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# USO DE TRIGLICERÍDEOS DE CADEIA MÉDIA NA DIETA, DO JEJUM INTERMITENTE E SEUS EFEITOS SOBRE A DIGESTIBILIDADE DA DIETA, METABOLISMO E COGNIÇÃO EM CÃES ADULTOS

Porto Alegre
2020

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Dissertação apresentada como requisito à obtenção do Grau de Mestre em Zootecnia, na Faculdade de Agronomia, da Universidade Federal do Rio Grande do Sul.

Orientador: Luciano Trevizan

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# USO DE TRIGLICERÍDEOS DE CADEIA MÉDIA NA DIETA, DO JEJUM INTERMITENTE E SEUS EFEITOS SOBRE A DIGESTIBILIDADE DA DIETA, METABOLISMO E COGNIÇÃO EM CÃES ADULTOS

Autor: Aline Kummer de Souza
Orientador: Prof. Dr. Luciano Trevizan

**RESUMO:** Este estudo foi desenvolvido para avaliar as respostas metabólicas e a capacidade de aprendizado de cães alimentados com dietas contendo triglicerídeos de cadeia média (TCM) ou mantidos em jejum de 48h. Doze cães Beagle adultos foram utilizados em um delineado em quadrado latino balanceado e incompleto, composto por 3 tratamentos (8 repetições) e 2 blocos de 40 dias. Duas dietas experimentais foram formuladas para atender todas as necessidades nutricionais de cães adultos: dieta controle - 11% de gordura de aves foi utilizada para cobrir a dieta; dieta TCM – metade da gordura de aves foi substituída por TCM purificados. Os tratamentos foram três programas de alimentação: T1 - dieta controle ofertada duas vezes ao dia; T2 - dieta controle ofertada a cada 48 horas em única refeição no período da manhã; e T3 - dieta TCM ofertada duas vezes ao dia. Os cães foram adaptados ao regime alimentar e às dietas por 10 dias. Após este período, foi realizado o teste de digestibilidade com coleta total de fezes e urina por 6 dias e coleta de sangue para avaliação de glicose, triglicerídeos, β-hidroxibutirato e ácidos graxos não esterificados (NEFA) a cada 12 horas por 48 horas. Durante o período experimental foram testados 4 condicionamentos de aprendizado, com aumento do grau de dificuldade, para avaliação da cognição em uma adaptação da caixa teste Wisconsin General Test Apparatus. Não houve diferença nos coeficientes de digestibilidade das dietas entre os regimes de alimentação nem em relação a dieta contendo TCM. Os tratamentos apresentaram efeito ($P < 0,05$) sobre glicose, triglicerídeos e ácidos graxos não esterificados. No entanto, não houve efeito dos tratamentos sobre o nível de β-hidroxibutirato. O jejum foi mais eficiente que o TCM e controle para melhorar a capacidade de aprendizado dos cães nas tarefas certas e erradas de condicionamento, clicker e target ($P < 0,05$). Não houve diferença entre os tratamentos para a tarefa gestual. Foi possível concluir que o tratamento em jejum melhora a capacidade de aprendizado dos cães e aumenta o nível de AGNE, mas não altera o nível de β-hidroxibutirato. O tratamento com TCM em estudo de curto prazo parece não ser capaz de aumentar AGNE e β-hidroxibutirato e não tem efeito sobre a capacidade de aprendizado.

**Palavras-chave**: comportamento; corpos cetônicos; jejum; nutrição; triglicerídeos

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[1]Dissertação de Mestrado em Zootecnia - Produção Animal ou Plantas Forrageiras, Faculdade de Agronomia, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brasil. (82p.) Abril, 2020.

# USE OF MEDIUM-CHAIN TRIGLYCERIDES IN THE DIET, INTERMITTENT FASTING AND ITS EFFECTS ON DIET DIGESTIBILITY, METABOLISM AND COGNITION IN ADULT DOGS[2]

Author: Aline Kummer de Souza
Advisor: Prof. Dr. Luciano Trevizan

**ABSTRACT:** This study was developed to evaluate the metabolic responses and the learning capacity of dogs fed diets containing medium chain triglycerides (TCM) or fasted for 48 hours. Twelve adult Beagle dogs were used in a balanced and incomplete Latin square design, composed of 3 treatments (8 repetitions) and 2 blocks of 40 days. Two experimental diets were formulated to meet all the nutritional needs of adult dogs: control diet - 11% poultry fat was used to cover the diet; TCM diet - half of the poultry fat was replaced by TCM. The treatments were three feeding programs: T1 - control diet provided twice a day; T2 - control diet was offered every 48 hours in a single meal in the morning; and T3 - TCM diet provided twice a day. The dogs were adapted to the diet and diet for 10 days, then the digestibility test was performed with collection of feces and urine for 6 days, blood collection for glucose, triglycerides, β-hydroxybutyrate and non-esterified fatty acids (NEFA) every 12 hours for 48 hours. During the experimental period, 4 learning skills were tested, with an increase in the degree of difficulty, to assess cognition in an adaptation of the Wisconsin General Test Apparatus test box. There was no difference in the digestibility coefficients of the diets between the diets nor in relation to a diet containing TCM ($P > 0.05$). The treatments had an effect ($P < 0.05$) on glucose, triglycerides and NEFA. However, there was no effect of treatments ($P > 0.05$) on the level of β-hydroxybutyrate. Fasting was more efficient ($P <0.05$) than TCM and control to improve the dogs' ability to learn the right and wrong conditioning, clicker and target tasks. There was no difference between treatments ($P> 0.05$) for the gestural task. It was possible to conclude that fasting treatment improves the dogs' learning capacity and increases the level of NEFA, but does not alter the level of β-hydroxybutyrate. TCM treatment in a short-term study does not seem to be able to increase NEFA and β-hydroxybutyrate and does not increase learning ability.

**Key words**: behavior; ketone bodies; fasting; nutrition, triglycerides.

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## RELAÇÃO DE ABREVIATURAS

**AGCM:** Ácidos graxos de cadeia média
**ASH**: mineral matter
**BHB**: β-hydroxybutyrate
**CF**: crude fiber
**CP**: crude protein
**DM**: dry matter
**EE**: ether extract
**GE**: gross energy
**LHS**: hormone-sensitive lipase
**MCT**: medium chain triglycerides
**ME**: metabolizable energy
**MER**: maintenance energy requirement
**NEFA**: non-esterified fatty acids
**OM**: organic matter
**SNC**: Sistema nervosa central
**TCM**: triglicerídeos de cadeia média
**WGTA**: Wisconsin General Test Apparatus

# CAPÍTULO I

# 1. INTRODUÇÃO

Os cães possuem um processo de aprendizagem que se torna mais facilitado enquanto ainda filhotes, mas na fase adulta e senil, ainda é possível que haja novos aprendizados. Na fase adulta os cães necessitam de um estímulo que os chame a atenção como gestos, luz, barulho e petisco. A cognição é a capacidade de nova aprendizagem através destes estímulos, desenvolvendo um novo conhecimento a partir de um preexistente no cão (Snitcofsky, 2013). Sendo assim, quando estimulados por algo e principalmente pela convivência social com humanos, os cães demonstram uma relação sociocognitva de habilidades de comportamento e aprendizagem tornando este o primeiro passo para a formação de uma memória.

Os cães são utilizados como modelo de aprendizagem e cognição, principalmente para o estudo do envelhecimento cerebral de humanos (Bunford et al., 2017, Giusto et al., 2002). Perante um novo aprendizado, o desenvolvimento cognitivo dos cães é testado para mensurar este desempenho através de diferentes sistemas de memórias dos cães. Estes testes também podem elucidar as diferenças de aprendizagem entre cães jovens, adultos e senis (Landsberg, 2005). Para o aprendizado, os cães utilizam a memória e seu cérebro está condicionado a utilização de substratos energéticos, como a glicose, sendo este o principal substrato para energia cerebral, porém, em situações como fome em longo prazo (jejum), outro substrato pode ser utilizado pelos tecidos para produção de energia além da glicose, são estes os ácidos graxos. Estes triacilglicerois também podem ser fornecidos através de dietas aos animais, que os torna mais ativos, sugerindo que a suplementação possa agir na redução o declínio cognitivo de cães enquanto a estimulação cerebral pela interação social e ambiente possa ser mais efetiva no aprendizado. O cérebro adulto é capaz, quando necessário, passar da dependência quase completa da glicose à utilização de corpos cetônicos, estes podem ser fornecidos através da dieta cetogênica com a inclusão de ácidos graxos ou pelo jejum através de metabolismo, ativando o processo de lipólise no tecido adiposo, ao melhorar o direcionamento a produção de corpos cetônicos.

Este estudo visa demonstrar a aprendizagem de cães adultos pelo teste de cognição Wisconsin General Test Apparatus (adaptado por Landsberg, 2005), e com o fornecimento de uma dieta cetogênica com inclusão de triglicerídeos de cadeia média (TCM) purificados, e um regime dietético que promova jejum para produção de corpos cetônicos circulantes, promovendo a substituição da glicose como substrato energético cerebral e utilização dos corpos cetônicos como nova fonte energética para melhora cognitiva e de aprendizagem dos cães.

# 2. REVISÃO BIBLIOGRÁFICA

## 2.1. **Memória e aprendizado**

O termo cognição é definido como o processo de aquisição do conhecimento através da percepção, atenção, associação, memória, raciocínio e linguagem (Teixeira, 2015). Segundo Snitcofsky (2013) a cognição é demonstrada através da atenção, reconhecimento espacial, uso de ferramentas, raciocínio, tomada de decisões, comunicação e “linguagem” ou cognição social, temporalidade: capacidade de medir o tempo. Está ligada ao comportamento dos animais quando há um estímulo ou prova, em que se estuda a percepção, atenção, motivação, memória, formação de conceitos, raciocínio e comunicação ou cognição social nas espécies animais (Fujita et al., 2012). Os cães são animais sociais que convivem com os humanos, e são modelos de aprendizagem cognitiva.

A comunicação entre homens e cães mostra habilidades sociocognitivas complexas por parte dos cães (Lampe et al., 2017) Cães possuem grande habilidade em tarefas de busca por objetos devido à comunicação frequente com os humanos (Call et al. 2003). Riedel et al. (2008), em estudo com cães de diferentes idades, os quais teriam de achar alimentos escondidos e com ajuda de sinais de comunicação dos humanos para facilitar a busca, concluíram que os filhotes demonstraram uma melhor utilização dos comandos fornecidos pelos humanos para concluir a tarefa. O processo de domesticação desenvolveu as habilidades cognitivas nos cães (Reid, 2009), resultando na seleção dos comportamentos que exibem um menor medo de alguma agressão advinda dos seres humanos (Hare & Tomasello, 2005). Através deste processo evolutivo o ambiente humano tornou-se natural aos cães.

Os cães aprendem através da socialização e observação de outros cães e dos humanos (Miklósi, 2007; Kubinyi et al., 2003, Fugazza et al., 2016). A aprendizagem pode ser definida como o primeiro contato com uma informação e, portanto, o primeiro passo na formação da memória (Snitcofsky, 2013), pode

ocorrer de duas formas, associativa e não associativa (Snitcofsky, 2013). Na aprendizagem não associativa, o indivíduo é exposto a um estímulo, o qual gera modificações no comportamento do animal (Squirre & Kandel, 1999). A associativa, se baseia na relação entre estímulos e reforços positivos (recompensas) ou negativos (punições), através de condicionamento (Matthews & Matthews, 2010).

A aprendizagem associativa que ocorre por meio do condicionamento pavloviano, que consiste em ensinar relações de contingência ou dependência entre dois estímulos. No condicionamento pavloviano, a relação de contingência entre o estímulo neutro e o estímulo incondicionado é autônoma da resposta: havendo ou não resposta, o estímulo incondicionado será apresentado depois da apresentação de um estímulo condicionado.

Para elucidar este condicionamento o experimento de Pavlov demonstrou que o reflexo de salivação do cão doméstico ocorre pela presença de uma substância comestível (estímulo incondicionado) na sua boca (Snitcosfky, 2013). O organismo aprende que os eventos do ambiente antecipam a ocorrência de um estímulo. Primeiramente se identifica qual estímulo induz certo comportamento, utiliza como exemplo: acariciar a cabeça do cão, e verificaríamos que o cão não abana o rabo quando dizemos “abana”. Depois, no treino, falaríamos “abana” sempre antes de acariciar a cabeça. Se, depois de fazer isso várias vezes, verificarmos que o cão abana o rabo cada vez que falamos “abana”, podemos dizer que a resposta de abanar o rabo foi condicionada de forma pavloviana ao comando “abana”.

A memória é considerada uma representação interna de uma experiência comportamental, codificada espaço-temporariamente em circuitos neuronais (Siwak-Tapp et al., 2007). Para Cummings et al. (1996a), cães são excelentes modelos para estudo do envelhecimento cerebral em humanos, colabora também para achados em relação a memória de aprendizagem e as funções cognitivas. O aprendizado sob efeito do consumo de uma dieta torna os cães mais ativos, sugerindo que os alimentos atuam reduzindo os efeitos negativos que retardam o processo de cognição associado ao envelhecimento em cães sem sintomas de alguma doença. No entanto, esses alimentos apenas adiam

parcialmente o nível do declínio cognitivo de cães idosos, enquanto a estimulação cerebral por interação social e ambiente pode ser mais efetiva (Beynen, 2017). A adição de 5,5% de triglicerídeos de cadeia média em dietas secas reduziu os erros cometidos por cães idosos em tarefas de aprendizagem (Pan et al., 2010).

## 2.2. **Testes de aprendizagem**

Vários testes foram desenvolvidos para mensurar este desempenho através de diferentes sistemas de memória dos cães. Os testes também podem elucidar as diferenças de aprendizagem entre cães jovens, adultos e senis (Milgram, et al., 1994; 2002a; 2002b; Landsberg, 2005). Os testes cognitivos foram desenvolvidos para avaliar diversos aspectos da cognição que correspondem a capacidade do cão baseada no conhecimento (discriminação, aprendizado reverso, reconhecimento do objeto e aprendizado espacial) e na habilidade (abordagem da recompensa e do objeto) (Cummings et al., 1996b).

No teste de abordagem à recompensa, esta é colocada à esquerda ou à direita. Através de sugestão visual, o animal deve aprender a escolher somente o local que contém a recompensa (Cummings et al., 1996b). Geralmente, os cães realizam 10, 20 ou até 40 tentativas por dia, dependendo da metodologia utilizada. O intervalo entre as tentativas pode variar de 30 a 90 segundos (Milgram, et al., 1994, Cummings et al., 1996b). Normalmente, os cães são julgados como aprendido a tarefa quando atingem um nível de 90% de acertos, ou um nível de 80% por dois dias consecutivos e então mantêm um nível de 70% ao longo das próximas três sessões (Landsberg, 2005). Quando comparados cães jovens a idosos, são encontradas diferenças em favor dos jovens nos testes de cognição, indicando sensíveis alterações cognitivas dependentes da idade (Milgram et al., 2002 b, Pekcec et al., 2008).

## 2.3. **Wisconsin General Test Aparattus**

Alguns testes cognitivos são realizados em aparelhos adaptados, como o Wisconsin General Test Apparatus (Fox, 1971), onde o animal de estudo é

alocado em uma câmara de madeira adequada ao seu tamanho, separado por barras de ferro como portas que se abrem e fecham tipo guilhotinas, variando a quantidade de portas com a quantidade de opções ofertadas ao animal (objeto, alimento, recompensa, etc.). A frente do animal será apresentada uma bandeja com as opções, havendo somente uma opção correta (ou porta correta), e então as portas irão se abrir para que o animal faça a escolha de um dos lados. A porta irá se fechar sempre do mesmo lado (esquerda ou direita) para o animal quando a escolha estiver incorreta, até que ele aprenda a escolher a correta, e então validar o aprendizado do animal em erros e acertos, até que o animal tenha completado a tarefa ao acertar todas as tentativas (Landsberg, 2005, Milgram, et al., 1994; 2002a; 2002b).

