UNIVERSIDADE ESTADUAL PAULISTA - UNESP

CAMPUS DE JABOTICABAL

INFLUÊNCIA DO PERFIL DE AMINOÁCIDOS DO ALIMENTO NA COMPOSIÇÃO CORPORAL, GASTO ENERGÉTICO E METABOLISMO DE PROTEÍNAS DE CÃES EM REGIME PARA PERDA DE PESO

LETICIA WARDE LUIS

Médica Veterinária

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Leticia Warde Luis

Orientador: Prof. Dr. Aulus Cavalieri Carciofi

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DADOS CURRICULARES DA AUTORA

Leticia Warde Luis nasceu em Curitiba-PR no ano de 1993. Iniciou os estudos no Colégio Marista Paranaense (Curitiba-PR), concluindo o ensino médio no ano de 2010. Em 2011 ingressou no curso de Graduação em Medicina Veterinária na Universidade Federal do Paraná (UFPR). Durante a graduação foi aluna de projeto de extensão e de iniciação científica, monitora nas disciplinas de Ornitopatologia e Odontologia Veterinária. Concluiu a graduação em fevereiro de 2017. Realizou Residência em Nutrição e Nutrição Clínica de Cães e Gatos pelo Programa de Residência Profissional em Medicina Veterinária na Universidade Estadual Paulista – UNESP, Campus de Jaboticabal, de 2017 a 2019, sob orientação do Prof. Dr. Aulus Cavalieri Carciofi. Atualmente é Mestranda no Programa de Pós-Graduação em Medicina Veterinária na área de Clínica Médica Veterinária com ênfase em Nutrição de Cães e Gatos, sob orientação do Prof. Dr. Aulus Cavalieri Carciofi.

“Sempre que me sinto muito sozinho, sento e observo o céu á noite. Penso que uma dessas estrelas é a minha estrela. E em momentos assim, sei que a minha estrela estará sempre lá por mim, com uma voz calmante dizendo: Não desista, garoto.”

(Charlie Brown)

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CERTIFICADO

Certificamos que o projeto de pesquisa intitulado **"Influência do Perfil de Aminoácidos do Alimento na Composição Corporal e Gasto Energético de Cães em Regime de Perda de Peso"**, protocolo nº 07196/19, sob a responsabilidade do Prof. Dr. Aulus Cavalieri Carciofi, que envolve a produção, manutenção e/ou utilização de animais pertencentes ao Filo Chordata, subfilo Vertebrata (exceto o homem), para fins de pesquisa científica (ou ensino) - encontra-se de acordo com os preceitos da lei nº 11.794, de 08 de outubro de 2008, no decreto 6.899, de 15 de julho de 2009, e com as normas editadas pelo Conselho Nacional de Controle de Experimentação Animal (CONCEA), e foi aprovado pela COMISSÃO DE ÉTICA NO USO DE ANIMAIS (CEUA), da FACULDADE DE CIÊNCIAS AGRÁRIAS E VETERINÁRIAS, UNESP - CÂMPUS DE JABOTICABAL-SP, em reunião ordinária de 13 de junho de 2019.

Vigência do Projeto	05/08/2019 a 28/02/2021
Espécie / Linhagem	Cães domésticos
Nº de animais	40
Peso / Idade	5 a 30 kgs / 2 a 8 anos
Sexo	Fêmeas e machos
Origem	Animais domiciliados

Vigência do Projeto	05/08/2019 a 28/02/2019
Espécie / Linhagem	Cães domésticos
Nº de animais	6 (para avaliação da digestibilidade da dieta)
Peso / Idade	13 – 15 kgs / 2 – 6 anos
Sexo	Machos e fêmeas
Origem	Laboratório de Pesquisa em Nutrição e Doenças Nutricionais de Cães e Gatos – FCAV / UNESP Jaboticabal

Jaboticabal, 13 de junho de 2019.

Prof.ª Dr.ª Fabiana Pilarski
Coordenadora – CEUA

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INFLUÊNCIA DO PERFIL DE AMINOÁCIDOS DO ALIMENTO NA COMPOSIÇÃO CORPORAL, GASTO ENERGÉTICO E METABOLISMO PROTEICO DE CÃES EM REGIME PARA PERDA DE PESO

RESUMO: Obesidade canina é a doença nutricional mais frequente, com aumento contínuo de sua incidência. Dentre os desafios de seu tratamento, formulações adequadas que maximizem a redução da massa gorda, mas evitem perda de massa magra e mantenham saúde são necessárias. Estudos relacionam o maior teor de proteína na dieta com a manutenção da massa magra, entretanto, não há evidências das implicações da formulação de aminoácidos sobre o emagrecimento e metabolismo proteico de cães em regime de perda de peso. Ainda, não se conhece com precisão o gasto energético diário (GED) de cães obesos para perda de peso. Desta forma, objetivou-se comparar o GED de manutenção de cães obesos domiciliados com o de cães não obesos e avaliar o efeito da suplementação de metionina, triptofano, treonina e valina em alimento para perda de peso sobre o metabolismo proteico, gasto energético para emagrecimento e composição corporal de cães obesos ao início e após perda de 20% do peso corporal. Na Fase 1 (fase de peso estável) o estudo incluiu 20 cães não obesos e 20 obesos domiciliados, distribuídos em tratamento fatorial 2 (condições corporais) x 2 (rações: controle e suplementada com os aminoácidos), em delineamento inteiramente casualizado. Na Fase 2 (fase de perda de peso) os cães obesos foram divididos em dois grupos de acordo com a dieta utilizada e entraram em regime para perda controlada de 20% do peso corporal. O GED e a composição corporal (CC) dos cães foram determinados pelo método da água duplamente marcada, o metabolismo proteico pelo método do precursor e mensuração dos aminoácidos livres no plasma. A hipótese de que cães obesos apresentam menor necessidade energética de manutenção (NEM) não foi confirmada, uma vez que a ingestão calórica para peso constante foi semelhante entre os obesos e não obesos no presente estudo (P=0,119). Durante o emagrecimento a ingestão de metionina + cistina foi menor que o recomendado para cães alimentados com a ração controle, tendo esta atendido à recomendação no grupo suplementado com aminoácidos. Durante o emagrecimento, cães alimentados com a ração suplementada com aminoácidos

apresentaram tendência a emagrecerem mais rápido (P=0,083), mesmo apresentando mesmo consumo energético (P=0,682). Após 20% de perda de peso, cães no tratamento com suplementação de aminoácidos mantiveram a quantidade de massa magra do início, enquanto no grupo controle os cães apresentaram redução média de 6,5% (900g) da massa magra inicial, apesar destes valores serem estatisticamente semelhantes (P=0,531).

Palavras-chave: aminoácido livre, deutério, emagrecimento, isótopos estáveis, massa magra, obesidade

INFLUENCE OF THE FOOD AMINO ACID PROFILE IN THE BODY COMPOSITION, ENERGY EXPENDITURE AND PROTEIN FLUX OF DOGS IN A WEIGHT LOSS PROGRAM

ABSTRACT: Canine obesity is the most frequent nutritional disease, with a growing incidence. Among the challenges of its treatment, formulations that maximize the reduction of fat mass, but prevent loss of lean mass and maintain health during the process of weight loss are necessary. Several studies relate a higher level of protein in the diet with maintenance of lean mass, however, the implications of amino acid formulation on weight loss and protein metabolism in dogs undergoing regimen have not been studied. Still, the daily energy expenditure (DEE) of obese dogs for a healthy weight loss is not precisely known. Therefore, the aim of this study was to compare the maintenance energy expenditure of client-owned obese dogs with non-obese dogs and to evaluate the effect of methionine, tryptophan, threonine and valine supplementation in food for weight loss on protein metabolism, energy expenditure for weight loss and body composition of obese dogs at the beginning and after a 20% loss of body weight. In Phase 1 (static body weight phase), the study included 20 non-obese and 20 obese client-owned dogs, distributed in a 2 (body condition) x 2 (diet: control and amino acid) factorial treatment, in a completely randomized design. In Phase 2 (weight loss phase), obese dogs underwent a regimen for controlled loss of 20% of body weight. The DEE and body composition (CC) of dogs were determined by the doubly-labeled water method, protein metabolism by the precursor method and measurement of free amino acids in plasma. The hypothesis that obese dogs have a lower energy requirement for maintenance was not confirmed, since caloric intake for weight maintenance was similar between obese and non-obese dogs in the present study (P=0.119). During weight loss, the intake of methionine + cystine was lower than recommended for dogs fed the control diet, which, in the group supplemented with amino acids, met the recommendations. During weight loss, dogs fed the amino acid diet tended to lose weight faster (P=0.083), even with the same energy intake (P=0.682). After 20% of weight loss, dogs in the treatment with amino acid supplementation showed the same amount of lean

mass, while control group dogs showed a reduction of 6.5% (900g) of the initial lean mass, although these values are statistically similar (P=0.531).

