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Biodisponibilidade da gordura em dietas para carnívoros: Efeitos dietéticos e da espécie animal

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BIODISPONIBILIDADE DA GORDURA EM DIETAS PARA CARNÍVOROS: EFEITOS DIETÉTICOS E DA ESPÉCIE ANIMAL[1]

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Orientador: Alexandre de Mello Kessler

RESUMO

As gorduras são componentes importantes em dietas para carnívoros, porém o aproveitamento pelos animais sofre influência de fatores inerentes à composição das dietas, principalmente fibras e minerais, que precisam ser melhor estudados. Os trabalhos que avaliam as perdas endógenas de gordura nas fezes (PEGF) e os efeitos na digestibilidade aparente da gordura são escassos e divergentes. Desta forma, os objetivos destes estudos foram: determinar as PEGF em cães e mink; utilizar as PEGF para obtenção dos valores de digestibilidade verdadeira da gordura; além de avaliar os efeitos da composição de fibras dietéticas e a inclusão de emulsificantes no aproveitamento da gordura por cães. No primeiro estudo, as PEGF foram mensuradas em cães adultos. Dietas com níveis crescentes de gordura de frango foram utilizadas e as PEGF dos cães foram determinadas a partir de análises de regressão. As PEGF foram expressas em relação ao peso corporal (PC) dos animais, 155 mg/kg PV/dia, ou em relação ao consumo de gordura na matéria seca (MS), 7,9 g/kg MS consumida. A digestibilidade aparente total da gordura apresentou aumento conforme o consumo. A digestibilidade verdadeira total da gordura, corrigida para PEGF, não variou mediante o consumo de gordura. A relação entre PEGF e consumo de gordura foi maior em dietas com baixa gordura e explica o aumento na digestibilidade aparente da gordura quando concentrações maiores são consumidas. No segundo estudo, as PEGF foram determinadas em mink. Dietas com níveis crescente de óleo de soja foram formuladas e as PEGF avaliadas por regressão. Foi obtido o valor de 0,5 g/100g MS consumida, próximo ao relatado em cães. No terceiro estudo foi avaliada a influência das fibras dietéticas e da lecitina de soja na digestibilidade aparente da gordura em dietas para cães adultos. Foram avaliadas três fontes de carboidratos, amido de milho, celulose e polpa de beterraba, combinadas ou não à lecitina de soja. A polpa de beterraba reduziu a digestibilidade aparente e verdadeira da gordura em cães, aumentou o teor de umidade e elevou o escore fecal, porém ainda dentro da faixa considerada ideal. A lecitina de soja não apresentou efeito de melhora na digestibilidade dos macronutrientes e da energia das dietas.

Palavras-chave: cães, celulose, gordura de frango, mink, perda endógena de gordura, polpa de beterraba

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BIODISPONIBILITY OF FATS IN CARNIVORES DIETS: DIETARY EFFECTS AND ANIMAL SPECIE[2]

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ABSTRACT

Fats are important components of carnivores diets, however inherent factors of diets composition, mainly fibers and minerals, may affect its use by the animals, requiring further studies. Studies evaluating endogenous fat loss (EFL) and the effects on apparent total tract digestibility of fat are limited and divergent. The objectives of these studies were: to determine the EFL in dogs and mink; apply the EFL to calculated the true total tract digestibility of fats; and to evaluate the effects of dietary fiber composition and an emulsifying agent on fats digestibility in dogs. On the first study, the EFL was evaluated in adult dogs. Diets with increasing levels of poultry fat were used and the EFL was determined by regression analysis. The EFL was obtained according to the dogs' body weight (BW) as 155 mg/kg BW/day, or according to dry matter (DM) fat intake, as 7.9 g/kg DM intake. The apparent total tract digestibility of fat increases according to the intake level. However, the true total tract digestibility of fat, EFL corrected, remained unaffected by fat intake. The relation between EFL and fat intake is higher for low fat diets and explains why the apparent digestibility increases according to intake. On the second study, the EFL was measured in mink. Diets with graded levels of soybean oil were offered and the EFL evaluated by regression. The value of 0.5 g/100 g DM intake was obtained, close to the reported with dogs. The third study assessed the influences of dietary fibers and soy lecithin on apparent total tract digestibility of fat in adult dog diets. Were evaluated three carbohydrate sources; corn starch, cellulose and beet pulp; combined or not with soy lecithin. The beet pulp decreased apparent and true total tract digestibility of fat in dogs, increased the moisture level of faeces and the faecal score, yet the faeces still had good quality. The soy lecithin did not improve digestibility of macronutrients and energy of diets.

Keywords: beet pulp, cellulose, dogs, endogenous fat loss, mink, poultry fat

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RELAÇÃO DE ABREVIATURAS E SÍMBOLOS

ADF	Acid detergent fiber
AG	Ácidos graxos
ANOVA	Analysis of variance
ATTD	Apparent total tract digestibility
BW	Body weight
CCK	Colecistoquinina
r^2	Coeficient of determination
CETP	Proteína de transferência do colesterol esterificado
CP	Crude protein
d	Day
DM	Dry matter
EFL	Endogenous fat loss
EM	Energia metabolizável
Fe_2O_3	Iron oxide
g/d	Grams per day
GE	Gross energy
GLM	General linear models
GSH	Glutationa reduzida
HDL	Lipoproteína de alta densidade
LCAT	Lecitina-colesterol aciltransferase
LDL	Lipoproteína de baixa densidade
ME	Metabolizable energy
MER	Maintenance energy requirements
NDF	Neutral detergent fiber
OM	Organic matter
PEGF	Perdas endógenas de gordura nas fezes
PC	Peso corporal
SE	Standard error
SEM	Standard error of the mean
TTTD	True total tract digestibility
VLDL	Lipoproteína de muito baixa densidade

CAPÍTULO I

1. INTRODUÇÃO

Cães, gatos e mink (*Neovison vison*), são mamíferos classificados dentro da ordem *Carnivora*. Estes animais são caracterizados por apresentarem um intestino curto, com alta taxa de passagem do alimento ao longo do trato gastrintestinal e adaptação fisiológica ao consumo de dietas com alta densidade energética e altos níveis de gordura. Dietas que possuam o conteúdo energético distribuídos com relações próximas a 30:63:7 para proteína, gordura e carboidratos, respectivamente, são preferidas por cães (Hewson-Hughes et al., 2013).

A quantidade de gordura consumida possui efeito importante na digestibilidade aparente para carnívoros. O maior consumo de gordura, tanto em cães quanto em gatos, aumenta a digestibilidade aparente da gordura de forma hiperbólica (Kendall, 1984). Porém, acredita-se que este comportamento decorra apenas da diminuição na relação entre o consumo de gordura e as perdas endógenas fecais de gordura, conforme o aumento da gordura dietética (Jørgensen et al., 1993).

O estudo do conteúdo de componentes de origem endógena presente nas fezes de animais permite a determinação dos valores de digestibilidade verdadeira total de nutrientes presentes em dietas e matérias-primas. Na nutrição animal, em especial, na nutrição de cães, as pesquisas de perdas endógenas fecais estão concentradas principalmente em perdas de proteínas e aminoácidos (Hendricks & Sritharan, 2002; Hendricks et al., 2002; Hendricks et al. 2013), já que, quantitativamente, estas perdas são mais significantes do que as perdas de gorduras e demais componentes.

A determinação do conteúdo endógeno fecal de gorduras, também pode revelar informações importantes para a compreensão e interpretação do metabolismo dos lipídeos, principalmente em dietas com baixos níveis de gordura. Entretanto, as pesquisas realizadas para medição das perdas endógenas fecais de gorduras em cães até o momento são escassas (Sperry, 1926; Kendall, 1984), além de apresentarem metodologias diversas.

Sabe-se que a digestibilidade das gorduras depende do perfil de ácidos graxos (AG) (Pontieri, 2008; Marx et al., 2015), assim como também pode ser influenciada pela composição do restante da dieta. As fontes de fibras e alguns minerais, (Ca e Mg), são os principais componentes que podem influenciar negativamente a digestibilidade das gorduras (Freeman, 1984; Muir et al., 1996; Burkhalter et al., 2001; Bosch et al., 2009). Por outro lado, também existem componentes dietéticos que possuem potencial para aumentar o aproveitamento das gorduras. Um bom exemplo é a lecitina de soja, que pode atuar como agente emulsificante para promover a incorporação de AG na fase micelar da digestão das gorduras (Øverland et al., 1994).

O conhecimento de informações referentes ao aproveitamento de dietas e matérias-primas pelos animais, depende da realização de ensaios de digestibilidade. Existe uma carência de informações referentes a valores de digestibilidade e energia metabolizável de fontes gordura para cães, em comparação a estudos com outros não-ruminantes, como aves e suínos.

2. REVISÃO BIBLIOGRÁFICA

2.1 Gordura em dietas para carnívoros

A gordura da dieta é o componente que mais concentra energia, fornece AG essenciais e é determinante para transportar vitaminas lipossolúveis, além de ter grande importância para a palatabilidade e a textura em *pet food* (NRC, 2006). Dietas ricas em gordura são normalmente mais palatáveis e a inclusão de gordura de recobrimento em dietas secas extrusadas é um procedimento padrão utilizado pela indústria de alimentação de cães e gatos como forma de elevar a palatabilidade e a densidade energética.

A palatabilidade de uma dieta é ponto chave na nutrição de cães. A dieta deve ser atrativa para que o animal consuma o alimento na quantidade correta para atender às suas necessidades nutricionais. Uma dieta não palatável será rejeitada pelos animais, independente do seu conteúdo nutricional, sendo balanceada ou não (Case et al., 2011).

2.1.1 Cães

Para humanos, o consumo elevado de gorduras eleva o fator de risco para o desenvolvimento de doenças cardiovasculares, porém cães, quando alimentados com dietas contendo elevados teores de gordura não apresentam esta propensão (Kronfeld et al., 1977). Uma das possíveis explicações para tal, se dá pelo fato de que em cães a principal lipoproteína transportadora presente no plasma é a lipoproteína de alta densidade (HDL), fundamental para o transporte de fosfolipídios, ésteres de colesterol e colesterol livre no plasma (Maldonado et al., 2001). Cães também possuem uma particularidade pois são a única espécie em que a subclasse, HDL_1, além das subclasses, HDL_2 e HDL_3, é observada (Xenoulis & Steiner, 2010).

A formação de HDL_1 em cães, ocorre devido ao contínuo acúmulo de ésteres de colesterol produzidos pela enzima lecitina-colesterol aciltransferase (LCAT), nas moléculas de HDL_2. A LCAT está presente na circulação sanguínea ligada à HDL e exerce papel fundamental na conversão do colesterol em ésteres de colesterol nas moléculas de HDL, rota metabólica conhecida como transporte reverso do colesterol (Xenoulis & Steiner, 2010).

Outra particularidade no metabolismo lipídico em cães é a ausência de atividade enzimática da proteína de transferência do colesterol esterificado (CETP); enzima responsável pela transferência de triglicerídeos da lipoproteína de muito baixa densidade (VLDL) e dos quilomícrons para HDL_2 e ésteres de colesterol da HDL_2 para a VLDL e a lipoproteína de baixa densidade (LDL), o que resulta na formação das moléculas de HDL_1. Nas moléculas de HDL_1 os ésteres de colesterol são transferidos dos tecidos para o fígado para eliminação ou reutilização e não para moléculas LDL ou VLDL, como ocorre em humanos, que posteriormente transferem o colesterol para tecidos periféricos, desta forma, é proposto que a menor incidência de distúrbios ateroscleróticos em cães é devida a esta função do HDL_1 (Xenoulis & Steiner, 2010).

Apesar de apresentarem grande tolerância ao conteúdo de gordura nas dietas, a concentração de AG essenciais das fontes utilizadas nas formulações deve ser considerada para definir a quantidade mínima de gordura da dieta (NRC, 2006). As recomendações mínimas de gordura, determinadas pela AAFCO (2008), para cães adultos em manutenção são de 5%, enquanto que para animais em crescimento e reprodução devem ser de 8% na matéria seca (MS), fornecidas em dieta contendo 3500 kcal/kg de energia metabolizável (EM). Já os níveis mínimos de gordura recomendados pela FEDIAF (2013), para cães adultos em manutenção são de 5,5% e para animais em crescimento de 8,5%, fornecidos em dieta contendo 4000 kcal/kg de EM, em base seca. Estes valores são iguais aos estabelecidos pelo NRC (2006), para o fornecimento recomendado de gordura em ambas as fases fisiológicas. A maioria das dietas secas para cães adultos em mantença contém entre 5% e 13% de extrato etéreo em base seca (Case et al., 2011).

2.1.2 Mink

O mink (*Neovison vison*) é a principal espécie animal criada com o intuito de produção de pele (NRC, 1982). Ensaios de digestibilidade realizados com mink visavam apenas a obtenção de resultados para otimizar os limites de utilização de nutrientes nas dietas, de forma que tornasse a produção de peles mais eficiente e economicamente viável possível.

Porém, estudos comparativos entre cães e mink tem demonstrado alta correlação entre os valores de digestibilidade obtidos entre estas espécies (Ahlstrøm & Skrede, 1998; Vhile et al., 2005). Desta forma, a utilização de mink como modelo em ensaios de digestibilidade para cães torna-se favorável, pois facilita a padronização dos ensaios, possibilita o uso de número maior e mais uniforme de animais por grupo, além de apresentar benefícios econômicos (Vhile et al., 2005).

Tradicionalmente dietas para mink possuem altos valores de gordura. Dietas com 35 a 40% de gordura em base seca na dieta são reportadas como satisfatórias. Porém, dietas com 25% de gordura em base seca são recomendadas (NRC, 1982).

Assim como para cães, a digestibilidade das gorduras para mink é alta, aproximadamente 85%, podendo passar de 90% em alguns casos, dependendo da fonte (NRC, 1982). O principal fator que afeta a digestibilidade das gorduras para estes animais é a composição do AG esteárico (C18:0) das fontes. Sendo possível predizer, através de uma equação de regressão, $Y = 96{,}4 - 0{,}298x$ ($r^2 = 0{,}93$), a digestibilidade das fontes de gorduras, baseando-se apenas no seu conteúdo deste AG em questão (Rouvinen et al. 1988).

2.2 Digestibilidade da gordura

Os valores de energia metabolizável (EM) e os coeficientes de digestibilidade de uma mesma fonte de gordura variam conforme a espécie animal. A composição de uma matriz nutricional para formulação de dietas deve considerar estes valores de forma individual. Um compilado de valores de digestibilidade aparente da gordura (%) e EM (kcal/kg) das fontes de gordura utilizadas na nutrição animal estão apresentadas na Tabela 1.

Tabela 1. Valores de digestibilidade aparente total da gordura e energia metabolizável de diferentes matérias-primas usadas como fontes de gordura, conforme vários autores[1]

Autores	Espécie	Ingrediente	Coeficiente de Digestibilidade Aparente da Gordura (%)	Energia Metabolizável (kcal/kg)
Austreng et al. (1979)	Mink	Óleo de Soja	95,5	9052
		Óleo de Bacalhau	94,4	9075
		Óleo de Capelin	94,4	9050
NRC (1982)	Mink	Óleo de Soja	--	8790
		Sebo Bovino	--	8750
		Óleo de Peixe	--	9120
NRC (1986)	Gatos	Óleo de Soja	--	7283
		Sebo Bovino	--	8343
		Gordura de Frango	--	8044
		Óleo de Coco	--	8047
		Banha Suína	--	7850
NRC (1994)	Aves	Óleo de Soja[2]	--	8952
		Sebo Bovino[2]	--	7313
		Gordura de Frango[2]	--	9272
		Óleo de Menhaden	--	8450
Gröner & Pfeffer (1997)[3]	Cães	Óleo de Soja	98,0	9138
		Sebo Bovino	98,0	9186
		Banha Suína	98,0	8923
NRC (1998)	Suínos	Óleo de Soja	--	8400
		Sebo Bovino	--	7680
		Gordura de Frango	--	8180
		Óleo de Arenque	--	8330
Pontieri (2008)	Gatos	Óleo de Soja	92,9	8850
		Sebo Bovino	77,6	8032

(continuação Tabela 1.)

Autores	Espécie	Ingrediente	Coeficiente de Digestibilidade Aparente da Gordura (%)	Energia Metabolizável (kcal/kg)
Pontieri (2008)	Gatos	Gordura de Frango	92,3	9088
Rostagno et al. (2011)	Aves	Óleo de Soja	95,0	8790
		Sebo Bovino	80,0	7401
		Gordura de Frango	94,4	8681
Rostagno et al. (2011)	Suínos	Óleo de Soja	91,5	8300
		Sebo Bovino	87,1	7886
		Gordura de Frango	91,5	8228
Marx et al. (2015)	Cães	Óleo de Soja	99,1	9292
		Sebo Bovino	92,9	8692

[1]Valores expressos em base natural.
[2]Valores médios calculados a partir da base de dados.
[3]Valores de energia apresentados com energia digestível pelos autores.

2.2.1 Fatores que afetam a digestibilidade da gordura

A digestibilidade aparente total das gorduras varia conforme: grau de saturação, concentração de AG livres, posição dos AG na molécula de glicerol, tamanho da cadeia carbonada, concentração na dieta, além de poder sofrer interação com ingredientes fibrosos e minerais presentes nas dietas (Renner & Hill, 1961; Freeman, 1984; Kendall, 1984; Rouvinen et al., 1988; Rouvinen, 1990; Muir et al., 1996; Burkhalter et al., 2001; Gurr et al., 2002; Pontieri, 2008; Bosch et al., 2009; Marx et al., 2015).

2.2.1.1 Perfil lipídico

O perfil de AG de uma gordura reflete diretamente a sua digestibilidade e por consequência exerce efeito na metabolizabilidade da energia de dietas para animais de companhia. Gorduras cuja composição predominante é de AG insaturados propiciam melhor aproveitamento por cães (Marx et al., 2015), gatos (Pontieri, 2008) e mink (Rouvinen et al., 1988; Rouvinen, 1990).

Gorduras com maior grau de insaturação dos AG possuem maior potencial de formação de micelas no lúmen intestinal, já que a verdadeira hidrólise e emulsificação das gorduras, em não-ruminantes, ocorre no duodeno, devido a ação interligada dos fatores: secreção de bile pelo fígado, secreção da enzima lipase e seu cofator colipase pelo pâncreas e pela ação mecânica dos movimentos peristálticos do intestino (González & Silva, 2006).

Os sais biliares que participam no processo de digestão dos lipídeos são sintetizados a partir do colesterol, sendo produzidos no fígado, estocados na vesícula biliar e secretados no início do intestino delgado. Eles possuem diversas funções e uma delas é agir como agentes emulsificantes, preparando os triglicerídeos da dieta para hidrólise pela lipase pancreática no processo de digestão das gorduras. Eles também agem facilitando a absorção de vitaminas lipossolúveis pelo trato digestório (McDonald et al., 2002).

Cães suplementados com dietas contendo níveis elevados de AG poli-insaturados devem receber atenção especial com relação ao aumento da suscetibilidade a oxidação de radicais livres. O potencial para o desenvolvimento de quadros de peroxidação lipídica varia conforme o AG ofertado aos animais, sendo observada diminuição das concentrações de glutationa reduzida (GSH) dentro dos eritrócitos (Walters et al., 2010). Por outro lado, a fornecimento de dietas para cães, com conteúdo insuficiente de AG, principalmente poli-insaturados, podem propiciar o retardo no crescimento dos animais, aumento na susceptibilidade a infecções, pelagem áspera e descamação da pele (Rivers, 1982).

2.2.1.2 Nível de inclusão na dieta

O conteúdo de gordura na dieta influencia a taxa de passagem pelo trato gastrointestinal. Dietas ricas em gordura tendem a produzir menor taxa de esvaziamento gástrico (Bourreau et al., 2004). Lipídeos de 12 a 18 carbonos são estimuladores da liberação de colecistoquinina (CCK) que é um hormônio potente para inibir o esvaziamento gástrico (Argenzio, 1996). Um tempo maior de retenção do alimento no estômago pode permitir que haja melhor ação enzimática.

