original matter intake was calculated daily and individually using the differences between the amounts of diet offered and refused. The average daily gain (ADG; g/day) was calculated considering the intervals of each weighing and an average value was finally considered for each treatment. After this determinations, the feed conversion (FCR) was calculated from ratio between the total DMI and total BW gain.

Table 17 *Composition and nutritive value of the experimental diets*

	Treatments			
Nutrient	CTL	STA	STA+Cr	STA+RPF
Dry Matter. %	88.36	88.78	88.91	88.79
Crude Protein. % of DM	14.87	15.00	14.49	14.87
NDF. % of DM	26.88	17.89	18.50	20.30
ADF. % of DM	16.91	10.69	10.84	11.89
ME. Mcal/kg	2.83	3.00	3.00	3.00
EE. % of DM	2.90	3.33	3.11	4.73
Mineral material. % of DM	5.17	4.49	4.20	4.52
Calcium. % of DM	0.78	0.77	0.76	0.91
Phosphor. % of DM	0.33	0.34	0.31	0.33

CTL: diet energy level according to the NRC (2007); STA: diet with 10% more energy, the source being the starch; STA +Cr: diet with 10% more energy, the source being the starch and chromium; STA +RPF: diet with 10% more energy, the source being the starch and rumen protected fat; EE = ether extract; ME = metabolizable energy; ADF = acid detergent fiber; NDF = neutral detergent fiber.

After 45 days, the animals were fasted for 16h and the slaughtered following the methods of Welfare Animals. Ewe lambs were rended unconscious by electric shock and bled. After bleeding, skinning and gutting of the carcass, the hot carcass weight and dressing percentage (percentage ratio of carcass weight to live BW at slaughter) were evaluated.

The economic evaluations were analyzed according to Gross Profit Margin (revenues – diet costs) and the partial budget methodology (HOFFMAN et al. 1992) witch evaluate whether a change in production practices will increase or decrease profit. The analysis considered only fed costs and compared the Control (CTL) and the other scenarios (STA, STA+Cr and STA+RPF) as follows:

Net change in partial budgets = [1+2]-[3+4]

where 1 is the increase of revenues, 2 is the decrease of expenses, 3 is the decrease of revenues and 4 is the increase of expenses.

The database source to obtain feed ingredient prices (in Brazilian currency, Real) was the *Instituto de Economia Agrícola* (Agricultural Economics Institute) (IEA), considering a historical series (April 2019 to April 2020). Prices not found in this database were obtained in agricultural supply stores in the region of São Paulo and the price of the rumen protected fat and chromium propionate were provide by the supplier (Kemin Industries Inc). All series prices were adjusted to the inflation effect for April 2020, using the General Price Index (IGP - DI/FGV) as reference.

The revenues were measured by sale of live (US$1.79/kgBW) or slaughtered (US$3.60/kg carcass) animals, where US$1.00 equals R$ 5.32

(Brazil). These values were similar to those of São Paulo State (CEPEA, April 2020).

Statistical analysis were made using the PROC GLM (General Linear Model) of the SAS software (SAS 9.1, SAS Institute Cary, NC, USA) and the means compared by Tukey´s test with 5% of significance. The experimental design was a completely randomized with four treatments and three degrees of freedom. The model was $Y_{ij} = \mu + T_i + e_{ij}$, where Yij is the dependent variable (i = treatment and j=repetition), μ is the overall mean, T_i is the fixed effect of treatment (i = 1–4), and e_{ij} is the residual error.

Results

Animal performance is shown on Table 18. There was no differences for DMI (P=0.6559), initial (P=0.8158) or final body weight (P=0.9249), FCR (P=0.4250), TWG (P=0.2505) and carcass weight (P=0.6803), but there was significant differences for ADG (P=0.0011) and dressing percentage (P=0.0465). CTL had the highest average daily gain (0.1098g/day) but similar than STA (0.1074g/day), while STA+Cr and STA+RPF had the smallest weight gain (0.1046g/day and 0.1055g/day, respectively). However, CTL had the lower dressing percentage (45.04%) and STA+RPF had the highest (47.42%). Dressing percentage for STA and STA+Cr were 46.63% and 46.60%, respectively and did not differ from others treatments.