Key Words: amino acids, deuterium, weight loss, stable isotopes, lean body mass, obesity

LISTA DE ABREVIATURAS

CEUA: Comissão de Ética no Uso de Animais

PB: Proteína bruta

MS: Matéria seca

^{2}H: Deutério

^{18}O: Oxigênio 18

CO_2: Gás carbônico

ECC: Escore de condição corporal

TSH: Hormônio estimulante da tireóide

GED: Gasto energético diário

Kcal: Quilocalorias

$Kg^{0,75}$: Peso metabólico

EM: Energia metabolizável

EE: Extrato etéreo

MM: Matéria mineral

ECF: Escore de condição fecal

PC: Peso corporal

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1. REVISÃO DE LITERATURA

1.1 Obesidade

A obesidade é uma das afecções de origem nutricional mais comuns em cães e gatos. Pesquisas destacaram que 35 a 59% dos cães e gatos apresentam sobrepeso ou obesidade (Brooks et al., 2014; German et al., 2015a; Chandler et al., 2017; Porsani et al., 2020). Esta ocorre quando o animal estabelece balanço energético positivo prolongado, sendo reconhecida como doença multissistêmica grave, que pode desencadear ou agravar diversas comorbidades que causam impactos importantes na qualidade e expectativa de vida dos animais (Chandler et al., 2017).

As principais causas de obesidade são alimentação inapropriada (excesso de calorias), pouca prática de atividade física (baixo gasto energético), predisposições raciais e genéticas, castração e doenças que causem indisponibilidade para a prática de atividade física ou reduzam o gasto energético, por exemplo hipotireoidismo (German, 2006; Laflamme, 2012; Chandler et al., 2017). Além disso, o estudo epidemiológico de Coucier et al. (2010), assim como o de Colliard (2006), revelaram que a idade e o sexo em cães podem ser fatores predisponentes à obesidade, sendo os animais mais velhos de ambos os sexos e as fêmeas castradas mais predispostos ao ganho excessivo de peso (Mao, 2013).

O excesso de adiposidade está associado a efeitos adversos sobre a saúde, qualidade de vida e comprovadamente responsável por diminuir a expectativa de vida dos cães (Kealy et al., 2002; German et al., 2006; Flanagan et al., 2017). As implicações sistêmicas da obesidade têm sido bastante investigadas, pois esta condição se caracteriza por estado pró-inflamatório (Das, 2001; Laflamme, 2012) com relevantes alterações imunológicas (Da Silveira et al., 2009; German et al., 2010) e no metabolismo de lipídeos e carboidratos (Laflamme, 2012).

O tratamento da obesidade consiste em colocar o animal em situação de balanço energético negativo, por meio de diminuição da ingestão calórica e

aumento do gasto energético diário (Carciofi et al., 2005). Isto resulta em mobilização de tecido adiposo e consequente perda de peso (Laflamme, 2006). O desafio, no entanto, é se estabelecer composição alimentar que favoreça a saciedade frente ao déficit calórico instituído, bem como garantir ingestão suficiente de nutrientes essenciais de modo a não se impor no paciente perda de massa magra ou comprometimento do funcionamento de órgãos ou sistemas, como o tegumentar e o imunológico (Diez; Nguyen, 2006; Yamka et al., 2007).

Durante o processo de perda de peso o animal sofre mudanças em seu metabolismo energético que ainda não estão bem estabelecidas na literatura (NRC, 2006). Os estudos sobre metabolismo energético de cães ainda são insuficientes, existindo controvérsia inclusive sobre a necessidade energética de manutenção de cães domiciliados, sejam não obesos ou obesos (Bermingham et al., 2014). A falta de consenso sobre a necessidade energética pode, inclusive, ser parte das causas de obesidade, pois recomendações imprecisas da quantidade de alimentos podem resultar em ganho de peso (FEDIAF, 2020).

Não se conhecendo com exatidão a necessidade energética de manutenção, será imprecisa a proposição de restrição calórica para perda de peso. Publicações a respeito de protocolos para perda de peso em cães têm proposto diversas abordagens para se estabelecer inicialmente o fornecimento de calorias. Carciofi et al. (2005) sugerem o fornecimento da taxa metabólica basal do animal calculada para seu peso meta (70 kcal/kg peso meta^{0,75}/dia). Outros autores têm proposto diferentes formas de cálculo. Case et al. (2011) utilizam da mesma equação, porém com o peso corporal atual do cão. Já German et al. (2007), propõem redução de 40% a 50% da energia de manutenção proposta pelo NRC (2006; 132kcal/kg peso corporal^{0,75}/dia), calculada a partir do peso meta ao invés do peso corporal atual do animal. Porém, em estudo mais recente German et al. (2015b), sugere-se a mesma redução tendo como referência para cálculo da energia para manutenção 105 kcal/kg^{0,75}. Flanagan et al. (2017), por sua vez, recomendam 60 a 80 kcal/kg peso meta^{0,75}.

Desta forma, as proposições de calorias para emagrecimento são sempre adaptadas à taxa de emagrecimento alcançada pelo paciente. O veterinário

responsável deve ajustar o fornecimento de calorias e alimentos buscando-se manter emagrecimento em torno de 1% a 2%do peso corporal por semana (German et al., 2007; Case, et al., 2011; Brooks et al, 2014).

Estudos demonstram elevadas taxas de insucesso em programas de perda de peso realizados com cães domiciliados, com taxas de emagrecimento mais baixas do que o proposto na literatura. Carciofi et al. (2005) compararam o emagrecimento de cães obesos em condições experimentais com um grupo de cães obesos domiciliados, até perda de 15% do peso corporal, e observaram para o primeiro grupo a média de 1,39% de perda semanal de peso enquanto o grupo domiciliado teve média de 0,75% de perda semanal. German et al. (2015a), realizaram estudo que submeteu cães domiciliados a perda controlada de peso para determinar fatores associados com o sucesso do emagrecimento. Encontraram taxa de perda de peso semanal próxima ao estudo anterior, de 0,7% (0,1-1,7%), com média de consumo de 62,3 (44,0-92,9 kcal/kg^{0,75}) e perda média de 19,5% do peso corporal inicial. Flanagan et al. (2017), em outro estudo com cães domiciliados, encontraram taxa de perda de peso semanal de 0,9 ± 0,45%, no entanto, apenas 8,4% dos casos estudados (78/926) atingiram perda de 20% do peso corporal e o tempo de regime foi de 12 semanas, com evidências de redução nas taxas de perda de peso ao longo do período de acompanhamento. Em estudo retrospectivo de nosso grupo de pesquisa (Luis et al., dados não publicados) foram avaliados 94 programas de emagrecimento de cães atendidos pelo Serviço de Nutrição Clínica de Cães e Gatos do Hospital Veterinário da UNESP/Jaboticabal. No programa obteve-se taxa média de emagrecimento de 0,83 (0,27-1,65%) por semana. Ao final os cães estavam recebendo 58,9 (32,5-75,4) kcal/kg^{0,75}/dia, aproximadamente 62% da sua necessidade energética de manutenção (95 kcal/kg^{0,75}, corroborando com German et al. (2007) que refere resultados de restrições de 50 a 82% da necessidade de manutenção e apresentando taxa de emagrecimento.

A reduzida ingestão de alimentos, necessária para se alcançar o déficit de calorias e consequente consumo da massa gorda corporal, pode ocasionar ingestão limitada de nutrientes importantes para o animal, caso a dieta não seja adequadamente formulada para perda de peso. Esta preocupação já motivou

publicações que avaliaram o risco de deficiência nutricional em cães. Linder et al. (2012) avaliaram cinco alimentos comerciais (dois para perda de peso e três alimentos de manutenção) considerando sua oferta para cães com sobrepeso. Na quantidade de energia proposta no estudo para manutenção desses cães (87 kcal/kg^{0,75}), encontrou deficiência nos nutrientes colina, selênio, magnésio, riboflavina, niacina, metionina/cistina, triptofano, cloro e vitamina D. Em estudo posterior, a mesma autora acessou a concentração plasmática dos nutrientes previamente identificados como deficientes e dos aminoácidos essenciais em cães submetidos à perda controlada de 15% do seu peso corporal. Apesar de nenhum cão apresentar sinais clínicos de deficiência nutricional ao final do estudo, que teve duração média de 250 (91-674) dias, a concentração plasmática de treonina, glicina, colina e a concentração urinária de selênio diminuíram com a restrição calórica (Linder et al., 2013).