## 2.4. **Corpos cetônicos: fonte energética cerebral**

Durante o aprendizado os cães utilizam a memória e seu cérebro está condicionado a utilização de substratos energéticos, os neurônios são dependentes quase que exclusivamente de glicose (Lee, et al., 2000). A glicose é o principal substrato para energia cerebral de um mamífero adulto, porém em jejum, outro substrato pode ser utilizado pelos tecidos para produção de energia além da glicose, são os ácidos graxos. Apesar da baixa concentração, os ácidos graxos plasmáticos têm uma taxa de renovação alta, e a quantidade de calorias derivadas de sua oxidação maior que a da glicose (Aires, 2008). Porém, os ácidos graxos liberados no plasma não são utilizados pelo cérebro, sendo dependente quase que exclusivamente da glicose. Todavia, em certas condições, a fonte energética de subsídio para o cérebro é adquirida pela oxidação dos ácidos graxos para formação de corpos cetônicos (Nehlig, 2004). As cetonas são capazes de atravessar a barreira hematoencefálica e resultar em até 20% de economia da glicose (Studzinski et al., 2008) para o metabolismo energético do cérebro.

Com a oxidação dos corpos cetônicos se produz uma grande quantidade de ATP, sugerindo o aumento das reservas energéticas cerebrais (Pereira et al., 2010). Moore et al. (1976) estudaram o transporte e metabolismo da glicose em

cérebro de ratos amamentados em diferentes idades, descobrindo que há pouca utilização da glicose em cérebro de ratos neonatos, sugerindo o uso predominante de outras fontes energéticas que não fosse a glicose. A oxidação de ácidos graxos se desenvolve rapidamente depois do nascimento, em muitos tecidos, incluindo o fígado, onde são usados como precursores para síntese de corpos cetônicos (Girard et al., 1992). Autores mostraram que o sistema nervoso central (SNC) é capaz de utilizar outros substratos energéticos além de glicose, como manose (Dringen et al., 1994), frutose (Wada et al., 1998), glicerol e glutamina (Mckenna et al., 1986), corpos cetônicos (Williamson, 1985; Nehlig & Vasconcelos, 1993) e lactato (Tabernero, Vicario & Medina, 1996; Griffin et al., 1999).

De acordo com Nehlig (2004), o cérebro adulto é capaz, sempre que necessário, de passar da dependência quase completa da glicose à utilização de corpos cetônicos em quantidades mais ou menos importantes em proporção aos níveis circulantes. O controle da utilização dos corpos cetônicos no cérebro adulto é alcançado pela concentração do substrato e o grau de saturação das enzimas na utilização dos corpos cetônicos (Nehlig, 2004). O metabolismo da glicose do cérebro torna-se menos eficiente com a idade, de modo que fornecer fontes alternativas de combustíveis pode ser benéfico (Studzinski et al., 2008).

No cérebro, os corpos cetônicos são metabolizados por duas rotas diferentes, localizadas no citosol e nas mitocôndrias. O β-hidroxibutirato é degradado nas mitocôndrias por três enzimas, a β-hidroxibutirato desidrogenase, 3-cetoácido-CoA transferase, que estão localizadas nas mitocôndrias e acetoacetil-CoA tioliase, encontrada nas mitocôndrias e no citosol (Nehlig, 2004). O produto final destas reações é acetil-CoA, o primeiro intermediário em comum com a via do metabolismo da glicose.

A via mitocondrial do metabolismo de corpos cetônicos tem propósitos oxidativos e gera moléculas de acetil-CoA para a biossíntese de aminoácidos, enquanto a via citosólica que possui acetoacetato apenas para precursor está envolvida de forma proeminente na biossíntese de lipídios e colesterol (Nehlig, 2004). No fígado pode-se formar acetoacetato a partir de acetoacetil-CoA e acetil-CoA que são sintetizados durante a oxidação de ácidos graxos. Devido a

estas propriedades, o fígado é fonte de corpos de cetônicos no sangue e sua taxa de produção é aumentada com a utilização de ácidos graxos (Nehlig, 2004). O β-hidroxibutirato é predominantemente usado pelo cérebro em estados de cetose. Os corpos cetônicos podem participar no fornecimento de precursores para os processos biossintéticos associados ao crescimento e ao desenvolvimento do sistema nervoso central (Spitzer & Weng, 1972).

## 2.5. **Dieta cetogênica e/ou programa alimentar**

O intervalo prolongado entre refeições, o jejum ou a utilização de dietas cetogênicas podem ser uma forma de indução da formação de corpos cetônicos. A mudança nutricional pode influenciar a capacidade de transporte do carreador monocarboxilato (Cremer et al., 1979), encontrando aumento no transporte de β-hidroxibutirato, acetoacetato e de lactato (Cremer, Braum & Oldendorf, 1976) em cérebros de ratos adultos submetidos a jejum de vários dias. A queda da relação insulina/glucagon durante o jejum, ativa o processo de lipólise no tecido adiposo, o que favorece o direcionamento à produção de corpos cetônicos. Nos músculos, que representam cerca de 40% do peso corporal total, a utilização aumentada dos ácidos graxos livres inibe a utilização de glicose, substituindo, dessa forma, o consumo de glicose pelo dos ácidos graxos livres (Aires, 2008). Em cetose sanguínea contínua, há uma fase de adaptação do metabolismo cerebral estimada em até 20 dias, depois da qual os neurônios passam a utilizar os corpos cetônicos em lugar da glicose como principal gerador de energia. A cetonemia prolongada, seja por qualquer causa: amamentação, fome ou cetoacidose diabética, induz um aumento na atividade do mecanismo de transporte dos corpos cetônicos para o cérebro (Nehlig, 2004).

Durante a fome prolongada, em que os níveis circulantes de glicose são geralmente reduzidos e as reservas de glicogênio podem se esgotar, os corpos cetônicos, mais notavelmente β-hidroxibutirato, serve como combustível fisiológico crítico para vários tecidos extra-hepáticos (Masoro, 1968).

A dieta cetogênica consiste em alto teor de gordura, baixo teor de proteína e carboidratos, tipicamente com proporções de até 4:1 de gorduras para

proteínas e carboidratos. Foi introduzida pela primeira vez na década de 1920 para uso em pacientes com epilepsia infantil (Ferreira et al., 2003). Além de ser aplicada no tratamento da epilepsia (Cheng et al., 2003, Adibhatla & Hatcher, 2007), auxilia no controle de peso e na melhora do desempenho no exercício, aplicações nutricionais, preparações farmacêuticas humanas e veterinárias, e na cosmética, devido sua alta adsorção sobre a pele (Ferreira et al., 2003). A dieta cetogênica foi proposta como estratégia alternativa de tratamento para epilepsia canina (Martlé et al., 2014), e melhora do desempenho cognitivo (Snigdha et al., 2012).

As dietas cetogênicas constituídas de ácidos graxos de cadeia média apresentam digestão e absorção mais rápida, devido ao comprimento de cadeia e grau de saturação (6-12 carbonos). Uma dieta cetogênica apresenta cerca de 60% do valor energético total proveniente de triglicerídeos de cadeia média (Pereira, et al., 2010). Os triacilgliceróis que contém ácidos graxos de cadeia média são rapidamente hidrolisados pelas lipases lingual e gástrica, e os seus ácidos graxos são absorvidos pelas células intestinais, difundem-se pela circulação portal até chegarem ao fígado onde sofrem β-oxidação, formando acetil coenzima A. Ácidos graxos de cadeia média são misturados em uma proporção desejada e esterificados com o glicerol para formar triacilgliceróis em preparações comerciais (Cater et al., 1997). Kaplan & Greenwood (1998) mostram que dietas compostas de ácidos graxos influenciam alguns comportamentos, incluindo regulação da temperatura corporal, sensibilidade à dor, comportamento alimentar, seleção de macronutrientes e desempenho cognitivo.

## 2.6. **Triglicerideos de cadeia média (TCM)**

Denominam-se ácidos graxos de cadeia média (AGCM), moléculas apolares formadas por três ácidos graxos saturados que contêm de 6 a 12 átomos de carbono (Bhatnagar et al., 2009; Dayrit, 2014; Zentek et al., 2011) que estão esterificados no glicerol. Na década de 1980, apenas ácidos com 8 a 10 átomos de carbono eram considerados como ácidos graxos de cadeia média. Contudo, observou-se que aqueles com 6 a 12 átomos reuniam metabolismo

hepático e funções metabólicas e fisiológicas em comum (Dayrit, 2014), o que justificou a inserção deles nesse grupo. Triglicerídeos de cadeia média são, dentre as gorduras, as mais eficientes em produzir cetose, pois são rapidamente metabolizadas. A suplementação com triglicerídeos de cadeia média resultou em melhor desempenho cognitivo em cães idosos, em comparação com controles não suplementados (Pan et al., 2010).

### 2.6.1 Absorção dos TCM

São hidrolizados pela ação da lipase pancreática, transformados em ácidos graxos livres e absorvidos no duodeno mais rapidamente e eficiente, AGCMconstituem uma fonte rápida de energia, e são absorvidos diretamente na corrente sanguínea, a velocidade de absorção dos a AGCM no intestino é similar à da glicose (Colleone, 2002). Após passar pelos enterócitos, estes ácidos graxos atingem a circulação portal, transportados ao fígado ligados fracamente a albumina (Oliveira & Gazzola, 2002). Cerca de 80 a 100% dos AGCM presentes em todo o fluxo portal, são captutados pelo fígado e a parcela remanescente segue pela corrente sanguínea, e se torna disponível aos tecidos periféricos (Hirata &Hirata, 2002).

Parte dos AGCM é também diretamente solubilizada na fração aquosa do plasma. O metabolismo lipídico, composto pela digestão e absorção, pode ser dividido em duas vias básicas de acordo com (Bauer, 2004): representa o transporte dos lipídeos provenientes da dieta, do intestino para o fígado (Exógena) ou do próprio metabolismo lipídico no organismo (Endógeno) transporte das lipoproteínas sintetizadas nos hepatócitos, do fígado para os tecidos periféricos. Na fase pós absortiva, os AGCM têm seu transporte facilitado no plasma, por ligação a albumina e, pela veia porta, alcançando o fígado rapidamente.

Quanto maior é a cadeia carbônica dos ácidos graxos, mais este é encontado na linfa e menos no sangue portal. Na linfa os triglicerídeos circulam associados aos quilomícrons, já no sangue portal, estão ligados a albumina (Ferreira et al., 2003). Segundo Colleone (2002), em humanos, 8% dos AGCM

estão associados aos quilomícrons três horas após a ingestão de uma refeição com TCM em indivíduos saudáveis. Consumindo essa dieta por seis dias, esse valor atinge 15%.

## 3. HIPÓTESES E OBJETIVOS

Hipóteses:

1. A inclusão de triglicerídeos de cadeia média purificados na dieta de cães adultos é capaz de aumentar a produção de corpos cetônicos.
2. Baseado do hábito alimentar de canídeos selvagens, com grande intervalo entre refeições, é possível produzir em cães aumento de corpos cetônicos circulantes pelo maior espaçamento entre refeições.
3. O aprendizado dos cães adultos é melhorado quando há elevação dos corpos cetônicos circulantes.
4. Regime alimentar baseado em jejum pode reduzir a digestibilidade dos nutrientes e da energia das dietas.

Objetivo

1. Observar se a inclusão de 5,5% de TCM purificado na dieta de cães pode aumentar a circulação de corpo cetônicos.
2. Observar se o espaçamento entre refeições de 48 h pode ser um indutor da formaçao de corpos cetônicos.
3. Observar se há melhora da cognição de cães adultos quando TCM ou jejum é implementado na alimentação de cães
4. Observar se a digestibilidade dos nutrientes e da energia é afetada pelo regime dietético e pela presença de TCM.

## CAPÍTULO II

Effects of fasting and medium chain triglycerides in the diet on metabolism and learning ability of young adult Beagle dogs

Este capítulo é apresentado de acordo com as normas de publicação do periódico **Animal**

# Effects of fasting and medium chain triglycerides in the diet on metabolism and learning ability of young adult Beagle dogs

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Ketogenesis and learning ability in dogs

## Abstract

This study was designed to evaluate metabolic responses and the learning ability of dogs that were submitted to a medium chain triglycerides (**MCT**) containing diet and 48h fasting. Twelve adult Beagle dogs were allocated in metabolic cages during assays and had access to yards during the day. The study was a balanced incomplete latin square design, composed of 3 treatments (8 repetitions) and 2 blocks of 40 days. A diet was formulated within the nutritional needs of adult dogs and divided into: a control diet with 11% poultry fat and a MCT diet with 5.5% of poultry fat and 5.5% MCT. Treatments were three feeding programs: T1) the control diet was provided twice a day; T2) the control diet was offered every 48 hours in a single morning meal; and T3) the MCT diet was provided twice a day. A digestibility assay was performed and

blood was collected for analysis of glucose, triglycerides, non-esterified fatty acids (**NEFA**), and β-hydroxybutyrate. Dogs were subjected to four cognition tests with increasing levels of difficulty. There was no difference in the digestibility coefficients ($P > 0.05$). The treatments presented effect ($P < 0.05$) on the glucose, triglycerides and NEFA. However, there was no effect of treatments ($P > 0.05$) on the level of β-hydroxybutyrate. The fasting treatment was more efficient ($P < 0.05$) than the MCT and control treatments to increase the dogs' learning ability in the right and wrong conditioning, clicker and target tasks. There was no difference between treatments ($P > 0.05$) for the gesture task. It was possible to conclude that fasting treatment improves the dogs' learning ability and increases the NEFA level but does not alter the level of β-hydroxybutyrate. The MCT treatment in a short-term study seems like do not increase NEFA and β-hydroxybutyrate and does not increase learning ability.

**Keywords:** behavior, fasting, ketone bodies**,** nutrition, triglycerides.

**Implications**

Teaching dogs is essential to maintain the close relationship with humans. It is known that cognition can be improved in old dogs fed diets containing medium chain fatty acids, which increases the level of blood ketonic bodies; and fasting is another way to increase this cellular fuel. The results of this study will assist in choosing new ingredients to include in diets or a new feeding program that may improve learning capacity in young adult dogs, as well as understanding the metabolic responses associated with this process. It will directly benefit dogs, owners and trainers, as trainers will have more skills to adjust the lessons to the dogs learning.

## Introduction

There is a great advance in terms of understanding the skills that dogs have regarding learning, memory formation, information processing and other brain functions (Fujita et al., 2012; Fugazza et al., 2016; Lampe et al., 2018). Allied to this knowledge there is the need to develop methods and nutritional strategies that assist in improving, increasing and maintaining the dogs' cognitive functions. The brain of adult dogs continuously produces new cells, including neurons from progenitor cells in specific regions. However, over the years of the animal's life, there is a reduction in the proliferation of progenitor cells and in the survival of new neurons, and elderly dogs show a significant reduction in the formation of neurons when compared to adult and young dogs (Siwak-Tapp et al., 2007; Pekcec et al., 2008). The number of newly formed neurons and neurons in the hippocampus is significantly correlated with cognitive functions, including learning and memory in dogs, suggesting that neurogenesis is involved in the process of learning and forming memory (Siwak-Tapp et al., 2007).

The production of ATP needed to supply brain functions can be derived from the metabolism of dietary nutrients or from the metabolism of ketone bodies. Lipids play a fundamental role in brain structure and function, as they affect cell membrane structures and protein-membrane interactions, as well as serving as signaling molecules, binding to the plasma membrane or nuclear receptors and mediating transmembrane signaling and cell-to-cell communication (Adibhatla and Hatcher, 2007). In addition, several lipid constituents of cell membranes

have been shown to have a significant impact on receptors, glutamate transporters and ion channels (Giusto et al., 2002; Meves, 2008).

Medium chain triglycerides are formed by 6, 8, 10 and 12 carbon fatty acids and have some distinct characteristics, such as being absorbed more efficiently than long chain fatty acids. They are transported in the portal blood directly to the liver without resynthesis of triglycerides, and enter the mitochondria independently of carnitine palmitoyl transferase, being quickly converted into ketone bodies (Walther and Farese, 2012). This has been demonstrated in some studies, such as in the research by Pan et al. (2010), which supplementation of medium chain triglycerides for 8 months in elderly dogs significantly increased blood concentrations of ketone bodies and improved age-related decline in cognitive function.