O nível de inclusão de gorduras nas dietas para cães e gatos tem sido relacionado com alterações nos valores de digestibilidade aparente total de uma mesma fonte de gordura. O aumento do conteúdo de gordura é apontado por ocasionar um comportamento de aumento hiperbólico na sua digestibilidade (Kendall, 1984). Entretanto, acredita-se que este comportamento seja apenas o reflexo da diminuição da relação entre gordura consumida e as perdas endógenas de gordura nas fezes (PEGF) dos animais que consomem dieta com altos níveis de gordura (Jørgensen et al., 1993).

2.2.1.3 Perda endógena fecal

De forma geral, gorduras são altamente digestíveis e, consequentemente, estão presentes em baixas concentrações nas fezes de cães. Mesmo assim, o conteúdo de gordura fecal não é totalmente resultante de frações indigestíveis da dieta. Parte deste conteúdo pode ser decorrente de bactérias que habitam o cólon dos animais; da descamação epitelial, secreções biliares ou por meio de síntese “de novo” de AG pela população microbiana (Nutrition Reviews, 1955; Clement, 1975).

A determinação do conteúdo endógeno de gordura nas fezes dos animais pode ser realizada através do uso de diferentes técnicas: (1) pela adição de níveis crescentes de gordura a uma dieta; (2) utilização de uma dieta sintética livre de gorduras; ou (3) por meio do uso de isótopos. Cada uma das técnicas possui suas vantagens e desvantagens.

As estimativas de PEGF, por meio da técnica de adição crescente de gordura considera a passagem do conteúdo de medição pelo intestino grosso e pode sofrer influência considerável de microrganismos na composição das gorduras e dos AG fecais (Bayley & Lewis, 1965). Porém, em dietas com baixos níveis de fibra, principal componente dietético utilizado como substrato para fermentação no intestino grosso, a fermentação bacteriana é menor e, consequentemente, ocorre redução da presença deste conteúdo nas fezes dos animais.

A utilização de cânulas no intestino delgado dos animais propicia a retirada de amostras antes que ocorra o contato com as bactérias que habitam o intestino grosso. Porém o grau de representatividade da amostra referente a digesta após o ponto de coleta pode ficar comprometida, já que o ponto em que a cânula é inserida pode variar. Outro problema é a possível ocorrência de sedimentação ao redor da cânula, que torna a amostragem não uniforme com relação a digesta luminal (Low, 1980). Mas a maior problemática com relação ao uso de cânulas em animais recaí sobre as questões de bem-estar animal e ética na condução destes experimentos.

Outra forma de contornar os possíveis problemas das técnicas de medição de PEGF é a utilização de animais como mink (*Neovison vison*), que apresentam intestino grosso curto, ausência de ceco e possuem mínima atividade microbiana (Skrede, 1979). Os valores de digestibilidade dos nutrientes por estes animais possuem correlação com valores obtidos em estudos comparativos com cães (Ahlstrøm & Skrede, 1998; Vhile et al., 2005).

A técnica de utilização de isótopos para medição de conteúdo de origem endógena nas fezes, pode ser outra alternativa, mas possui custos bastante superiores as demais técnicas, principalmente quando comparada a técnica de adição de níveis crescentes do nutriente na dieta.

A determinação dos valores de PEGF em cães permite que sejam realizadas correções para a obtenção de valores de digestibilidade verdadeira total de gorduras em matérias-primas e dietas. Desta forma o comportamento hiperbólico de aumento na digestibilidade de uma mesma fonte de gordura, que normalmente é observado mediante aumento no consumo, pode ser corrigido.

A quantidade de trabalhos realizados para obtenção de valores de PEGF em diferentes espécies animais é pequena, além de apresentarem diferentes metodologias para obtenção dos resultados e divergirem na forma de apresentação dos valores obtidos. Na Tabela 2, são apresentados valores de PEGF em diferentes espécies animais.

Tabela 2. Perda endógena fecal de gordura de diferentes espécies, conforme vários autores

Autores	Espécie	Unidade		
		mg/kg PV/dia	mg/dia	g/100 g gordura consumida em base seca
Hill & Bloor (1922)	Gatos		251	
Sperry (1926)	Cães	22,0		
Lewis & Partin (1954)[1]	Homem		1000 – 3000	
Norcia & Lundberg (1954)	Ratos		3,4 – 6,3	
Freeman et al. (1968)	Suínos		5000 – 32900	
Rimeslåtten & Jørgesen (1971)[2]	Mink			0,8
Austreng (1979)	Truta arco-íris			0,4
Adams & Jensen (1984)[3]	Suínos			0,61 – 0,87
Kendall (1984)	Cães	242		
Kendall (1984)	Gatos	150		
Jørgensen et al. (1993)	Suínos			0,44
Kil et al. (2010)	Suínos			0,37 – 1,2

[1]Citado por Webb et al. (1963).
[2]Citado por Austreng et al. (1979).
[3]Valor médio obtido a partir dos dados apresentados.

2.3 Influência das fibras em dietas para cães

Apesar da fibra dietética não ser digerida por enzimas intestinais, é necessário que esta seja fornecida aos cães com a finalidade de promover a saúde e o funcionamento normal do trato gastrintestinal dos animais (Case et al. 2011). A fibra é considerada um componente heterogêneo do ponto de vista nutricional, químico e físico. Este componente pode ser classificado em duas subclasses; fibras insolúveis e fibras solúveis. As duas subclasses desempenham diferentes funções nos processos digestivos e absortivos no trato gastrintestinal. A relação entre fibra insolúvel e fibra solúvel dentro das fontes de fibra utilizadas em dietas pode afetar o aproveitamento (Burkhalter et al., 2001).

A polpa de beterraba e celulose microcristalina são fontes comuns de fibras utilizadas em dietas para cães. Estas fontes de fibras possuem diferentes propriedades físico-químicas, que acabam por determinar a sua fermentabilidade e afetar resposta fisiológicas nos animais (De Godoy et al., 2013). Existem também fontes comerciais de celulose não purificadas, que podem incluir componentes não padronizados de celulose e outras substâncias. Porém sua composição e características nutricionais são próximas.

A polpa de beterraba é composta de aproximadamente 17-20% de fibra solúvel (Fahey et al., 1990a) e possuí fermentabilidade *in vitro* de 33-38% para cães (Sunvold et al., 1995). A celulose por sua vez, possui fermentabilidade de 2-4% para cães, reflexo da sua alta concentração de fibra insolúvel, mais de 92% (Sunvold et al., 1995; De Godoy et al., 2013). A composição química destas duas fontes de fibras dietéticas é apresentada na Tabela 3.

Tabela 3. Composição química da polpa de beterraba e da celulose microcristalina, de acordo com revisão de literatura por De Godoy et al. (2013)

Componente (%)	Polpa de Beterraba	Celulose Microcristalina
Matéria seca	87,6 – 92,3	93,0 – 96,6
Matéria orgânica	90,4 – 95,4	99,4 – 100
Proteína bruta	7,5 – 16,3	0,0 – 2,0
Fibra dietética total	57,0 – 82,6	91,6 – 99,9
Fibra solúvel	46,9 – 68,9	92,0 – 97,0
Fibra insolúvel	13,1 – 28,6	2,3 – 3,5

Sunvold et al. (1995) observaram que dietas compostas pela mistura de várias fontes de fibras na proporção de 40% fonte de pectina e 60% mistura de gomas, apresentam digestibilidade da matéria seca superior, quando comparada a dietas contendo apenas uma fonte de fibras (polpa de beterraba, celulose ou polpa de citrus). Estes autores também apontam que dietas contendo apenas celulose ou 75% de celulose e 25% de goma arábica como fonte de fibras apresentam baixa digestibilidade da fibra dietética total.

A polpa de beterraba, quando adicionada em dietas para cães, tem sido reportada por conferir boa qualidade de fezes ao animais, além de afetar de forma pouco acentuada a digestibilidade das dietas (Fahey et al., 1990a,b). Já Bosch et al. (2009) demonstraram que a polpa de beterraba exerce efeito negativo no aproveitamento da gordura, inclusive de forma mais pronunciada que a celulose, ao avaliaram dietas para cães contendo polpa de beterraba ou celulose. Este efeito também foi encontrado por Muir et al. (1996) que ao testarem a inclusão destas mesmas fontes de fibra em dietas com alta gordura (23%) para cães, observaram que a dieta contendo polpa de beterraba apresentou maior redução da digestibilidade ileal da gordura, que as dietas contendo diferentes níveis de celulose. Burkhalter et al. (2001) ao testarem dietas com 20% de gordura para cães combinada ou não a inclusão de polpa de beterraba, também encontraram menores coeficientes de digestibilidade ileal e total da matéria seca, gordura e energia nas dietas contento polpa.

Burrows et al. (1982), avaliaram a inclusão crescente de celulose (0, 3, 6, 9%) em dietas úmidas para cães e observaram redução linear na digestibilidade da matéria seca e aumento linear no peso e na umidade das fezes. A digestibilidade da gordura também foi reduzida, porém de forma pouco acentuada. Através de regressão também estimaram a digestibilidade da celulose para cães como sendo de 6%. Cole et al. (1999) testaram a inclusão de casca de soja em dietas para cães e também encontraram efeito linear negativo na digestibilidade da matéria seca, matéria orgânica e da energia conforme a inclusão desta fibra.

Zentek (1996) avaliou a inclusão, de forma individual, de 10% de celulose, pectina ou goma guar em dietas para cães. Os resultados encontrados de digestibilidade comprovam a variação no aproveitamento dos nutrientes, conforme a fonte de fibra utilizada. A inclusão de fibra insolúvel na dieta afetou de forma mais pronunciada a digestibilidade, pois a dieta contendo celulose apresentou o menor coeficiente de digestibilidade da matéria seca. Já Merritt et al. (1979) reportam que quando cães são alimentados com dietas comerciais secas contendo 5% de fibra bruta, possuem menor aproveitamento da gordura e maior excreção fecal do que animais alimentados com dietas úmidas a base de carne.

2.4 Inclusão de emulsificantes em dietas

De acordo com a terminologia clássica, dois líquidos não miscíveis, quando misturados, podem formar dois tipos de emulsões: óleo em água e água em óleo. Emulsificantes, como as lecitinas, possuem segmentos hidrofílicos e hidrofóbicos em sua estrutura molecular e concentram-se na interface entre óleo e água, subsequentemente reduzem a tensão interfacial (Van Nieuwenhuyzen & Tomás, 2008).

A lecitina de soja, um coproduto obtido por meio do processamento dos grãos para obtenção do óleo, é comercialmente o principal emulsificante utilizado na alimentação animal. Este coproduto é composto principalmente de fosfolipídios, como a fosfatidilcolina, fosfatidiletanolamina e fosfatidilinositol; além de AG insaturados, com destaque para o AG linoleico (18:2) (Scholfield, 1981; Van Nieuwenhuyzen & Tomás, 2008). A composição típica dos principais componentes da lecitina de soja é apresentada na Tabela 4. Na alimentação animal as lecitinas possuem grande potencial de utilização, sendo aplicadas em várias frentes, como: emulsificantes econômicos, agentes de instantaneização, suplemento de colina ou como fonte de AG essenciais.

Tabela 4. Composição típica da lecitina de soja, conforme a literatura

Componente (%)	**Scholfield (1981)[1]**	**Van Nieuwenhuyzen & Tomás (2008)[2]**	**Van Nieuwenhuyzen & Tomás (2008)[3]**
Umidade	1,0	< 1,0	< 1,0
Óleo de soja	33,0 – 35,0	37,0	3,0
Fosfatidilcolina	19,0 – 21,0	15,0	24,0
Fosfatidiletanolamina	8,0 – 20,0	11,0	17,0
Fosfatidilinositol	20,0 – 21,0	10,0	16,0
Outros fosfolipídios	5,0 – 11,0	11,0	17,0
Esteróis	2,0 – 5,0	7,0	11,0
Carboidratos livres	5,0	3,5	5,5

[1]Lecitina líquida
[2]Lecitina líquida
[3]Lecitina em pó

Porém, a inclusão de lecitina de soja em dietas para animais nem sempre acaba por promover aumento na emulsificação das gorduras, melhora nos coeficientes de digestibilidade e índices de desempenho zootécnico (Øverland et al., 1994; Azman & Ciftci, 2004). Em estudos com frangos de corte em que a lecitina de soja foi utilizada como substituto do óleo de soja ou sebo bovino em dosagens de 1,3% a 2% da dieta, não foram observados efeitos significativos no crescimento e desempenho dos animais (Azman & Ciftci, 2004).

Em dietas para leitões em crescimento, a inclusão menor (0,24% de lecitina de soja) não foi eficaz em aumentar a emulsificação e consequentemente a digestibilidade ileal ou total da mistura da fonte de gorduras (banha suína, 65-70% e sebo bovino, 30-35%) utilizada na dieta (Øverland et al., 1994).

A lecitina de soja é usada em dietas para camarões e peixes, pois imprime características importantes às dietas, devido a sua alta composição de fosfolipídios. Estes nutrientes são essenciais em dietas para camarões e larvas de peixes, pois atuam tanto de forma funcional; melhora na estabilização, flutuação e na densidade dos grânulos de rações peletizadas e extrusadas; como de forma nutricional, por atuarem na absorção de gorduras e vitaminas lipossolúveis e por serem intermediários do metabolismo de lipídeos (Van Nieuwenhuyzen & Tomás, 2008).

A utilização de lecitina de soja pela indústria de rações para cães e gatos tem sido impulsionada pelas alegações de propriedades funcionais que este ingrediente pode agregar ao processo de extrusão dos alimentos, como ganhos de produtividade e diminuição de gastos energéticos. Yang et al. (2015) observaram que a inclusão de 0,12% de lecitina em pó à uma massa de amido de milho submetida a extrusão promoveu redução na viscosidade e na entalpia de gelatinização dos extrusados, conforme o aumento da temperatura do canhão.

3. HIPÓTESES E OBJETIVOS

As hipóteses estabelecidas foram as seguintes:

EXPERIMENTO 1

1. A excreção endógena fecal de gordura de cães é baixa, porém não pode ser desprezada, pois exerce efeito na digestibilidade aparente total da gordura, principalmente de dietas com baixa inclusão;
2. A digestibilidade verdadeira total das gorduras independe do seu valor dietético;
3. A digestibilidade aparente das gorduras é menor em dietas com menor inclusão de gordura devido ao conteúdo endógeno.

EXPERIMENTO 2

1. A excreção endógena fecal de gorduras de mink (*Neovison vison*) é semelhante à de cães;
2. A digestibilidade verdadeira total das gorduras não é afetada pela concentração de gordura dietética.

EXPERIMENTO 3

1. A composição da fibra dietética exerce efeito na digestibilidade aparente total das gorduras em dietas para cães.
2. A lecitina de soja ao ser incluída em dietas para cães adultos, melhora a emulsificação das gorduras e consequentemente aumenta a digestibilidade aparente total da gordura.
3. A composição da fibra dietética exerce efeito na umidade e escore fecal dos animais.

Os objetivos destes estudos foram: determinar a PEGF de cães e mink; demonstrar os efeitos que estas perdas fecais causam na digestibilidade aparente total da gordura dietética; determinar o valor de digestibilidade verdadeira total da gordura; quantificar os possíveis efeitos causados por componentes fibrosos das dietas na digestibilidade aparente total da gordura, efeitos na composição fecal dos animais; além de avaliar o uso da lecitina de soja como agente emulsificante em dietas para cães adultos.

CAPÍTULO II[3]

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Endogenous fat loss and true total tract digestibility of poultry fat in adult dogs

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Abstract: The objectives of this study were to determine endogenous fat loss (EFL) and true total tract digestibility (TTTD) of poultry fat in dogs using diets with different fat:carbohydrate ratio. Complementary assessments were apparent total tract digestibility (ATTD) of macronutrient and energy, food intakes, faecal outputs and stool quality. Twelve adult Beagle dogs were applied in a complete randomized block Design consisting of three 10 days periods. Dietary treatments included a low-fat basal diet (Basal; 3.4% fat) added by six poultry fat levels (1%, 4%, 10%, 12%, 16% or 20%). The dogs were housed individually in metabolic cages and fed to meet maintenance energy requirements. Diets ATTD of dry matter (DM), organic matter, fat and gross energy were improved according to fat inclusion ($p < 0.001$). The ATTD of crude protein was not affected by dietary fat increase ($p > 0.05$). The ME intake was similar among diets, despite the clearly low palatability of the 1% and 4% fat coated diets. The faecal outputs decreased according to fat inclusion and the faecal score was not affected by dietary treatment. The stool quality was close to the ideal for all diets. The EFL were estimated by two regression methods, and can be expressed as 155 mg/kg body weight/day or 7.9 g/kg of DM intake. The TTTD of poultry fat is the same independently of dietary fat level. Poultry fat is 99.3% digestible for dogs. The relation

between fat intake and EFL is higher for low fat diets and explains why the ATTD of fat increase according to dietary fat inclusion.

Key words: fats metabolism; metabolizable energy; regression equations

INTRODUCTION

Dogs have a high tolerance for dietary fat consumption and dietary levels ranging from 5% up to 66% fat have been reported (Kendall, 1984), without propensity for cardiovascular diseases development (Kronfeld et al., 1977). Although, the fat content of commercial dry extruded diets for adult dogs ranges between 5% and 13% on a dry basis; being carbohydrates the main source of energy, even when there is a clear preference by dogs for diets with fats as the main source of energy (Hewson-Hughes et al., 2013).

The dietary fat level affects apparent total tract digestibility (ATTD) of fats, duo to the hyperbolic behavior assumed by increasing its content (Kendall, 1984). However, this behavior may be due to a decrease on the relation between fat intake and fat endogenous loss of animals fed high fat diets. Reflecting in a lower ATTD of fats in low fat diets (Jørgensen et al., 1993), other than an improvement in digestibility of fats in high fat diets.

The measurement of dogs' endogenous fat losses (EFL) permits adjustments for the true total tract digestibility (TTTD) of fats present in raw materials and diets. The studies of endogenous faecal losses are mainly focus on protein loss rather than fat. Several studies evaluating protein and amino acids losses in dogs have been performed (Hendricks & Sritharan, 2002; Hendricks et al., 2002; Hendricks et al. 2013) however the studies with fats are limited and the data obtained by divergent methodology

(Sperry, 1926; Kendall, 1984). The evaluation of EFL in pigs is well documented and still the reported data has a wide range between studies (Carlson & Bayley, 1968; Freeman et al. 1968; Adams & Jensen, 1984; Jørgensen et al., 1993; Kil et al., 2010), besides significant individual effects be pointed out to influence animals' EFL (Freeman et al. 1968).

The present study aimed to evaluate differences in ATTD of fat, EFL and TTTD of fat by adult dogs fed dry extruded diets coated with poultry fat in increasing levels; measurement of the metabolizable energy (ME) content (kcal/kg) of poultry fat and the effects of increasing fat supplementation on dogs' faecal characteristics.

MATERIAL AND METHODS

All animal care and handling procedures were approved by Animal Ethical Committee of the Federal University of Lavras, protocol number 053/14.

Animals and Experimental Design

Twelve healthy, intact adult Beagle, six males and six females, were distributed in a complete randomized block Design consisting of three 10 days periods, with six observations per treatment. Dogs were blocked by sex in pairs, at each experimental period. Each period included an adaptation phase (d 1 to 5) followed consecutively by a total faecal collection phase (d 6 to 10). During the adaptation and collection phases the dogs were housed in individual metabolic cages and had free access to drinking water. Body weights (BW) were measured at d 1 and d 10. Temperature and daylight fluctuated with external conditions. Between periods, the dogs were housed individually in kennels for five days, and fed a commercial dry premium diet.

Diets

A low fat diet, 3.4% fat in dry matter (DM) basis, was used as a basal diet, (Table 1). The six treatment diets were made by adding increasing amounts of fat to the basal diet. Poultry fat was added at 1%, 4%, 10%, 12%, 16% or 20% by coating (Table 2). To ensure a proper consumption and avoid fat losses by leakage, the coating was performed daily immediately before each meal in the bowl subsequently offered to each dog. All diets were added 1% powder palatability enhancer (swine liver).

The dogs were fed twice a day and the food offer was calculated to meet their maintenance energy requirement (MER) estimated at 130 kcal ME x BW(kg)$^{-0.75}$ day^{-1} (FEDIAF, 2013). The fat content of the experimental diets comprehends the range of the majority commercial dry extruded diets for dogs.