Aqui falar do estudo do german e gaylord tb

1.2 Perda de massa magra durante o emagrecimento

Tradicionalmente, as dietas para perda de peso em cães contêm baixa densidade energética, concentração elevada de proteína e fibra e enriquecimento de microelementos e vitaminas (Carciofi et al., 2005; German et al., 2010; Laflamme, 2012). Entretanto, mesmo com o elevado teor proteico, a depender da qualidade da proteína e especificamente de seu perfil de aminoácidos, esta pode não suprir adequadamente a quantidade necessária de aminoácidos para manter a síntese e o *turn-over* proteico no organismo durante a restrição energética imposta para o emagrecimento (Yamka et al., 2007). Caso isto ocorra, haverá perda de massa muscular, com potencial prejuízo ao sistema imunológico, estrutura da pele e pelos e comprometimento da saúde geral (Diez; Nguyen, 2006; German et al., 2007; Yamka et al., 2007; Case et al., 2011).

Estudo publicado por German et al. (2007), demonstrou perda significativa de aproximadamente 15 ± 15,4% da massa magra ao longo do emagrecimento de cães, que durou 177 ± 98,2 dias. No estudo foram empregados dois alimentos, uma ração comercial seca e uma úmida, contendo 37,1% e 55% de proteína bruta na matéria seca e 3275 e 3670 (matéria seca) kcal/kg

respectivamente. Diez et al. (2002) compararam duas dietas de alta fibra (FDT teste: 30,8%; controle: 38,6%) , sendo a teste elevada proteína (47,5% MS) e a controle, dieta comercial para perda de peso com proteína moderada (23,8% PB na MS). A composição corporal foi avaliada pelo método da água marcada com deutério. Os grupos tiveram perda de peso corporal semelhante, no entanto, no grupo alimentado com a ração com alta proteína 80,4±3,1% desta perda foi correspondente a massa gorda, enquanto no grupo ingerindo dieta comercial, 70,0±3,1% foi massa gorda com maior perda proporcional de massa magra. Esses estudos corroboram com outros que indicam que ingestão elevada de proteína durante a perda de peso auxilia na manutenção da massa magra, facilita a perda de gordura corporal e previne novo ganho de peso (Hannah, 1999; Weber et al., 2007; Yamka et al. 2007; German et.al., 2010).

1.3 Ingestão de proteína e aminoácidos

No estudo retrospectivo, anteriormente mencionado, do nosso grupo de pesquisa (Luis et al., dados não publicados) foram avaliados cinco alimentos comerciais coadjuvantes específicos para perda de peso disponíveis no Brasil. Estes apresentavam, em média, 33,2 (29,5-38,8) % de proteína bruta (valores analisados), o que está acima do recomendado para cães adultos em manutenção (FEDIAF, 2020; NRC, 2006). No entanto, devido à restrição alimentar imposta (consumo médio de 17,8 (10,0-27,6) g ração/kg^{0,75}/dia), o consumo de proteína médio dos cães foi de 6,5 (3,6-8,3) g/kg^{0,75}/dia. Considerando os valores mínimos recomendados pelo FEDIAF (2020; 4,95 g/kg^{0,75}/dia) e NRC (2006; 3,28 g/kg^{0,75}/dia), verificou-se que apesar do consumo médio de proteína ter sido adequado, a depender do paciente, de sua necessidade energética individual e do alimento utilizado, entre 1,1% e 20,2% dos cães ingeriram menos proteína do que o recomendado pelo FEDIAF (2020) e pelo NRC (2006).

Mais importante que a baixa ingestão de proteína bruta, para um dos alimentos avaliados consumo insuficiente de triptofano foi verificado em 92,6% dos pacientes, de metionina em 38,3%, de metionina mais cistina em 10,6%, além de ingestão marginal de treonina e valina pelos cães que necessitaram de maior restrição alimentar. A reduzida ou mesmo deficiente ingestão de metionina

e triptofano frente ao emagrecimento com alimentos comerciais já havia sido identificada em publicações anteriores, uma delas é o estudo de Linder et al. (2012), previamente citado que, dentre outros nutrientes, identificou como insuficiente a ingestão de metionina+cistina e triptofano. German et al. (2015b) realizaram estudo retrospectivo de 27 casos de cães obesos submetidos à perda de peso controlada com ração comercial seca para obesidade. A média de perda foi 28,3 (16-40)% e a ingestão média de energia foi de 61 kcal/kg^{0,75} (44–74 kcal/kg^{0,75}). Assim como no estudo anterior, neste os cães não apresentaram evidências clínicas de deficiências nutricionais, no entanto, para selênio, colina, metionina/cistina, triptofano, magnésio e potássio, a ingestão foi menor do que a recomendada pelo NRC (2006). Destacam ainda que, em relação aos aminoácidos, 44% dos cães ingeriram quantidade insuficiente de metionina/cistina e 7% dos cães ingeriram quantidade de triptofano também menor do que a recomendação do NRC (2006). Gaylord et al. (2018) avaliaram através de questionário aplicado a 178 médicos veterinários do estado da Carolina do Norte (EUA) a eficácia do programa de perda de peso e adequação da ingestão de nutrientes de cães através da porcentagem de restrição calórica a qual os animais eram expostos. Foram avaliados 31 alimentos, dentre eles, rações comerciais para manutenção e alimentos para perda de peso. Para ingestão média de 60 kcal/kg^{0,75}, todos os produtos apresentaram três ou mais nutrientes sob risco de deficiência de acordo com as recomendações do NRC (2006), dentre os principais estavam os aminoácidos metionina, metionina/cistina e triptofano.

Assim, verifica-se a necessidade não somente de melhor caracterização da necessidade energética para perda de peso, para com base nela se ter melhor conhecimento e previsão da quantidade de alimento a ser oferecida, como também se estudar as implicações deste baixo consumo de matéria seca sobre as características das formulações dos alimentos coadjuvantes para perda de peso, garantindo assim o estabelecimento do déficit energético com ingestão suficiente de aminoácidos e demais nutrientes para suporte à manutenção da massa muscular e saúde dos cães (Linder et al., 2013; German et al., 2015).

Dentre os aminoácidos com baixo consumo decorrente à restrição energética para perda de peso (Luis et al., dados não publicados; Linder et al., 2012; German et al., 2015b), a metionina é normalmente considerada um dos aminoácidos limitantes na formulação de dietas para cães (NRC, 2006). Sua deficiência pode resultar em diversos problemas metabólicos como redução na replicação celular e na síntese de fosfolipídios. Menor síntese de fosfolipídios acarreta acúmulo de lipídeos no fígado, uma vez que este aminoácido é doador de grupo metil, componente da coenzima s-adenosilmetionina. Metionina é, ainda, importante componente das proteínas estruturais do organismo. Além disso, sua deficiência causa anorexia e perda de peso (NRC, 2006). Este aminoácido também é importante fonte de enxofre, sendo utilizado na síntese de outros componentes que apresentam este elemento em sua composição, como os demais aminoácidos sulfurados. Assim, possui papel essencial na síntese da cisteína, aminoácido utilizado na síntese da proteína corporal, formação da pele e pelos e da glutationa, componente muito importante na resposta ao estresse e estados inflamatórios (NRC, 2006). A partir da metionina no organismo é utilizada para síntese da taurina, aminoácido fundamental a vários processos metabólicos. Desta forma o cão não necessita de cisteína e taurina *per si*, mas necessita de metionina para síntese destes aminoácidos não essenciais. A deficiência destes é descrita em cães alimentados com pouca metionina. Dentre os sintomas verificados da deficiência de taurina em cães destaca-se a cardiomiopatia dilatada (Kittleson et al., 1997; Fascetti et al., 2003; Tôrres et al., 2003; Sanderson, 2006).