The prolonged interval between meals can be a way of inducing the formation of ketone bodies. When glucose levels are low, free fatty acids are released from triglycerides and transported through the blood to other tissues where they are oxidized to provide energy through the mitochondrial beta-oxidation pathway (Nehlig, 2004). Ketone bodies, such as acetoacetate, acetone and beta hydroxybutyrate, resulting from the Krebs' cycle, are transported to tissues such as the heart and brain as an energy source (Cheng et al., 2003). Thus, the objective of this study was to evaluate metabolic responses and the learning ability of young adult Beagle dogs that were submitted to a medium-chain triglycerides containing diet and fasting for 48 hours as a way to increase circulating ketone bodies.

## Material and methods

***Animals management and experimental design***

Twelve, healthy, adult (aged 4 and 5 years old) Beagle dogs (6 males and 6 females, body weight between 10 and 14 kg), with a body condition score between 4 and 6 based on the Laflamme (1997) classification were used in the study. All dogs were vaccinated and submitted to a complete blood and parasitological tests and clinical examinations to ensure their health to start the experiment. The dogs were allocated in metabolic cages (0.80 × 0.70 × 0.90 m) equipped with feeders and drinkers in an air-conditioned room with controlled temperature (24°C) and photoperiod (14 h light and 10 h dark). The dogs were kept in the cages during the digestibility analysis period and during the night, only. In other periods, the dogs were separated by gender and set free in two yards (400 $m^2$) with grass without access to any food, but drink water *ad libitum*. The amount of food supplied was calculated based on the content of metabolizable energy (**ME**) estimated from the chemical composition of the diets (NRC, 2006) and dogs were fed maintenance energy requirement of 120 kcal ME/$kg^{0.75}$/day **(MER)**, and had free access to the drink water during the entire experiment.

The study was designed in a balanced incomplete latin square design, composed of three treatments with 4 replications each and 2 blocks in a total of 100 days of experimental period (Figure 1). The cognition tests were carried out to increase complexity. Then in the first block 4 replications were acquired to the first test. In the second block, 3 cognition tests were performed in sequence, then 4 replications for each test were obtained. Each experimental block started with an adaptation phase (10 days) to the diets, digestibility assay, blood

collection and the management for carrying out the learning activities, totaling 40 days. During the adaptation all dogs received diets according to the feed program designed to the experimental period.

### *Diets and treatments*

A diet was formulated with ingredients traditionally used in commercial formulations to meet the nutritional needs of adult dogs (Table 1) (FEDIAF, 2018). The diet was extruded, but the covering fat was added separately in order to produce two different experimental diets: Control diet, 11% poultry fat was used as a cover for the extruded feed; MCT diet**,** poultry fat was a half replaced by 5.5% of purified medium chain triglycerides (Concepta Ingredients®) to cover the extruded feed.

The treatments were made based on the feeding program. The control diet was provided twice a day, 50% MER in the morning and 50% MER in the afternoon, with an interval between meals of 12 hours (Treatment 1 – T1). The control diet was used to compose the fasting program treatment. The diet was offered every two days (interval of 48 hours) in a single morning meal, with 200% of the MER (Treatment 2 – T2). The last treatment (T3) was made by offering the MCT diet 50% of the MER in the morning and 50% of the MER in the afternoon, with an interval between meals of 12 hours.

### *Digestibility assay and analysis*

Dogs were fed MER during all assay according to the treatments. A gelatin capsule containing 1,000 mg of iron (III) $Fe_2O_3$ oxide was provided orally to

147 separate the beginning and end of faecal collection periods. The dogs were
148 weighed weekly.
149 The test was conducted according to the guidelines recommended by AFFCO
150 (2008). Each digestibility period was determined in 8 days (Figure 1) after 10
151 days of adaptation to the diets and feed program, with three days of adaptation
152 to the cages, and five days of total collection of feces and urine. Urine was
153 collected for pH and density analysis. Diet samples were collected and stored
154 during the trial.
155 Faecal samples were scored after each defecation as follows: 1) hard dry and
156 crumbly, “bullet like”; 2) well formed, does not leave a mark when picked up,
157 kickable; 3) moist beginning to lose form, leaving a definite mark when picked
158 up; 4) the majority, if not all the form is lost, poor consistency, viscous and 5)
159 watery diarrhea (Moxham, 2001). The faeces were collected shortly after
160 defecation and stored at -20°C. Total faecal outputs from each animal and block
161 were weighted and mixed then samples were taken and dried at 55°C in a
162 forced-air oven for 72 hours according to AOAC (1995) followed by grinding in a
163 Willey mill in a 1-mm screen.
164 Analysis of dry matter (**DM**; 934.01), organic matter (**OM**; 920.36), mineral
165 matter (**MM**; 942.05), crude protein (**CP**; 954.01) (Model TE 036/2, Tecnal,
166 Piracicaba, Brazil), crude fiber (**CF**; 962.10) and etheric extract in acid
167 hydrolysis (**EE**; 954.02 ) (Model 170/3, Fanem, São Paulo, Brazil) and gross
168 energy (**GE**; isoperibolic calorimetric bomb model C2000 - IKA Werke GmbH &
169 Co. KG, Staufen, Germany) were conducted according to AOAC (1995).

The following equation was used to determine apparent total tract digestibility of nutrients and energy (ATTD): [intake (g/d) – faecal output (g/d) / intake (g/d)] x 100. The organic matter content was calculated by: OM (%) = %DM – %Ash. The dietary ME was calculated by different factors for comparison: ME, kcal/kg = [(GE intake – faecal GE) – (CP intake grams – faecal CP grams) x 1,25] / DM intake (FEDIAF, 2018).

***Blood collection and analysis***

After 13 days od adaptation to the treatments, blood samples were collected over a total period of 48 hours, with 12 hours interval between each collection, in each experimental block. Samples were collected after at least 12-hour overnight fast and before night feeding. The procedure was performed by venipuncture of the cephalic vein and vacuum tubes containing EDTA were used. The blood was centrifuged at 3500 rpm × 10 minutes for plasma separation. Samples were stored at -20°C.

Glucose, triglycerides, non-esterified fatty acids (**NEFA**) and β-hydroxybutyrate (**BHB**) were analyzed. For analyzes of blood, the plasma was thawed at room temperature at the time of testing and processed immediately. A spectrophotometer (Wiener Lab, CM200, Argentina) and a set of commercial reagents (Ranbut D3hidroxibutirate Randox laboratories, Crumlin, UK; NEFA Randox, Crumlin, UK) were used. The procedures were performed according to the manufacturer's recommendations.

***Cognition tests***

***Wisconsin General Test Apparatus (WGTA)***

All dogs were adapted to WGTA over a period of 15 days prior the test (Figure1). The dogs underwent daily cognitive tests in the morning and afternoon throughout the experimental period carried out in an air-conditioned room with minimal external interference. An apparatus adapted from the WGTA (Fox, 1971) was used to perform the cognitive tests and tests with different levels of difficulty were adopted, increasing complexity over the experimental period.

The WGTA consisted of a wooden box compatible with the size of the dogs, equipped with hollow guillotine doors on the front. These doors adapted to the size of the animal. In addition to the doors, there was a tray with holes where the rewards were placed (snack). The experimenter was separated from the dog by a false mirror, to avoid eye contact between them. Below the mirror, there was a hinged wooden door, through which the tray with objects was passed (Milgram, et al., 1994, 2008; Landsberg, et al., 2005). The overview of the test box can be observed in the Supplementary material 1.

All dogs were allocated within the WGTA individually, remaining for 1 minute for recognition, right after the cognition test started. In the center of the box was a grid that separated the dog and the evaluator, and below the grid were two guillotine doors (one on the left and one on the right side). Through a movable door under the false mirror, the evaluator placed two identical snacks behind each guillotine door. The dog could see only the evaluator's hands placing the snacks. The guillotine doors opened at the same time, leaving the snacks exposed to the dog.

An animal behavior specialist assisted the evaluator. The specialist taught the evaluator all the learning activities developed with the dogs and judged that all procedures were performed correctly throughout the experimental period.

The objective of the first learning test was that the dog should learn to choose the food always on the same door (left or right). Two snaks were placed in front of the doors - one snak in front of the right door and one in front of the left door. The doors were open and the animal could choose which snak to take. When choosing the door correctely, the dog could take the snak, and this was counted as doing the task correctly. If the animal chose the wrong side door, the door was closed, which made it impossible for the animal to take the snak, and this was considered an error when performing the task.

The evaluations were carried out twice a day, immediately after the provision of the morning diets and before the evening meal. The procedure was performed 10 times a day for each dog, with intervals of 5 seconds between each procedure.

***Conditioning right and wrong***

The objective of the test was to condition the dog which side was correct to seize the snack. Each dog already had a pre-definition of which side (left or right) could seize the snack with its mouth or paw. When the dog chose the right side, the dog could seize the snack, and the attitude counted as correct. If the dog chose the wrong side, the two doors were closed and the dog did not seize the snack, and the attitude was counted as a mistake. The number of correct answers was used to evaluate the dogs' learnability.

Within the first 40 days block, for 8 days the dogs were evaluated daily, i.e., 20 evaluations a day, 10 in the morning and 10 in the afternoon. Subsequently, for 9 days, the dogs were evaluated daily, with 10 evaluations only in one shift. Finally, for 6 days, the dogs were evaluated on alternate days, with 10 evaluations in a single shift. Each dog was evaluated a total of 330 times.

***Gesture conditioning***

The objective of the test was conditioning the dog to stand on all fours looking at the evaluator's hand. The evaluator placed his hand through the WGTA's movable door, being held flat in front of the dog, at the same height as his head, and the dog observed the evaluator's hand through a hollow grid. To perform the learning, the dog should stand on all fours and stare at the evaluator's hand. From the moment the evaluator placed his hand inside the WGTA, a stopwatch was used to measure the time of each learning attempt. The task execution time was used to evaluate the dogs' learning ability. A snack was offered to the dog each time the movement was performed correctly. Within the second 40 days block, during 12-day period, 10 attempts were performed daily. Each dog was evaluated a total of 120 times.

***Clicker***

The objective of the test was teaching the dog standing on all fours, looking straight ahead with sound of the clicker. Once in the cage, dog's name was spoken before each attempt and a stopwatch was started to measure the learning execution time. Once the learning was carried out, the evaluator pressed the clicker with two quick clicks and a snack was offered to fix the

learning. The task execution time was used to evaluate the dogs' learning ability. Within the second 40 days block, during a period of 13 days, 10 attempts were made daily. Each dog was evaluated a total of 130 times.

***Target***

The purpose of the test was designed to the dog associate touching a black circle with its snout under clicker sound. A solid black circle (10 cm in diameter) was glued to a wall inside the WGTA. During learning, the dog should touch the black circle with the snout. After touching black circle, the evaluator used a clicker and after two consecutive clicks a snack was offered to the dog to fix the learning. The dog's name was spoken before starting each attempt, and a stopwatch was used to measure the time of execution of each attempt. The task execution time was used to evaluate the dogs' learning ability. Within the second 40 days block, during a period of 14 days, 10 attempts were made daily per dog. Each dog was evaluated a total of 140 times.

***Statistical Analysis***

The experimental design used was a balanced incomplete Latin square, considering the two periods (40 days each) simultaneously, in a structure of repeated measures over time. Three treatments were used, composed of 8 repetitions each and the dog was considered an experimental unit. The digestibility results were submitted to ANOVA and when a significant difference was observed, the means were compared by Tukey's test ($P < 0.05$). For biochemical analysis data, an ANOVA was performed considering a mixed model structure and with repeated measures over time, considering the two

292 evaluation periods, gender, time of blood collection and treatments, in the
293 presence of a significant F, the means were compared by LS means. For the
294 data of the learning ability analyzes, the number of repetitions used were the
295 total evaluation times in each learning test and the dog was considered an
296 experimental unit. An ANOVA was performed considering a mixed model
297 structure and with repeated measures over time, considering the factors day,
298 gender and treatments, in the presence of a significant F, the means were
299 compared by LS means. In addition, a regression analysis was performed,
300 considering the gender as a restriction factor and day as a quantitative variable
301 to observe on which day the dogs reached 80% of learning. Accepting that
302 when reaching 80% the test was really learned (Landsberg, 2005).

303
304 **Results**

305 ***Digestibility***

306 The dogs body weight was not influenced by the diets and the evaluation period
307 ($P > 0.05$; Table 2). The intake of ME was not influenced by treatments ($P >$
308 0.05). Daily fat intake was affected by treatments: the dogs fed with the fasting
309 treatment consumed less fat than the others in block 1 and block 2 ($P < 0.05$).
310 The intake of other nutrients was not affected by treatments and periods ($P >$
311 0.05). The estimated digestible and metabolizable energy of DM were not
312 influenced ($P > 0.05$) by treatments, periods and animals. Likewise, the
313 apparent digestibility coefficients of crude energy, crude protein, carbohydrates,
314 fat and organic matter showed no difference ($P > 0.05$) for diet, period and
315 animal. Fecal characteristics were not affected by treatments ($P > 0.05$)

## *Blood analysis*

### *Glucose*

The differences among treatments depend on the hour of blood collection, because there was a difference in the interaction between treatment and hour ($P = 0.0345$). The treatments presented effect on the glucose at 12 and 24 hours after the meal ($P < 0.05$). At hour 12, the dogs fed the fasting treatment had higher glucose (96.6 mg/dL; Figure 2 A), than dogs of the control treatment, which presented intermediate glucose level (81.7 mg/dL) and the dogs of the MCT treatment, which presented lower glucose level (69.6 mg/dL). At hour 24, glucose was higher for dogs that were fed the MCT treatment (118 mg/dL), intermediate for dogs fed the fasting treatment (95.2 mg/dL) and lower in dogs fed the control treatment (92.6 mg/dL).

### *Triglycerides*

There were significant differences among treatments and these differences depend on the hour of blood collection, because there was an interaction between treatment and hour ($P = 0.0060$). The treatments had effect ($P < 0.05$) at 12, 24 and 48 hours after meal, but there was no effect at the 36 hours ($P > 0.05$). There was a relevant response due to the hour of blood collection only for fasting treatment ($P < 0.0001$). At all times when there was a significant difference between treatments, the fasting treatment showed higher triglyceride values and was distinguished from the other treatments (Figure 2 B).

### *Non-esterified fatty acids*

The differences among treatments were dependent on the hour of blood collection, because there was an interaction between treatment and time (P = 0.0810). The treatments had an effect (P = 0.0241) in the evaluation of 36 hours after the meal. There were significant differences among the hours for fasting treatment (P < 0.0001; Figure 2 C). At hour 36 after meal, the NEFA level was higher in dogs that were fed the fasting treatment (0.996 mg/dL), intermediate in dogs fed the control treatment (0.608 mg/dL) and lower levels in dogs fed the MCT treatment (0.447 mg/dL).

#### *β-hydroxybutyrate*

The feeding programs showed no effect on the β-hydroxybutyrate levels (P > 0.05), as well as there was no interaction among any factors evaluated (Figure 2 D).

### *Cognition tests*

#### *Conditioning right and wrong*

The day factor showed significant difference (P < 0.0001). The feeding programs and their interactions with the day and gender factors did not show significant effects (P > 0.05). Observing the averages of the treatments it is possible to observe that the dogs fed control treatment reached the maximum of 100% of learning at the end of the period (day 30), while the dogs fed each 48 hours reached the maximum of 100% on day 22, and the dogs fed MCT treatment did not reach the maximum learning until the end of the experimental period (Figure 3).

Evaluating data by linear regression regardless of gender, considering 80% of correct answers as if the task was learned, dogs fed with the MCT treatment learn avidity on the 13th day, dogs fed control treatment learn the task on 12th day and dogs that were fed with the fasting treatment showed best results, learning the task on the 9th day.