Sample Collection

Diet subsamples were collected and stored during the trial. Total faecal collection was adopted. Faecal samples were scored after each defecation by a same person as follows; 1 = hard dry and crumbly, “bullet like”; 2 = well formed, does not leave a mark when picked up, kickable; 3 = moist beginning to lose form, leaving a definite mark when picked up; 4 = the majority, if not all the form is lost, poor consistency, viscous and 5 = watery diarrhea (Moxham, 2001). At the start and at the end of each faecal collection period 1000 mg gelatin capsules containing iron oxide (III) Fe_2O_3 were given orally to the animals, to separate the faecal collection periods. The faeces were collected shortly after defecation and stored at -20 °C. Total faecal outputs from each animal and period were weighted and mixed then samples were taken and dried at 55 °C in a forced-air oven for 72 hours according to AOAC (1995) followed by grinding in a Willey mill in a 1-mm screen.

Chemical Analysis

Diets and faeces were analyzed for DM, ash, crude protein (CP) and fat concentration by acid hydrolysis method according to AOAC (1995). The diets were also analyzed for crude fiber content (AOAC, 1995). Dietary and faecal gross energy (GE) were determined by bomb calorimeter (IKA® – WERKE model C2000 basic). The fatty acid profile of the poultry fat was analyzed by capillary gas chromatography (CGC AGILENT 68650 SERIES GC SYSTEM) by AOCS (2009) and the results are shown at Table 3.

Calculations

The following equation was used to determine ATTD: [nutrient intake (g/d) – faecal output (g/d) / nutrient intake (g/d)] x 100. The organic matter (OM) content was calculate by: OM (%) = %DM – %Ash. The dietary ME was calculated by different factors for comparison: ME_{FEDIAF} = [(GE intake – faecal GE) – (CP intake grams – faecal CP grams) x 1,25] / DM intake (FEDIAF, 2013). The Association of American Feed Control Officials (AAFCO) apply dietary composition and fixed ME factors: ME_{AAFCO} = 8.5 kcal/g of fat + 3.5 kcal/g of CP + 3.5 kcal/g of N-free extract (AAFCO, 2008). The dietary protein:fat:carbohydrate ratio as % of ME was also calculated.

The EFL was estimated by two regressing methods; according to dog's BW (mg of fat/kg BW/day) or fat intake (g/kg of DM). The first equation related the ATTD of acid hydrolyzed fat (%) on the daily fat intake (g/kg BW) using hyperbolic non-linear regression model. The EFL (g/kg BW/day) was considered as the intercept on the *x*-

axis. While the second estimative related the apparently total digested fat (g/kg of DM intake) on the dietary fat intake (g/kg of DM), whereas the true digestibility of fat is estimated from the slope of the regression equation and the EFL (g/kg of DM intake) the intercept value. The diets TTTD of fat were calculated separately after deducting EFL values by both methods. The ME content (kcal/kg) of poultry fat was calculated multiplying the GE content (kcal/kg) by the slope of the EFL linear equation.

An estimative equation for the ME content (kcal/kg) of dry extruded diets for dogs was also proposed by a simple regression of the ME content (kcal/kg) on the fat content (%) of the diets.

Statistical Analysis

Differences in the ATTD of macronutrients and energy, TTTD of fat, intakes and faecal outputs between the dietary treatments were tested for significance using ANOVA by GLM procedure (Statgraphics Plus for Windows 4.1) and the means were compared by Tukey test at 5% probability. The faecal score was analyzed by Kruskal-Wallis test at 5% probability once the data did not respect a normal distribution. The EFL was estimated by hyperbolic and linear regressions using the “Advanced Regression” module of Statgraphics Plus for Windows 4.1 software.

RESULTS

The poultry fat has a predominant unsaturated fatty acid profile, almost 70% of total fatty acid content, being the major component, the monounsaturated fatty acid, oleic acid (18:1), Table 3. The low melting point of poultry fat, assured by the high presence of unsaturated fatty acids, has desirable characteristics for feed manufacturing.

All dogs remained healthy during the study; and did not have significant BW changes during the experimental periods, 15.6±0.02 kg. The low fat diets were less palatable and the dogs spent more time to consume the 1% and 4% fat added diets. Nevertheless, the animals had similar energy intakes (ME kcal/day; kcal ME x BW(kg)$^{-0.75}$ day) among diets and close to daily recommendations (FEDIAF, 2013), Table 4.

The daily faecal outputs (g as-is/day; g of DM/day) decrease according to fat coating increase ($p < 0.0001$). The faecal fat and DM content increased by fat coating, yet without showing significant changes on dogs' faecal scores. All diets promoted good stool quality by the dogs (Table 4). As expected, the ATTD of DM, OM, fat and GE of the diets increased according to the fat coating ($p < 0.0001$). However, the ATTD of CP ($p = 0.68$) was not influenced by fat coating resulting in similar values among diets (Table 5).

The endogenous faecal fat content was given by two estimative methods; first by the hyperbolic regression: Y = 100.5 – 15.5/x ($r^2 = 0.95$; SE = 1.03), whereas Y= fat digestibility (%) and x= daily fat intake (g/kg of BW), Figure 1; Assuming a null fat digestibility the intercept on the *x*-axis of the hyperbolic equation, it generates a value of 155 mg of fat/kg BW/day, and is assumed to be from endogenous origin. Also, the apparently total digested fat (g/kg of DM intake) was linearly related to dietary fat intake (g/kg of DM), as follows: Y = 0.993x – 7.90 ($r^2 = 0.99$; SE = 1.08), Figure 2. The true digestibility of fat was estimated from the slope of the regression equation and the intercept value considered being the EFL (g/kg of DM intake).

The TTTD of fat was assayed and showed similar values for all diets for both regression methods (Table 5). The TTTD of fat had values close to 100% independently of the dietary fat level and estimative method employed. Poultry fat was almost totally digestible by dogs, with 99.3% digestibility, and has a ME content of 9087 kcal/kg.

A prediction equation for dietary ME (kcal/kg) based on the fat content (%) was also proposed, once most commercial dry extruded diets for dogs are within the dietary fat range of the dietary treatments. The relation between dietary ME (kcal/kg) content and dietary fat level (%) was given by a linear equation, as follows: Y (ME; kcal/kg) = 3288 + 53.7x (dietary fat level; %) (r^2 = 0.98; SE = 49.8), Figure 3.

The comparison between the ME (kcal/kg) estimates methods for diets content are shown at Table 6. The diets ME (kcal/kg) contents remained similar for the three methods, however the AAFCO standard factors slightly underestimated the ME (kcal/kg) of the diets.

DISCUSSION

Food Intakes

The dietary fat level has a significant influence on palatability which can lead to animals' daily food overconsumption if offered free-choice feeding, leading in short to medium-term period to overweight and obesity (Toll et al., 2010). However, if high fat and energy density diets are offered according to the dog daily recommendations, no increase in BW is observed and the high digestibility of those diets reflects in good faecal characteristics by the dogs.

In our study we offered a wide range of energy density and fat level diets to the dogs, although the daily energy intakes were restricted to their MER, one could expect a lower ME (kcal/kg of $BW^{-0.75}$ day) intake by the low fat diets, once those diets were clearly less palatable, however dogs consumed the same amount of energy [kcal ME/day and kcal ME x $BW(kg)^{-0.75}$ day] for all diets, despite the higher fat intake (g/day) of the high fat diets.

The dogs' BW maintenance through the experimental periods can be interpreted as a result of a correct balance between daily energy intake and physical activity developed; also shows that the MER of adult kennel dogs recommended by FEDIAF (2013) was well applicable to the experimental conditions.

Faecal characteristics

During the entire study were not reported any cases of steatorrhea by the dogs. The stool quality was not affected by dietary fat level ($p > 0.05$) and remains between the ideal ranges of 2.5-2.8. However, the DM faecal content increased according to the fat addition in the diet, as a result of higher proportion of highly digestible content in the diet. The poultry fat when coated up to 20% do not promotes any detrimental effects on dogs' faecal outputs, and resulted in faecal characteristics desired by dog owners.

Similar results regarding good stool quality have already been reported by previous experiment with dogs fed diets coated up to 13% with soybean oil or beef tallow (Marx et al., 2015). The maximum inclusion level of fat in dry extruded diets for adult dogs without causing detrimental effects on stool characteristics still needs to be study.

Apparent total tract digestibility

The diets showed significant higher ATTD of macronutrients according to the inclusion of poultry fat, excepts for CP. Similar results have already been reported in diets with increasing levels of soybean oil and beef tallow (Marx et al., 2015).

The high ATTD of DM of the 1% fat coated diet, almost 84%, shows the good capacity of digestion of carbohydrates by dogs. The ME (kcal/kg) content from carbohydrates accounts for 62% of this diet. The extrusion process, mainly by submitting the diet to high temperature and pressure, allows the cooking of the starches and increases its digestibility.

EFL

Fats are usually highly digestible and consecutively have a low faecal excretion. Even so, not all faecal fat content has dietary origin, being present contents arising from bacteria at the colon, epithelial debris and the *de novo* synthesis of fatty acids by microbial population (Nutrition Reviews, 1955).

The EFL value obtained in this study, of 155 mg/kg BW/day is lower than the previous reported in dogs (242 mg/kg BW/day), but similar to that described by Kendall et al. (1984) for cats (150 mg/kg BW/day). Sperry (1926) obtained even lower EFL values by dogs when fed a lipid free diet of 22 mg/kg BW/day. The method used to access the EFL as well as the chemical analysis applied to determine the dietary and faecal fat content may explain partly the large differences between studies.

The 7.9 g/kg of DM intake considered to be from endogenous origin are within the range of values reported in pigs fed diets containing as fat source, soybean oil or corn oil, of 3.8 to 22.4 g/kg of DM intake (Adams and Jensen, 1984; Jørgensen et al., 1993; Kil et al. 2010).

The EFL of dogs weighting 15.5 kg was estimated to be 2.4 g/day. The dietary macronutrient composition may exert direct effects on EFL, especially the fiber content. The absorption of dietary fat and resorption of endogenous fat (e.g., bile acids) has been reported to be decreased by high fiber diets in pigs (Bach Knudsen & Hansen, 1991). The fermentation of soluble fiber on pig's hindgut can generate volatile fatty acids and promote the growth of bacterial populations that can be shed in the faeces and account as faecal fat content, increasing the EFL losses (Bach Knudsen et al., 1991). One can expect that dogs fed high fiber diets present similar hindgut behavior.

However, the crude fiber content of the experimental diets was low and mainly arising from insoluble fiber sources (e.g. cellulose; wheat bran) or from soluble and insoluble fiber mix sources (e.g. soybean meal; beet pulp). Thus, the faecal fat content arising from bacterial fermentation can probably be neglect in this case.

True total tract digestibility

The differences on ATTD of fats as a result of dietary fat concentrations was not observed on TTTD of fats. The rise on ATTD of fat by the addition of dietary fat is given by the decrease on the relation between fat intake and EFL (Freeman et al., 1968; Adams and Jensen, 1984; Jørgensen et al., 1993; Kil et al. 2010).

The TTTD of fats obtained by the two EFL estimates, generate similar results and not significantly different among diets. The TTTD of fats calculated using the EFL predicted by the hyperbolic regression, presented slightly higher than 100% digestibility values of almost all diets. This can be due the standard error of the estimate or the small BW changes by dogs during experimental periods, once the mean BW value of initial and final measurements were used to estimate the EFL in the hyperbolic equation. The TTTD of fats of all diets given by the linear regression has shown values between 98-100% and close to 99.3% given by the slope value of the equation.

Although it is known that the dietary fatty acid profile directly affects its melting point and consecutively determine differences in digestibility of fat sources (Renner & Hill, 1961; Rouvinen et al., 1988; Rouvinen et al., 1990; Marx et al, 2015), a similar pattern is expected when fat sources, other than poultry fat, are fed to dogs. In a previous study, the digestibility of soybean oil and beef tallow by dogs proved to be 99% and 92%, respectively (Marx et al., 2015).

Metabolizable energy

Fats have the highest energy content per unit and therefore can be primarily compounds to determine foods caloric density (Toll et al., 2010). The ME content of a diet can be estimated by different equations (NRC, 2006; AAFCO, 2008), that considered the content of three macronutrients; protein, fats and carbohydrates and their respective caloric contributions. In this study the dietary fat levels varied from 4.9% to 23.8% among treatments in DM basis; and it is safe to affirm that almost the totality of commercial dry extruded diets for dogs presents dietary fat level within this range. The dietary fiber content is known to exert a strong influence on dog feed’s digestibility by decreasing the ATTD of macronutrients, and thus lowering the ME (kcal/kg) contents (Fahey et al., 1990a; Fahey et al., 1990b; Muir et al., 1996). Even so, the fiber contents of the dietary treatments were low and close to practical levels of commercial diets for dogs.

With this idea in mind, a linear relation between dietary fat content and ME energy content was generated as follows; Dietary ME (kcal/kg) = 3316.4 + 53.46 · Dietary fat level (%) (r^2 = 0.98; SE = 49.7). This equation can be used to predict the ME (kcal/kg) content of low fiber dry extruded diets for dogs very accurately.

The slightly lower ME (kcal/kg) estimates of experimental diets given by the AAFCO (2008) equation when compared to the other two equations, suggest that the digestibility values for macronutrient assumed for dry extruded diets for dogs may be underestimated and need to be revised.

CONCLUSION

The EFL of adult dogs can be expressed as 155 mg/kg BW/day or 7.9 g/kg of DM intake. Poultry fat is 99.3% digestible for dogs. The relation between fat intake and EFL is higher for low fat diets and explains why the ATTD of fat increase according to dietary fat inclusion.

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Table 1. Basal diet ingredients composition as-is and chemical analysis

Ingredient	Unit/kg	
Brewers' Rice	g	350.0
Wheat Flour	g	268.2
Soybean Meal	g	100.0
Wheat Bran	g	50.0
Meat and Bone Meal	g	50.0
Corn Gluten	g	30.0
Blood Plasma	g	30.0
Soya Isolated Protein	g	30.0
Beet Pulp	g	20.0
Brewer's Dried Yeast	g	20.0
Dicalcium Phosphate	g	14.7
Celulose	g	10.0
Sodium Chloride	mg	6000
Premix Mineral/Vitamins*	mg	4000
Potassium Chloride	mg	1100
Choline Chloride	mg	900
Chemical Composition[1]	**Unit/kg**	
Dry Matter	g	917.1
Organic Matter	g	940.5
Crude Protein	g	259.8
Crude Fiber	g	29.9
N-free Extract	g	616.4
Acid hydrolyzed Fat	g	34.4
Ash	g	59.5
ME_{AAFCO}[2]	kcal	3359

[1]Values in dry basis, except for dry matter.
[2] ME_{AAFCO} = 8.5 kcal of ME/g of fat + 3.5 kcal of ME/g of CP + 3.5 kcal of ME/g of N-free extract.
*Provided the following per kilogram of diet: Vitamin A, 7000 IU; Vitamin B1, 2 mg; Vitamin B12, 25 mcg; Vitamin B2, 4 mg; Vitamin B6, 2 mg; Vitamin D3, 600 IU; Vitamin E, 50 IU; Vitamin K3, 1 mg; Folic Acid, 0.2 mg; Pantothenic Acid, 10 mg; Biotin, 0.03 mg; Niacin, 30 mg; Cobalt, 10 mg; Copper, 7 mg; Iron, 80 mg; Iodine, 1.5 mg; Manganese, 5 mg; Selenium, 0.2 mg; Zinc, 100 mg; Antioxidant (BHT), 150 mg.

Table 2. Chemical composition of the experimental diets (DM basis except for DM)

	Basal Diet +					
	1% FAT	4% FAT	10% FAT	12% FAT	16% FAT	20% FAT
DM, %	91.7	91.7	91.8	91.9	92.4	92.0
Ash, %	5.7	5.6	5.5	5.3	5.0	4.9
Crude Protein, %	25.7	25.8	24.1	24.2	22.9	22.0
Crude Fiber, %	2.4	2.4	2.3	2.3	2.3	2.2
N-free Extract, %	61.3	58.5	54.9	52.6	50.1	47.1
Acid hydrolyzed fat, %	4.9	7.8	13.2	15.7	19.7	23.8
Gross Energy, kcal/kg	4447	4584	4789	4976	5140	5285
ME_{AAFCO}[1], kcal/kg	3464	3609	3885	4018	4228	4443
%ME[2] Crude Protein	26	25	22	21	19	17
%ME Fat	12	18	29	33	40	46
%ME N-free Extract	62	57	49	46	41	37

[1]ME_{AAFCO} = 8.5 kcal of ME/g of fat + 3.5 kcal of ME/g of CP + 3.5 kcal of ME/g of N-free extract.
[2]%ME = Calculated from AAFCO (2008) conversion factors.

Table 3. Fatty acid profile, (% total fatty acid) and gross energy, (kcal/kg) of poultry fat

Fatty Acids		Total fatty acid (% m/m)
C 10:0	Capric	0.03
C 12:0	Lauric	0.05
C 14:0	Myristic	0.72
C 15:0	Pentadecanoic	0.11
C 16:0	Palmitic	23.57
C 16:1	Palmitoleic	7.27
C 17:0	Margaric	0.16
C 17:1	cis-10-heptadecenoic	0.12
C 18:0	Stearic	5.99
C 18:1 trans	Elaidic	0.27
C 18:1	Oleic	40.90
C 18:2	Linoleic	19.05
C 18:3 trans	t-linolenic	0.23
C 18:3	Linolenic	1.15
C 20:0	Arachidic	0.08
C 20:1	Eicosenoic	0.26
C 22:0	Behenic	0.05
∑ Saturated		30.75
∑ Unsaturated		69.25
Gross Energy		9151

Table 4. Food intake, faecal output and stool quality of adult Beagle dogs fed diets with increasing poultry fat levels

	Basal + 1% FAT	Basal + 4% FAT	Basal + 10% FAT	Basal + 12% FAT	Basal + 16% FAT	Basal + 20% FAT	SEM	*P*-value
Intake								
Food intake, g of DM/day	282.1^{a}	272.1^{a}	260.8^{ab}	231.8^{cd}	236.5^{bc}	212.1^{d}	2.39	<0.0001
Fat intake, g/day	13.4^{d}	21.2^{c}	34.2^{b}	36.1^{b}	47.0^{a}	51.7^{a}	0.67	<0.0001
Caloric intake, kcal ME/day	999.7	1004.0	1029.9	964.0	1035.1	969.9	9.10	0.2710
Caloric intake, kcal ME x BW(kg)$^{-0.75}$ day	128.3	129.2	132.5	124.0	132.3	125.0	0.98	0.1853
Faecal output								
Faecal output, g as-is/day	151.3^{a}	137.0^{ab}	120.7^{bc}	97.8^{cd}	97.5^{cd}	80.9^{d}	2.27	<0.0001
Faecal output, g of DM/day	45.7^{a}	42.9^{ab}	39.4^{bc}	33.1^{cd}	31.8^{de}	27.0^{e}	0.59	<0.0001
Faecal Fat, % of DM	4.9^{b}	5.0^{b}	6.6^{a}	6.7^{a}	6.4^{a}	7.2^{a}	0.11	<0.0001
Faecal DM, %	30.4^{c}	31.7^{bc}	32.2^{bc}	34.4^{a}	33.2^{ab}	33.4^{ab}	0.19	0.0002
Faecal Score[1]	2.8	2.5	2.6	2.4	2.6	2.6	0.03	0.0678

$^{a-e}$Within a row, means lacking a common superscript differ ($P \leq 0.05$).

[1]Faecal score based on the following scale: 1 = hard dry and crumbly, "bullet like"; 2 = well formed, does not leave a mark when picked up, kickable; 3 = moist beginning to lose form, leaving a definite mark when picked up; 4 = the majority, if not all the form is lost, poor consistency, viscous and 5 = watery diarrhea.