O triptofano, outro aminoácido com incidência importante de deficiência na restrição calórica severa para perda de peso, é precursor da síntese de proteínas, da vitamina niacina e dos neurotransmissores serotonina, melatonina e 5-hidroxitriptofano no organismo. Sua deficiência, assim como de outros aminoácidos, causa anorexia e perda de peso (NRC, 2006). Além disso, há evidências de que dietas suplementadas com altas concentrações de triptofano possam ter efeitos neurocomportamentais, como diminuição da agressividade em cães pelo aumento da síntese dos neurotransmissores (Denapoli et at., 2000). A treonina é um aminoácido alifático que tem como principal função a participação na formação da glicina (NRC, 2006), outro aminoácido não

essencial que atua como antioxidante celular e auxilia no controle de processos inflamatórios sistêmicos (Matilla et al., 2002). A treonina atua, também, na formação de proteínas no organismo e sua deficiência está associada, principalmente, a anorexia e perda de peso em cães (NRC, 2006). A valina também se mostrou marginal em algumas dietas do estudo (Luis, et al., dados não publicados), ela é um aminoácido de cadeia ramificada, precursor de glicose no organismo e essencial à síntese proteica. Assim como a treonina, sua deficiência está associada à anorexia e perda de peso em cães (NRC, 2006).

1.4 Método da água duplamente marcada

Para o estudo da composição corporal dos animais, quantificando-se sua massa corporal magra e gorda, bem como para determinação do gasto energético pode-se empregar o método da água duplamente marcada com isótopos estáveis de deutério (^{2}H) e oxigênio 18 (^{18}O) (Belislle, 2001; Diez; Nguyen, 2006; Goloni, 2020). Esse método é considerado padrão ouro para avaliar a composição corporal e o gasto energético dos animais simultaneamente (Guidotti et al., 2013). Ele é baseado na inoculação de isótopos estáveis por via subcutânea e utiliza amostras de sangue venoso para avaliar a incorporação e o decaimento dos isótopos ^{18}O e ^{2}H no contingente de água corporal (Ducatti, 2011; Park, et.al., 2014). Por meio da concentração basal e após enriquecimento dos isótopos se pode determinar a quantidade de água corporal. Com base nesta informação e na constante de hidratação da massa magra se estabelece o contingente de massa magra e, por diferença com o peso corporal se estima a massa gorda corporal (Schierbeek et al., 2009). O ^{18}O é perdido do corpo em forma de água e dióxido de carbono (CO_2), enquanto o ^{2}H é perdido somente na forma de água. Com base nisto, tem-se que a diferença do desaparecimento de ambos reflete a produção de CO_2 resultante da oxidação de gorduras, carboidratos e proteínas (Belislle, 2001; Park, et.al., 2014). Empregando-se o volume de CO_2 e o coeficiente do alimento se estima o gasto energético total do animal durante o período (Elia; Livesey, 1992). Importante vantagem do método é que não existe necessidade de se restringir a atividade muscular voluntária, permanecendo o animal com sua rotina normal,

representando assim o gasto energético de manutenção no período (NRC, 2006; Ballevre et al., 1994).

1.5 Método do precursor (^{13}C-Leucina)

O metabolismo proteico e dos aminoácidos em cães é pouco estudado, sobretudo em se tratando da manutenção de adultos (Humbert et al., 2001). As possíveis modificações resultantes da obesidade, especialmente durante programas de emagrecimento, ainda não foram exploradas por publicações científicas. Segundo Humbert e colaboradores (2002), o metabolismo de proteína sofre interferência de vários fatores, variando de acordo com a ingestão calórica e proteica do indivíduo, sendo dessa forma afetado na restrição calórica severa. Métodos isotópicos podem ser utilizados para medir o fluxo de aminoácidos e a degradação e síntese proteica, sendo o método do precursor (^{13}C-leucina), referência na determinação do metabolismo proteico dos animais (Bier, 1989; Humbert et al., 2001). Isótopos estáveis são átomos que representam um mesmo elemento químico, mas variam no número de nêutrons sem afetar suas propriedades químicas, sendo excelentes traçadores biológicos para estudo da cinética, turnover e metabolismo de nutrientes (Kim et al., 2016). No método do precursor a ^{13}C-leucina é administrada, através da coleta de plasma e gás expirado, os parâmetros de enriquecimento são obtidos e aplicados em equações de fluxo, síntese e degradação proteica (Goudoever et al., 1995).

2. OBJETIVOS

Diante do exposto o presente estudo tem por objetivos: a) comparar o gasto energético diário, composição corporal e metabolismo proteico de cães domiciliados em fase estática da obesidade com os de cães não obesos, nas mesmas condições de vida; b) comparar o efeito de duas dietas semelhantes em proteína bruta, energia e fibra dietética, sendo uma suplementada com os aminoácidos metionina, triptofano, treonina e valina e outra não, sobre o gasto energético para perda de peso, a composição corporal e o metabolismo proteico de cães obesos ao início e após perda de 20% do peso corporal inicial.

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CAPÍTULO 2 - Influence of the Food Amino Acid Profile in the Body Composition, Energy Expenditure and Protein metabolism of Obese Dogs in a Weight Loss Program

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Influence of the Food Amino Acid Profile in the Body Composition, Energy Expenditure and Protein metabolism of Obese Dogs in a Weight Loss Program

Short title: Amino acid composition in weight loss diet for dogs

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Abstract

Diet formulation to support a healthy weight loss in dogs is still challenging, as adequate amino acids and other nutrients intake must be assured in a situation of low dry matter and energy consumption, necessary to induce a negative energy balance. The aim of the study was to compare daily energy expenditure (DEE) of client-owned obese and non-obese dogs and evaluate the supplementation of methionine, tryptophan, threonine, and valine in a high protein low energy formulation on protein metabolism, DEE for weight loss and body composition (BC) of obese dogs after weight loss. In Phase 1, the study included 20 non-obese and 20 obese client-owned dogs, organized in a 2 (non-obese and obese) x 2 (control and amino acid supplemented diets) factorial arrangement. In Phase 2, dogs were submitted to controlled loss of 20% of the initial body weight (BW), and randomly sorted to control or amino acid supplemented diets. The DEE and BC were determined by the double-labeled water method, and protein metabolism with the ^{13}C-leucine tracer and free plasma amino acid concentrations. For Phase 1 data were evaluated by variance analysis in a 2x2 factorial arrangement, and in Phase 2, repeated measures analysis of variance was used, considering the effects of diet, time, and diet*time ($P<0.05$). Body fat was 23.4±1.4% and 43.1±1.3% ($P<0.001$), and energy consumption (EC) to stable body weight was 91.5±2.4 kcal/kg$^{0.75}$/day and 85.2±2.0 kcal/kg$^{0.75}$/day ($P<0.044$) for non-obese and obese dogs, respectively. During Phase 2, dogs lost 20.0±3.4% of the initial BW in 23.2±2.2 weeks, without a diet effect ($P>0.05$). The EC for weight loss was similar between diets (Control: 57.3±1.0 kcal/kg$^{0.75}$/day; Amino acid: 56.7±0.9 kcal/kg$^{0.75}$/day; $P=0.682$), but the weight loss rate per week tended to be higher ($P=0.083$) for the Amino acid (0.83±0.07% loss per week) than Control (0.63±0.08%/week). Both groups lost the same amount of body fat (Control: 4.2±1.3 kg; Amino acid: 4.3±1.0 kg; $P=0.990$), but dogs on Amino acid diet did not lost lean mass, while Control diet fed dogs lost -0.9±0.8kg of lean mass, although values were not significant ($P=0.531$). Protein intake during weight loss was similar among treatments ($P=0.870$), but higher methionine, methionine + cystine, and tryptophan was observed for Amino acid ($P<0.05$). Methionine + cystine intake on Control was lower than recommended, might explaining the lean mass loss.

Key-words: obesity, lean body mass, methionine, tryptophan

1. INTRODUCTION

Obesity induces several adverse health effects on dogs and is responsible to decrease their life expectancy and quality of life (Kealy et al., 2002; German, 2006). Several publications explored its negative results on health, including increased inflammation, disturbed glucose metabolism (Gayet et al., 2004; German et al., 2009; Brunetto et al., 2011; Jeremias et al., 2020), and higher incidence of joint diseases, among others (German, 2006; Marshall et al., 2009).

Despite the described adverse effects, not always obesity is recognized and treated (German & Morgan, 2008; Porsani et al., 2020). Its treatment consists of inducing a negative energy balance, most frequentlyusing a low energy and high content fiber and protein formulation (Carciofi et al., 2005; Laflamme, 2012) intended to support satiety and limit energy digestibility, assuring adequate intake of amino acids and other essential nutrients (Diez & Nguyen, 2006; Yamka, Frantz, Friesen, 2007). However, elevated unsuccessful rates have been described on weight loss programs, with low compliance of owners and veterinarians, and reduced rates of weight loss (Carciofi et al., 2005; German et al., 2015a; Flanagan et al., 2017).