When gender was used as a classification factor, a significant effect for gender was observed (P = 0.0114). The linear and quadratic components of time (days) are significant, as well as their interaction with treatments and gender (P <0.05). This result indicates different treatment responses depending on the days for different genders (Figure 4). Females achieve 80% of learning faster than males. The fasting treatment was more effective to increase the dogs' learning capacity (females 7 days; males 12 days), the basal treatment was intermediate (females 9 days; males 15 days) and the MCT treatment was less effective (females 10 days; males 16 days).

### *Gesture conditioning*

The treatments had no effect on gesture learning (P > 0.05). No effect on the interaction among treatments and the gender and day factors (P > 0.05) was observed.

### *Clicker*

The differences among treatments for Clicker learning were associated with gender and day, because there was interaction among the three factors (P = 0.0028; Figure 5). On the first day, MTC treatment was less efficient to improve

the learning for female dogs, followed by control and fasting treatments ($P < 0.05$). MCT treatment was less efficient to improve the learning for males on days 6 and 7, followed by control and fasting treatments.

### *Target*

The differences between treatments for target learning are associated with the day, because there was significance for the interaction between the day and treatment ($P = 0.043$; Figure 6). There are significant differences ($P = 0.0031$) among treatments on the second day of evaluation. The fasting treatment proved to be more efficient to improve the dogs' learning, the control treatment was intermediate and the MCT treatment was less efficient.

## Discussion

All dogs remained healthy and active throughout the experimental period. Dogs have adapted well to diets, alimentary program, metabolic cages, and to the box used to measure behavior. No leftovers were observed during all the study.

The study was designed to adapt dogs to the diets and the feed programs, then measure the digestibility, and metabolic changes and its effect on cognition. We hypothesized that MCT containing diet and dogs fasted for 48 hours would be a way to induce metabolic changes, especially increasing the ketone bodies in the blood circulation then, improving learning ability of young Beagle dogs. It is already known that feeding elderly dogs with MCT increases ketone bodies in blood, and it produces benefits on cognition (Pan et al., 2010). The idea of this proposal arose from the need to increase cognitive ability in young adult dogs to

make easier to teach them. In wild canids, fasting is a natural feeling present before hunting. In this stage dogs must be hungry, in catabolic state, and perhaps with high concentration of ketone bodies which may have some impact on strategies to hunt the pray.

Large intervals between meals may impact on intestinal passage rate and absorption could be overpassed, reducing digestibility. Also, the MCT replacing long chain triglycerides must have some impact, improving fat digestibility. However, digestibility coefficients were not affected in our study neither by the type of fat nor for the fast interval. Beynen et al. (2002) and Fragua et al. (2015) reported improvements only in the fat digestibility supplementing medium chain fatty acids in the diet of dogs. Fragua et al. (2015) supplement 2.35% and 3.65% of the total diet with coconut oil, an inclusion that is still lower than 5.66% (11% of ME) used by Beynen et al. (2002). In our study we used higher inclusion (5.5% purified MCT in the diet), once purified triglycerides were used instead of coconut oil which contains close to 60% of medium chain triglycerides.

The dogs had no change on body weight throughout the study. Dogs ingested the same content of macronutrients and energy in the control and MCT treatments, that met the MER. After digestibility assay been performed the content of energy from the diets was recalculated. It shows that dogs had the same ingestion of energy among treatments. Fasting has been associated with weight loss in several species (Secor et al., 2016). It was expected that dogs 48 hours fasted would lose weight. Low carbohydrate diets are more effective to induce ketogenesis, but extruded diet contains considerable amount of soluble

carbohydrates which can produce some effects on metabolism. Carbohydrates in the diet maintain the levels of glucose in circulation and no drastic withdrawal of lipid storage during the fasting period occurs. When there is a reduction in circulating glucose and, consequently, a reduction in insulin, the activation of the intracellular enzyme hormone-sensitive lipase (**LHS**) occurs. LHS hydrolyzes triglycerides stored in free fatty acids and glycerol (Nelson et al., 2010). The speed at which this process occurs and the amount of lipid removed from stores may be associated with the amount of energy provided in the diet during fasting. In the Leung et al. (2020) study, dogs were fed intermittent fasting (48h) and the dogs lost more weight when intermittently fasted on a low fat diet, but there was no difference in the percentage of body weight change when the dogs were daily fed compared to when they were intermittently fasted on a high fat diet.

In our study, dogs 48 h fasted had plasmatic glucose concentrations within the normal reference range, however high values of triglycerides and glucose were obtained at 12h. This reflects the consumption of 200% of ME in a single meal rather than the macronutrient composition of the diets or the fasting time. It is possible to observe the reduction in glucose and triglycerides over the fasting hours, associated with an increase in the circulating NEFA from 24 hours, however there was no change in the level of β-hydroxybutyrate (ketone body). The accumulation of hepatic triglycerides is physiological, but when the NEFA intake exceeds the liver's ability to oxidize fatty acids, there is an accumulation of intermediate metabolites, known as ketone bodies, including β-hydroxybutyrate (Palmquist & Mattos, 2011). Fasting is characterized by low

glucose concentrations and low levels of insulin and high glucagon. NEFA released in response to fasting promotes maintenance of whole-body energy homeostasis in the absence of an external energy supply (Bertolucci et al., 2008). The increase in NEFA concentrations during fasting may reflect the mobilization of adipose tissue mediated by the decrease in insulin, which leads to inhibitory effects on lipogenesis and lipolysis stimulants (Bertolucci et al., 2008; Desvergne et al., 2006). A discussao está parecendo a revisao bibliográfica. Muito longa e distante ds resultados

The amount of β-hydroxybutyrate was not influenced by the treatments, even with the MCT enrichment or in fasting period. This result may be associated with the level of inclusion and the triglyceride profile of the product used to supplement MCT in the diet, and the presence of carbohydrates in the fasting treatment. The concurrent switch from carbohydrate to lipid metabolism stems from the near depletion of glycogen stores and a new reliance on the catabolism of energy-dense lipid stores (Secor, 2016). Leung et al. (2020) found that dogs fasted for 48 h on a high fat diet enriched in medium chain triglycerides (14.7% of the total calories in the diet) promoted higher blood β-hydroxybutyrate. Leung et al. (2020) and Pan et al. (2010) and other authors substituted fat for coconut oil, which is composed of fatty acids: C: 6, C: 8, C: 10, C: 12, C: 14, C: 16, C: 18, and C: 20, containing higher concentrations of lauric (C: 12 - 48%) and myristic (C: 14 - 19%) acids (Senphan & Benjakul, 2016). In our study, triglycerides came from a purified product, with higher concentrations of caprylic fatty acid (C: 8 – 50-60%). It is known that medium chain fatty acids have distinct metabolisms in the brain, for example, C10: 0

promotes glycolysis and lactate formation, while C8:0 increases the ketogenesis rates of astrocytes. These effects can activate transport systems that supply nutrients to neighboring neurons in the form of lactate and ketone bodies (Thevenet et al., 2016).

Other factors to be considered are how long MCT was supplied in diet and the dogs age. It was established that dogs show marked age-dependent decline in learning and memory, which varies as a function of task (Adams et al., 2000; Chang et al., 2002). According to Pan et al. (2010), they offered to the elderly dogs a diet 5·5% purified MCT replacing beef tallow for 8 months, and the dogs fed the MCT diet showed significantly elevated levels of β-hydroxybutyrate in the fourth and eighth month of the experiment. In addition, although a single blood sample is indicative of the concentration of a metabolite at that moment, it does not describe its flux of production and utilization (Reichard, 1974)

The brain does not use only glucose as an exclusive energy source but can also use ketone bodies as energy when it is available. The β-hydroxybutyrate provide an alternative source of energy for neurons and is preferentially utilized over lactate and pyruvate by neurons as an energy substrate (Valente-Silva et al., 2015). During learning, dogs use memory and their brain is conditioned to the use of energetic substrates. In the present study, dogs went through a learning process with increasing levels of learning.

The first teaching task was to condition the dog to learn which side of the WGTA should choose the snack. The dogs that were fed the control and fasting treatments achieved 100% learning and the dogs that received the MCT

508 treatment did not reach 100% within the testing period. Landsberg et al. (2005)
509 considers that dogs typically are judged to have learned a task if they achieve a
510 score higher of 90%, or scores of 80% over 2 days and then maintain a score of
511 70% over the next 3 testing sessions. Considering this information, when
512 observing the learning graph (Figure 3) of the dogs, it is noticeable that dogs of
513 the control and fasting treatments remained above 80% from day 18, whereas
514 the dogs of the MCT treatment reduced the level of correct answers. The results
515 of the present study are contrary to the results found by Pan et al. (2010), they
516 observed that MCT supplementation tended to improve spatial learning and
517 memory and visual-spatial attention within 2 weeks after the MCT
518 supplementation in old dogs. It is necessary to emphasize that in the present
519 study, the MCT treatment did not increase the levels of β-hydroxybutyrate,
520 which may reflect a great difference in the result between the studies. Another
521 significant difference between the studies is the age of the dogs. Results found
522 by Milgram et al. (2005) suggest that young dogs reach maximal performance
523 of learning between about two and four years of age, and that performance
524 begins to fall off around five years of age. By eight years of age, there are clear
525 and consistent age-dependent impairments.

526 The second task, which was performed at the beginning of the second trial
527 period, aimed conditioning the dog to stand on all fours looking at the
528 evaluator's hand. Despite having a higher level of difficulty than the previous
529 task, in this task the treatments did not affect the dogs' behavior and they
530 performed the task at similar times. However, in the third task, fasting treatment
531 was effective in improving the dog's learning, as they took less time to stand on

all fours, looking forward when they heard the clicker sound. In this task, a difference was also observed between genders. This result can be explained by the presence of ovarian hormones in the adult dog as the main determinant of the observed differences in learning (Mongilo et al. (2017). Fluctuations in reproductive hormones across the estrous cycle have been shown to bias learning strategies through the activation of different memory systems (Korol et al. 2004), and females are more prone to memorize sequences of motor actions (Waller & Nadel, 2013).

There is a strong relationship between neuronal activity and the use of cerebral glucose. The maintenance of neuronal activity at a high level depends on the increase in ATP production (Magistretti, 2009). Fasting animals utilize endogenous glucose, lipids (e.g., glycerol, fatty acids, and ketone bodies), and amino acids to produce the ATP necessary to fuel cellular processes (Secor et al., 2016). Even though the treatments did not increase the level of β-hydroxybutyrate, it was observed that the NEFA level increased significantly in the fasting treatment, which may explain the better cognitive result in the tasks 1 and 3. This explanation also applies to the last and most difficult task.

In the last task, the purpose of the test was for the dog associate touching a black circle with its snout with the sound of the clicker, and fasting treatment proved to be more efficient in promoting improvement in dogs' learning. Throughout the experimental period, the evaluator noticed that the dogs of the fasting treatment were more attentive and performed the activities with greater precision than the others. In addition to the explanations given by the results found in the study itself, some other factors can also be considered for the best

learning result in the fasting treatment. Lactate is transported by the same monocarboxylate transporters as β-hydroxybutyrate and serve as an energy source for cells during fast (Bouzier-Sore et al., 2003). Lactate concentrations are increased in the brain of humans and rats fasted for 2 days (Leino et al., 2002; Pan et al., 2000). Leung et al. (2020) justify that the reduction of lactate in dogs with intermittent fasting (48 h) may be due to an increase in absorption by the liver, brain and kidneys. Fasting may confer cognitive benefit also by increasing brain GABA levels, which, in turn, can counteract the neurotoxic effect of excessive glutamate (Wang et al., 2003). The mechanism has been associated to the induction of glutamate decarboxylase, an enzyme that facilitates the conversion of glutamate into GABA (Cheng et al., 2004). As well as, intermittent fasting has been reported to induce the production of brain-derived neurotrophic factor (Martin et al., 2006), which is associated with neurogenesis and molecular learning and memory, particularly in the hippocampus (Vedovelli et al., 2011; Rault et al., 2018).

Collectively, the cognitive assessment data demonstrate that fasting treatment is more effective in increasing learning ability than control and MCT treatments, however it is necessary to emphasize that the result of MCT supplementation may be closely associated with the dose used and profile of fatty acids compounding MCT.

## Conclusions

Fasting for 48 hours increased the amount of non-esterified fatty acids in the blood and increase the learn ability of healthy adult dogs, but it is not possible to

observe increase of β-hydroxybutyrate in circulation. The substitution of 5.5% of the feed coverage by MCT is not able to promote the increase in the level of non-esterified fatty acids and β-hydroxybutyrate, as well as it is not able to improve the learn ability of dogs.

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## Declaration of interest

We declare no conflict of interest, including financial, personal, or other relationships with other people or organizations that could inappropriately influence this work**.**

## Ethics Committee

All procedures performed in this study were approved by the ethics committee of the Federal University of Rio Grande do Sul under protocol number 36138 and were conducted in accordance with ethical and animal welfare standards.

## Software and data repository resources

None of the data were deposited in an official repository.

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744 **Table 1.** Composition of experimental diets (as fed basis) provided to dogs
745 (Beagle breed)

| Ingrediets, % | Control* Diet | Fasting diet | MCT diet |
|---|---|---|---|
| Corn grain | 16,7 | 16,7 | 16,7 |
| Brewers rice | 24,4 | 24,4 | 24,4 |
| Wheat bran | 6,23 | 6,23 | 6,23 |
| Corn gluten meal | 7,65 | 7,65 | 7,65 |
| Poultry fat | 11,0 | 11,0 | 5,50 |
| Medium chain tryglicerides | 0,00 | 0,00 | 5,50 |
| Sugar cane fiber | 0,98 | 0,98 | 0,98 |
| Meat and bone meal | 0,98 | 0,98 | 0,98 |
| Chicken byproducts meal | 30,2 | 30,2 | 30,2 |
| Salt | 0,45 | 0,45 | 0,45 |
| Potassium chloride | 0,18 | 0,18 | 0,18 |
| Premix[1] | 0,36 | 0,36 | 0,36 |
| Yucca schidigera extract | 0,03 | 0,03 | 0,03 |
| Liquid caramel dye | 0,89 | 0,89 | 0,89 |
| Analyzed composition, % | | | |
| Dry matter | 91,5 | 91,5 | 91,9 |
| Organic matter | 93,4 | 93,4 | 93,4 |
| Moisture | 10 | 10 | 10 |
| Crude protein | 31,5 | 31,5 | 31,1 |

| | | | |
|---|---|---|---|
| Crude fiber | 1,05 | 1,05 | 0,94 |
| Ether extract acid hidrolysis | 14,3 | 14,3 | 15,1 |
| Ash | 6,63 | 6,63 | 6,56 |
| Gross energy, kcal/kg | 4810 | 4811 | 4762 |
| Metabolizable energy, kcal/kg | 4177 | 4037 | 4081 |

[1]Addition per kilogram of product: Vitamin A (min) 10,800 IU; Vitamin B1 (min) 8.4 mg; Vitamin B12 (min) 30 mcg; Vitamin B2 (min) 6 mg; Vitamin B6 (min) 6 mg; Vitamin D3 (min) 940 IU; Vitamin E (min) 60 IU; Vitamin K3 (min) 4.5 mg; Niacin (min) 55 mg; Folic acid (min) 1.2 mg; Pantothenic acid (min) 12 mg; Biotin (min) 0.08 mg; Cobalt (min) 10 mg; Copper (min) 7 mg; Iron (min) 80 mg; Iodine (min) 1.5 mg; Manganese (min) 7.5 mg; Selenium (min) 0.35 mg; Zinc (min) 100 mg; Taurine (min) 400 mg; BHT (antioxidant, min) 155 mg.
*Control and fasting diets: 11% poultry fat was used as a cover for the extruded feed; MCT diet: poultry fat was a half replaced by 5.5% of medium chain triglycerides (Concepta Ingredients®) to cover the extruded feed. Concepta Ingredients composition (%): C 6:0 - max 1,0; C 8:0 - 50,0 to 65,0; C 10:0 - 34,0 to 45,0; C 12:0 - max 2,0; C 14:0 - max 1,0.