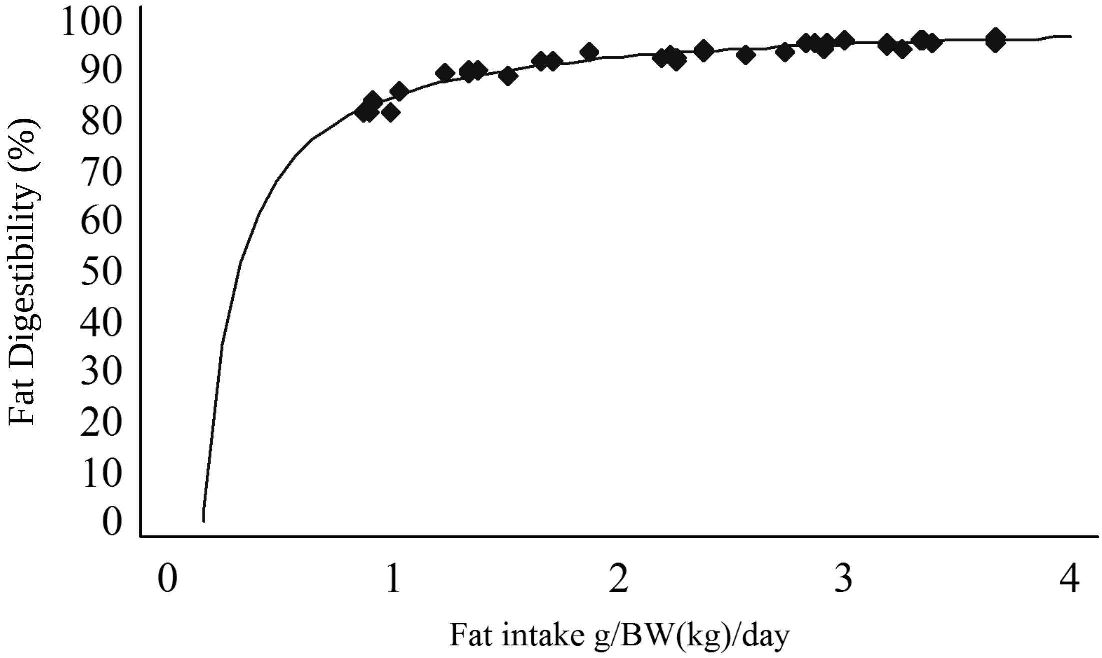

Figure 1. Relation between fat digestibility and fat intake is explained by the hyperbolic regression as follows: Y = 100.5 – 15.5/x (r^2 = 0.95; SE = 1.03).

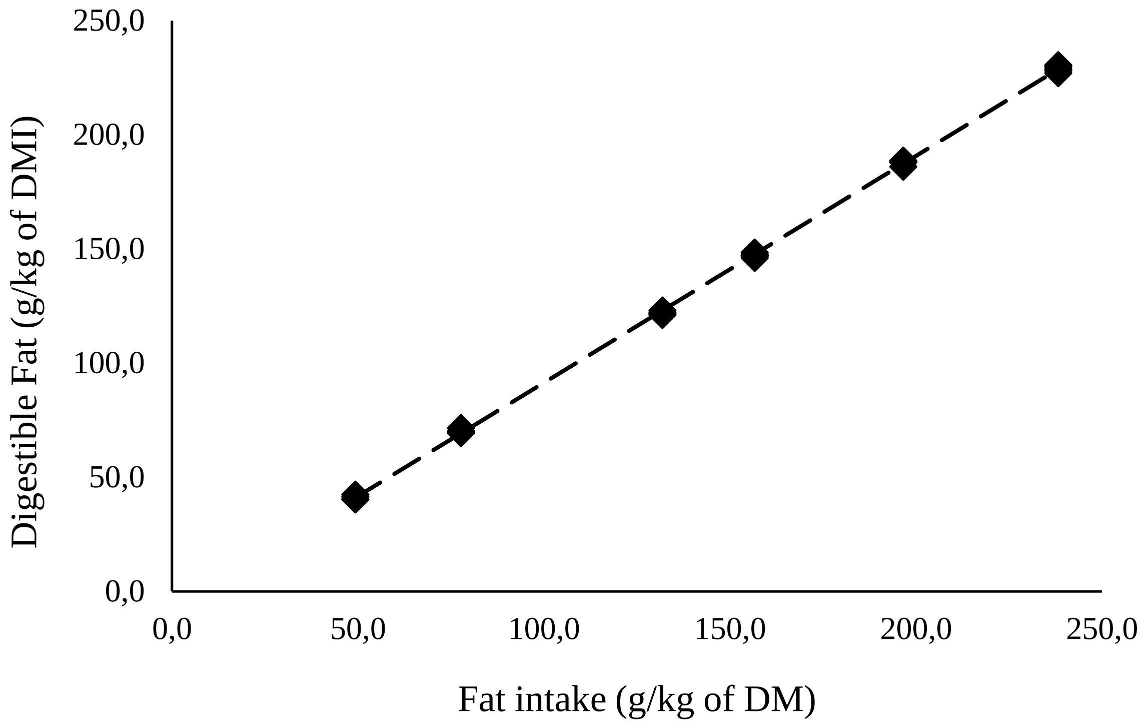

Figure 2. Regression of total tract apparently digested fat on dietary fat intake as follows: Y = 0.993x – 7.90 (r^2 = 0.99; SE = 1.08).

Table 5. Apparent total tract digestibility (ATTD) of macronutrient and energy, true total tract digestibility (TTTD) of fat and endogenous fat losses (EFL) of adult Beagle dogs fed diets with increasing poultry fat levels

	Basal + 1% FAT	Basal + 4% FAT	Basal + 10% FAT	Basal + 12% FAT	Basal + 16% FAT	Basal + 20% FAT	SEM	*P*-value
ATTD, %								
DM	83.8^{d}	84.4^{cd}	85.0^{cd}	85.7^{bc}	86.5^{ab}	87.2^{a}	0.14	<0.0001
OM	87.0^{d}	87.3^{d}	87.9^{cd}	88.5^{bc}	89.2^{ab}	89.7^{a}	0.11	<0.0001
CP	87.0	88.3	88.1	86.1	87.0	87.4	0.38	0.6783
Fat	83.5^{d}	89.9^{c}	92.6^{b}	93.6^{b}	95.6^{a}	96.2^{a}	0.16	<0.0001
Energy	86.1^{d}	86.7^{d}	88.1^{c}	88.8^{bc}	89.7^{ab}	90.4^{a}	0.11	<0.0001
TTTD of Fat, %								
EFL corrected by mg/kg BW day	100.3	101.1	99.8	100.3	100.8	100.8	0.15	0.4734
EFL corrected by g/kg DMI	99.4	100.0	99.0	98.6	99.6	99.4	0.16	0.2644
EFL								
Adult Beagle 15.6 kg BW, g/day	2.4	2.4	2.4	2.4	2.4	2.4	0.004	0.6687
Relative to fat intake, %	16.9^{a}	11.3^{b}	6.8^{c}	6.8^{c}	5.1^{d}	4.7^{d}	0.09	<0.0001

[a-d]Within a row, means lacking a common superscript differ ($P \leq 0.05$).

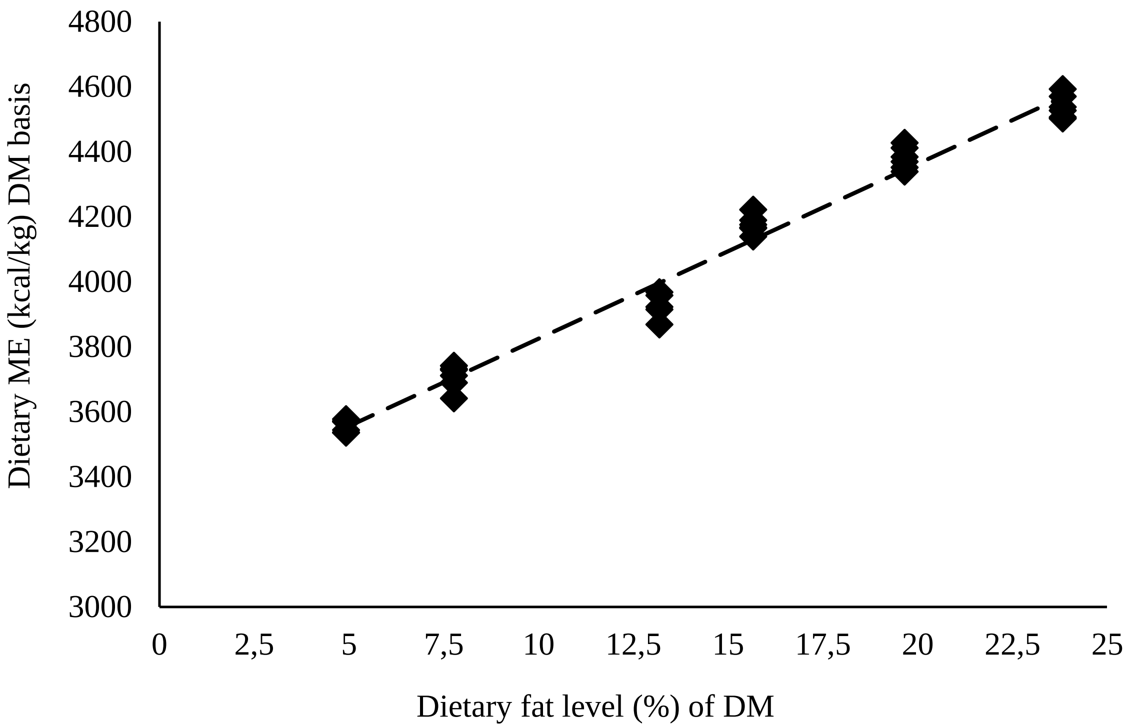

Figure 3. Relation between dietary metabolizable energy content and dietary total fat level given by a linear equation as follows: Y = 3288 + 53.7x (r^2 = 0.98; SE = 49.8).

Table 6. Comparison between dietary metabolizable energy (kcal/kg) of experimental diets according to different prediction equations

	Basal Diet +					
	1% FAT	4% FAT	10% FAT	12% FAT	16% FAT	20% FAT
ME content, DM basis						
ME_{FEDIAF}[1], kcal/kg	3550	3689	3954	4161	4365	4539
ME_{AAFCO}[2], kcal/kg	3464	3609	3885	4018	4228	4443
$ME_{fat\ estimative}$[3], kcal/kg	3553	3705	3996	4129	4344	4568
ME content, as-is basis						
ME_{FEDIAF}[1], kcal/kg	3256	3383	3629	3824	4035	4176
ME_{AAFCO}[2], kcal/kg	3178	3309	3566	3691	3909	4088
$ME_{fat\ estimative}$[3], kcal/kg	3259	3398	3667	3794	4016	4203

[1]ME_{FEDIAF} = [(GE intake – faecal GE) – (CP intake grams – faecal CP grams) x 1,25] / DM intake.
[2]ME_{AAFCO} = 8.5 kcal of ME/g of fat + 3.5 kcal of ME/g of CP + 3.5 kcal of ME/g of N-free extract.
[3]$ME_{fat\ estimative}$ = 3288 + 53.7 ·Dietary fat (%)

CAPÍTULO III[4]

[4]Artigo escrito conforme as normas da Canadian Journal of Animal Science – no formato de Short Communication.

Determination of endogenous fat loss and true total tract digestibility of fats in mink (*Neovison vison*)

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Abstract: The objective of this study was to determine endogenous fat loss (EFL) and true total tract digestibility (TTTD) of soybean oil in mink (*Neovison* vison) using diets with different fat:carbohydrate ratios. The apparent total tract digestibility (ATTD) of macronutrient and energy was also evaluated. Four diets with 6.3, 13.9, 22.0 and 34% fat in dry matter basis were used in a digestibility assay. Sixteen male mink were distributed in a Complete Randomized Design. The study was conducted in two experimental phases, first adaptation to the diets and cages (d 1 to 3), followed by the total faecal collection period (d 4 to 7). The ATTD of fat values were 90.8, 95.9, 96.9 and 97.8%, respectively. From the results, EFL was determined to be 0.5 g/100 g DM consumed. This is of similar magnitude as for other species. The relation between fat excretion and dietary fat level using soybean oil could be determined from the equation: Excretion (g) = 0.5 g + 0.0075 x g dietary fat/100 g DM consumed. The TTTD of soybean oil was determined to be 99%. In practice, true fat digestibility values will have

negligible impact in feed formulation as they are close to apparent values with the fat levels normally used for mink.

Key words: fats metabolism; metabolizable energy; regression equations

INTRODUCTION

In animal nutrition research, endogenous losses of protein and amino acids are more important and of main concern since these losses quantitatively are higher than for fat and fatty acids. The reason is that digestive enzymes are made of proteins and therefore the difference between apparent and true protein digestibility of amino acid digestibility is quite high. Endogenous fat in the digestive tract originate mainly from bile and from cells shedding of the mucosa of the digestive tract (Nutrition Reviews, 1955; Clement, 1975). The impact of the endogenous losses on apparent total tract digestibility (ATTD) of both protein and fat will be dependent on the dietary level, so with low dietary content of protein or fat the endogenous losses will make out a larger proportion of the protein and fat in the faeces. One method to determine endogenous losses is therefore to give the animal a protein-free or a fat-free diet and measure the content of the nutrient in the faeces, which is equal to the endogenous losses. The other common method is to give graded dietary levels of the nutrient and make a linear regression.

Apparent digestibility of fat is high in mink, 70-96%, and the fatty acid composition of the dietary fat source will be the main factor affecting apparent digestibility (Austreng et al., 1979; Rouvinen, 1990). Excretion of endogenous fat is therefore ignored in fat digestibility calculations. However, endogenous fat loss (EFL) in interspecies comparisons is interesting to have knowledge on, as well as for establishing a standard dietary fat level in studies determining fat digestibility.

Previous studies have found that the EFL in mink is 0.8 g/100 g dry matter (DM) consumed (Rimeslåtten and Jørgensen, 1971). In an unpublished study with dogs, a very similar relation was found, 0.79 g/100 g DM consumed (Marx, unpublished). We wanted to confirm this result since mink is applied as model for digestibility in dogs.

MATERIAL AND METHODS

Animals and Diets

Four diets with 6.3, 13.9, 22.0 and 34% of fat on DM basis were given to adult male mink with body weight (BW) of 2.5 – 3.0 kg to measure fat digestibility using four mink for each diet. The animals were housed inside in laboratory conditions in metabolic cages designed for collection of faeces and separation of urine. The digestibility assay was carried in a seven days period; the first three days for adaptation, and the last four days for faeces collection and precise feed intake registration. Diets were prepared by adding all ingredients to a bakery mixer and adding water up to the desired consistency. The animals were offered, once a day, about 65 g of feed DM daily corresponding to 286, 317, 318, 383 kcal/day of gross energy (GE), for the four diets, respectively from the order given in Table 1.

Chemical analyses

Diets and faeces were analyzed for DM, ash, crude protein (CP), starch and fat concentration by acid hydrolysis method according to AOAC (1995). Dietary and faecal gross energy (GE) were determined by bomb calorimeter (IKA® – WERKE model C2000 basic).

Calculations

The following equation was used to determine ATTD: [nutrient intake (g/d) – faecal output (g/d)/nutrient intake (g/d)] x 100. The organic matter (OM) content was calculate by: OM (%) = %DM – %Ash. The carbohydrate content was calculated by difference as follows: Carbohydrates (%) = %DM – (%Ash + %Fat + %CP).

The EFL was estimated by relation between fat excretion and dietary fat level whereas the EFL (g/kg of DM intake) was estimated from the intercept value.

Statistical Analysis

Differences in the ATTD of macronutrients and energy among the dietary treatments were tested for significance using ANOVA by GLM procedure (Statgraphics Plus for Windows 4.1). The EFL was estimated by linear regression using the “Advanced Regression” module of Statgraphics Plus for Windows 4.1 software.

RESULTS AND DISCUSSION

Feed intakes were adequate, but varied among groups and within each group, 82, 68, 69 and 99% of the feed offered. Digestibility values are presented in Table 2, showing that ATTD of fat increased with inclusion level from 90.8 % to 97.8.

The regression equation plot showed that the endogenous fat excretion would be 0.5 g fat /100 g DM with a fat free diet and that the relation between faecal fat excretion and dietary fat level using soybean oil could be determined from the equation:

Excretion (g) = 0.5 g + 0.0075 x g dietary fat/100 g DM consumed

Because of variation within the group given 13.9% fat, the R-square was as low as 0.44. However, the EFL obtained in this study (0.5 g/100 g) was of the same magnitude as Rimeslåtten and Jørgensen, (1971) (refered to in Austreng et al., 1979) and from unpublished data by Marx with dogs, showing both around 0.8g/100g DM consumed.

The EFL measured in mg/kg BW/d in 2.5 – 3.0 kg male mink, by our data would give an amount of 100–120 mg/day of EFL. Kendall (1984) determined EFL in cats to be 150 mg/kg BW/d and 242 mg/kg BW/d in dogs, while Marx (unpublished) determined 155 mg/kg BW/d in dogs. Jørgensen et al. (1993) determined EFL to be 0.47 g/100 g dietary dry matter at distal ileum and 0.44 g/100 g dietary dry matter at faeces in pigs.

If true fat digestibility values are calculated with the correction factor found in the present study the values would be very similar; 98.9 (1.2), 99.5 (0.8), 99.1 (0.3), 99.2% (0.1), respectively for the experimental diets. In practice, true fat digestibility values will have negligible impact in feed formulation as they are close to apparent values with the fat levels normally used for mink. For the low fat diet in the present study, that showed the highest difference between apparent and true fat digestibility, about 8%, the ME content on DM basis was 326 kcal/100g and the ME distribution from protein, fat and carbohydrates was 50, 16 and 34 %. In practice this distribution of main nutrients is not usually seen for mink because of the low fat content.

In conclusion, the study showed that endogenous fat loss in mink is about 0.5 g/100 g DM consumed corresponding to 100-120 mg/kg BW/d. This is of the same magnitude as in other species.

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Table 1. Diet composition and chemical content

	Diet 6.3 % Fat	Diet 13.9 % Fat	Diet 23.0 % Fat	Diet 34 % Fat
Ingredients, %				
Soybean oil	-	2.4	5.3	9.0
Fishmeal	15.5	19.0	21.3	24.0
Corn starch	8.4	7.9	6.2	4.0
Cellulose powder	1.9	2.4	2.7	3.0
Vitamins/mineral Premix	0.01	0.01	0.01	0.01
Water	74.2	68.3	64.5	60.0
Total	100	100	100	100
Chemical Composition[1]				
Ash, %	9.8	9.4	9.5	9.3
Crude protein, %	43.5	43.8	43.0	42.9
Fat, %	6.3	13.9	22.0	34.0
Carbohydrate[2], %	40.4	32.9	25.5	13.8
Starch, %	26.9	20.4	14.7	8.9
GE, kcal/kg	4,512	4,907	5,337	5,772

[1]Values in dry basis.

[2]Carbohydrate calculated by difference as follows: Carbohydrate (%) = %DM – (%CP + %Fat + %Ash).

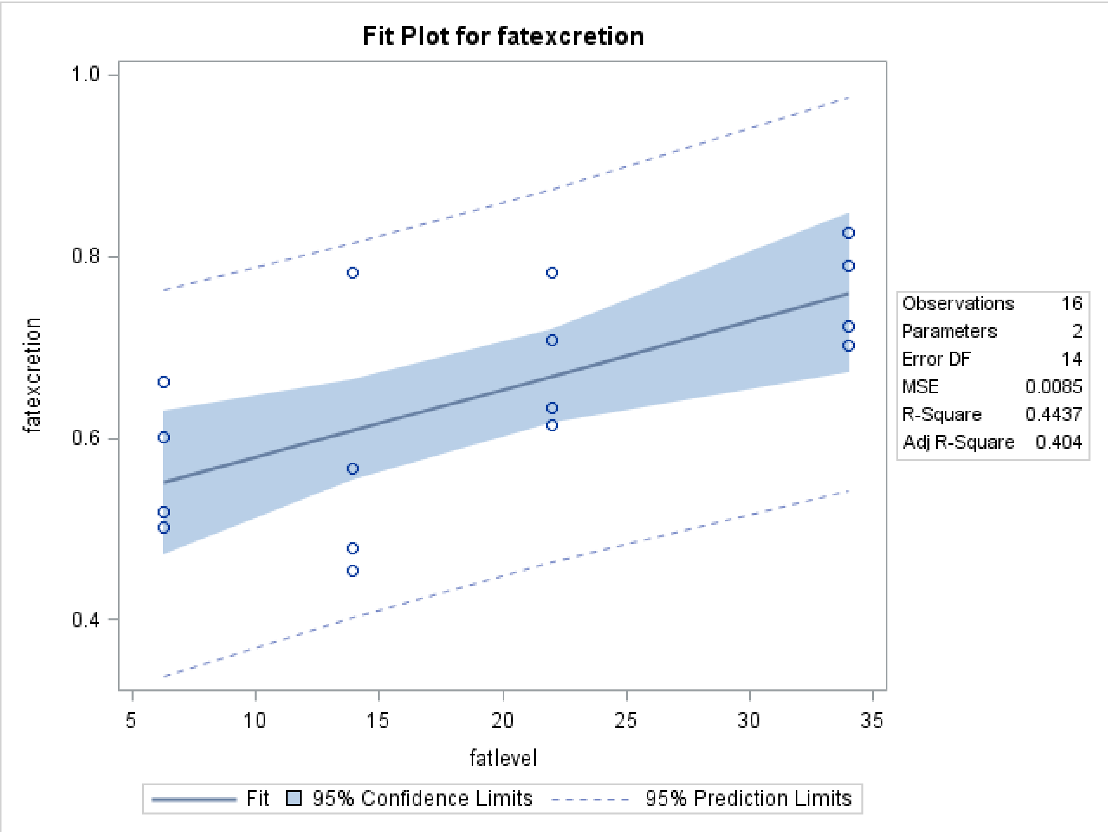

Figure 1. Regression equation of faecal fat excretion (g/100 g) on dietary fat level (%).