Economic evaluation (Table 19) shows a difference in the cost of diet per animal between treatments (P=0.0147). CTL was US$1.42 more expensive per animal (US$8.21) than STA+RPF (US$6.79). However, there was no differences for revenues, considering live animal (P=0.9249) or slaughter

(*P*=0.6803) and for gross profit margin considering live animal (*P*=0.9654) or slaughter (*P*=0.6137).

***Table 18** Performance of ewe lambs fed with differents levels and source of energy finished in feedlot*

	Treatments						
	CTL	STA	STA+Cr	STA+RPF	MEAN	SEM	p-value
DMI (kg/day)	0.94	0.95	0.92	0.89	0.93	0.0185	0.6559
Body Weight (kg)							
Initial	23.25	22.93	24	23.42	23.40	0.3844	0.8158
Final	32.46	32.93	32.83	32.17	32.59	0.4309	0.9249
ADG (g/day)	0.109 a	0.107 ab	0.106 B	0.105 B	0.106	0.0005	0.0011
FCR	4.53	4.21	4.64	4.51	4.48	0.0922	0.4250
Total Weight Gain	9.212	10.000	8.825	8.755	9.184	0.2417	0.2505
Carcass weight	14.63	15.36	15.30	15.26	15.14	0.2291	0.6803
Dressing (%)	45.04B	46.63AB	46.60AB	47.42A	46.45	0.3141	0.0465

CTL: diet energy level according to the NRC (2007); STA: diet with 6% more energy, the source being the starch; STA +Cr: diet with 6% more energy, the source being the starch and chromium; STA +RPF: diet with 6% more energy, the source being the starch and rumen protected fat; DMI = dry matter intake; ADG = average daily gain; FCR = Feed conversion ratio;

The partial budget evaluation (Table 19) revealed that the best treatment relative to Control treatment was the STA+Cr, considering live animals sales and STA+RPF, considering slaughter animals sales. On the first scenario (live animal sales), all treatments were better than control resulting in profitability of US$1.73, US$1.74 and US$ 0.96 for treatments STA, STA+CR and STA+RPF, respectively. On the second scenario (slaughter animals sales), all treatments were also better than Control and resulted in profitability above US$3.49. ST+RPF had the highest gain with US$3.69/per animal more than control.

Table 19 *Economic evaluation and partial budges resulting of lamb production fed with different levels and source of energy finished in feedlot*

	Treatments						
	CTL	STA	STA+Cr	STA+RPF	MEAN	SEM	p-Value
Diet Cost							
US$/kg DM							
US$/day							
US$/animal	5.86	6.11	5.91	5.57	5.85	0.1195	0.4501
Revenues source (US$/head)							
Live animal	58.37	59.23	59.05	57.86	58.60	0.7748	0.9248
Slaughtered	52.94	55.55	55.34	55.22	54.77	0.8280	0.6805
Gross profit margin							
US$/live animal – Diet cost	52.50	53.12	53.14	52.29	52.76	3.6732	0.9654
US$/ Slaughtered animal – Diet cost	47.08	49.44	49.43	49.65	48.74	4.0434	0.6137
Partial budgets (US$/head)	Diet-CTL						
NC1[1]		0.62	0.64	-0.21			
NC2[2]		2.36	2.35	2.57			

CTL: diet energy level according to the NRC (2007); STA: diet with 6% more energy, the source being the starch; STA +Cr: diet with 6% more energy, the source being the starch and chromium; STA +RPF: diet with 6% more energy, the source being the starch and rumen protected fat;
[1]Net change ofpartial budgets − live animal sale
[2]Net change ofpartial budgets − slaughtered animal sale

Discussion

To formulate the diets, it was considered the animal requirements for a daily gain of 300 g with a DMI close to 1.20 kg, according no NRC (2007). Despite not reaching these values, the animals still remained within the expected consumption of 3% of live weight on average (NRC, 2007).