Diet formulation to support a healthy weight loss in dogs is still a challenge, as adequate amino acids and other nutrients intake must be assured in a situation of low dry matter and energy consumption, necessary to induce a negative energy balance (Diez et al., 2002; German et al., 2015b). The lack of more precise ways to determine energy intake to weight maintenance and loss (German et al., 2007; Bermingham et al., 2014) opens possibility to more errors on food consumption estimation that, added to formulations not always based on expected nutrient consumption throughout the regimen, might lead animals to be exposed to nutrient deficiencies. Reported energy allowances to induce body weight loss in dogs varied from 58,9 kcal to 63 kcal/kg of current body weigth$^{0.75}$/day (Luis et al., data not published; German et al., 2007; German et al., 2015a; Flanagan et al., 2017). This energy allowance induces fatty mass utilization in animals that usually have lower MER, due to association of factors

including neutering, old age, diseases that reduces exercise possibility, and others (German, 2006; Zoran, 2010).

When facing high caloric restrictions, animals can often have loss of lean mass, with possible negative effects to immune system and the general health (Diez & Nguyen, 2006; German et al., 2007; Yamka et al., 2007). Additionally, deficiencies in methionine and tryptophan intake in dogs undergoing energy restriction has also been reported (Linder et al., 2012; German et al., 2015; Gaylord, Remillard & Saker, 2018). In the authors personal experience, dogs undergoing weight loss using commercial diets for obesity treatment available in Brazil might experience amino acid deficiency. Depending on the brand, based on analyzed amino acid composition, up to 40% of dogs might have lower than the recommended allowance intake, according to NRC (2006) of tryptophan, methionine, and methionine plus cystine, with marginal intake to threonine and valine, reinforcing the necessity to study the better amino acid profile in diets for weight loss (Luis et al., unpublished data).

Additionally, there are few studies on protein and amino acid metabolism in adult dogs (NRC, 2006; Shoveller et al., 2017), especially when it comes to obesity. Considering that calories and protein intake directly affects protein and amino acid metabolism (Humbert, Martin, Dumon, Darmaun, & Nguyen, 2002) studies on amino acid and protein requirement during weight loss are required to better balance their diets, may be contributing to avoid the observed loss on lean mass favoring health.

Considering the available information, are hypothesis of the present study that obese client-owned dogs have lower metabolizable energy requirements to weight maintenance than non-obese client-owned dogs, and during weight loss a decrease in energy requirement resulting from metabolic adaptations makes necessary to reduce the energy supply to animals continue reducing body weight. It is also expected that the supplementation of the limiting amino acids methionine, tryptophan, valine and threonine would reduce the lean mass loss favoring a healthier weigh loss program. Due this, the objectives of the present study were to compare the energy expenditure to stable body weight, and protein metabolism in client-owned obese and non-obese dogs, and to evaluate the supplementation of methionine, tryptophan, valine and threonine in a high fiber, and protein, low energy diet on the energy expenditure, protein metabolism, and

body composition of obese dogs after 20% of a controlled reduction in body weight.

2. MATERIAL AND METHODS

Place of study

The study was performed at the Veterinary Hospital “Governador Laudo Natel” from Faculdade de Ciências Agrárias e Veterinárias da Universidade Estadual Paulista (UNESP), Jaboticabal, São Paulo, Brazil and at the Laboratório de Pesquisa em Nutrição e Doenças Nutricionais de Cães e Gatos “Prof. Dr. Flávio Prada” from the same institution. The experimental diets were produced at the Extrusion Laboratory of the same institution. The analyzes of deuterium and ^{18}O were performed at the Laboratório de Espectrometria de Massas de Razão Isotópica of the Faculdade de Medicina da USP de Ribeirão Preto, São Paulo, Brazil. The analysis of ^{13}C on expired gas was performed on the Centro de Isótopos Estáveis “Professor Dr. Carlos Ducatti”, Instituto de Biociências, UNESP, Botucatu, São Paulo, Brazil. The concentration of free amino acids in the plasma was caried out at the Amino Acid Laboratory, Department of Molecular Biosciences, School of Veterinary Medicine, University of California, Davis, USA. The experimental procedures with animals were previously approved by the Ethics in the Use of Animals Committee under the number 07196/19 and the participation of domiciled animals was in agreement with their owners through a previously signed authorization term.

Animals’ selection

In Phase 1 (static body weight phase) of the study were selected 20 non-obese client-owned dogs, with body condition score (BCS) between 4 and 5 of 9 (Laflamme, 1997), and 20 client-owned obese dogs, with BCS between 8 to 9, from 3 to 10 years old, females and males, totalizing 40 animals. The two groups were homogenized to present the same proportion of males and females, and to have similar mean age. All animals were previously assessed through anamnesis, physical examination and complementary exams (blood count and serum biochemistry: alanine aminotransferase, creatinine, albumin, total protein, cholesterol and triglycerides). Additionally, the obese dogs group undergone

screening tests to detect diabetes mellitus (blood and urinary glucose), hyperadrenocorticism (small dose dexamethasone suppression test), and hypothyroidism (serum TSH and free T4). Only healthy animals, free of endocrinopathies and fed with commercial dry kibble foods were included in the study. Additional criterial of inclusion for the non-obese dogs were stable body weight for at least 12 months and had never been obese before. For the obese group, animals need to be in a stable obese body weight for at least 12 months. The non-obese dogs participated only in Phase 1 of the study and obese dogs in Phase 1 (static body weight) and Phase 2 (weight loss program).

Experimental diets

The experiment included two low-energy diets balanced for weight loss, formulated according to FEDIAF (2018) recommendations for adult dogs. A Control diet formulation with high fiber and high crude protein, low fat and low energy was developed. This same formulation was supplemented with the amino acids methionine, tryptophan, threonine and valine to produce the Amino acid treatment. The other ingredients remained unchanged (Table 1).

The diet ingredients were mixed, grounded in a hammer mill with a 1.0 mm screen sieve size (Moinhos Tigre, São Paulo, Brazil) and then extruded in a single screw extruder (MEX 250, Manzoni, Campinas, Brazil), with processing capacity of 250kg/h. During the extrusion process the amperage of the extruder motor was constantly monitored. The extrudates density (g/L) was determined only when the system reached stability. The temperature of the pre-conditioner was maintained by injecting direct steam above 90°C. Water, steam, screw speed and raw material flow were adjusted according to the food formulation. After extrusion, the extrudates were dried in a forced two-pass air dryer, heated to 105°C and, subsequently coated with poultry fat, liquid and powder palatants.

Table 1. Analyzed chemical composition of the diets[1]. Values are expressed as mean ± standard deviation of two production batches, in a dry-matter basis.

Item	Control	Amino acid
Moisture (%)	5.70±0.82	5.86±0.86
Crude protein (%)	31.6±0.92	31.8± 0.37
Essential amino acids		
Arginine (%)	1.97±0.14	1.98±0.14
Histidine (%)	0.76±0.03	0.77±0.03
Isoleucine (%)	1.24±0.06	1.23±0.04
Leucine (%)	2.65±0.10	2.76±0.18
Lysine (%)	1.81±0.04	1.88±0.09
Methionine (%)	0.62±0.03	0.93±0.09
Phenylalanine (%)	1.33±0.02	1.36±0.03
Tryptophan (%)	0.37±0.05	0.46±0.06
Threonine (%)	1.32±0.07	1.49±0.10
Valine (%)	1.52±0.03	1.59±0.04
Non-essential amino acids		
Alanine (%)	2.18±0.15	2.19±0.15
Aspartic acid (%)	2.79±0.01	2.87±0.01
Proline (%)	2.02±0.21	2.03±0.22
Glutamic acid (%)	4.53±0.25	4.58±0.30
Glycine (%)	2.39±0.34	2.42±0.36
Serine (%)	1.44±0.02	1.46±0.01
Taurine (%)	0.23±0.01	0.26±0.03
Cystine (%)	0.24±0.09	0.26±0.07
Tyrosine (%)	1.02±0.06	0.99±0.07
Acid hydrolyzed fat (%)	10.67±0.42	10.08±0.01
Starch (%)	24.08±0.11	23.41±0.51
Crude fiber (%)	10.48±0.64	9.96±0.29
Total dietary fiber (%)	25.21±2.85	25.23±1.19
Ash (%)	6.77±0.35	6.76±0.34
Calcium (%)	1.03±0.17	1.03±0.19
Phosphorus (%)	0.89±0.06	0.90±0.09

1 Diets have the same basic formulation, but the Amino acid treatment have added DL-Methionine, L-Tryptophan, L-Threonine, and L-Valine. Ingredient list: Poultry by-product meal, sorghum, sugarcane fiber, sweet potato flour, corn gluten meal 60%, chicken fat, beet pulp, natural flavor, brewer's yeast, barley, potassium chloride, mannan-oligo-saccharide, fructo-oligosaccharides, vitamin and mineral premix, sodium chloride, seaweed meal, choline chloride, and antioxidants.