Table 2. Apparent total tract digestibility (ATTD) of macronutrient and energy of adult mink fed diets with increasing soybean oil levels. Standard deviation in parentheses

	Diet 6.3 % Fat	Diet 13.9 % Fat	Diet 23.0 % Fat	Diet 34 % Fat
ATTD, %				
DM	75.7 (1.0)	77.6 (0.9)	76.4 (1.2)	78.1 (0.1)
CP	86.5 (1.0	87.8 (0.8)	86.3 (0.9)	86.7 (0.1)
Fat	**90.8 (1.2)**	**95.9 (0.8)**	**96.9 (0.3)**	**97.8 (0.1)**
Carbohydrate[1]	68.6 (1.7	65.2 (1.0)	55.8 (2.7)	29.2 (0.7)
Starch	95.1 (3.1)	97.0 (0.2)	96.4 (0.3)	95.9 (0.3)
Energy	81.1 (1.7)	83.8 (0.7)	84.4 (0.9)	86.7 (0.1)

[1]Carbohydrate calculated by difference as follows: Carbohydrate (%) = %DM – (%CP + %Fat + %Ash).

CAPÍTULO IV[5]

[5]Artigo escrito conforme as normas da revista Archives of Animal Nutrition.

Dietary fiber composition influences macronutrients and energy digestibility in dogs

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Abstract: Soluble and insoluble fiber content in the diets may influence differently the digestion and absorption of nutrients. The objective of this study was to determine the effects of different fiber sources, combined or not with soy lecithin, on apparent total tract digestibility (ATTD) of macronutrients and energy and the true total tract digestibility (TTTD) of fat; complementary assessments were energy intake and stool quality. Eighteen healthy adult dogs from different breeds were applied in a 3x2 factorial Design, consisting of two 10 days periods, with a total of six observations per treatment. Dietary treatments included three basal diets, formulated with corn starch, cellulose powder or beet pulp, up to 10% of its content. Each one of the basal diets was coated with 10% of poultry fat or 9% of poultry fat + 1% of soy lecithin, totalizing six experimental diets. All diets had the same fat content and the total fiber content of the cellulose and beet pulp diets was equal. The dogs were housed individually in kennels and fed to meet maintenance energy requirements. The diet intake (kcal metabolizable energy/kg body weight$^{-0.75}$ day) was the same for all dietary treatments. The cellulose and beet pulp inclusion decreased ATTD of dry matter (DM), organic matter, neutral detergent fiber (NDF), carbohydrates and energy. The ATTD of fat, crude protein and energy and the TTTD of fat was lower for the beet pulp diets, while the ATTD of acid detergent fiber was lower for the cellulose diets. The soy lecithin did not improve macronutrients and energy digestibility in dog diets. Beet pulp affected negatively the faecal score and increased the faecal moisture, however the results remained within the standard levels.

Key words: beet pulp; cellulose; emulsifier; soy lecithin

INTRODUCTION

Dietary fats and fibers are essential components in dog diets for different reasons. Fat is a source of essential fatty acids, assisting fat soluble vitamins transport and absorption and as important energy source. Fiber is important to regulate gastrointestinal transit time, works as a prebiotic to the microbial populations and improves gut health and may change the stool consistence.

However, a negative effect caused by dietary fibers on apparent total tract digestibility (ATTD) of macronutrients and energy in dog diets have already been reported by several studies (Burrows et al., 1982; Fahey et al. 1990a; Fahey et al. 1990b; Sunvold et al., 1995; Muir et al., 1996; Burkhalter et al., 2001; Bosch et al. 2009). This effect seems to be depended of fibers composition. Fiber sources vary according to their soluble and insoluble composition. Beet pulp and cellulose are common fibers used in commercial pet foods worldwide. Their different chemical composition and physiochemical properties, reflect in different fiber fermentability patterns and physiological outcomes (De Godoy et al., 2013).

The digestibility of fats can be influenced by their composition itself or due other dietary components, mainly fibers and minerals (Renner & Hill, 1961; Freeman, 1984; Kendall, 1984; Muir et al., 1996; Burkhalter et al., 2001; Gurr et al., 2002; Bosch et al., 2009; Marx et al., 2015). Emulsifying agents, such as soy lecithin, have been pointed out to have potential use in animal nutrition, such as: instantisation agents, choline supplement or as essential fatty acid source. Soy lecithin has hydrophilic and a lipophilic segments in their molecular structure and concentrate at the interface between oil and water subsequently reducing the interfacial tension (Van Nieuwenhuyzen & Tomás, 2008).

Therefore, the present study aimed to evaluate the effects of different fiber sources, combined or not with soy lecithin, on ATTD of macronutrients and energy and the true total tract digestibility (TTTD) of fat; complementary assessments were energy intake and stool quality.

MATERIAL AND METHODS

All animal care and handling procedures were approved by Animal Ethical Committee of the Federal University of Lavras, protocol number 053/14.

Animals and Experimental Design

Eighteen healthy, intact adult dogs, were distributed in a 3x2 factorial Design, consisting of two 10 days periods, with a total of six observations per treatment. Each period included an adaptation phase (d 1 to 5) followed by a total faecal collection phase (d 6 to 10). During the adaptation and collection phases the dogs were housed in individual kennels and had free access to drinking water. Body weights (BW) were measured at d 1 and d 10. Temperature and daylight fluctuated with external conditions. Between periods, the dogs were housed in the same kennels for five days, and fed a commercial dry premium diet.

Diets

Three similar basal extruded diets were formulated and extruded with up to 10% of its content being from different carbohydrate sources (corn starch, cellulose powder or beet pulp). The corn starch diet was formulated to have a lower fiber content while the other two diets were formulated to have a higher and similar dietary fiber content. All diets were formulated to have the same fat content.

Each one of the basal diets was coated with 10% of poultry fat or 9% of poultry fat plus 1% of soy lecithin. All diets were added by 1.5% powder palatability enhancer (swine liver). The ingredient and chemical compositions of the six experimental diets are shown at Table 1.

The dogs were fed twice a day and the food offer was calculated to meet their maintenance energy requirement (MER) estimated at 130 kcal metabolizable energy (ME) x $BW(kg)^{-0.75}$ day^{-1} (FEDIAF, 2013).

Sample Collection

Diet subsamples were collected and stored during the trial. Total faecal collection was adopted. Faecal samples were scored after each defecation by a same person as follows; 1 = hard dry and crumbly, “bullet like”; 2 = well formed, does not leave a mark when picked up, kickable; 3 = moist beginning to lose form, leaving a definite mark when picked up; 4 = the majority, if not all the form is lost, poor consistency, viscous and 5 = watery diarrhea (Moxham, 2001). At the start and at the end of each faecal collection period 1000 mg gelatin capsules containing iron oxide (III) Fe_2O_3 were given orally to the dogs, to separate the faecal collection periods. The faeces were collected shortly after defecation and stored at -20 °C. Total faecal outputs from each dog and period were weighted and mixed then samples were taken and dried at 55 °C in a forced-air oven for 72 hours according AOAC (1995) followed by grinding in a Willey mill in a 1-mm screen.

Chemical Analysis

Diets and faeces were analyzed for DM, ash, crude protein (CP) and fat concentration by acid hydrolysis method according to AOAC (1995); neutral detergent fiber (NDF) and acid detergent fiber (ADF) were analyzed according to Silva & Queiroz (2002). Dietary and faecal gross energy (GE) were determined by bomb calorimeter (Parr Instrument Co., model 1261, Moline, IL, USA).

Calculations

The dietary ME was calculated according to the follow equation: ME_{FEDIAF} = [(GE intake – faecal GE) – (CP intake grams – faecal CP grams) x 1,25] / DM intake (FEDIAF, 2013).

The following equation was used to determine ATTD: [nutrient intake (g/d) – faecal output (g/d)/nutrient intake (g/d)] x 100. The organic matter (OM) content was calculate by: OM (%) = %DM – %Ash. The carbohydrate content was calculated by difference as follows: Carbohydrates (%) = %DM – (%Ash + %Fat + %CP).

The endogenous fat loss (EFL) of dogs were considered as 155 mg/kg BW/day (Marx, unpublished). The TTTD of fat was determined according the equation: TTTD of fat: [fat intake (g/d) – {faecal output (g/d) – 155 (mg/d)}/fat intake (g/d)] x 100.

Statistical Analysis

Differences in the ATTD of macronutrients and energy, TTTD of fat, energy intake and faecal outputs between the dietary treatments were tested for significance using ANOVA by GLM procedure (Statgraphics Plus for Windows 4.1), considering a factorial decomposition of treatments (3 fiber sources x 2 lecithin levels). Preplanned contrasts were used to evaluate the effects of dietary fiber sources, soy lecithin and the interactions between both on intake, digestibility parameters and dogs' faecal characteristics. The means were compared by Student-Newman-Keuls test at 5% probability.

RESULTS

All dogs remained healthy throughout the study. The dogs had a similar intake (kcal ME x BW $kg^{-0.75}$ day), for all experimental diets. The fiber inclusion did not decrease diets acceptance, despite decreasing energy density (kcal ME/kg) by the inclusion of fiber. The diets with cellulose and beet pulp had lower ATTD of DM, OM, NDF, carbohydrates and energy ($p < 0.05$), compared to the corn starch diet. In addition, the cellulose diet had similar ATTD of CP, fat and for the TTTD of fat, compared to the corn starch diet. The ATTD of ADF of cellulose diets was the lowest ($p < 0.05$). Finally, the beet pulp diets increased faecal moisture and impair the faecal score, but it remained within the standard levels. The comparison of fiber sources and soy lecithin effect on intake, ATTD of macronutrients and energy, TTTD of fat and faecal characteristics are showed at Table 2. The effects of fiber sources, soy lecithin and the interaction between them on the evaluated parameters are showed at Figures 1 to 12.

The inclusion of 1% soy lecithin on dog diets was not effective to increase emulsification and consequently ATTD of macronutrients and energy. However, interactions between fiber source and soy lecithin were observed for ATTD of CP (p = 0.0026) and energy (p = 0.0274), due to a crossed effect of lecithin in cellulose and beet pulp diets. Soy lecithin increased ATTD of CP and energy in cellulose diets and decreased in beet pulp diets. The lack of a physiological explanation for this behavior, demands further studies to understand the results.

DISCUSSION

Food intake

The ME content (kcal/kg) decreased in the cellulose and beet pulp diets (Table 1). This effect was predicted and corrections for food offer were made before starting the trial. However, one may expect that the dietary fiber inclusion could depress food intake. This behavior was not observed, once dogs had similar energy intakes (kcal ME x BW $kg^{-0.75}$ day). This result can be interpreted so that the inclusion of dietary fibers, in diets with reasonable amounts of fat (19% DM basis), do not reduce acceptance by dogs.

Apparent total tract digestibility

Both fiber sources decreased the ATTD of the majority of macronutrientes evaluated. Data indicate that ATTD of macronutrients and energy is affected, at least in part, by increasing the fermentability of the dietary fiber component (Sunvold et al, 1995). However, the only components with lower ATTD for beet pulp diets than cellulose were CP and fat, also the TTTD was lower for beet pulp.

Some researchers have already reported decrease in ATTD of nitrogen for beet pulp diets compared to a supplemental fiber control diet or cellulose diets (Sunvold et al., 1995; Fahey et al., 1990a; Fahey et al., 1990b; Bosch et al, 2009). The dietary inclusion of beet pulp or other fermentable fibers, and the subsequent fermentation in the colon, may enhance the fermentation of protein or enhance production of microbial nitrogen due to increased energy availability. By providing energy, fermentable fibers encourage microbial growth and, thus, contribute to the production of nitrogenous constituents (Sunvold et al., 1995; De Godoy et al., 2013).

Thus, the greater ATTD of CP by dogs consuming the corn starch and cellulose diets may not actually reflect differences among diets in small intestinal absorption of CP but may reflect the lower microbial protein present in their faeces.

The lower ATTD of fats could be explain from similar reasons, the larger microbial population on large intestine of dogs fed fermentable fibers, such as beet pulp, could reflect on a higher EFL arising from phospholipid content of bacteria cells shed in faeces. However, after the EFL corrections, the TTTD of fat still showed significant differences between cellulose and beet pulp.

The lower ATTD and TTTD of fat by beet pulp diets may be due the lower mean retention time of digesta associated to the inclusion of this fiber source on dog diets (Fahey et al., 1990a). The emulsification of fat may be compromised due to the lower retention time of food on stomach and small intestine, causing a reduction on micelles formation and a decrease on activity of the digestive enzymes. The 1% soy lecithin inclusion did not have significant results on increasing dietary fats emulsification, once no differences on ATTD of macronutrients and energy and TTTD of fat were observed in the soy lecithin added diets.

The ATTD of NDF and carbohydrates was greater for the corn starch diets. This result was expected, since the starch is almost completely digestible for dogs (Fortes et al., 2010).

The ATTD of ADF was the lowest for cellulose diets since the concentration of insoluble fibers on cellulose is high. This was the only ATTD parameter lower for cellulose in comparison with beet pulp.

True total tract digestibility of fat

The EFL content in dogs faeces of 155 mg/kg BW/day (Marx, unpublished) was applied for TTTD of fat calculations. The TTTD of fat increased for all diets, but the significant differences remained, with lower values for the beet pulp diets. As mentioned before, the lower retention time promoted by beet pulp was probably the may reason of the lower apparent and true digestibility of fat.

Faecal characteristics

The evaluation of stool quality is an important tool for vets and nutritionist, once diarrhea and constipation are undesirable conditions. Also, stool quality is important for dog owners because it is often used as an indication of animal health. The optimal stool quality range vary from faeces firm enough to prevent diarrhea until faeces soft enough to prevent constipation.

In our study, diets with 10% corn starch and 6.5% cellulose, induced a greater DM faecal content and lower faecal scores than diets with 10% beet pulp, thus reflecting in harder stools, easier to pick up. Sunvold et al. (1995) observed similar faecal characteristics in dogs fed diets with approximately 8% cellulose or 12.5% beet pulp. The authors reported faecal DM content of 46.6% and 24.7%, and faecal scores of 2.4 and 2.8, for cellulose and beet pulp diets, respectively.

Bosch et al. (2009) evaluated diets with 8.5% cellulose or beet pulp content and their results corroborate these findings. Dogs fed cellulose had higher DM faecal content, 37.9% and lower faecal score 2.44, while dogs fed beet pulp had lower DM faecal content, 23.1% and slightly higher faecal score 2.5. Burkhalter et al. (2001), reported the DM faecal content of 30% and faecal score of 3.1, for dogs fed diets with 7.5% beet pulp. These results showed a minor discrepancy towards the previously mentioned and ours, but still with similar values.

The higher fermentability of beet pulp induces the increase of water binding on faeces; while the low fermentability of cellulose induces opposite effect. However, both fiber sources may be included, according to tested levels, in adult dog diets without causing any significant detrimental effect on stool quality.

CONCLUSION

The ATTD of macronutrients and energy decreases by including fibrous components, especially beet pulp, in dog diets. The cellulose inclusion did not affect ATTD or TTTD of fat by dogs. The use of soy lecithin was not effective to improve ATTD of macronutrients and energy. Beet pulp supplementation induces looser and high moisture faecal outputs.

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Table 1. Composition of experimental diets as-is and chemical analysis

Ingredients (%)	Diets					
	Corn Starch	Corn Starch + Soy Lecithin	Cellulose	Cellulose + Soy Lecithin	Beet Pulp	Beet Pulp + Soy Lecithin
Poultry Viscera Meal	24.10	24.10	24.10	24.10	24.10	24.10
Brewers' Rice	23.34	23.34	23.34	23.34	23.34	23.34
Rice Bran	12.00	12.00	12.00	12.00	12.00	12.00
Corn Grain	10.00	10.00	10.00	10.00	10.00	10.00
Corn Starch	10.00	10.00	3.50	3.50	-	-
Cellulose Powder	-	-	6.50	6.50	-	-
Beet Pulp	-	-	-	-	10.00	10.00
Poultry Fat	10.00	9.00	10.00	9.00	10.00	9.00
Corn Gluten Meal	5.00	5.00	5.00	5.00	5.00	5.00
Meat and Bone Meal	3.00	3.00	3.00	3.00	3.00	3.00
Palatability Enhancer	1.50	1.50	1.50	1.50	1.50	1.50
Soy Lecithin	-	1.00	-	1.00	-	1.00
Sodium Chloride	0.50	0.50	0.50	0.50	0.50	0.50
Vitamins and Minerals*	0.40	0.40	0.40	0.40	0.40	0.40
Potassium Chloride	0.15	0.15	0.15	0.15	0.15	0.15
Choline Chloride	0.01	0.01	0.01	0.01	0.01	0.01
Chemical Composition[1]	Corn Starch	Corn Starch + Soy Lecithin	Cellulose	Cellulose + Soy Lecithin	Beet Pulp	Beet Pulp + Soy Lecithin
Dry Matter (%)	93.6	94.6	93.9	93.9	96.0	95.8
Organic Matter (%)	93.1	93.3	92.9	93.3	92.8	92.7
Crude Protein (%)	30.1	28.9	28.2	29.1	29.9	28.9
NDF (%)	22.2	21.5	26.7	25.2	26.7	26.1
ADF (%)	4.0	3.5	5.8	5.0	6.1	5.9
Carbohydrates[2] (%)	44.0	45.1	46.0	44.8	43.5	44.6
Acid Hydrolyzed Fat (%)	19.1	19.4	18.7	19.3	19.3	19.3
Ash (%)	6.8	6.7	7.1	6.7	7.2	7.3
ME_{FEDIAF}[3] (kcal/kg)	4,256	4,269	4,039	4,062	4,045	3,916

[1]Dry matter basis, except for dry matter.
[2]Carbohydrates = %DM – (%Ash + %Fat + %CP).
[3]ME_{FEDIAF} = [(GE intake – faecal GE) – (CP intake grams – faecal CP grams) x 1,25] / DM intake.
*Provided the following per kilogram of diet: Vitamin A, 7000 IU; Vitamin B1, 2 mg; Vitamin B12, 25 mcg; Vitamin B2, 4 mg; Vitamin B6, 2 mg; Vitamin D3, 600 IU; Vitamin E, 50 IU; Vitamin K3, 1 mg; Folic Acid, 0.2 mg; Pantothenic Acid, 10 mg; Biotin, 0.03 mg; Niacin, 30 mg; Cobalt, 10 mg; Copper, 7 mg; Iron, 80 mg; Iodine, 1.5 mg; Manganese, 5 mg; Selenium, 0.2 mg; Zinc, 100 mg; Antioxidant (BHT), 150 mg.