**Table 2.** Animal weight, nutrient intake, digestibility coefficients and fecal characteristics of the experimental feed programs provided to dogs (Beagle breed) in the two 40-day experimental blocks

| **Variable** | **Treatments** | | | | | **Dog** | **Block** |
|---|---|---|---|---|---|---|---|
| | **Control diet 50/50%[1]** | **Control diet Fasting[2]** | **MCT diet 50/50%[3]** | **SEM[4]** | **P value[5]** | **P value[5]** | **P value[5]** |
| Body weight. Kg | 13.2 | 13.3 | 14.2 | | 0.669 | 13.5 | 13.7 |
| Factor. kcal ME/kg$^{0.75}$ | 112 | 102 | 107 | 2.35 | 0.285 | 0.315 | 0.418 |
| Daily intake. g/dia | | | | | | | |
| DM | 156 | 136 | 147 | 4.83 | 0.262 | 0.205 | 0.599 |
| OM | 136 | 131 | 135 | 4.41 | 0.071 | 0.001 | 0.025 |
| CP | 64.5 | 62.0 | 63.1 | 2.06 | 0.079 | 0.001 | 0.031 |
| EEHA | 21.0[a] | 20.2[b] | 21.8[a] | 0.72 | 0.013 | 0.001 | 0.012 |
| NFE | 48.4 | 46.6 | 47.9 | 15.6 | 0.063 | 0.001 | 0.030 |
| MM | 9.91 | 9.45 | 9.54 | 0.31 | 0.154 | 0.006 | 0.020 |
| ME. kcal/dia | 777 | 724 | 775 | 26.3 | 0.055 | 0.004 | 0.020 |
| Apparent digestibility. % | | | | | | | |
| Natural matter | 55.4 | 44.8 | 56.6 | 1.69 | 0.123 | 0.538 | 0.182 |
| DM | 80.3 | 76.9 | 81.9 | 0.74 | 0.210 | 0.545 | 0.092 |
| OM | 85.0 | 82.2 | 86.6 | 0.57 | 0.157 | 0.486 | 0.106 |
| CP | 85.6 | 82.2 | 85.9 | 0.69 | 0.381 | 0.680 | 0.127 |
| NFE | 83.3 | 81.3 | 84.7 | 0.56 | 0.255 | 0.405 | 0.121 |
| EEHA | 89.8 | 88.3 | 92.0 | 0.41 | 0.146 | 0.466 | 0.115 |
| MM | 16.1 | 3.88 | 16.8 | 3.45 | 0.436 | 0.671 | 0.052 |
| Digestibility of energy | 86.1 | 83.4 | 87.1 | 0.55 | 0.193 | 0.500 | 0.115 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Metabolizability of energy | 79.1 | 76.7 | 80.0 | 0.49 | 0.205 | 0.488 | 0.115 |
| Fecal characteristics | | | | | | | |
| Fecal Score[6] 1 to 5 | | | | | | | |
| Fecal DM % | 40.5 | 38.4 | 38.0 | 0.48 | 0.257 | 0.601 | 0.081 |
| Faeces g/d (As is) | 99.7 | 119 | 94.1 | 4.62 | 0.157 | 0.158 | 0.275 |
| Faeces g/d (DM) | 40.5 | 45.9 | 35.7 | 1.88 | 0.202 | 0.149 | 0.108 |

[1]Control Diet - 11% poultry fat was used as a cover for the extruded diet and it was provided 50% of the MER in the morning and 50% in the afternoon. [2]Control diet Fasting - The diet was offered every two days. with 200% of the MER in only one meal. 3 MCT diet 50/50% - a half of the poultry fat from the control diet was substituted with purified medium chain triglycerides and the diet was provided twice a day. SEM[4] - Standard error of the mean; P value[5] - Probability; [a, b] Means followed by different letters differ statistically by the Tukey test (P < 0.05); Number of repetitions: 8.; [6]Fecal score; 1 = hard: dry pellet; 2 = hard. formed. and dry stool remains firm and smooth. 3 = soft. formed. and moist: softer stool that retains shape; 4 = soft: formless stool assumes shape of container and 5 = watery: liquid that can be poured.

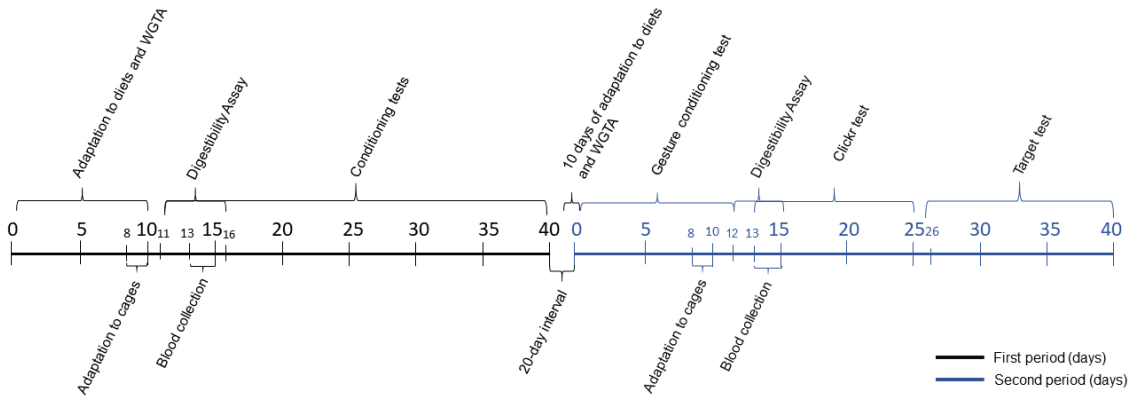



**Figure 1.** Timeline of experimental periods of dogs (Beagle breed) fed with three feeding programs

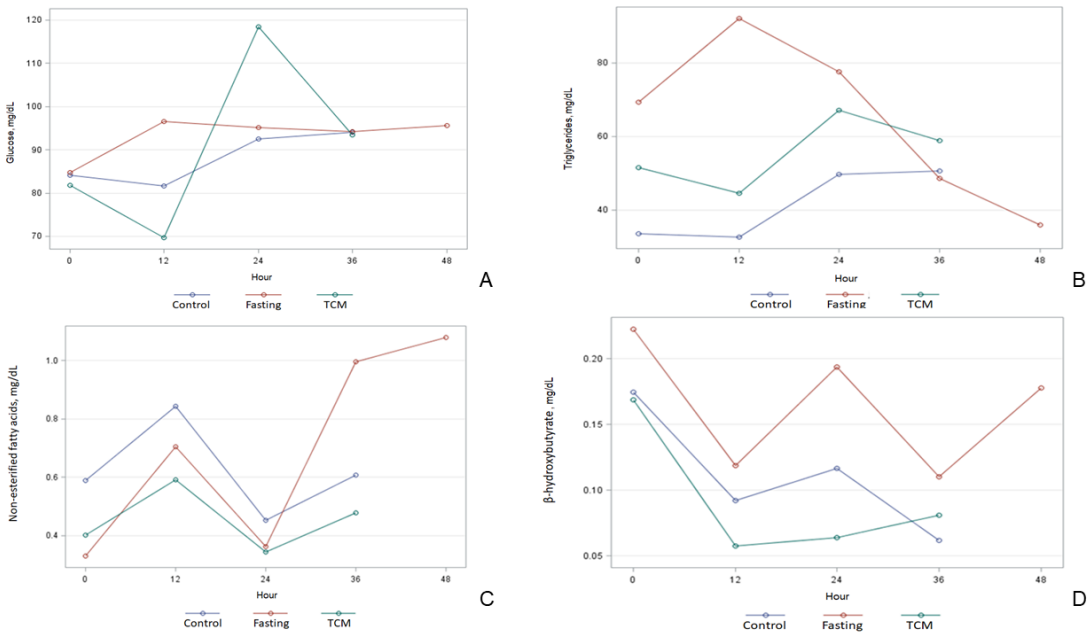



**Figure 2.** Glucose (A), triglycerides (B), non-esterified fatty acids (C) and β-hydroxybutyrate (D) levels of dogs (Beagle breed) fed with three feeding program. Each point of the graphic is a mean of 8 dogs.

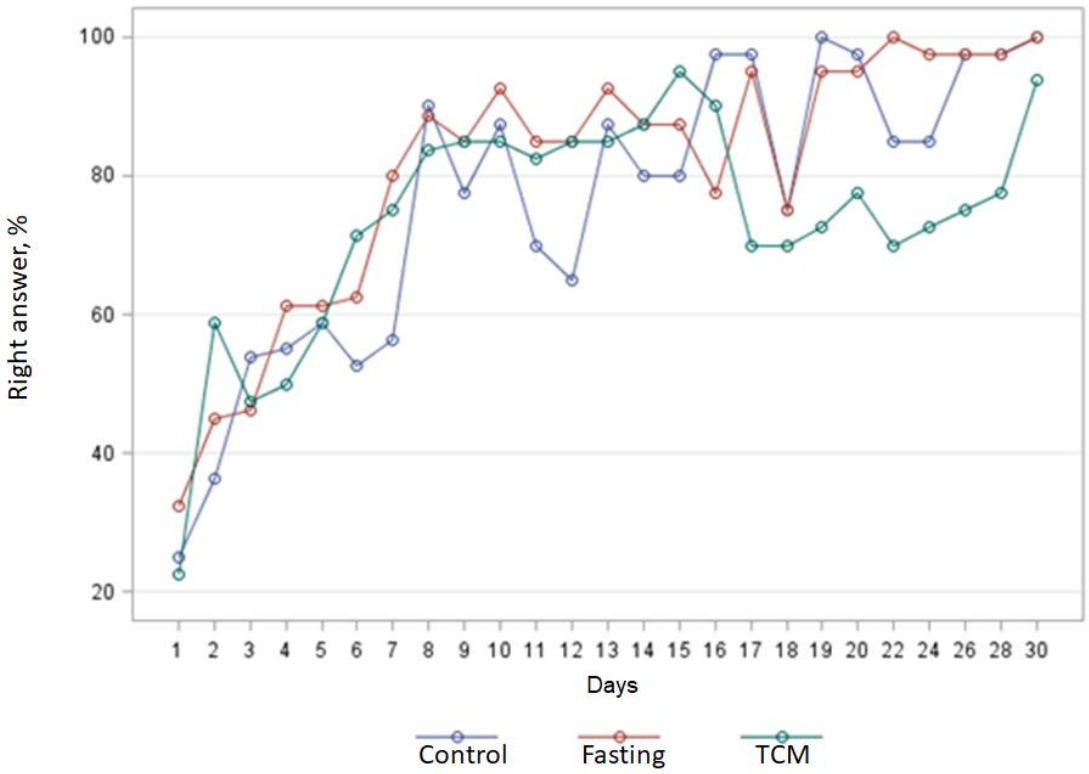
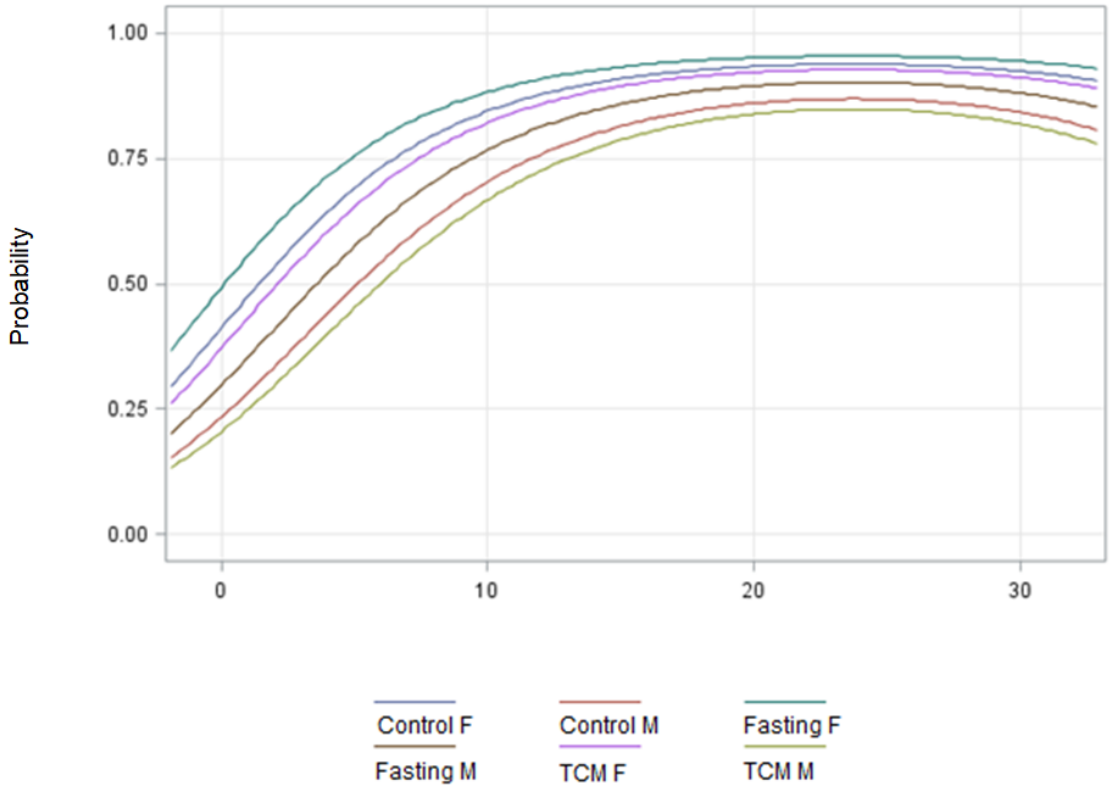



**Figure 3.** Learning behavior of right and wrong conditioning of dogs (Beagle breed) fed with three feeding programs over 30 days; Each point is a mean of 330 evaluations.




790
791 **Figure 4.** Learning behavior of right and wrong conditioning of dogs (Beagle
792 breed) according to female (F) and male (M) fed with three feeding programs