Before tested on client-owned dogs, the total tract apparent digestibility coefficients of nutrients and the metabolizable energy (ME) content of the diets were determined by the method of total feces collection (FEDIAF, 2018). The study was conducted using six dogs per food (beagles, 11,9±1,1 kg, 4,5±1,2 years). Adaptation to diets was performed for 5 days, with dogs housed on a 1.5m to 4m kennels with solarium and daily access to a playground. Total feces collection was conducted for 5 days, with animals individually housed in 1.0m x 1.0m x 1.0m metabolic cages. Animals were fed daily at 9:00 am during 30min, in sufficient quantity to meet their maintenance energy requirement (NRC, 2006). Offered and refused food was weighted, and the intake recorded. Feces were collected at the mealtime, weighted and frozen (-15°C). The quality of the feces was evaluated using a score from 0 to 5 (CARCIOFI et al., 2008), being: 0 - liquid feces; 1 - pasty and shapeless stools; 2 - soft, malformed stools that take the shape of the collection container; 3 - soft, formed, and moist stools that mark the floor; 4 - well-formed and consistent stools, which do not mark the floor; 5 well formed, but hard and dry. At the end of the collection period, the feces were tawed and homogenized, composing one sample per animal, and then dried in a forced ventilation oven at 55°C for 72h.

Dried feces and food samples were grounded in a knife mill (MOD 340, ART LAB, São Paulo, Brazil), with a 1mm sieve, and then analyzed according to the procedures described by the AOAC (2010) for dry matter, ash, crude protein, crude fiber, dietary fiber and ether extract after acid hydrolysis. The amount of starch was determined according to Hendrix (1993), and the gross energy content in a calorimetric pump (IKA Calorimeter System C 2000, USA). All analyzes were conducted in duplicate and repeated if a coefficient of variation greater than 5% was verified between replicates. On diets, amino acid composition was analyzed according to the Pico-Tag method (White, Hart & Fry, 1986), except for tryptophan, analyzed by enzymatic alkaline hydrolysis (Lucas & Sotelo, 1980). The calcium content was determined by atomic absorption method described as described in the Compêndio Brasileiro de Alimentação Animal (2017), method n.40. Phosphorus content was analyzed by official method 965.17 from AOAC (2010). Digestibility results, food ME content and fecal characteristics are shown in Table 2.

Table 2. Total tract apparent digestibility coefficient of nutrients, metabolizable energy content and feces characteristics of dogs fed the experimental diets (mean ± standard deviation).

Item	Diets		P Value
	Control	Amino acid	
Body weight (kg)	11.8±1.26	11.9±1.1	0.897
	Nutrient intake (g/dog/day)		
Dry matter	166.43±13.1	165.74±13.0	0.950
	Total tract apparent digestibility coefficient (%)		
Dry matter	66.7±4.0	64.1±2.6	0.341
Organic matter	69.0±3.8	66.7±2.2	0.323
Crude protein	82.8±1.7	82.4±1.9	0.747
Acid-hydrolyzed fat	88.3±1.6	86.6±2.3	0.284
Gross energy	71.2±3.5	68.7±2.1	0.279
Metabolizable energy (kcal/g as fed basis)	3.10	3.0	0.192
	Feces characteristics		
g/dog/day	173.8±19.6	173.5±17.3	0.984
g/dog/day (DM)	59.1±6.2	63.7±5.3	0.317
Dry matter (%)	34.0±1.3[a]	36.8±1.2[b]	0.013
Fecal condition score	4.0±0.1	4.0±0.05	0.793

Experimental design

In the Phase 1, or the static weight phase, DEE (daily energy expenditure), BC (body composition), plasma amino acid profile and protein metabolism were determined in obese and non-obese dogs after 15 days of diet intake to constant body weight. The study was organized in a completely randomized design. The experimental unit was considered each dog. Treatments were organized in a 2 (obese and non-obese dogs) x 2 (Control and Amino acid diets) factorial arrangement, totalizing 4 experimental treatments. The obese and non-obese dogs were randomly distributed between diets, totalizing 10 experimental units for each combination of diet and body composition. The groups were balanced to present similar proportions of males and females and age. Initially, dogs were fed an amount of food to maintain a constant body weight (based on owner

information or estimated as 95 kcal/kg$^{0.75}$/day). Owners weighted daily the offered and refused food in a scale and animals were then weighted weekly and the amount provide adjusted to achieve constant body weight. After 15 days of food intake under constant body weight, DEE and BC were determined by the doubly labeled water method, plasma was obtained to measure free amino acids, and protein metabolism was studied with the ^{13}C-Leucine method.

After this, the obese dogs underwent to Phase 2 of the study, or the weight loss phase. A standardized weight loss program was conducted and the effects of the consumption of the two experimental diets were compared regarding their implications on DEE, BC, plasma amino acid profile and protein metabolism of dogs. The study followed a completely randomized design with two diets and 20 obese dogs, totalizing 10 dogs (experimental unit) per diet. Dogs remained on the diets sorted in the Phase 1. Initially, the amount of food offered was readjusted to 60 kcal/kg of current body weight$^{0.75}$/day. Owners weighted daily the offered and refused food in a scale. Dogs were then weighed every two weeks and the amount of food provided adjusted when necessary to achieve a body weight loss of approximately 1% per week. A guide with recommendations for weight loss was provided to owners, with information such as feeding frequency, estimated time to reach the target weight and tips for controlling the animal's appetite. When dogs achieved approximately 10% and 20% of body weight loss, DEE and BC was determined by the doubly labeled water method. When dogs lost 20% of body weight, additionally plasma was obtained to measure free amino acids, and protein metabolism was studied with the ^{13}C-Leucine method.

Doubly labeled water method application

The method was applied at the end of phase 1 in obese and non-obese dogs, and in phase 2, when dogs reached 10% and 20% reduction in initial body weight. After 12-hour fasting and four-hour with no water, blood collection was performed to determine basal body water concentration of the isotopes. Immediately afterwards, each dog received an isotope solution at an approximate dose of 0.12g of ^{2}H at 99.9 atm% per kg of body water and 2g of ^{18}O at 10 atm% per kg of body water (Sercon Limited, Unit 3B Crewe Trade Park, Gateway, Crewe, Cheshire, UK). For this a 6:100 (w/w) solution of ^{2}H at 99.9 atm% and ^{18}O

at 10 atm% was prepared. This was hypodermic inoculated between the scapulae. At 2 hours after the application, a new blood sample was collected to determine the isotopic enrichment in body fluids. Blood samples were then collected after 3, 5 and 7 days of inoculation to assess the elimination of isotopes in body water. In all collections, approximately 3 mL of blood were removed by direct puncture of the jugular vein and stored in vacutainer tubes with ethylenediamine tetraacetic acid (EDTA). The serum was separated, collected, and stored at -20°C in cryogenic tubes sealed with plastic paraffin for later analysis. Aiming accuracy in the application and precision in the knowledge of the administered dose, the syringes were weighed on a precision scale before being filled, the syringe plus the isotope solution and, finally, the syringe immediately after inoculation of the isotopes in the animals (Ferriolli, Pfrimer & Cruz, 2008). The body mass of the dogs was established on scales calibrated using certified standard weights (INMETRO: standard weight from 10 g to 1 kg - Accuracy Class OIML E1; Uncertainty: 1/3 of the maximum allowable error for accuracy class OIML E1/2004).

Isotopes were analyzed by Isotope Ratio Mass Spectometry determination (IRMS) in a Hydra System equipment (ANCA 20-20, Europa Scientific, Cheshire, UK), according to procedures described by Ferriolli et al., (2008). For ^{2}H the samples were processed in duplicate (200 µL per replication) with platinum in vacutainers, followed by six hours of rest. For ^{18}O the samples were processed in triplicate (150 µL per replication), filled with CO_2 and left to rest for 24 hours, later isolating and purifying the compound.