Table 2. Food intake, faecal output, apparent total tract digestibility (ATTD) of macronutrient and energy, true total tract digestibility (TTTD) of fat and stool quality of adult dogs fed diets with different fiber sources and soy lecithin

	Fiber Sources			Soy Lecithin		SEM	*P*-value		
	Corn Starch	Cellulose	Beet Pulp	0%	1%		Fiber Source (FS)	Soy Lecithin (SL)	FS vs. SL
Intake									
Food intake, kcal ME x BW(kg)$^{-0.75}$ day	131.1	131.3	127.8	131.6	128.6	1.6074	0.6050	0.3581	0.4310
ATTD, %									
DM	83.3[a]	78.7[b]	78.0[b]	79.7	80.3	0.3274	<0.0001	0.3139	0.2940
OM	87.9[a]	82.7[b]	82.1[b]	84.0	84.4	0.2790	<0.0001	0.4626	0.2655
CP	86.2[a]	85.7[a]	82.8[b]	84.7	85.2	0.3327	0.0005	0.4826	0.0026
NDF	76.9[a]	68.6[b]	71.4[b]	71.5	73.0	0.6268	0.0001	0.2478	0.9490
ADF	34.0[a]	10.0[b]	27.0[a]	25.5	21.8	1.7117	<0.0001	0.2824	0.4453
Carbohydrates[1]	87.3[a]	76.9[b]	78.1[b]	80.5	81.0	0.3736	<0.0001	0.5357	0.8633
Fat	91.8[a]	91.8[a]	89.8[b]	91.1	91.1	0.2703	0.0049	0.9419	0.3482
Energy	87.7[a]	83.7[b]	82.5[b]	84.5	84.8	0.2867	<0.0001	0.6322	0.0274
TTTD of Fat, %									
EFL corrected by mg/kg BW day	97.0[a]	96.9[a]	94.8[b]	96.2	96.3	0.2980	0.0075	0.9608	0.4465
Faecal output									
Faecal DM, %	38.1[a]	40.1[a]	27.4[b]	34.9	35.6	0.6852	<0.0001	0.6061	0.7551
Faecal Score[2]	2.5[ab]	2.4[a]	2.7[b]	2.5	2.5	0.0367	0.0099	0.8533	0.6895

[a-b]Within a row, means lacking a common superscript differ ($P \leq 0.05$).

[1]Carbohydrates = %DM – (%Ash + %Fat + %CP).

[2]Faecal score based on the following scale: 1 = hard dry and crumbly, "bullet like"; 2 = well formed, does not leave a mark when picked up, kickable; 3 = moist beginning to lose form, leaving a definite mark when picked up; 4 = the majority, if not all the form is lost, poor consistency, viscous and 5 = watery diarrhea.

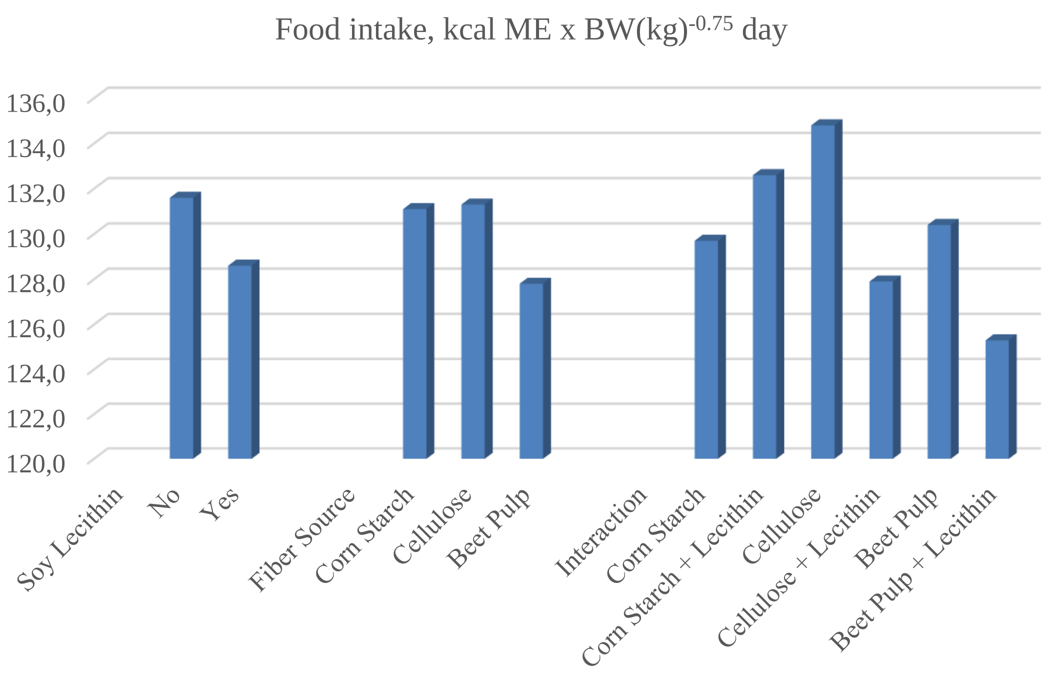

Figure 1. Food intake (kcal ME x BW(kg)$^{-0.75}$ day, according to each group.

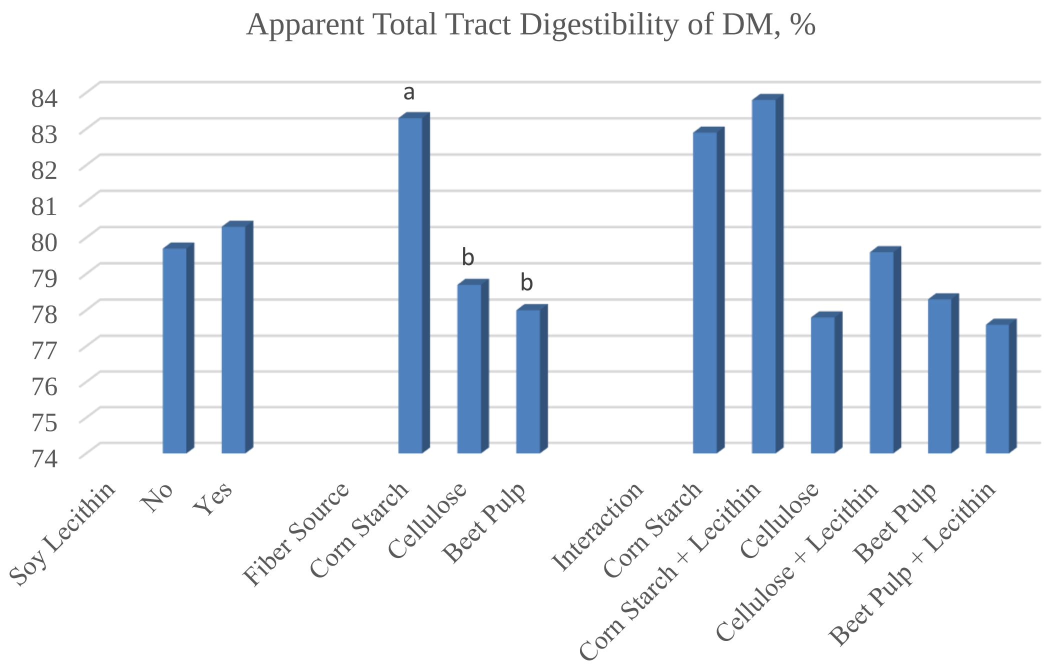

Figure 2. Apparent total tract digestibility of DM according to each group. [a-b]Within fiber source group, means lacking a common superscript differ ($P < 0.0001$).

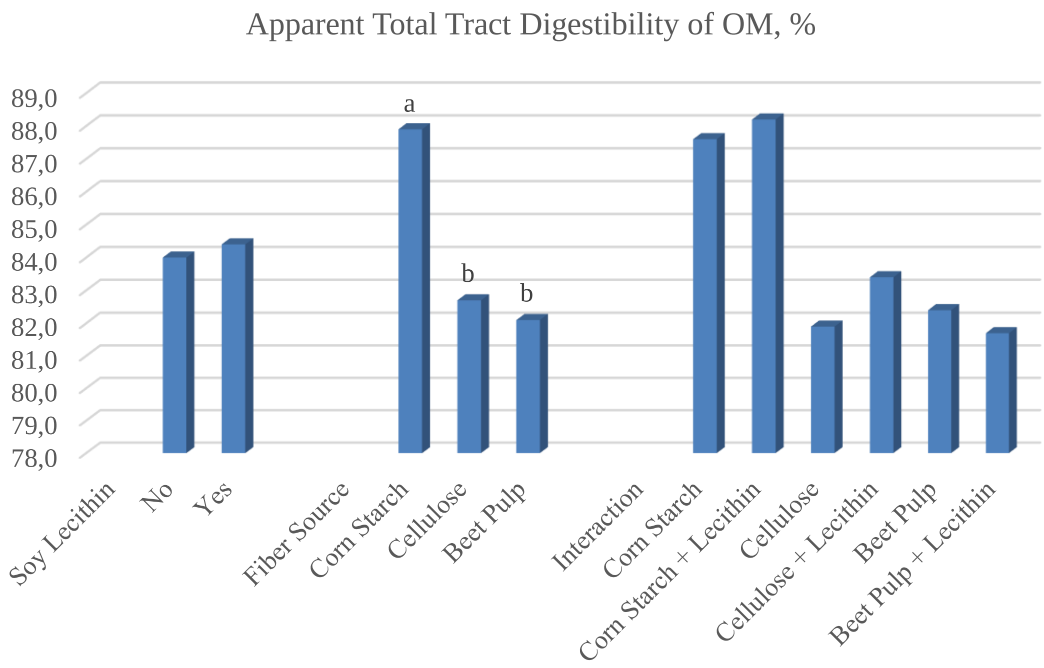

Figure 3. Apparent total tract digestibility of OM according to each group. [a-b]Within fiber source group, means lacking a common superscript differ ($P < 0.0001$).

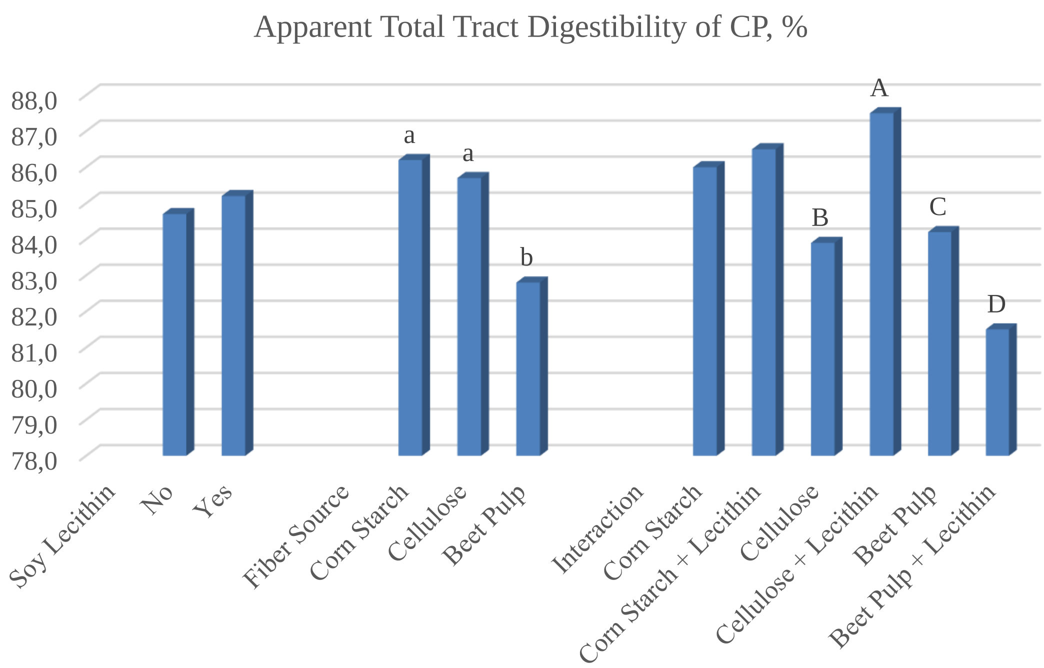

Figure 4. Apparent total tract digestibility of CP according to each group. [a-b]Within fiber source group, means lacking a common superscript differ ($P = 0.0005$). [A-B]Within cellulose based diets on interaction group, means lacking a common superscript differ ($P \leq 0.05$). [C-D]Within beet pulp based diets on interaction group, means lacking a common superscript differ ($P = 0.0026$).

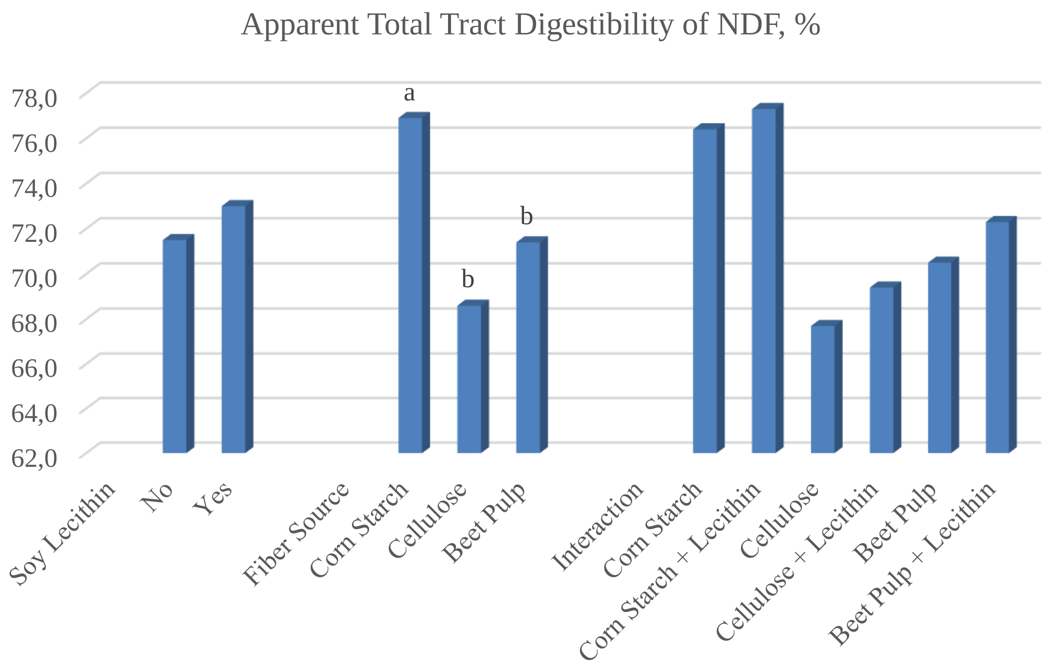

Figure 5. Apparent total tract digestibility of NDF according to each group. [a-b]Within fiber source group, means lacking a common superscript differ ($P = 0.0001$).

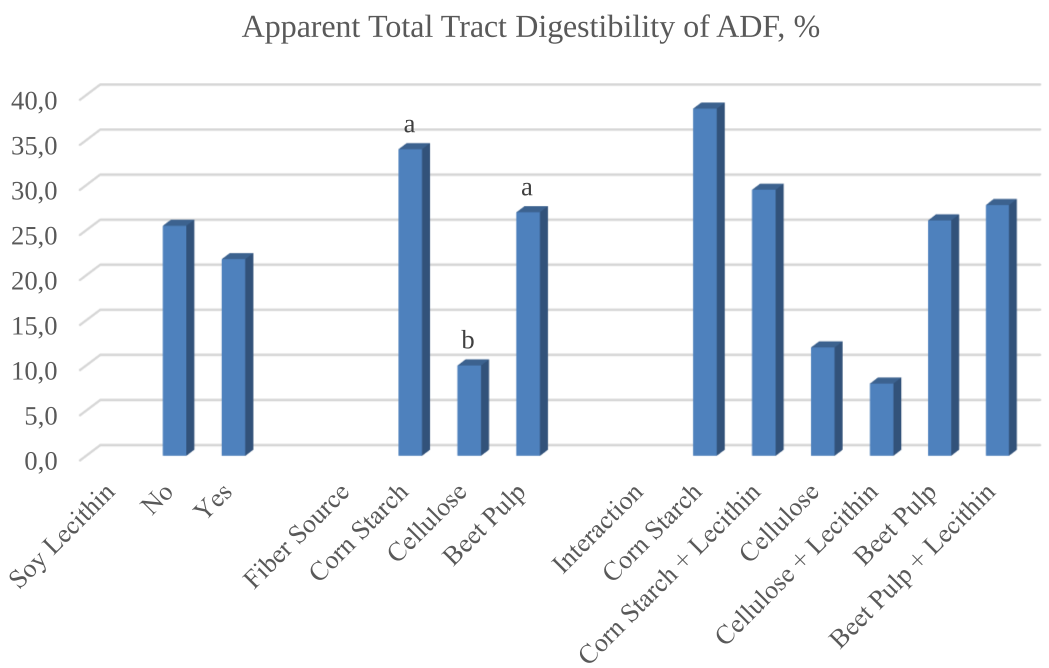

Figure 6. Apparent total tract digestibility of ADF according to each group. [a-b]Within fiber source group, means lacking a common superscript differ ($P < 0.0001$).

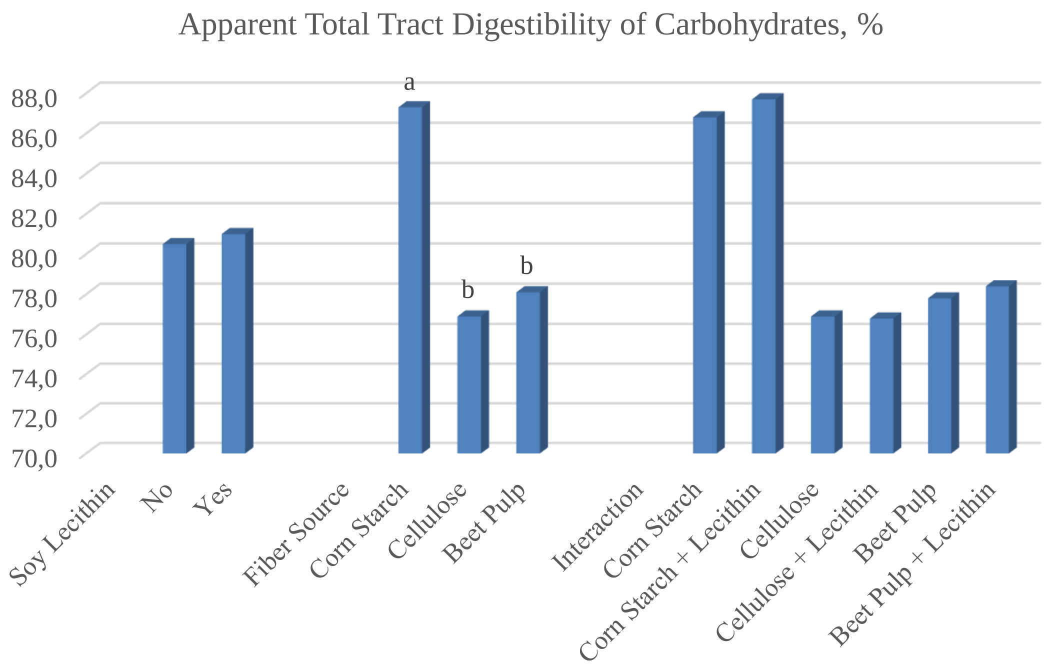

Figure 7. Apparent total tract digestibility of Carbohydrates[1] according to each group. [a-b]Within fiber source group, means lacking a common superscript differ ($P < 0.0001$). [1]Carbohydrates = %DM – (%Ash + %Fat + %CP).