It is well established that diets with fat above 5% is inhibitory to microbial function resulting in decreased nutrient digestion and DMI (CHALUPA et al., 1986) and only lambs fed a ration containing more than 10% of protected-fat can get lower DMI (KUMAR et al., 2006; SEABROOK; PEEL; ENGLE, 2011b). On this trial, STA+RPF had less than 5% of EE in the diet and that wasn't enough to cause a significant effect on DMI. However, lambs fed STA+RPF consumed approximately 5% less feed than lambs fed the CTL and that could explain the differences on ADG. Even with different ADG and same initial BW, final BW had no significant differences between treatments and ranged from 32.17 (STA+RPF) to 32.93 (STA).

Feed conversion ratio (FCR) is defined as the amount of feed consumed to gain a unit of body weight and it is considered optimal a FCR of 5.0 kg feed/kg weight gain. Feed conversion rates lower than 4.5 feed/kg weight gain during the finishing period are regarded as exceptional performances (Van Der Merwe et al., 2020). There was no differences between treatments in this trial and the FCR was lower than 5.0 kg feed/kg weight gain. STA diet had a FCR of 4.21.

Chromium could be considered as a metabolic modifier with potential to increase growth rate and improve productivity due to a better energy metabolism (DIKEMAN, 2007; MORENO-CAMARENA et al., 2015). However, chromium supplementation did not have an effect on growth performance, similar to the findings of Domínguez-Vara et al. (2009b), Arvizu et al. (2011a), Moreno-Camarena et al. (2015) and Griss et al. (2020b).

Hot carcass weight was not different between treatments, similar to Seabrook et al. (2011) that found no differences on HCW between control lambs and lambs fed until 7% of protected fat. Moreno-Camarena et al. (2015) used three levels of supplemental Cr (0.0, 0.2 and 0.4 mg kg of dry matter) to finishing lambs and also did not found differences for carcass weight and dressing percentage.

Different from Dutta et al. (2008), Manso et al. (2009) and Seabrook et al. (2011), lambs supplemented with protected fat had higher dressing percentage than control (47.42 vs 45.04).

Profitability is the main driver in a feedlot finishing operation (VAN DER MERWE; BRAND; HOFFMAN, 2020) and nutrition in an intensive feeding system accounts for approximately 70% of the capital inputs (LIMA et al., 2017). The use of 5% of palm oil on a lambs diets have been economically beneficial (DUTTA; AGNIHOTRI; RAO, 2008).

Even if there was no statistical difference for gross margin, costs per animal or revenues per animal, the partial budgets methodology shows that diets with more energy can be economically viable if the producers use the slaughter animal revenue. Using the selling price per kg of live animal, STA+RPF diet had a loss of US$0.21 while treatments STA and STA+Cr gain US$0.62 and US$0.64 per animal, comparing with CTL.

However, when considering the selling price per kg of animal slaughter, STA+RPF had the highest gain with US$2.57 more per animal than CTL and US$0.22 more per animal than STA and STA+Cr. This may have occurred as a result of the higher dressing percentage that the inclusion of protected fat provided.

Conclusion

In summary, it is economically beneficial to use diets with more energy for feedlot lambs. The addition of chromium has no negative or positive effect on performance and economic evaluation. On the other hand, the addition of rumen protected fat of palm oil had a positive effect on dressing percentage witch had economic gain.

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CONSIDERAÇÕES FINAIS

A nutrição afeta a reprodução e o desempenho produtivo não só das ovelhas, mas também da sua prole, tornando possível programar um melhor desempenho dos cordeiros, por meio da manipulação da dieta materna. Assim, a nutrição pode ser considerada fator determinante para a viabilidade econômica na produção de ovinos.

Fornecer dietas com energia abaixo da recomendação para cada fase produtiva tem menor custo de produção. Contudo, também promove piores índices zootécnicos, com alta mortalidade de cordeiro e produção de cordeiros e mães mais fracas após o parto, o que pode enfraquecer o vínculo materno-filial, além de atrasar tanto o retorno ao estro de ovelhas recém-paridas quanto a puberdade da sua prole. Portanto, não se torna um projeto economicamente viável em longo prazo.

Dieta com nível nutricional de acordo com as recomendações do NRC (2007), apesar de promover um desempenho satisfatório, teve maior consumo de silagem de milho, não sendo viável economicamente. Já o fornecimento de dietas com energia acima das recomendações promove uma melhora no desempenho de ovelhas e de suas filhas, tornando o projeto viável em longo prazo e, portanto, sendo recomendada para produtores.