The pool size for ^{18}O was used to estimate total body water (Schoeller & Santen, 1996):

$$\mathrm{N\ (moles)} = \left(\frac{WA}{18{,}02a}\right) \times \frac{(\delta a - \delta t)}{(\delta s - \delta p)}$$

Where: N = pool size of body water; W= amount of water used to dilute the labelled water dose; A= the weight of labelled water administration (grams); a= the diluted dose for analysis; δ= enrichment of dose (a), dilution water (t), post dose sample (s) and pre dose baseline (p) samples.

The determination of lean mass was based on the principle of lean mass hydration constant of 73.2% for mammals, with the following equation: Lean body mass (LM, kg) = body water, kg ÷ 0.732. The difference between total body mass (kg) and LM (kg) was used to estimate the fat mass content (Rathbun & Pace, 1945).

The isotopes enrichment on body fluids was established on the sample collected after 2h of inoculation. To calculate the elimination rate of ^{2}H and ^{18}O, the two-point formula (Schoeller & Santen, 1996) was used, obtaining a constant for each isotope. The mean value calculated with samplings at 3, 5 and 7 days was used for each animal:

$$K = \frac{LN[X(t2) - X(t1)]}{t2 - t1}$$

Where: K = rate constants for ^{2}H (Kd) and ^{18}O (Ko); LN = natural logarithm; X(t2) = sampling point of isotope elimination; X(t1) = sampling point of the isotope enrichment; t1= day of isotope enrichment sampling; t2= day of isotope elimination sampling.

The calculation of the amount of CO_2 produced was established in two manners. For dogs with more than 10kg of body weight the formula of Ballevre et al., (1994) was used:

$$\mathrm{rCO2}\ \left(\frac{\mathrm{mol}}{\mathrm{day}}\right) = \left(\frac{1}{2.08}\right) * (\mathrm{No} * \mathrm{Ko}) - (\mathrm{Nd}.\mathrm{Kd}) - 0.0258 * (\mathrm{No} * \mathrm{Kd}) - (\mathrm{Nd} * \mathrm{Kd})$$

Where: rCO_2 = CO_2 production; No = body water contingent with oxygen; Ko = constant of elimination of ^{18}O in body water; Nd = estimated body water contingent with deuterium; Kd = constant of elimination of deuterium in body water.

For dogs with less than 10kg of body of body weight the equation proposed by Lifson & McClintock (1966) was utilized:

$$\mathrm{rCO2}\ \left(\frac{\mathrm{mol}}{\mathrm{day}}\right) = \left(\frac{\mathrm{N}}{2.08}\right) * (\mathrm{Ko} - \mathrm{Kd}) - 0.015 * \mathrm{Kd} * \mathrm{N}$$

Where: rCO_2 = CO_2 production; N = dilution space for ^{18}O; Ko = constant of elimination of ^{18}O in body water; Kd = constant of elimination of ^{2}H in body water.

To determine the energy expenditure of the dogs, the equation described by Elia & Livesey (1992) was used:

$$DEE\ \left(\frac{\text{kj}}{\text{day}}\right) = \text{rCO2} * 22.4\left(\frac{3.7}{\text{FQ}} + 1.326\right) * 4.18$$

Where: DEE = daily energy expenditure; rCO_2 = CO_2 production; FQ = food quotient

To apply the last equation the food quotient (FQ) was calculated from the digestible nutrient composition of the experimental diets, according to the coefficients of apparent digestibility of crude protein, ether extract and starch determined in the digestibility test performed, as previously described (Black, Prentice & Coward, 1986):

$$FQ = \frac{(P * 0.781) + (F * 1.427) + (S * 0.746)}{(P * 0.996) + (F * 2.019) + (S * 0.746)}$$

Where: FQ = food quotient; P = digestible protein; F = digestible fat (acid hydrolyzed ether extract); S = digestible starch.

^{13}C-leucine method application

The method was applied at the end of Phase 1 (the static body weight phase) and the end of Phase 2 (after 20% body weight loss). After 12 hours of food fasting and 4 hours of water deprivation, each animal was cannulated with an intravenous catheter (Descarpack®, size: 24G, 22G or 20G according to the size of the dogs), placed on the chephalic vein. The tracers used were L-[1-^{13}C] Leucine (132.17mg, 99 atm%, 15N, Cambridge Isotope Laboratories®) and $NaH^{13}CO_3$ (85mg, 98 atm%, ^{13}C, Cambridge Isotope Laboratories®) in solutions made with 0.9% sodium chloride and water. After the collections of blood through the catheter and basal exhaled gas using a spirometry mask, a priming dose of labeled compounds was administered via catheter (combination of 4.64 mg/kg of L-[1-^{13}C] Leucine and 0.20 mg/kg of [^{13}C] $NaH^{13}CO_3$) and after twenty minutes

the intermittent infusion started through the flush of 1.4 mg/kg of L-[1-^{13}C] Leucine every 20 minutes for a total period of 140 minutes.

Plasma collection was performed at the following intervals (in minutes): 0, 80, 100, 120, 140, 160 and 180. The expired gas was collected using a specific spirometry mask at the following intervals (in minutes): 0, 80, 100, 120, 160, and 200. Each dog was previously adapted and trained to breast on the mask. The expired gas was collected through a tube coupled to the spirometry mask, as the gas was "blown" into the vial by the dog. The expired gas samples were kept at ambient temperature (≈28°C) until the analysis. Blood samples were placed in Vacutainers tubes with EDTA K2 anticoagulant and later centrifuged to separate the plasma, stored in microtubes, and stored in a freezer (-20º C).

The expired gas was analyzed in an isotope ratio mass spectrometer, specific for $^{13}CO_2/^{12}CO_2$ analysis (ABCA-IRMS, SerCon, Gateway, UK). The plasma samples were analyzed in an isotope ratio mass spectrometer coupled to an element analyzer (Thermo Fisher Scientific, Waltham, MA, USA).

Determination of protein turnover:

The fluxes of labeled compounds and rates of protein synthesis and degradation were calculated according to the model proposed by Picou & Taylor-Roberts (1969):

$$Q = O(\text{ou } E) + S = I + B$$

Where: Q= body protein turnover rate or tracer flux (µmol.kg^{-1}. d^{-1}); O= amino acid oxidation measured by expired gas; S= protein synthesis; I= tracer dose (0,0057mmol.kg-0,75.h-1); B= protein degradation.

Flux determination, synthesis and degradation:

$$Q\ (\mu\text{mol.kg} - 1.\text{d} - 1) = i\ (\frac{Ei}{Ep * 1{,}25} - 1)$$

Where: Q= amino acid flux, i= tracer infusion rate (µmol.kg-1.h-1); Ei= natural enrichment of plasma (atm%), basal point, Ep= plasma enrichment (atm%) plateau after infusion of the marked compound; factor 1.25 corrects intracellular leucine values.

To convert leucine flux/turnover to whole-body protein values, the average body protein leucine content of 8g/100g protein is used (Matthews et al., 1980):

$$O_{leu}\ (\mu mol.kg^{-1}.d^{-1}) = {}^{13}CO_2 * VCO_2 * NaH^{13}CO_3$$

Where: L-[1-^{13}C] leucine oxidation (Lox) is quantified by measuring $^{13}CO_2$ enrichment in exhaled air and multiplying it by total CO_2 production and a bicarbonate correction factor for CO_2 losses between oxidation and respiration (Van Hall, 1999).

$$S\ (\mu mol.kg^{-1}.d^{-1}) = Q - O$$

Where: Protein synthesis (S) can be calculated from flux and oxidation of [1-^{13}C] leucine, since other parameters are calculated in the plateau.

$$S\ (\mu mol.kg^{-1}.d^{-1}) = Q - Eu$$

Where: S= protein synthesis; Q= flux and e_{ep} and total ^{15}N excreted from the final component (µmol.kg-1.d-1, urea or ammonia or average) discounting the basal enrichment.

$$B\ (\mu mol.kg^{-1}.d^{-1}) = Q - I$$

Where: Protein degradation (B) can be calculated from the difference between the flux and the administration of L-[1-^{13}C] leucine. In this case, the ingestion is zero, as they are studies carried out on an empty stomach, the degradation is equal to the flow.

$$BN\ (\mu mol.kg^{-1}.d^{-1}) = S - B$$

Where: BN= nitrogen balance (^{15}N) (difference between synthesis and degradation).