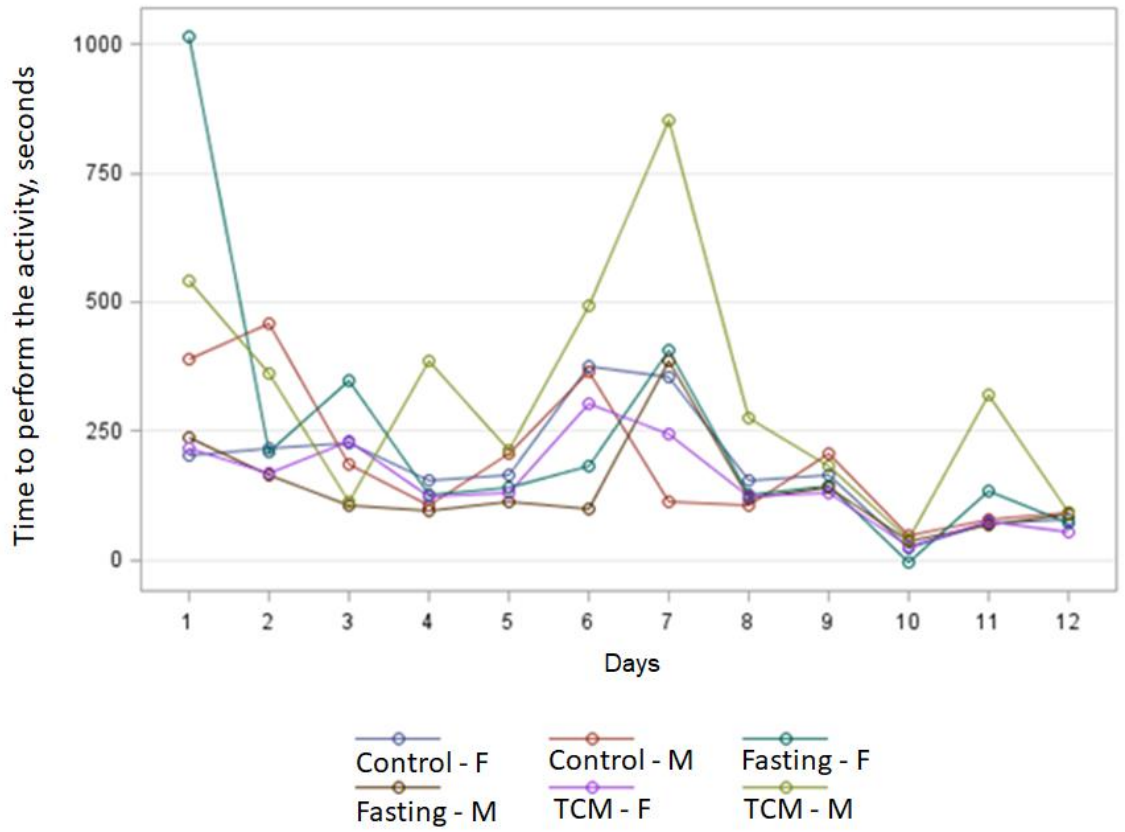
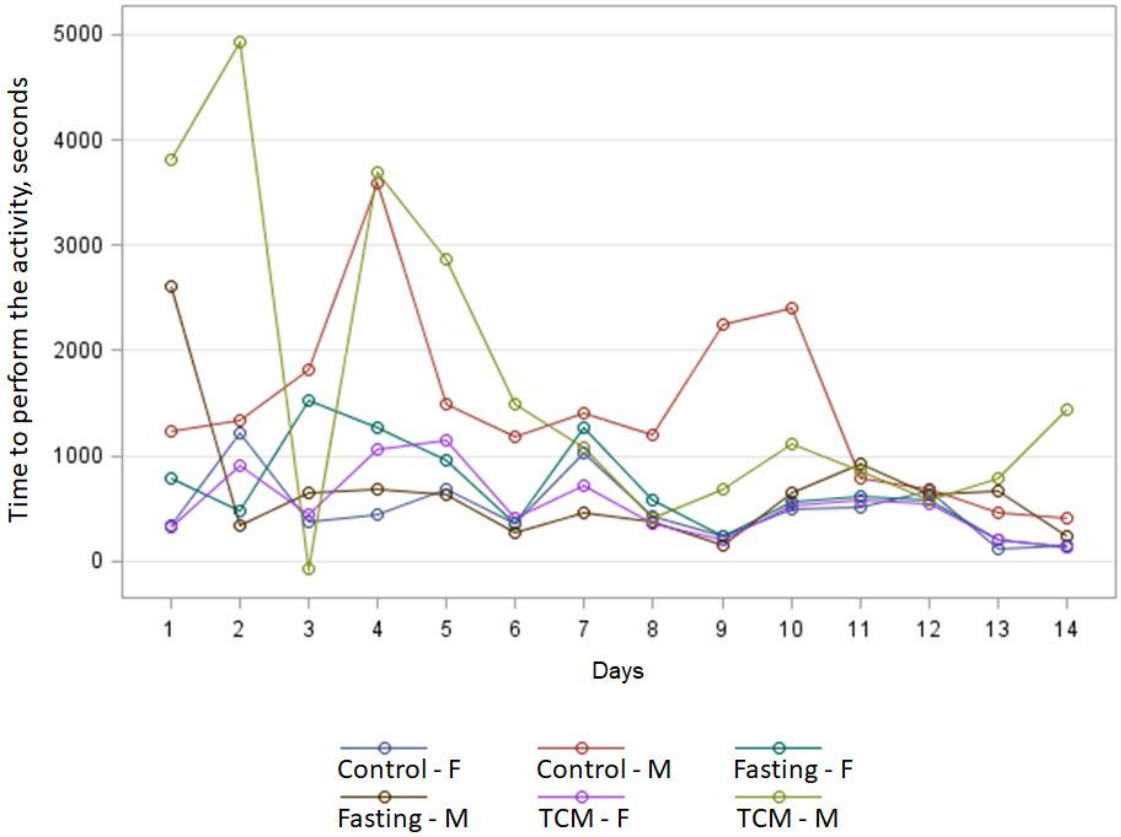



**Figure 5.** Time that dogs (Beagle breed) took to perform the task with the Clicker according to female (F) and male (M) fed with three feeding programs; Each point is a mean of 130 evaluations.




798
799 **Figure 6.** Time that dogs (Beagle breed) took to perform the task with the Target
800 according to female (F) and male (M) fed with three feeding programs; Each point
801 is a mean of 140 evaluations.

# CAPÍTULO II

## CONSIDERAÇÕES FINAIS

Os cães estão alcançando idades mais avançadas e as expectativas de vida estão cada vez mais longas. A longevidade do cão é acompanhada pelos mesmos transtornos observado em humanos. Conforme a idade avança a capacidade de aprendizado já não pode ser comparada a de um cão jovem. Alguns estudos atribuem às dietas, ingredientes, programas alimentares e atividade física a melhora cognitiva de cães. Apesar de se conhecer alguns nutrientes capazes de melhorar a cognição de cães em idades mais avançadas não se sabe se estes mesmos ingredientes podem melhorar a capacidade de aprendizados em cães mais jovens. De fato, se isto fosse possível, a administração de nutrientes específicos favoreceria os treinamentos de animais adultos, por exemplo.

Neste contexto, a formação de corpos cetônicos em cães aprece ser uma promissora via para estimular a cognição. O aumento de corpos cetônicos pode ser proporcionado com maior aporte de ácidos graxos de cadeia média via dieta ou pelo jejum. Porém não se conhece o efeito em cães jovens.

O programa alimentar utilizando o jejum por 48 horas, parece se mostrou mais efetivo na melhora cognitva de cães adultos, neste estudo. Os cães em jejum se mostraram, visivelmente, mais atentos durante a tarefa, buscando a execução de forma ágil e correta. Os testes de aprendizagem devem ser efetuados em tempo curto e com diversas tentativas, assim, evitando a aversão à aprendizagem e tornando a tarefa algo agradável.

Estudos futuros que possibilitem a exploração cognitiva de cães adultos de forma direcionada, ensinando tarefas com gradativo grau de dificuldade podem melhorar a discriminação dos resultados. É importante inserir uma serie de aprendizados em ordem crescente de dificuldade ao longo do tempo. Cada indivíduo deve ser observado com atenção, afinal o comportamento possui um fator de personalidade bastante forte e pode influenciar nos resultados. O uso de quadrado latino completo e crossover podem ser delineamentos importantes para minimizar o efeito do indivíduo.

Os testes que avaliam corpos cetônicos são pouco sensíveis a variações plasmáticas, especialmente àqueles que utilizam química seca. Estes testes servem para evidenciar variações grandes nas concentrações plasmáticas. Testes laboratoriais utilizando química úmida são os mais adequados pois revelam valores contínuos e permitem maior distanciamento entre os níveis de corpos cetônicos circulantes.

A suplementação de triglicerídeos de cadeia média pode ser feita de várias formas na alimentação. Eles podem ser provenientes dos ingredientes da dieta ou podem ser provenientes de formas purificadas associados ou não ao glicerol, na forma de triglicerídeo. Ainda, podemos ter triglicerídeos contendo um mesmo ácido graxo de cadeia média esterificado, formando um triglicerídeo puro. A forma com que são administrados aos animais podem alterar as rotas oxidativas e o resultado de suas avaliações devem ser considerados.

Os efeitos esperados para a suplementação de ácidos graxos de cadeia média em cães foram observados com período mais longo de suplementação.

Neste estudo utilizamos 40 dias e talvez esta possa ter sido uma das causas por não observarmos aumento significativo dos níveis de corpos cetônicos.

O jejum pareceu ter sido um fator alimentar importante. Cães alimentados neste regime parecem oxidar mais lipídeos que os cães alimentados duas vezes ao dia. Ao mesmo tempo, a proposta do jejum deve ser vista como uma proposta para entender o metabolismo animal sob diferentes programas alimentares. Aqui, não está sendo proposta a alimentação de cães a cada 48 horas, mas estamos tentando entender como o regime alimentar afeta o metabolismo. Cães de raças grande e gigantes em regime alimentar em que grandes refeições são fornecidas com intervalos longos podem cursar com volvo gástrico devido ao tamanho da refeição. Portanto, mais estudos são necessários para entender como diferentes raças são afetadas pelos programas alimentares e dietas.

Neste estudo não foi observada a taxa de esvaziamento gástrico e a taxa de passagem do alimento no trato gastrintestinal. Esta seria uma avaliação importante, uma vez que a digestibilidade da matéria seca e dos nutrientes da dieta não foram afetados pelo programa alimentar. Entender se grandes refeições produzem lentidão no esvaziamento gástrico podem ser um ponto central para entender o perfil hormonal pós-prandial dos hormônios e a influência sobre a saciedade. Observando o comportamento dos cães ancestrais, grandes refeições fazem parte dos hábitos alimentares e talvez cães possam ainda ser influenciados por regimes dietéticos mais espaçados. Entender a influencia destes regimes alimentares e de nutrientes específicos sobre a resposta digestiva, metabólica e cognitiva de cães continua sendo objetivo da pesquisa.

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# APÊNDICES

## Apêndice A – Carta de aprovação do Comitê de ética no uso de animais

U F R G S
UNIVERSIDADE FEDERAL
DO RIO GRANDE DO SUL

**PRÓ-REITORIA DE PESQUISA**

**Comissão De Ética No Uso De Animais**

CEUA UFRGS

***CARTA DE APROVAÇÃO***

**Comissão De Ética No Uso De Animais analisou o projeto:**

**Número:** 36138
**Título:** COMPORTAMENTO INGESTIVO E DE SACIEDADE EM SUÍNOS E CÃES

**Vigência:** 01/01/2019 à 01/01/2022

**Pesquisadores:**

**Equipe UFRGS:**

LUCIANO TREVIZAN - coordenador desde 01/01/2019
PEDRO HENRIQUE SESSEGOLO FERZOLA - Outra Função desde 01/01/2019
Bruna Cristina Kuhn Gomes - Aluno de Doutorado desde 01/01/2019
Caroline Fredrich Dourado Pinto - Aluno de Mestrado desde 01/01/2019
Aline Kummer de Souza - Aluno de Mestrado desde 01/01/2019

***Comissão De Ética No Uso De Animais aprovou o mesmo , em reunião realizada em 14/01/2019 - Sala 330 do anexo I do prédio da Reitoria, Campus Centro. , em seus aspectos éticos e metodológicos, para a utilização de provenientes de 12 cães da raça Beagle, seis machos e seis fêmeas com 10 a 14 Kg e idade entre 4 e 5 anos, provenientes do canil experimental da Faculdade de Agronomia da UFRGS, de acordo com os preceitos das Diretrizes e Normas Nacionais e Internacionais, especialmente a Lei 11.794 de 08 de novembro de 2008, o Decreto 6899 de 15 de julho de 2009, e as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), que disciplinam a produção, manutenção e/ou utilização de animais do filo Chordata, subfilo Vertebrata (exceto o homem) em atividade de ensino ou pesquisa.***

Porto Alegre, Sexta-Feira, 8 de Fevereiro de 2019

ALEXANDRE TAVARES DUARTE DE OLIVEIRA
Vice Coordenador da comissão de ética

## Apêndice B – Normas para redigir o capítulo III – Publicação no periódico (Animal)

*animal*
**An International Journal of Animal Bioscience**

# Instructions for authors

**Last updated June 2018**

## Introduction

***animal*** – an International Journal of Animal Bioscience is a peer-reviewed journal, published monthly in English, in both print and online formats (12 issues making a volume). Special issues or supplements may also be produced upon agreement with the Editorial Board. There are no page charges, except for reproduction of illustrations printed in colour and for the Open Access option that requires payment of an Article Processing charge.

The scope of the journal, the expected standards of published articles, the article types published by *animal*, the ethics policy, the evaluation procedures and peer-review criteria, the handling of misconducts as well as procedures for complaints and appeals are presented in the Publication policies available at https://www.cambridge.org/core/journals/animal/information/instructions-contributors.

Submitted manuscripts should not have been published previously, except in a limited form (e.g. abstract or short communication to a symposium or part of MSc or PhD theses) and should not be under consideration for publication by another journal. Book reviews are not accepted.

## General specifications for different types of article

**Table 1** *Specifications for the articles published in* ***animal***

| Article type | Maximum length (all text except figures) | Maximum number of tables plus figures | Maximum number of references | Additional information |
|---|---|---|---|---|
| Original research | 7 000 words (= 9 journal pages) | 8 | 35 | |
| Short communications | 3 000 words | 3 | 10 | |
| Reviews | 9 500 words (= 12 journal pages) | 10 | 50 | |
| Opinion papers | 1700 words (= 2 journal pages) or 1 200 if a figure is submitted | 1 | 5 | |
| All article types | | | 5 references per 1000 words | Supplementary material can be proposed and will be made available online |

## Recommendations for preparation of papers

**The responsibility for the preparation of a paper in a form suitable for publication lies with the author**. Authors should consult recent articles of *animal*, available at https://www.cambridge.org/core/journals/animal, to make themselves familiar with the layout and style of *animal*. A **style sheet** summarising these indications is available on our website at https://www.cambridge.org/core/journals/animal/information/instructions-contributors.

**Before submitting your manuscript, you should consult the pre-submission checklist at** (https://www.cambridge.org/core/journals/animal/information/instructions-contributors). **Manuscripts that do not comply with the specifications described in Table 1 or with the directions detailed below will not be accepted for peer-review**. Compliance with instructions will ensure that manuscripts are peer reviewed exclusively on academic merit. Any deviations from these instructions will be at the discretion of the Editor-in-Chief.

All co-authors must agree with the content of the manuscript. Authors must have obtained permission to use copyrighted material in the manuscript prior to submission. Work described in the manuscript must comply with ethical guidelines available on the website https://www.cambridge.org/core/journals/animal/information/instructions-contributors and be reported according to "The ARRIVE Guidelines for Reporting Animal Research" detailed in Kilkenny *et al.* (2010)[1] and summarised at www.nc3rs.org.uk.

***Scientific writing***

A good quality of scientific writing is required. The research must be understandable by a general scientific readership and by specialists. The research problem is identified, existing knowledge relevant to the problem is analysed, the hypothesis is clear. The reporting is complete. The central message is identified. Arguments and evidence are presented in a clear, logical and balanced way from the most general to the specific points. Discussion connects all results obtained in an organised and proper way with a clear interpretation. Sentences are simple, short and direct, the style is concise and precise.

***English***

A good quality of written English is required. Spelling may be in British or American English, but must be consistent throughout the paper. Care should be exercised in the use of agricultural terminology that is ill-defined or of local familiarity. If the English is not good enough, the manuscript will be sent back to the authors with a recommendation that authors have their manuscripts checked by an English language native speaker before re-submission. Cambridge University Press lists a number of third-party services specialising in language editing and / or translation at: https://www.cambridge.org/core/services/authors/language-services and suggests that authors contact them as appropriate. Use of any of these services is at the author's own expense. The copy-editor will not perform language editing.

***Manuscript layout***

Manuscripts should be prepared using a standard word processing programme such as Microsoft Word, and presented in a clear, readable format with easily identified sections and headings. A style sheet is available on our website at https://www.cambridge.org/core/journals/animal/information/instructions-contributors.

*Manuscript layout directions*

- Typed with double-line spacing with wide margins (2.5 cm)
- Lines must be continuously numbered; the pages must also be numbered
- Arial 12 should be used for the text, and Arial 11 for tables and references
- Sections should typically be assembled in the following order: Title, Authors, Authors' affiliations including department and post/zip codes, Corresponding author, Short title, Abstract, Keywords, Implications, Introduction, Material and methods, Results, Discussion, Acknowledgements, Declaration of interest, Ethics committee, Software and data repository resources, References, Tables, List of figure captions

[1] Kilkenny C, Browne WJ, Cuthill IC, Emerson M and Altman DG 2010. Improving bioscience research reporting: The ARRIVE guidelines for reporting animal research. PLoS Biology 8, e1000412. doi: 10.1371/journal.pbio.1000412.

- Use of small paragraphs with less than 6 to 8 lines must be avoided
- Footnotes in the main text are to be avoided
- The manuscript complies with the section specific requirements set out below

***Full title***
The title needs to be concise and informative. It should:
(*a*) attract the attention of a potential reader scanning a journal or a list of titles;
(*b*) provide sufficient information to allow the reader to judge the relevance of a paper to his/her interests;
(*c*) incorporate keywords or phrases that can be used in indexing and information retrieval, especially **the animal species** on which the experiment has been carried out;
(*d*) avoid inessentials such as 'A detailed study of ...', or 'Contribution to ...';
(*e*) not include the name of the country or of the region where the experiment took place;
(*f*) not include Latin names, if there is a common name, or abbreviations.