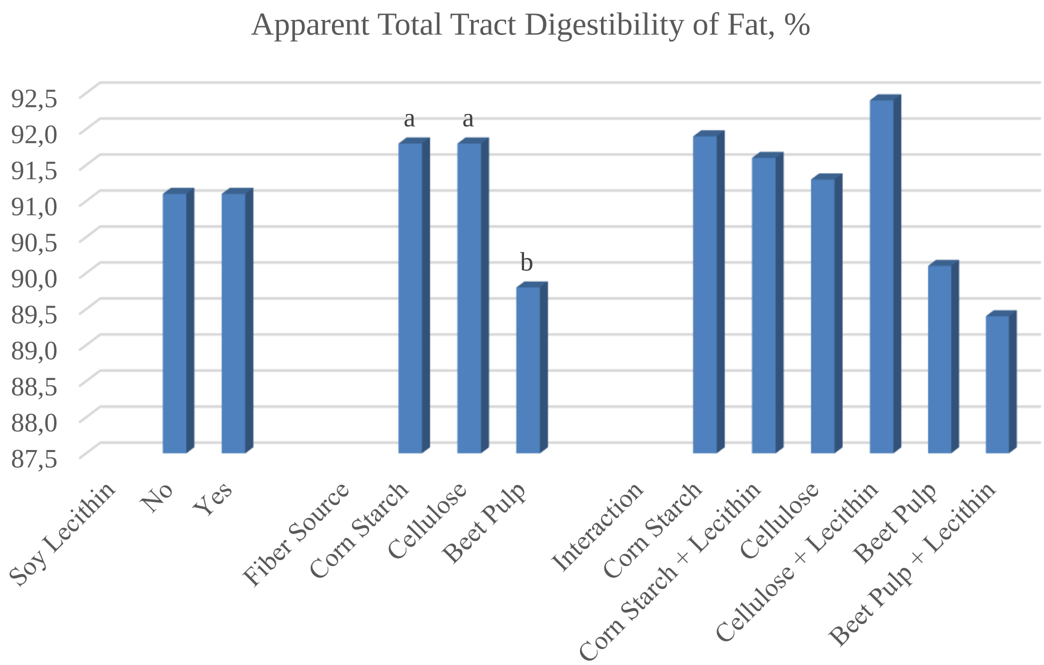

Figure 8. Apparent total tract digestibility of Fat according to each group. [a-b]Within fiber source group, means lacking a common superscript differ ($P = 0.0049$).

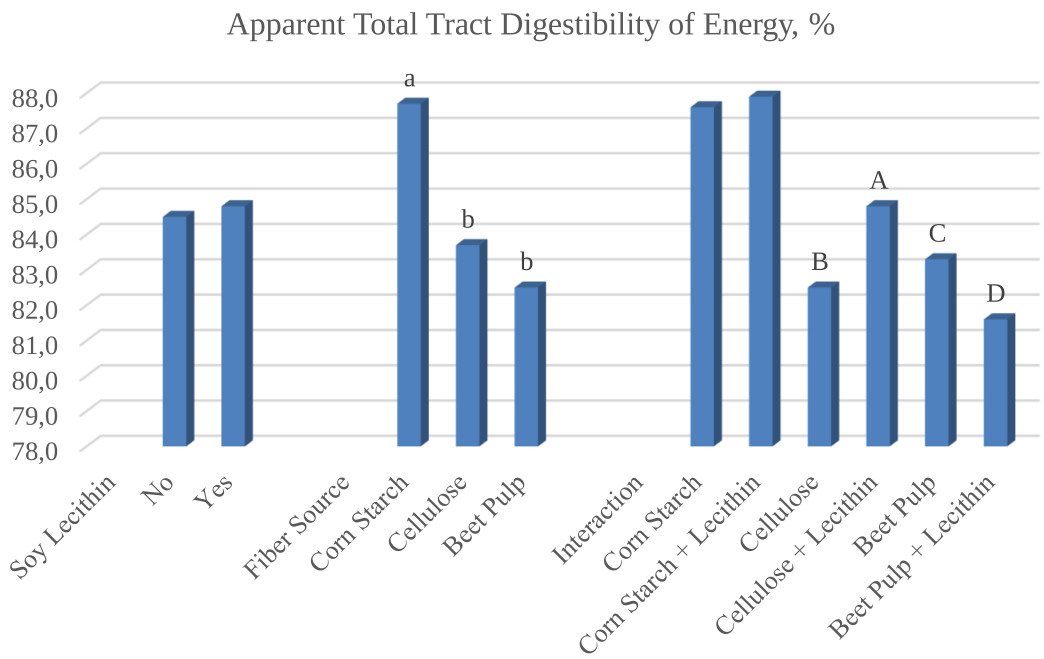

Figure 9. Apparent total tract digestibility of Energy according to each group. [a-b]Within fiber source group, means lacking a common superscript differ (*P* < 0.0001). [A-B]Within cellulose based diets on interaction group, means lacking a common superscript differ ($P \leq 0.05$). [C-D]Within beet pulp based diets on interaction group, means lacking a common superscript differ (*P* = 0.0274).

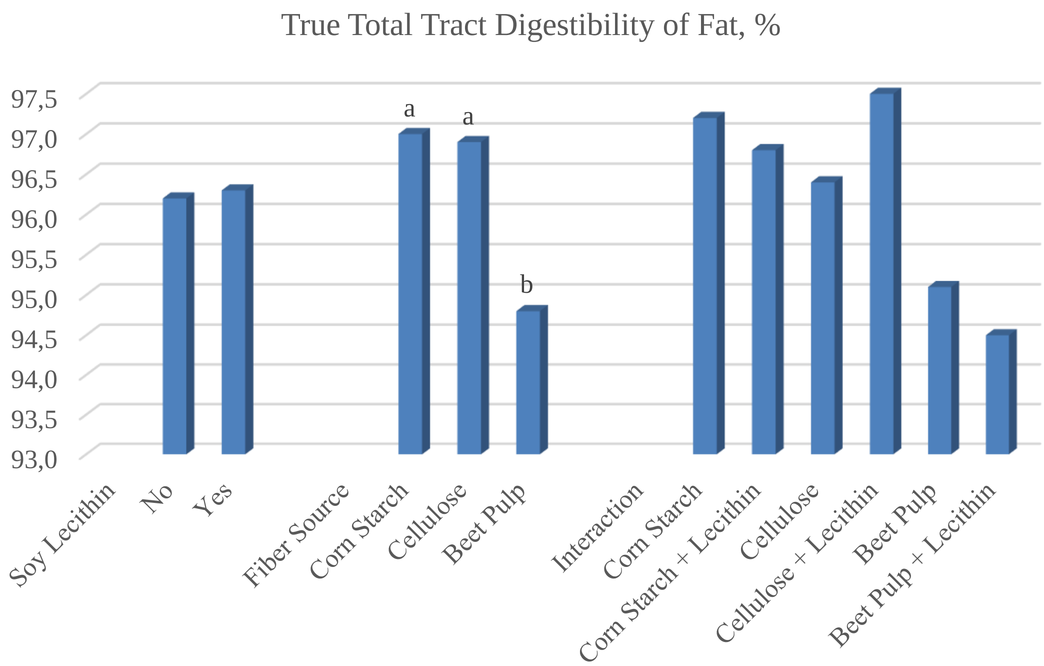

Figure 10. True total tract digestibility of Fat according to each group. [a-b]Within fiber source group, means lacking a common superscript differ (*P* = 0.0075).

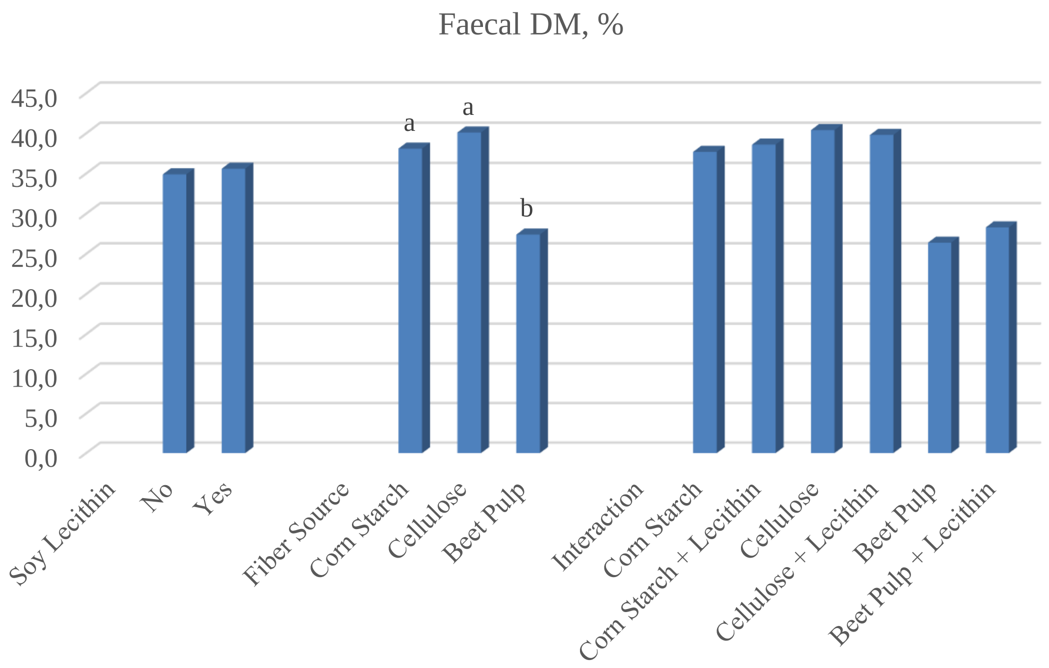

Figure 11. Faecal DM content according to each group. [a-b]Within fiber source group, means lacking a common superscript differ ($P < 0.0001$).

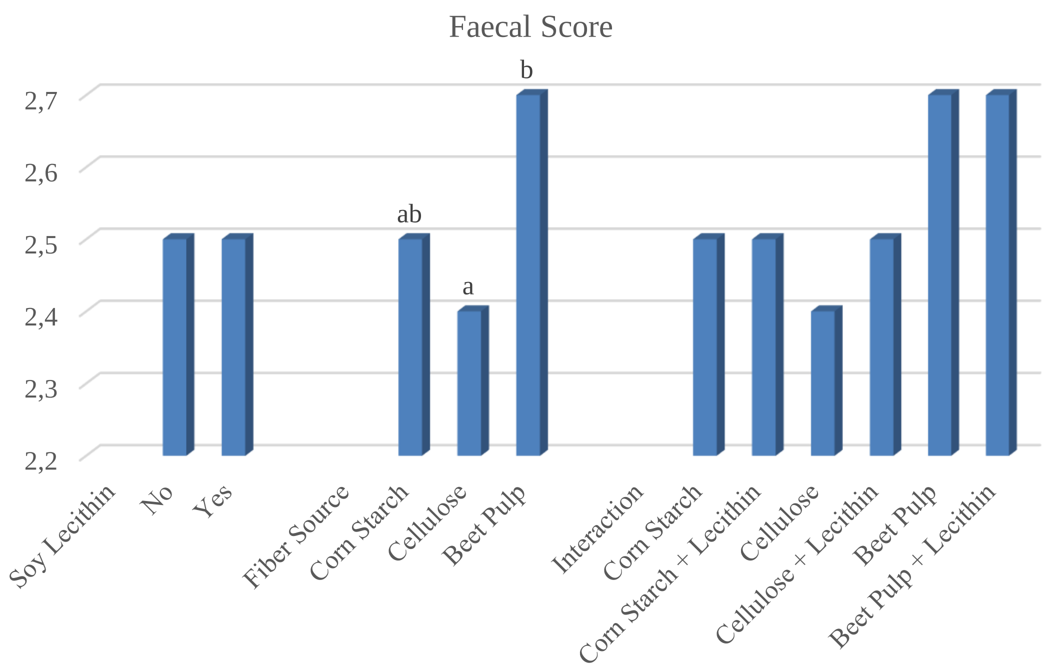

Figure 12. Faecal score content according to each group. [a-b]Within fiber source group, means lacking a common superscript differ ($P = 0.0099$).

CAPÍTULO V

4. CONSIDERAÇÕES FINAIS

As excreções endógenas de gordura nas fezes de carnívoros, apesar de baixas, não podem ser menosprezadas. A partir da determinação do conteúdo de origem endógena nas fezes, correções para digestibilidade verdadeira total podem ser realizadas. A digestibilidade aparente total da gordura em dietas para cães apresenta comportamento de aumento hiperbólico mediante o consumo. Este comportamento é decorrente da diminuição da relação entre o conteúdo de origem endógena e o consumo de gordura. Desta forma, em dietas com baixos níveis de gordura (e.g. dietas econômicas e dietas *light*), as perdas endógenas possuem participação proporcionalmente maior nas fezes; por consequência a digestibilidade aparente total é subestimada. A correção para as PEGF permite demonstrar que a digestibilidade verdadeira da gordura permanece a mesma, independentemente da quantidade consumida.

O metabolismo de cães e mink é similar. Desta forma, as PEGF destas duas espécies são próximas, porém com valores numericamente inferiores para a segunda espécie. A ausência de ceco e a baixa atividade microbiana no intestino grosso dos mink, provavelmente seja a principal causa da menor excreção endógena nas fezes destes animais. Dietas práticas para mink possuem níveis elevados de gordura, por conseguinte, as PEGF não impactam de forma tão acentuada nos ajustes de digestibilidade verdadeira da gordura, como ocorre em dietas comerciais para cães.

A celulose e a polpa de beterraba; fontes de fibras comumente utilizadas em dietas para cães; afetam negativamente a digestibilidade dos principais macronutrientes e da energia. A celulose, porém, não exerce efeito de redução na digestibilidade aparente e verdadeira da gordura. Com relação as características fecais, a polpa de beterraba aumenta a umidade fecal e eleva o escore fecal, apesar das fezes ainda manterem características desejáveis.

A adição de 1% de lecitina de soja em dietas para cães adultos não melhora a emulsificação das gorduras. A lecitina de soja não afetou positivamente a digestibilidade da gordura ou demais macronutrientes e da energia de dietas com baixa fibra. Assim como também não evitou a redução na digestibilidade dos macronutrientes e energia de dietas com celulose e a polpa de beterraba. A interação observada entre as fontes de fibra e a lecitina de soja para digestibilidade aparente da proteína bruta e energia, demanda maiores estudos para melhor interpretação deste resultado.

A compreensão das variáveis endógenas e dietéticas que afetam o aproveitamento das gorduras permite a realização de ajustes e correções importantes na formulação de dietas práticas para cães.

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6. APÊNDICES

Apêndices 1 – Instrução para autores da Revista Archives of Animal Nutrition

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Contents List

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Apêndices 2 – Instrução para autores da Revista Canadian Journal of Animal Science

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Association of Official Analytical Chemists. 1980. Official method of analysis. 13th ed. AOAC, Washington, DC.

Conference proceedings

Brouwer, R. and de Wit, C. 1968. A simulation model of plant growth. Proc. Easter School in Agricultural Science, University of Nottingham, UK. Butterworths, London, UK.

Thesis

Beck, M.J. 1980. The effects of kinetin and napthaleneacetic acid on in vitro shoot multiplication and rooting in fishtail fern. M.Sc. thesis, University of Tennessee, Knoxville, TN. 31 pp.

Charts

Royal Horticultural Society. 1966. RHS colour chart. RHS, London, UK.

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Raine, M. 1998. 24 Sept. Fall fertilizing should be as late as possible. The Western Producer [Online]. Available: http://www.producer.com/docs/news/Sep_24_98/prod3.htm [1998 Oct. 10].

Certain media have been omitted from these guidelines. Bulletin board listings, discussion group threads, electronic mail, etc. are not included because they usually represent nothing more than "personal communication" and can be cited as such according to present guidelines.

Refer to "Citing electronic publications in the *Canadian Journal of Plant Science*, Can. J. Plant Sci. 79: 473-474" and the journal's Operations Manual. Refer to "BIOSIS List of Serials with Title Abbreviations" (Thompson Scientific, 3501 Market Street, Philadelphia, PA 19104, USA) for accepted abbreviations for journals; only these may be used. Double check accuracy of all pages, volume numbers, and dates.

Trademarks

Authors are required to provide registered trademark notification for proprietary products.

Footnotes

Footnotes can be used and are required on the title page and in tables (see section on tables). On the title page, footnotes will generally fall into one of the following categories:

(a) Notes on the title indicating the paper is part of a thesis submitted by one of the authors, that the paper was presented at a symposium, etc.

(b) General disclaimer. If the use of trade or brand names is necessary in the test, the use of a disclaimer may be advisable. For example, "The use of trade names, proprietary produce, or vendor does not imply endorsement by the authors, nor criticism of similar products not mentioned."

(c) Current address of author(s) if it is not the one listed in the by-line or address for reprint requests.

(d) Indication that an author is deceased.

All footnotes on the title page are indicated by superscript Arabic number. **For table footnotes, see the section on Tables (below).**

Tables

Tables are used to present numerical data in a self-explanatory manner. They should be intelligible without consulting the text and should not duplicate data already given in the text or illustrations. Tables should be typed double-spaced, each table on a separate sheet. Use the Microsoft Word Table function to create tables. Each data point in a table should be in a separate cell. Do not format tables using tabs, spaces and hard returns. In these cases, tables will be re-keyed by typesetters leading to the possible introduction of errors. Paginate the tables in series with the text. Number tables using Arabic numbers and cite all tables in the text. Align horizontally parallel entries by their bottom lines. Do not use vertical rules in tables. Avoid the use of horizontal rules within the body of the table; if necessary, separate data sets by spaces.

Table heading

Describe the topic and general trends of the table. Capitalize in sentence format and end without a period. Do not indent second and subsequent lines. Do not include units of measurement in the heading; place them following column or stub headings within the table.

Column Headings

Capitalize only the first word, proper nouns, and capitalized abbreviations. Subheadings should be joined by a rule. Give units in parentheses on the last line of the column head. When several column headings share the same units of measurement, place the unit below the headings, centred, with rules on each side to indicate the headings to which they refer.

Body of the table

Headings used within the body to separate subject classes should be centred and *italicized*. Use sentence capitalization. Centre entries under column heads. Centre data within columns on decimal points, dashes, ± signs, etc. If data have been excluded from the table, (en dashes are used for minus signs). Do not use ditto marks to repeat data in columns.

Table footnotes

Use bold lowercased superscript letters in reverse starting with ^{z} (^{z}, ^{y}, ^{x}, ^{w}, etc.) to designate footnotes. Set each footnote on a separate line, flush with the left-hand margin of the table. Include footnotes on all tables to which they refer, do not say "see footnote Table 1". Place footnote symbols at the first occurrence in the table, working left to right, top to bottom. Explain in footnotes any unconventional abbreviations used in the table. The asterisk (*) is used only to designate, statistical significance, e.g., *, **, *** significant at $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively.

Statistical analysis in tables

If needed, to indicate statistical significance, use either lowercased letters (*a*,*b*,*c*, etc.) (italic, not superscript) or a single asterisk for the 5% level; uppercased letters (*A*,*B*,*C*) or a double asterisk for the 1% level; a triple asterisk for the 0.1% level. Do not leave a space between the entry and letter. Mean comparison tests should be supported by significant *F* value in ANOVA designs.

Illustrations

Illustrations should be planned to fit one-column (8.5 cm x 22.4 cm; 3.5 inches x 8.75 inches) or two-column (17.6 cm x 22.4 cm; 7 inches x 8.75 inches). Each figure should be numbered consecutively in Arabic numerals and must be referred to in the text. Abbreviations and units of measurement must correspond to those used in the text and journal style (use SI units).

Line drawings

Lines must be sufficiently thick (minimum 0.5 points) to reproduce clearly; lettering, including superscripts and subscripts, and symbols must be in proportion to the illustration and large enough to allow for reduction without loss of clarity. Use the same font for all figures. Use clear, bold patterns and avoid the use of fine grey-scale patterns. Labelling on graphs should be parallel to the graph's axes.

Photographs

Photographs should be high-quality, continuous tone with good tonal contrast. Use uppercased letters to mark subdivisions. Electron micrographs must include a scale bar on the illustration.

Colour illustrations

Colour figures and photographs are accepted but authors are expected to pay the full cost of reproduction **($750.00 per manuscript)**.

Electronic graphic files

Digital art is rendered in pixels (dots) per inch (dpi). The size at which digital art can be reproduced is limited by its resolution, measured in pixels per inch (ppi). Continuous tone images (photographs) should be a minimum of 300 ppi; line drawings should be 1200 ppi.

To check the resolution of a digital file on a PC: right click on the file, then select "Properties" / "Summary" / "Advanced". The width and height of the file will be shown in pixels and the horizontal and vertical resolution in dpi. Dividing the width and height in pixels by the horizontal and vertical resolution in dpi will give the maximum reproduction size of the file in inches.

JPEG images deteriorate each time they are opened and resaved. Rename JPEG files using the menu option rather than opening and resaving the file. If possible, EPS or PDF files are a better alternative to JPEG files.

When submitting images, ensure the file name includes the manuscript number and figure number (e.g., CJSS2012-001_Fig1.pdf). **We do not accept Powerpoint or Sigmaplot files**; however, these files can be copied, saved, and submitted as Word files.