Contudo, dieta com alta energia, mas utilizando como fonte sais de cálcio de ácidos graxos (C16:0), não é recomendada durante a gestação de ovelhas por diminuir os índices reprodutivos tanto da mãe quanto das filhas, principalmente diminuindo a taxa de prenhez e atrasando a puberdade, fazendo com que o projeto não se torne economicamente viável. O uso da gordura parece ser eficiente para manter a condição de escore corporal no pós-parto. Além disso, recomenda-se o uso da gordura protegida em fase de confinamento de fêmeas para o abate, pois proporciona um melhor rendimento de carcaça e, consequentemente, maior retorno financeiro.

Já a utilização de dietas com alta energia, sendo o amido a principal fonte de energia e suplementação com propionato de cromo durante a estação de monta, gestação e lactação, foi a dieta de maior viabilidade econômica em longo prazo. O cromo promoveu melhores índices reprodutivos tanto da ovelha quanto de suas filhas pela antecipação do retorno ao estro no pós-parto das ovelhas e da puberdade das cordeiras. Quando utilizado apenas no confinamento de fêmeas para engorda, o cromo não afetou o desempenho dos animais, não sendo recomendado seu uso para essa finalidade.

Apesar dos resultados positivos e promissores da utilização de cromo na dieta de ovelhas prenhes, pela revisão sistemática de literatura, nota-se que ainda há poucos experimentos e poucos resultados satisfatórios. Com isso, recomenda-se a utilização de cromo com cautela e incentiva-se que mais experimentos com essa categoria animal sejam realizados.

Em conclusão, recomenda-se a utilização de dietas com energia acima das recomendações do NRC (2007), sendo a principal fonte de energia o amido, para ovelhas em estação de monta, gestação e lactação. Para cordeiras confinadas para o abate, recomenda-se a utilização de dieta com energia acima da recomendação, com suplementação de gordura protegida de óleo de palma.

IMPLICAÇÕES

Apesar de existirem diversos trabalhos utilizando diferentes níveis de energia na dieta de ovinos, em diversas fases produtivas, este trabalho analisou, a curto e em longo prazo, a viabilidade econômica dessas dietas, comprovando que diminuir o custo total da produção economizando na dieta não vale a pena para o produtor, além de mostrar a viabilidade de dietas com suplementação de cromo e gordura protegida, o que não é amplamente encontrado na literatura.

O fornecimento da dieta total foi realizado a fim de mimetizar os acontecimentos a campo para que os resultados de desempenho encontrados fossem mais realistas. Contudo, o cálculo de consumo de matéria seca individual foi prejudicado, tendo que ser simulado por meio de equações e não calculado de forma direta.

A revisão sistemática sobre a utilização de cromo na dieta de ovinos junto com os resultados encontrados neste projeto são um passo importante para que seja possível recomendar a utilização do cromo. Mostra-se a necessidade de mais experimentos com ovelhas gestantes, a fim de recomendar a dose adequada.

Este projeto analisou a utilização do cromo e suas consequências em nível de desempenho produtivo e reprodutivo. Sugerem-se experimentos que testem a tolerância à glicose e sensibilidade a insulina para verificar os efeitos do cromo no metabolismo energético. Além disso, o cromo foi fornecido misturado na dieta total das ovelhas, também a fim de mimetizar o que ocorreria a campo. Para verificar a dose ideal e garantir a ingestão individual, outra forma de fornecimento deveria ser realizada, por exemplo: em cápsulas por ingestão forçada diária ou em mistura apenas com pequena quantidade de concentrado e fornecido individualmente, sendo a dieta total fornecida apenas após a ingestão dessa primeira mistura. De qualquer forma, os animais teriam que ficar em baias/piquetes individuais ou com 2-3 animais cada para facilitar o manejo.

Não foi avaliado neste projeto, mas para poder recomendar a utilização de cromo, também é importante que em próximos experimentos, verifiquem a capacidade de bioacumulação do cromo em tecidos destinados a alimentação humana, além de avaliar a excreção do cromo via urina e fezes e sua capacidade de contaminação do meio ambiente, se é ou não prejudicial às pastagens e aos animais.