*Full title directions*
- No more than 170 characters including spaces
- Include "Review:", "Invited review:" or "Animal board invited review:" before the full title if required (see Table 1)
- Title of an invited opinion paper should start with "Opinion paper:"
- Title of a short communication should start with "Short communication:"

***Authors and affiliations***
Information, such as author names and affiliations, may be automatically extracted at the time of submission. To take advantage of the extraction process, you must 1) use a superscript number after each author name and, 2) begin each full affiliation with the corresponding superscript number as follows:
Example

J. Smith[1,a], P.E. Jones[2], J.M. Garcia[1,3] and P.K. Martin Jr[4] [initials only for first names]
[1]*Department of Animal Nutrition, Scottish Agricultural College, West Main Road, Edinburgh EH9 3JG, UK*
[2]*Animal Science Department, North Carolina State University, Raleigh, NC 27695-7621, USA*
[3]*Laboratorio de Producción Animal, Facultad de Veterinaria, Universidad de Zaragoza, C. Miguel Servet, 177, 50013, Zaragoza, Spain*
[4]*Dairy Science Department, North Carolina State University, Raleigh, NC 27695-7621, USA*

[a]*Present address: Dairy Science Laboratory, AgResearch, Private Bag 11008, Palmerston North, New Zealand* (for any author of the list whose present address differs from that at which the work was done)

Corresponding author: John Smith. E-mail: John.Smith@univ.co.uk.

The corresponding author who submits and manages the manuscript during the submission/review process must be registered on Editorial Manager. He or she can be different from the corresponding author indicated in the manuscript who will be the correspondent for the published paper. Only one corresponding author is indicated in the manuscript.

***Short title (max 50 characters including spaces)***
Authors should provide a short title (after the corresponding author line) with the same specifications as the full title for use as a running head. If the short title is not appropriate, it could be modified by the Editorial Office, with the author's agreement.

***Abstract (max 400 words, single paragraph)***
The abstract should be complete and understandable, without reference to the paper. It is important to attract the attention of potential readers. The context and the rationale of the study are presented succinctly to support the objectives. Experimental methods and main results are summarised but should not be overburdened by numerical values or probability values. The abstract ends with a short and clear conclusion. Citations and references to tables and figures are not acceptable. Abbreviations used in the abstract must be defined in the abstract.

***Keywords (5 keywords)***
Keywords are essential in information retrieval and should not repeat words in the title with respect to indicating the subject of the paper.

*Keyword directions*

- Five keywords
- Keywords should be short and specific
- The animal species or type is among the keywords but differently from the title
- The use of non-standard abbreviations in the list of keywords is not allowed

***Implications (max 100 words)***

Implications must explain the expected impact that the results may have on practice, when they will be applied. Impact may be economic, environmental or social. Implications should not be limited to presenting the context and objectives, and should not be an "abstract of the abstract". They are written in simple English suitable for non-specialists or even non-science readers. Use of non-standard abbreviations is discouraged.

***Introduction***

The introduction briefly outlines the context of the work, presents the current issues that the authors are addressing and the rationale to support the objectives, and clearly defines the objectives. For hypothesis-driven research, the hypothesis under test should be clearly stated. Increasing the knowledge on a subject is not an objective *per se*.

***Material and methods***

Material and methods should be described in sufficient detail so that others can repeat the experiment. Reference to previously published work may be used to give details of methods, provided that references are readily accessible and in English.

Critical methodologies, including mathematical equations and statistical models must be described in detail either in the Material and Methods section or in the Supplementary Materials. For these critical methodologies, results from quality control tests must be reported (e.g. intra/inter-assay CV, recovery tests…).

If a proprietary product is used as a source of material in experimental comparisons, it should be described using the appropriate chemical name. If the trade name is helpful to the readers, provide it in parentheses after the first mention. Authors who have worked with proprietary products, including equipment, should ensure that the manufacturers or suppliers of these products have no objections to publication if the products, for the purpose of experimentation, were not used according to the manufacturer's instructions.

***Statistical analysis of results***

The statistical analysis of results should be presented in a separate sub-section of the "Material and methods" section. The statistical design and the models of statistical analysis must be described, as well as each of the statistical methods used. Sufficient statistical details must be given to allow replication of the statistical analysis. The experimental unit must be defined (e.g. individual animal, group/pen of animals). Generally, and when there are more than 2 treatments, an analysis of variance with F-tests is preferred to multiple *t*-tests. A statistical guide for authors is available on the website at https://www.cambridge.org/core/journals/animal/information/instructions-contributors. The publication of Lang and Altman (2013)[2] can also be used as a reference.

*Statistics directions*

- In the text, the probability of significance is indicated by the following conventional standard abbreviations (which need not be defined): $P > 0.05$ for non-significance and $P < 0.05$, $P < 0.01$ and $P < 0.001$ for significance at these levels. Exact level of probability (e.g. $P = 0.07$) can also be used
- When data are analysed by analysis of variance, a residual error term, such as the pooled standard error, the residual standard deviation (RSD), or the root mean square error (RMSE) is given for each criteria/item/variable/trait in a separate column (or line)

[2] Lang T and Altman D 2013. Basic statistical reporting for articles published in clinical medical journals: the SAMPL guidelines. In Science editors' handbook (ed. Smart P, Maisonneuve H and Polderman A), pp. 175-182. European Association of Science Editors, Exeter, UK. This document may be reprinted without charge but must include the original citation.

- Treatment means are reported with meaningful decimals. For guidance, the last digit corresponds to 1/10 of standard error (e.g., for a standard error of 1.2, the mean values should be reported as 15)
- In tables, probabilities are indicated in a separate column. The *P* values (e.g. $P = 0.07$) are reported or indicated by *, ** and *** for $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively
- In tables, differences between treatments (or comparison of mean values) are indicated using superscript letters with the following conventional standard: a, b for $P < 0.05$; A, B for $P < 0.01$; in most cases, the 0.05 level is sufficient

***Results - Discussion***
Separation between Results and Discussion is preferred to highlight the interpretation of results. Presentation of Results and Discussion in a single section is possible but discouraged.

***Acknowledgements***
In this section, the authors may acknowledge (briefly) their support staff, their funding sources (with research funder and/or grant number), their credits to companies or copyrighted material, etc.

***Declaration of interest.*** Papers with a potential conflict of interest must include a description/explanation of the conflict in the Declaration of interest section.

***Ethics statement.*** Where relevant, approval of the work by an ethics committee or compliance of the work with national legislation, as relevant, must be described in this section.

***Software and data repository resources.*** Authors must indicate whether their data or models are deposited in an official repository and give the full reference. They should also indicate the access rights.

***References***
Citations from international refereed journals or from national refereed journals with at least an English abstract are preferred. Citations from national abstracts/conference proceedings, MSc or PhD thesis, institutional/technical reports, non-English documents that cannot be obtained easily by the reader or that are not peer-reviewed should be minimized. In general, no more than 3 references can be given for the same statement (except for reviews and meta-analyses).

***Citation of references.*** In the text, references should be cited by the author(s) surname(s) and the year of publication (e.g. Smith, 2012). References with two authors should be cited with both surnames (e.g. Smith and Wright, 2013). References with three or more authors should be cited with the first author followed by *et al.* (in italics; e.g. Smith *et al.*). Multiple references from the same author(s) should be as follows: Wright *et al.* (1993 and 1994), Wright *et al.* (1993a and 1993b). Names of organisations used as authors (e.g. Agricultural and Food Research Council) should be written in full in the list of references and on first mention in the text. Subsequent mentions may be abbreviated (e.g. AFRC).
"Personal communication" or "unpublished results" should follow the name of the author in the text where appropriate. The author's initials but not his title should be included, and such citations are not needed in the reference list.

*In-text citation directions*

- Cite references by name(s) of author(s) and year of publication
- Use Doe (2014) or (Doe, 2014) for single authors
- Use Doe and Smith (2014) or (Doe and Smith, 2014) for two authors
- Use Doe *et al.* (2014) or (Doe *et al.*, 2014) for three or more authors
- "*et al.*" is in italics
- When multiple references are cited, rank them preferably by chronological order using commas and semicolons: (Doe, 1999; Smith and Doe, 2001; Doe *et al.*, 2014 and 2015)

***List of references.*** Literature cited should be listed in alphabetical order by authors' names and references should not be numbered. **It is the author's responsibility to ensure that all references are correct.**

*Journal article directions*

- References from journal articles are formatted as:

Author A, Author B, Author CD and Author E Year. Article title. Full Name of the Journal Volume, first-last page numbers.
Examples
- Berry DP, Wall E and Pryce JE 2014. Genetics and genomics of reproductive performance in dairy and beef cattle. Animal 8 (suppl. 1), 115–121.
- Knowles TG, Kestin SC, Haslam SM, Brown SN, Green LE, Butterworth A, Pope SJ, Dirk Pfeiffer D and Nicol CJ 2008. Leg disorders in broiler chickens: prevalence, risk factors and prevention. PLoS ONE 3, e1545.
- Martin C, Morgavi DP and Doreau M 2010. Methane mitigation in ruminants: from microbe to the farm scale. Animal 4, 351-365.
- Pérez-Enciso M, Rincón JC and Legarra A 2015. Sequence- vs. chip-assisted genomic selection: accurate biological information is advised. Genetics Selection Evolution 47, 43. doi:10.1186/s12711-015-0117-5.
- When the article is online but not yet printed, the right format is:
  Zamaratskaia G and Squires EJ 2008. Biochemical, nutritional and genetic effects on boar taint in entire male pigs. Animal, doi:10.1017/S1751731108003674, Published online by Cambridge University Press 17 December 2008.

- No punctuation (i.e. no comma or full stop or semicolon) between the surname and initials of an author, after initials, before publication years, after journal names and before volume numbers
- Include "and" (without comma) before the last author for multiple author references
- All authors' names are provided, do not use "*et al.*" in the reference list
- Publication years are included after the author list without parentheses
- No capitals for article titles except initial capital of the first word and words that ordinarily take capitals
- Journal names are given in full (not in abbreviated form) and the initial letter of all main words is capitalised (except little words such as "and", "of, "in", "the"...), e.g. Journal of Animal Science
- Issue numbers are not mentioned
- Use a comma (","), not a semicolon (";") before page numbers
- Page numbers are given in full (e.g. "1488-1496" not "1488-96")

*Book directions*

- References from books or official reports are formatted as:
  Author(s)/Editor(s)/Institution Year. Book title, volume number if more than 1, edition if applicable. Publisher's name, City, State (2-letter abbreviation) for US places, Country.
  Examples
  - Association of Official Analytical Chemists (AOAC) 2004. Official methods of analysis, volume 2, 18th edition. AOAC, Arlington, VA, USA.
  - Littell RC, Milliken GA, Stroup WW and Wolfinger RD 1996. SAS system for mixed models. Statistical Analysis Systems Institute Inc., Cary, NC, USA.
  - Martin P and Bateson P 2007. Measuring behaviour. Cambridge University Press, Cambridge, UK.
  - National Research Council (NRC) 2012. Nutrient requirements of swine, 11th revised edition. National Academy Press, Washington, DC, USA.
- The list of author or editor name(s) and publication years are written as for journal articles (all authors are provided; commas between authors, except for the last one; "and" before the last author where there are two or more authors; full stops after publication years)
  Example
  - Author A, Author B, Author CD and Author E Year.
- No capitals for book titles except initial capital of the first word and words that ordinarily take capitals
- Detailed publisher information is given and listed as:
  Publisher's name, City, State (2-letter abbreviation) for US places, Country.
  ***Please note – if a publisher is based in more than one place, use only the first one. If multiple publishers are list, it is acceptable to use only the first one.***
  Examples
  - AOCS Press, Champaign, IL, USA.
  - Cambridge University Press, Cambridge, UK.
  - International Organization for Standardization, Geneva, Switzerland.

- FAO, Rome, Italy.

*Book chapter directions*

- References from chapters or parts of books are formatted as:
  Author A, Author B, Author CD and Author E Year. Chapter title. In Title of book (ed. A Editor and B Editor), pp. first-last page numbers. Publisher's name, City, State (2-letter abbreviation) for US places, Country.
  Example
  - Nozière P and Hoch T 2006. Modelling fluxes of volatile fatty acids from rumen to portal blood. In Nutrient digestion and utilization in farm animals (ed. E Kebreab, J Dijkstra, A Bannink, WJJ Gerrits and J France), pp. 40–47. CABI Publishing, Wallingford, UK.
- The list of authors and publication years are written as for journal articles (all authors are provided; commas between authors, except for the last one; "and" before the last author where there are two or more authors; full stops after publication years)
  Example
  - Author A, Author B, Author CD and Author E Year.
- No capitals for chapter and book titles except initial capital of the first word and words that ordinarily take capitals
- Detailed publisher information are given and listed as:
  Publisher's name, City, State (2-letter abbreviation) for US places, Country.
  *Please note – if a publisher is based in more than one place, use only the first one. If multiple publishers are listed, it is acceptable to use only the first one.*
  Examples
  - AOCS Press, Champaign, IL, USA.
  - Cambridge University Press, Cambridge, UK.
  - Editions Quae, Versailles, France.

*Proceedings/Conference papers directions*

- References from proceedings or conference papers are formatted as:
  Author A, Author B, Author CD and Author E Year. Paper title. Proceedings of the (or Paper presented at the) XXth Conference title, date of the conference, location of the conference, pp. first-last page numbers or poster/article number.
  *Please note – If proceedings are published in a journal, the article should be formatted as for a journal article. If they have been published as chapters in a book, the article should be formatted as for a chapter in a book.*
  Examples
  - Bispo E, Franco D, Monserrat L, González L, Pérez N and Moreno T 2007. Economic considerations of cull dairy cows fattened for a special market. In Proceedings of the 53rd International Congress of Meat Science and Technology, 5-10 August 2007, Beijing, China, pp. 581–582.
  - Martuzzi F, Summer A, Malacarne M and Mariani P 2001. Main protein fractions and fatty acids composition of mare milk: some nutritional remarks with reference to woman and cow milk. Paper presented at the 52nd Annual Meeting of the European Association for Animal Production, 26-29 August 2001, Budapest, Hungary.
- The list of authors and publication years are written as for journal articles (all authors are provided; commas between authors, except for the last one; "and" before the last author where there are two or more authors; full stops after publication years)
  Example
  - Author A, Author B, Author CD and Author E Year.
- No capitals for paper titles except initial capital of the first word and words that ordinarily take capitals
- Conference dates are provided in the format: DD Month YYYY, e.g. 10 August 2014
- Conference locations are given and listed as:
  City, State (2-letter abbreviation) for US places, Country.
  Examples
  - Champaign, IL, USA.
  - Cambridge, UK.
  - Versailles, France.
  - Geneva, Switzerland.

*Website directions*

- References from websites are formatted as:
  Author(s)/Institution Year. Document/Page title. Retrieved on DD Month YYYY (i.e. accessed date) from http://www.web-page address (URL).
  Examples
  - Bryant P 1999. Biodiversity and Conservation. Retrieved on 4 October 1999, from http://darwin.bio.uci.edu/~sustain/bio65/Titlpage.htm
- The list of author name(s) and publication years are written as for journal articles (all authors are provided; commas between authors, except for the last one; "and" before the last author where there are two or more authors; full stops after publication years)
  Example
  - Author A, Author B, Author CD and Author E Year.
- No capitals for document/page titles except initial capital of the first word and words that ordinarily take capitals
- Dates when documents were retrieved are included in the format: DD Month YYYY, e.g. 10 August 2014
- Web-page addresses are provided

*Thesis directions*

- References from theses are formatted as:
  Author AB Year. Thesis title. Type of thesis, University with English name, location of the University (i.e. City, State (2-letter abbreviation) for US places, Country).
  Example
  - Vlaeminck B 2006. Milk odd- and branched-chain fatty acids: indicators of rumen digestion for optimisation of dairy cattle feeding. PhD thesis, Ghent University, Ghent, Belgium.
- Author's name and publication year are written as for journal articles
  Example
  - Author AB Year.
- No capitals for thesis titles except initial capital of the first word and words that ordinarily take capitals
- Degree levels are given, e.g. PhD, MSc
- University names and locations are given and listed as:
- University name, City, State (2-letter abbreviation) for US places, Country.
  Examples:
  - Louisiana State University, Baton Rouge, LA, USA.
  - Cambridge University, Cambridge, UK.