Short communications

Short communications of usually not more than 2000 words are open to short preliminary reports of important findings. They might contain research results that are complete but characterized by a rather limited area or scope of investigation, descriptions of new or improved techniques or equipment, including data on performance. Short communications should contain only a few (usually not more than nine) references. Consult a recent issue of the journal for format. Illustrative material should be kept to a minimum, usually not more than one table or figure. **Abstracts are obligatory and shall be no longer than 50 words**.

Animal care

If applicable, authors must certify that animals were cared for under guidelines comparable to those of the Canadian Council on Animal Care (Guide to the care and use of experimental animals, available from the Canadian Council on Animal Care (CCAC), Constitution Square, Tower 2, 1510-130 Albert Street, Ottawa, ON K1R 1B1, Canada)

Cultivar descriptions

For cultivar descriptions of agricultural crops, the cultivar must first be registered in Canada with the Variety Registration Office of the Canadian Food Inspection Agency (CFIA) where this requirement exists for Canadian release. The CFIA registration number must be mentioned in the description. The description of the cultivar (germplasm) must not have been published previously in a peer-reviewed journal. Cultivar descriptions will only be considered for crops intended for Canadian production. See the journal's Operations Manual for the standard format for the description of new cultivars.

Specific style guide

Units of Measure

SI System

The SI system of units (*Système international d'unités*) is used in the journal. The SI is based on seven base and two supplementary units (Table 1).

Table 1. SI base and supplementary units

Length	metre	m
Mass	kilogram	kg
Time	second	s
Electric current	ampere	A
Thermodynamic temperature	kelvin	K
Luminous intensity	candela	cd
Amount of substance	mole	mol
Plane angle	radian	rad
Solid angle	steradian	sr

One principal advantage of the SI is that all other units in the system can be derived from the base units by simple multiplication and division. Examples of derived units are the unit of area (m^3), the unit of volume (m^3), the unit of velocity ($m\ s^{-1}$), etc. Some derived units have been assigned special names (Table 2).

In addition, certain non-SI units are so widely used in everyday life that they have been retained for general use with the SI; a few others have been accepted for use with the SI "for a limited time" (Table 3).

Usage

On many occasions, the SI base and derived units are inconveniently large or small. Hence, prefixes are used to form multiples or submultiples of the units (Table 4). Prefixes are written immediately before the symbol of the units to which they apply with no intervening space or punctuation. They should never be used alone (e.g., µ for micron is unacceptable; rather µm), nor should compound prefixes ever be used (e.g., mµm for millimicrometre; instead use nm, nanometre).

It is preferable to use prefixes in multiples of 10^3 and therefore to avoid deci, centi, deca, and hecto whenever possible. Notable exceptions are the centimetre (cm) and the hectare (ha).

Units, however, should be chosen with prefixes so that the numerical component falls between 0.1 and 1000.

When a unit is in a fractional form, the prefix should be applied to the numerator only. (Because it is the only base unit defined with a prefix, the kilogram is of course an exception.)

Style and Format

Use the symbol for a standard unit of measurement only if it is preceded by a number. Spell out a unit's name if it follows a spelled out number (e.g., opening a sentence). Do not begin a sentence with a symbol or abbreviation.

Numbers and units that form compound adjectives should be hyphenated, e.g., 1000-kernel weight, 2-mo-old calf, 15-mm opening, 15-cm-deep layer.

The denominators of units derived by division should be written with negative indices. Do not use a solidus (/) unless the units are written in full, e.g., kg ha^{-1} yr^{-1}, W m^{-2}, but metre/second.

A space (not a dot) is left between the elements of a unit and between the numerical value and the first letter of the unit's symbol, except for the symbols of degree, minute, and second, e.g., 91 m (not 91m, which could mean 91 metres or 9 lumens), N m (newton-metre, not m N for metre-newton, which if the space were omitted is the symbol for millinewton), but 45°30", 25°C. When numerical values are less than one, a zero must be written before the decimal marker, e.g., 0.3. In a series of measurements, place the unit at the end, e.g., 3–10°C; 1, 4, and 8 µg L^{-1}. Units whose name is derived from a proper name are not capitalized when written out in full, but the first letter of the unit's symbol is, e.g., K (kelvin), Pa (pascal), but °C (degree Celsius).

Special Uses

The adoption of the SI has created difficulties, and some confusion, in reporting concentration and light.

(i) The amount of substance concentration, or, simply, concentration, can be expressed, for example, as concentration in HCl = 0.1 mol L^{-1} or 0.1 M HCl.

The base unit mole (mol) replaces such terms as gram-molecule and gram-equivalent. The concept of equivalence and the corresponding amount of substance concentration that it defines, i.e., normality (N), should therefore no longer be used. Concentration in mol L^{-1} can also be reported as molarity (M) of a solution, as in the above example.

Concentration can also be expressed on a mass or volume basis. This could be the case, e.g., for nutrient concentrations in plants, soils, and fertilizers, or for soil and plant water contents:

mmol kg^{-1}, mg kg^{-1}, g m^{-3} or g kg^{-1}

The use of percent (%) must be avoided whenever SI base or derived units can be used instead. Nevertheless, percent is acceptable for such quantities as coefficient of variation, plant or animal population estimates, increases or decreases in yield, fertilizer grades, relative humidity, soil texture, base saturation, land area estimates.

As with percentage, parts per million or billion (ppm or ppb) can be equally ambiguous and are therefore unacceptable. Use instead units such as L L^{-1}, mg L^{-1}, or mg kg^{-1}.

(ii) The purpose for which a study is undertaken determines the units that should be used for light intensity. In studies based on radiant energy received from a light source, the accepted SI units are the joule per square metre (J m^{-2}) for total radiant energy received or the watt per square metre (W m^{-2}), which expresses energy received per unit area or irradiance. Plants respond to photosynthetically active radiation (PAR) in the waveband 400 to 700 nm. The proper quantity to measure in this case is the flux density of quanta received per unit area, which is expressed in micromoles per square metre per second (mol m^{-2} s^{-1}). This latter unit replaces the non-SI Einstein (E), which is equivalent to 1 mole quanta. When other wavelengths are considered, the waveband should be specified.

In animal physiology studies, light measurements can be reported in lux (lx). This measurement refers to the intensity of light, or illuminations, as perceived by the human eye (and also by the animal, presumably). Thus, the lux is an inappropriate unit for use in the plant sciences.

Abbreviations

Generally, abbreviations in upper case do not have periods and are not letter-spaced, e.g., DM, RH. Abbreviations in lower case usually have periods, but no letter spacing, e.g., a.i. (active ingredient), i.d. (inside diameter).

A list of abbreviations used must be supplied, which will be printed on the first page of the paper. Abbreviations widely used throughout science, such as DNA, can be used in the title, abstract, and text without definition. Other abbreviations must not be used in titles, but may be used in the text if they are parenthetically defined at first use. An abbreviation should not be used unless the abbreviated term is used at least three times in the manuscript. Plural abbreviations do not require a final “s”. Refer to "Scientific Style and Format" (6th edition) for additional standard abbreviations.

Abbreviations of Frequently Cited Periodicals (Refer to "BIOSIS List of Serials with Title Abbreviations": http://www.biosis.org.uk)

International feed names and international feed numbers (IFN)

Each feedstuff referred to in the manuscript may be identified by the International Feed Number (IFN) and a simplified name of the feed. The IFN, when used, should be listed only once in the manuscript, preferably in tables if tables listing feeds are included. If no table of feed ingredients is included, the the IFN should be given in parentheses following the feed name in the Materials and Methods section. The use of the IFN makes the full description of the International Feed names redundant and space consuming. Thus, simplified names for the full description of the International Feed Names should be used in the text and tables as indicated in the examples for the following feeds: Clover, red, hay sun-cured, early bloom (IFN 1-02-400) Soybean, seeds, meal solvent extracted (IFN-5-04-604). In the text, they should be written as ... red clover hay (IFN 1-01-400), barley (IFN 4-00-549), and soybean meal (IFN 5-04-604) were fed. In tables, they should appear as shown below:

Ingredient (%) Barley (IFN 4-00-549) (78.6) Soybean meal (IFN 5-04-604) (18.0)

The international Feed Names and IFN are listed in "Utah Agricultural Experiment Station Bulletin 501" (1981). Copies of the bulletin may be obtained from: The Utah Agricultural Experiment Station, Bulletin Room, Utah State University, UMC 50, Logan, UT 84322 USA. Many different names are used in describing feedstuffs, but to establish a common basis for comparing nutritive values it is essential that the exact IFN given for the specific International Feed Name in this bulletin be used. If a feed ingredient is not exactly described in Bulletin 501, write to: Director, International Feedstuffs Institute, Utah State University, UMC 46, Logan, UT 84322 USA, and an International Feed Name and IFN will be provided.

Time

Use the 24-h clock system: 0930, 1340, etc. Give day length in quantitative hours (e.g., 2 h 16 min). Abbreviate the terms hours (h), minute (min), second (s), year (yr), month (mo), week (wk) when used with a number in the text but spell them out when they are used alone.

Dates

Use Arabic numerals for all dates. Abbreviate the month when it is used with the date (day) but spell out the name of the month when it is used alone or with the year. Do not put commas between day, month and year. Abbreviations are: Jan., Feb., Mar., Apr., Aug., Sep., Oct., Nov., Dec. Give dates in the order: year, month, day (2003 Sep. 01). When referring to a space of years given both years in full, e.g., 1980–1983 rather than 1980–83.

When referring to a group of continuous years, add the plural "s" without an apostrophe. e.g., 1970s.

Abbreviations BC, AD, BP should be capitalized.

Places

Spell out the names of countries, provinces, and states when they are used alone; abbreviate them when they are used with the city, in footnotes, and in references. Translate the names of foreign cities and countries into English in text and authors by-line. Street addresses, institutional names, etc. retain their foreign spelling.

Statistics

Place emphasis on the interpretation of the experimental results based on a sound statistical methodology.

Experimental designs, data, statistical model, and analyses must be clearly described, more fully in non-standard designs. Reference to a computer program used in analysis is not sufficient description of design. The experimental design should be appropriate to the objectives of the experiment and the statistical procedure(s) should be appropriate for the design. If necessary, data should be transformed to satisfy assumptions required for valid statistical analysis. In combined analyses, error variances should be homogeneous or heterogeneity should be taken into account in the analysis. Summary statistics should be accompanied by estimates of their precision. Means usually should be accompanied by either the standard error of the mean, the standard error of the difference between means, or a confidence interval. The number of replicates used or the degrees of freedom should be indicated. If emphasis is on the spread of the data values, then the standard deviation or the range may be given. If a mean (–x) and standard error of a mean (s–x) have been calculated on transformed data, back transformed values should be presented; one procedure of doing this is to transform the range of values (–x – s–x + s–x) back onto the original scale of measurement. The statistical procedure used for separation of means should be appropriate for the data being analyzed. For example, fitting response functions using regression techniques or using planned sets of contrasts among means or groups of means are appropriate where treatments are graded levels of a quantitative variable or where there are combinations of two or more factors at two or more levels, whereas multiple comparison tests are appropriate when unstructured qualitative treatments are involved. Statistical hypothesis testing is an important aspect of analyzing experimental results. The author(s) should indicate what probability level is being used for the rejection of a null hypothesis. Probability levels commonly used are $P < 0.05$ and $P < 0.01$, with $P < 0.001$ and $P < 0.1$ being used rarely. Rigid use of $P < 0.05$ or $P < 0.01$ will often form a dichotomy, which is artificial and unnecessary; an effect with probability of $P = 0.049$ might be declared significant but a second at $P = 0.051$ might not be. When the null hypothesis is not being rejected at a prespecified level, the observed probability level should be stated: $P = 0.056$, 0.078, 0.095, etc. Also, if acceptance of the hypothesis that there is no difference is of importance, it would be of value to state the observed probability level: $P = 0.65$, 0.92, etc. In presenting correlation coefficients and mean squares, $P < 0.05$(*), $P < 0.01$(**), and $P < 0.001$(NS) may be used since the reader can apply or determine other probability levels if desired. When relevant, mean squares should be presented rather than significance of F tests (i.e., *,**, and NS) since the latter alone does not provide the reader with basic information. As a matter of clarity and logic, when tests of significance indicate no difference, state that there was “no (significant) difference”, rather than saying “the difference was non-significant”. The GLM procedure of SAS has been widely used for analysis of variance; however, it was designed to analyze data having fixed effects only. Models that have both fixed and random effects should be analyzed using the MIXED procedure of SAS. This is also important in analyzing data sets with repeated observations on the same experimental unit that have heterogeneous variances over time and/or unequal within subject time-dependent correlations. The CJAS will not normally accept papers reporting the use of the GLM procedure to analyze datasets that include random effects or repeated measurements on the same experimental unit where the data show heterogeneous variances and/or unequal within subject time-dependent correlations. Avoid reporting a number of similar experiments separately; combine when possible. Omit raw data, information that can be calculated by the reader, and material irrelevant to the objectives. Results that are not significant may be included when relevant but may best be covered in the text rather than in tables or figures. Give only meaningful digits. A practical rule is to round so that the change caused by rounding is approximately one-tenth of the standard error. Such rounding increases the variance of the reported value by less than 1% so that less than 1% of the relevant information contained in the data is sacrificed.

Numbers

Follow the rules given below for writing numbers:

- Spell out numbers one through nine and use numerals for 10 and above and in instances given below.
- Use Arabic numerals when they precede abbreviated units of measure (2 g, 5 d, $4.00, 3%) and numerical designations in the text (exp. 1, group 3)
- Use Arabic numerals to express time and date: 2003 Sep. 01, 0800 (not 08:00 h), etc.
- In a series using some number less than 10 and some more than 10, use numerals for all: 2 Holsteins, 6 Charolais, and 15 Friesians.
- When using numbers of more than four digits, leave a space between each group of three going from the decimal point: 10 000, 450 000, etc.
- Numbers of two to four digits are run together: 3500, 1000, etc., except when they are used (e.g., in tables) in columns with numbers of more than four digits, when a space is inserted after the third digit from the decimal point:
  23 000
  450 000
  1 200
- When writing a large number ending in several zeros, use a word for part of the number: 1.8 million rather than 1 800 000.
- When two numbers appear adjacent to each other, spell out the first: ten 2-d-old chicks, rather than 10 2-d-old chicks.
- Do not begin a sentence with a numeral. If a number is spelled out at the beginning of a sentence, spell out its associated unit (Five millilitres of ... not Five mL of ...)
- Follow the same rules for ordinals as for whole number: first, third, 1st, 3rd.
- When enumerating a discussion, the use of secondly, thirdly, etc. is incorrect; use first, second, third, etc.

- Spell out units of measurement not associated with a number.

Equations

Use the simplest form of the equation possible especially in non- displayed equations in the text, e.g., use (*a* + *b*)/(*c* + *d*) rather than
a+b
c+d
Ensure that possibly ambiguous characters are clear (1, one/ell; 0, zero/oh; k/K; c/C; u/U/v/V, etc.). Single-letter mathematical abbreviations are usually set in italics; abbreviations of more

than one letter (max., ln, avg.), chemical symbols and numbers are not italicized. Number equations with Arabic numerals in parentheses at the right margin of the text; refer to equations in the text as “Eq. 4”.

Table 2. SI derived units with special names

Quantity	Name	Symbol	Expression in terms of	Expression in terms of
Frequency	hertz	Hz	B	SI s^{-l} b i
Force	newton	N	B	m^{-1} kg s^{-2}
Pressure, stress	pascal	Pa	N m^{-2}	m^{-1} kg s^{-2}
Work, energy, quantity of heat	joule	J	Nm	m^2kg s^{-2}
Power, radiant flux	watt	W	Js^{-1}	m^{-2} kg s^{-3}
Electric charge, quantity of electricity	coulomb	C	B	S A
Electric potential	volt	V	W A^{-1}	m^2kg s^{-3} A^{-1}
Electric capacitance	farad	F	C V^{-1}	m^2 kg^{-1} s^4A^2
Electric resistance	ohm	O	V A^{-1}	m^2 kg s^{-3} A^{-2}
Electric conductance	siemens	S	A V^{-1}	m^2 kg^{-1} s^3 A^2
Magnetic flux	weber	Wb	V s	m^2 kg s^{-2} A^{-1}
Magnetic flux density	tesla	T	Wb m^{-2}	kg s^{-2} A^{-1}
Inductance	henry	H	Wb A^{-1}	m^2 kg s^{-2} A^{-2}
Celsius temperature	degree Celsius	ºC	B	K
Luminous flux	lumen	lm	B	cd sr
Illumination	lux	lx	lm m^{-2}	m^{-2} cs sr
Activity (radionuclide)	becquerel	Bq	B	s^{-1}
Absorbed dose (radiation)	gray	Gy	J kg^{-1}	m^{-2} s^{-2}
Dose equivalent	sievert	Sv	J kg^{-1}	m^2 s^{-2}

Table 3. Non-SI units that are retained for general use or accepted for use with the SI

Quantity	Name	Symbol	Value in SI units
Time	minute	min	60 s
	hour	h	3 600 s
	day	d	86 400 s
	month	mo	604 800 s
	year	yr	–
Angle	degree	º	π/180 rad
	minute	'	π/10 800 rad
	second	"	π/648 000 rad
Mass	tonne	t L	1000 kg or 1Mg
Volume	litre	l	1 dm^3 or 10^{-3} m^3
Area	hectare	ha	10 000 m^2 or 10^{-2} km^2

Table 4. SI prefixes

Multiples			**Submultiples**		
Factor	Prefix	Symbol	Factor	Prefix	Symbol
10^{18}	exa	E	10^{-1}	deci	d
10^{15}	peta	P	10^{-2}	centi	c
10^{12}	tera	T	10^{-3}	milli	m
10^{9}	giga	G	10^{-6}	micro	µ
10^{6}	mega	M	10^{-9}	nano	n
10^{3}	kilo	k	10^{-12}	pico	p
10^{2}	hecto	h	10^{-15}	femto	f
10^{1}	deca	da	10^{-18}	atto	a

7. VITA

Fábio Ritter Marx, filho de Carlos Eduardo Marx e Ilse Maria Ritter, nasceu no dia 12 de fevereiro de 1986 em Cachoeira do Sul, Rio Grande do Sul.

Realizou seus estudos de ensino fundamental e médio na Escola Sinodal Barão do Rio Branco em Cachoeira do Sul, formou-se no ano de 2003. Ingressou no curso de Zootecnia na Universidade Federal de Santa Maria – UFSM, Santa Maria – RS, no ano de 2005. Desenvolveu atividades de iniciação científica com ênfase em nutrição e avaliação de alimentos para animais. Foi bolsista da Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul – FAPERGS, durante dois anos. Obteve o grau de Zootecnista no ano de 2009.

Ingressou no Programa de Pós-graduação em Zootecnia da Universidade Federal do Rio Grande do Sul – UFRGS no ano de 2010 sob a orientação do professor Dr. Alexandre de Mello Kessler. Período em que foi bolsista de mestrado do Conselho Nacional de Desenvolvimento Científico e Tecnológico – CNPq. Desenvolveu seu projeto de mestrado na área de nutrição de animais de companhia, intitulado: “Uso do óleo de soja e sebo bovino sobre a digestibilidade da dieta, perfil bioquímico e consistência fecal de cães adultos”. Durante o período do mestrado também participou de pesquisas paralelas na área de nutrição e avaliação de alimentos para aves e suínos. Obteve o grau de Mestre em Zootecnia no ano de 2012; ano em que iniciou os estudos de doutorado no mesmo programa de pós-graduação.

Em seu doutorado continuou a condução de experimentos no âmbito de avaliação de gorduras em dietas para cães. Foi bolsista de doutorado da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – CAPES. Neste período também realizou trabalho que objetivou avaliar o uso de ossos crus como agentes redutores de cálculo dentário em cães adultos. Além de conduzir trabalhos paralelos na área de nutrição e avaliação de alimentos para aves e suínos.

Durante o período de agosto de 2014 a março de 2015, participou do Programa de Doutorado Sanduíche no Exterior – CAPES, na Universidade Norueguesa de Ciências da Vida – NMBU, em Ås na Noruega, sob a orientação do professor Dr. Øystein Ahlstrøm. Neste período, participou de pesquisas relacionadas à avaliação de gorduras em dietas para cães. Adquiriu experiência na condução de pesquisas com mink (*Neovison vison*) como animal experimental. Além de ter participado de palestras, cursos e aulas relacionados a nutrição animal e a técnicas de processamento de alimentos para animais realizados no Departamento de Zootecnia da NMBU.