***Tables***

Tables should be simple. The same material should not be presented in tabular and graphical form. Please refer to the style sheet available at https://www.cambridge.org/core/journals/animal/information/instructions-contributors.

*Table directions*

- Each table is on a separate page at the end of the main text (one table per page)
- Tables are typed, preferably in double spacing. Single spacing is possible for long tables
- Tables are numbered consecutively using Arabic numbering. They are referred to as Table 1, Table 2, etc., with capital 'T', no italics
- Each table has its own explanatory caption. The caption is sufficient to permit the table to be understood without reference to the text. The animal species and the experimental treatments or the issue under study are indicated in each caption. The caption does not contain the protocol or the results
- Tables are created in Word using the table function within the programme (without using tabs). Layout can be portrait or landscape
- Large tables are discouraged in the manuscript but they may be submitted as Supplementary Material
- No vertical lines between columns and no horizontal lines between rows of data
- Generally, variables are in rows and treatments in columns
- Column headings are concise

- Separate columns are included to present the basic statistical results: error terms (preferably residual error terms) and probabilities
- Row items are organized with main items followed by indented sub-items in order, e.g. to group the criteria that share the same type of measurements or the same unit
- For any (sub-)item, only the first letter of the first word is in capitals
- Units are clearly stated either in the caption (only if a limited number of units are used), or for each (sub-)item. Standard abbreviations for units are used
- Footnotes are referenced using superscript numbers
- Abbreviations used in a table are defined as footnotes (preferred option) or in the caption
- Treatment means are reported with meaningful decimals. For guidance, the last digit corresponds to 1/10 of standard error
- Number of decimals for the indicators of residual variability (RSD, SEM, RMSE etc.) are either identical to that chosen for mean values or have one more decimal. The choice is consistent in all the tables
- See above (Statistics) for the presentation of statistical results in tables

***Figures***

Figures should be simple. The same material should not be presented in tabular and graphical form. Specific guidelines are provided for images (see Image Integrity and Standards).

*Figure directions*

- Figure captions are all listed on a separate page at the end of the main text
- Figures are numbered consecutively in the text. They are referred to as Figure 1, Figure 2, etc., the word 'Figure' being spelled out with capital 'F', no italics
- Captions begin as Figure 1, Figure 2, etc. They are sufficiently detailed to allow the figure to be understood without reference to the text ("Figure 1 Effect of fat source and animal breed on carcass composition in pigs" is preferred to "Figure 1 Carcass composition"). The animal species and the experimental treatments or the issue under study are indicated in each caption. The caption does not contain the protocol or the results. Abbreviations used in each figure have to be defined in the caption and kept to a minimum
- Figures are not inserted in the text. Each figure (without caption) is uploaded separately with **one separate file per figure and no embedded captions in these file**s
- Figure size should be readable in a width of approximately 175 mm (i.e. the maximum size of printing over two columns). Easy reading of the figure is required
- Ensure that the font size is large enough to be clearly readable at the final print size (should not be less than 8 point, or 2.8 mm, after reduction). We recommend you use the following fonts: Arial, Courier, Symbol, Times, Times New Roman and ensure that they are consistent throughout the figures. In addition, ensure that any fonts used to create or label figures are embedded if the application provides that option
- Symbols and line types should allow different elements to be easily distinguished (generally, solid symbols are used before open symbols, and continuous lines before dotted or dashed lines)
- Figures are usually supplied as black and white
- Colours can be used in figures if they are essential to understanding the figure. Publication charges are made for colour figures. The cost for reproducing figures in colour within the printed issue is £200.00 / $320.00 per figure
- If figures are to be printed in colour, use CMYK (instead of RGB) colour mode preferably
- Figures should be provided as TIFF or EPS files. Other formats, such as MS Word, MS Excel, MS PowerPoint, AI and layered PSD (up to CS5), are permitted, provided that figures have been originally created in these formats and that the embedded artwork is at a suitable resolution.
- Resolutions for TIFF figures at the estimated publication size must be:
    - for line figures (e.g. graphs) – 1200 dpi (6000 px for 1 column, 8400 px for 2 columns)
    - for figures with different shadings (e.g. bar charts) – 600 dpi (3000 px for 1 column, 4200 px for 2 columns)

  - for half tones (e.g. photographs) – 300 dpi (1500 px for 1 column, 2100 px for 2 columns)
- Images from the internet are unacceptable, because most of them have a resolution of only 72 dpi
- If your drawing/graphics application does not provide suitable 'export' options, then copy/paste or import the graphic into a Word document
- For further information, please refer to the Cambridge Journals Artwork Guide, which can be found online at: http://journals.cambridge.org/artworkguide

***Image Integrity and Standards***

Any image produced by an instrument (e.g. scanner, microscopy…) with the objective of being used to derive quantitative results is considered as original data. Manuscripts that report images without any quantitative findings are not acceptable. Digitalisation of an image converts the image into numerical values that can be analysed like any other numerical values. The full information may prove important beyond what the author would like to show. Hence images submitted with a manuscript should be minimally processed; some image processing is acceptable (and may be unavoidable), but the final image must accurately represent the original data and exclude any misinterpretation of the information present in the original image. If original data are used just to illustrate a point, this should be accompanied by a clear statement in the manuscript telling the reader this and explaining what is being demonstrated. Please refer to the Office of Research Integrity guidelines on image processing in scientific publication.

*Image Integrity and Standards directions*

- Image acquisition: Equipment and conditions of image acquisition and processing must be detailed in the Material and methods section. This includes the make and model of equipment, the acquisition and the image processing software, and the image treatment if any. If you export files from an acquisition device, make sure to use a format with no loss of information and do not file them into a higher resolution than that of acquisition. Authors have the responsibility to archive original images, with their metadata, in their original format without any compression or compressed without loss of information.
- Preparation of images for a manuscript: For guidance, we refer to the Journal of Cell Biology's instructions to authors (http://jcb.rupress.org/site/misc/ifora.xhtml#image_aquisition) which states:
  1) No specific feature within an image may be enhanced, obscured, moved, removed, or introduced.
  2) The grouping of images from different parts of the same gel, or from different gels, fields, or exposures must be made explicit by the arrangement of the figure (i.e., using dividing lines) and in the text of the figure legend.
  3) Adjustments of brightness, contrast, or color balance are acceptable if they are applied to every pixel in the image and as long as they do not obscure, eliminate, or misrepresent any information present in the original, including backgrounds. Non-linear adjustments (e.g., changes to gamma settings) must be disclosed in the figure legend.

  For further information, image examples, and more detailed guidance, we advise reading What's in a picture? The temptation of image manipulation (reprinted in the *Journal of Cell Biology* (2004) 166, 11-15).
- If a cropped image is included in the main text of a paper (e.g. a few lanes of a gel), display the full original image, including the appropriate controls, the molecular size ladder and/or the scale as relevant, as a single figure in a Supplementary Material file to facilitate peer-review and for subsequent on-line publication.
- The statistical analysis applied to the quantitative data associated with images must clearly define the statistical unit considered (e.g. the animal, the sample).
- Image screening prior to acceptance: Digital images from manuscripts nearing acceptance for publication will be screened for any evidence of improper manipulation or quality. If the original images cannot be supplied by authors on request, the journal reserves the right to reject the submission or to withdraw the published paper.

***Supplementary material***

Authors can include supplementary material in any type of article. Detailed description and results of quality control checks of critical methodologies should be reported in Supplementary materials if not included in the Material and methods section. Supplementary material will appear only in the electronic

version. A link to this on-line supplementary material will be included by the Copy Editor at the proof stage. Supplementary material will be peer-reviewed along with the rest of the manuscript. The main text of the article must stand alone without the supplementary material. Supplementary material should be presented according to the instructions for the main text. **It will not be copy-edited and authors are entirely responsible for the presentation of the supplementary material according to *animal*'s style.**

*Supplementary material directions*

- In the main text, supplementary material are referred to as:
  "Supplementary Table S1", "Supplementary Table S2", etc. for tables
  "Supplementary Figure S1", "Supplementary Figure S2", etc. for figures
  "Supplementary Material S1", "Supplementary Material S2", etc. for other material
  For example: "The list of references used for the meta-analysis is given in Supplementary Material S1 and Supplementary Table S1 reports, etc."
- Supplementary material is submitted along with the main manuscript in a separate file and identified at uploading as "Supplementary File – for Online Publication Only"
- The title of the article and the list of authors are included at the top of the supplementary material
- No line numbering
- Single spacing
- Unlike the figures included in the main text, each supplementary figure has its own title embedded below the figure

## Typographical conventions

***Title and headings***

As illustrated, and detailed above and in the style sheet (see https://www.cambridge.org/core/journals/animal/information/instructions-contributors), the *animal* conventions apply to (*a*) *Title* of the paper, Authors' names and addresses; (*b*) *Main section headings*, such as Abstract, Implications, Introduction, Material and methods, Results, Discussion, Acknowledgements, Declaration of interest, Ethics committee, Software and data repository resources, References; and (*c*) two levels of *Subheadings*.

*Title and heading directions*

- Title – use bold, with an initial capital for the first word only and for words that ordinarily take capitals
- Authors' names – use lower case with initials in capitals (e.g. J. Doe)
- Authors' addresses – use italics
- Headings are left aligned with an initial capital for the first word only, and not numbered
- Main section headings – use bold with no full stop at the end; text follows on the next line (e.g. **Abstract**)
- Subheading (level 1) – use italics with no full stop at the end; text follows on the next line (e.g. *Experimental design*)
- Sub-subheading (level 2) – use italics and end with a full stop; text follows on the same line (e.g. *Milk fatty acid composition.* The fatty acid…)

***Abbreviations***

Standard abbreviations (Table 2) are not defined. Non-standard abbreviations are defined at first use separately in the abstract and in the main text, they should be written in **bold capitals at first occurrence**. To facilitate understanding of the manuscript, the number of abbreviations should be kept to a minimum (not more than 10 non-standard abbreviations is advised). Abbreviations in the titles, (sub)headings or keywords are discouraged.

*Abbreviation directions*

- Define abbreviations at first appearance in the abstract and in the main text
- Authors should avoid excessive use of non-standard abbreviations (a maximum of 10 is advised)
- No author-defined abbreviation in the (short) titles, in (sub)headings or in keywords
- Abbreviations used in tables and figures must be defined either as footnotes or in the caption
- Do not start a sentence with an abbreviation

**Table 2** *Abbreviations that do not require definition*

| Item | Definition |
|---|---|
| Standard abbreviation | |
| ACTH | Adrenocorticotropic hormone |
| ADF | Acid detergent fibre |
| ADL | Acid detergent lignin |
| ADP | Adenosine diphosphate |
| ANOVA | Analysis of variance |
| ATP | Adenosine triphosphate |
| BLUP | Best linear unbiased prediction |
| BW | Body weight |
| CoA | Coenzyme A |
| CP | Crude protein |
| DM | Dry matter |
| DNA | Deoxyribonucleic acid |
| ELISA | Enzyme-linked immunosorbent assay |
| FSH | Follicle-stimulating hormone |
| GLC | Gas-liquid chromatography |
| GLM | General Linear Model |
| HPLC | High performance (pressure) liquid chromatography |
| IGF | Insulin-like growth factor |
| IR | Infrared |
| LH | Luteinising hormone |
| MS | Mass spectrometry |
| n | Number of samples |
| NAD | Nicotinamide adenine dinucleotide |
| NADP | Nicotinamide adenine dinucleotide phosphate |
| $NADPH_2$ | Reduced nicotinamide adenine dinucleotide phosphate |
| NDF | Neutral detergent fibre |
| NIRS | Near infrared stectrophotometry |
| PAGE | Polyacrylamide gel electrophoresis |
| PCR | Polymerase chain reaction |
| PMSG | Pregnant mare serum gonadotropin |
| RNA | Ribonucleic acid |
| SDS | Sodium dodecyl sulfate |
| UV | Ultraviolet |
| Standard statistical abbreviation | |
| CV | coefficient of variation |
| df | degrees of freedom |
| EMS | expectation of mean square |
| F | variance ratio |
| LSD | least significant difference |
| MS | mean square |
| *P* | probability |
| use ns | $P \leq 0.05$, in tables |
| use * | $P \leq 0.05$, in tables |
| use ** | $P \leq 0.01$, in tables |
| use *** | $P \leq 0.001$, in tables |
| *r* | simple correlation coefficient |
| *R* | multiple correlation coefficient |
| $R^2$ | coefficient of determination |
| rSD | residual standard deviation |
| RMSE | root mean square error |
| SD | standard deviation |
| SED | standard error of difference |
| SEM | standard error of mean |
| *Sy.x* | standard error of estimate |
| $\chi^2$ | chi square |

The names of the chemicals do not need to be written in full; chemical symbols are sufficient. Fatty acids are abbreviated using the rule: cis-18:1 for the sum of cis octadecenoic acids. When isomers are described, the double bond positions are identified by numbering from the carboxylic acid end: c9,t11-18:2; iso-15:0. The terms "omega 3" and "omega 6" are discouraged and replaced by "n-3" and "n-6", e.g. 18:3n-3. Trivial names can be used for most known fatty acids (myristic, palmitic, oleic, linoleic, linolenic) and abbreviations in some cases: CLA for conjugated linoleic acids, EPA for eicosapentaenoic acid, DHA for docosahexaenoic acid. Chemical names and trivial names cannot be mixed in a same table.

***Capitals***

*Capitals directions*

- Initial capitals are used for proper nouns, for adjectives formed from proper names, for generic names and for names of classes, orders and families
- Names of diseases are not normally capitalised

***Italics***

*Italics directions*

Use italics for:

- Authors' addresses (see above)
- Subheadings (see above)
- Titles for tables (but not captions for figures)
- Most foreign words, especially Latin words, e.g. *ad hoc, ad libitum, et al., in situ, inter alia, inter se, in vitro, per se, post mortem, post partum, m. biceps femoris*
  but no italics for c.f., corpus luteum, e.g., etc., i.e., NB, via
- Mathematical unknowns and constants
- Letters used as symbols for genes or alleles e.g. *HbA, Tf D* (but not chromosomes or phenotypes of blood groups, transferrins or haemoglobins, e.g. HbAA, TfDD)

***Numerals***

*Numerals directions*

- In text, use words for numbers zero to nine and numerals for higher numbers. In a series of two or more numbers, use numerals throughout irrespective of their magnitude
- Do not begin sentences with numerals
- For values less than unity, 0 is inserted before the decimal point
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- Use numerals if a number is followed by a standard unit of measurement (e.g. 100 g, 6 days, 4th week).
- Use numerals for dates, page numbers, class designations, fractions, expressions of time, e.g. 1 January 2007; type 2
- Dates are given with the month written in full and the day in numerals (i.e. 12 January *not* 12th January).
- For time use 24-h clock, e.g. 0905 h, 1320 h

***Units of measurement***

The International System of Units (SI) should be used. A list of units is found at http://physics.nist.gov/cuu/Units/units.html. Recommendations for conversions and nomenclature appeared in *Proceedings of the Nutrition Society* (1972) 31, 239-247. Some frequently used units that are not in the SI system are accepted: e.g. l for litre, ha for hectare, eV for electron-volt, Ci for curie. Day, week, month and year are not abbreviated. The international unit for energy (energy value of feeds, etc.) is Joule (or kJ or MJ).

A product of two units should be represented as N·m and a quotient as N/m (e.g. g/kg and not $g.kg^{-1}$). When there are two quotients, represent as: g/kg per day (not g/kg/day).

***Concentration or composition***
Composition is expressed as mass per unit mass or mass per unit volume. The term *content* should not be used for concentration or proportion.

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## VITA

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