Determination of plasma free amino acid concentration

Free amino acids concentration in the plasma was also measured at the end of Phase 1 and Phase 2. Samples were collected at each phase after a 12-hour fasting period and in the postprandial period, 6 hours after the meal. Three mL of blood was collected through jugular vein puncture and stored in heparinized tubes. Blood was centrifuged at 3500 rpm for ten minutes. After centrifugation the supernatant was separated for deproteinization. The samples were deproteinized using a 6% sulfosalicylic acid solution containing 400mMol/Ml of L-Norleucine in a 1:1 ratio (Spitze, Wong, Rogers & Fascetti, 2003). After homogenization of the tubes with sample and solution, they remained resting on the bench for fifteen minutes and then centrifuged for twenty minutes at 15000 rpm and at a temperature of 4°C. The supernatant containing the free amino acids was then collected, stored in 1.5 mL eppendorfs sealed with plastic parafilm and kept in a freezer at -80°C for further analysis. Free amino acids were determined by cation exchange and reactive ninhydrin HPLC (High Performance Liquid Chromatography) (Biochrom 30 Amino Acid Analyzer, Biochrom, Cambridge, UK).

Statistical analysis

Data were verified for homoscedasticity and normality of errors. In Phase 1, data were evaluated in a completely randomized design considering the effects of diet (Control and Amino acid), body composition (obese or non-obese) and the diet*body composition interaction, in a 2x2 factorial arrangement of treatments. In Phase 2, data were evaluated by repeated measures analysis of variance, with two factors between animals (diet and time) and one factor within the animals (time), considering the main effects of diet, time and the interaction time*diet.

The experimental unit considered was each dog, with 10 repetitions per diet in both Phase 1 and Phase 2. The digestibility trial was evaluated by variance analysis in a randomized block design. Analyzes were performed using the Proc MIXED of the SAS software (SAS Institute, Cary, NC, USA, 2003). Values of $P<0.05$ was considered significant and $P<0.10$ as a trend.

3. RESULTS

The chemical composition of the diets was similar, differing only on the supplemented amino acids concentration. Diets also showed similar values for apparent total tract digestibility of nutrients and metabolizable energy (ME) content.

To evaluate the experimental diets in domiciled dogs, in the group of non-obese 26 animals were assessed and only 20 were completely healthy and able to participate. Regarding the obese dog group, 63 owners were contacted and of these, 84% (53/63) dogs were assessed, 52% (33/63) had no alterations in clinical, physical and laboratory exams, and were submitted to the endocrine tests. Of the 33 obese dogs tested for endocrine diseases, 21% (7/33) showed results consistent with endocrine disorders and were removed from the project. Due this, from the total number of contacted owners, only 41% of the dogs started the project (26/63). Along the study, 19% (5/26) of the owners opted to interrupt the weight loss program before 10% of body weight loss and 15% (4/26) before completing 20% of body weight loss. So, completed the project achieving 20% weight loss 65% (17/26) of the obese dogs that were initially included. The characteristics of the dogs that participate on the study are described in the appendices.

The diets had a good acceptance by the client-owned dogs in both study Phases. During Phase 1, in which the recommended food amount was established to maintain a constant body weight, few dog owners in the non-obese group reported difficulties in the animals' acceptance of diets but reported adaptation within a short period of time. No animal had episodes of diarrhea or vomiting. Both the obese and the non-obese dog groups maintained a constant body weight along the Phase 1 ($P>0,05$ for body weight variation between the start and end of the phase; data now shown). No diet effect was observed for any parameter evaluated ($P>0,05$), as show in Table 3. However, between dog groups, obese animals presented higher BCS, body weight, and fat mass %, with lower lean mass % ($P<0.01$). The energy intake to constant body weight was also lower in obese dogs than in non-obese animals ($P=0.044$).

Table 3. Non-obese (n = 20) and obese (n = 23) owned dogs fed with the experimental diets during the static body weight phase. Values expressed as mean ± standard error.

Item		Control	Amino acid	Mean	P value		
					Diet	BC[1]	Diet*BC
Initial body weight (kg)[2]	**Non-obese**	12.8±2.9	15.6±3.3	14.2±2.5			
	Obese	28.3±3.1	24.8±2.9	26.5±2.1	0.914	<0.001	0.315
	Mean	20.5±2.4	20.2±2.2				
Body condition score[3]	**Non-obese**	4.6±0.01	4.6±0.01	4.6±0.01			
	Obese	8.4±0.01	8.3±0.01	8.3±0.01	0.861	<0.001	0.861
	Mean	6.6±0.01	6.6±0.01				
Energy consumption (kcal/kg^{0,75}/day)	**Non-obese**	92.3±3.2	90.7±3.2	91.5±2.4			
	Obese	86.2±2.8	84.2±2.6	85.2±1.9	0.774	0.044	0.706
	Mean	89.2±2.2	87.5±2.1				
Lean body mass (%)	**Non-obese**	76.6±1.9	76.6±1.9	76.6±1.4			
	Obese	56.1±1.9	57.6±1.8	56.9±1.3	0.691	<0.001	0.687
	Mean	66.4±1.4	67.1±1.3				
Fat body mass (%)	**Non-obese**	23.4±1.9	23.4±1.9	23.4±1.4			
	Obese	43.9±1.90	42.4±1.8	43.1±1.3	0.691	<0.001	0.687
	Mean	33.6±1.4	32.9±1.3				

1 BC = effect of body composition
2 The final body weight did not differ from the initial body weight of the dogs (P>0.05). Data not shown.
3 Body condition score (BCS) based on 9 points scale (Laflamme, 1997).

The weight loss phase (Phase 2) of obese animals was divided into two stages; first 10% of body weight loss; total of 20% of body weight loss (Table 4). No diet effect was observed for body weight, body condition score, and total body weight loss (P>0.05), as the groups were balanced to be similar, and dogs were submitted to a controlled weight loss program so these parameters did not interfere on results of body composition and energy expenditure. Although no diet effect was observed for time in regimen, marked time effect was observed (P<0.001) and dogs lasted approximately 13.3±1.7 weeks to lose the first 10% of body weight and 23.2±2.2 weeks to lose the second 10% loss of body weight. This is reinforced by the weight loss rate per week, with a mean of 0.91±0.07% per week in the first 10% of body weight loss but reduced to 0.55±0.08% per week on the second half of the regimen (P=0.004). Interesting, a tendency for a higher weight loss rate was observed for dogs fed the Amino acid diet, that lost body weight in a rate approximately 24% higher than animals in the Control diet (P=0.083).

Energy intake to achieve weight loss was similar among phases and diets (P>0.05), with a mean value of 57.4±4.6 kcal/kg of current body weight$^{0.75}$/day. Along the regimen, dogs lost a significant amount of fatty mass (P<0.001), but not of lean mass (P=0,536). Their mean fatty mass content reduced from 41.2±1.6% to 29.7±1.6%, without a diet effect (P=0.314). However, when the difference in fatty mass loss was observed by regimen time, higher fat amount was lost in the second (4.2±0.8 kg) than in the first period of weight loss (2.5±0.8 kg; P=0.021). It is interesting to observe also that although the lean mass loss was similar among regimen periods and diets (P>0.05), dogs fed the Control diet lost in total 0.9±0.8kg of lean mass after 20% body weight loss, while dogs on Amino acid diet did not lose lean mass, keeping the same amount of lean mass content from the beginning (11.9±2.0kg) until the end (11.9±2.0kg) of the regimen.

34 **Table 4.** Obese owned dogs fed with the control or amino acid experimental diets during the weight loss phase (values expressed
35 as mean ± standard error).

Item	Time	Control	Amino acid	Mean	P valor		
					Diet	Time	Diet*time
Body weight (kg)	**Initial**	25.5±2.2	22.7±2.0	24.1±1.5	0.439	<0.001	0.627
	10% BW loss	22.6±2.2	20.4±2.0	21.5±1.5			
	20% BW loss	19.8±2.2	17.8±2.0	18.8±1.5			
	Mean	22.6±2.1	20.3±2.0				
Body condition score[1]	**Initial**	8.3±0.7	8.2±0.6	8.2±0.6	0.647	<0.001	0.771
	10% BW loss	7.0±0.6	7.1±0.6	7.0±0.6			
	20% BW loss	5.7±0.8	5.6±0.7	5.6±0.7			
	Mean	7.2±1.3	7.0±1.2				
Time (weeks)	**Initial**	-	-	-	0.241	0.002	0.608
	10% BW loss	15.6±2.6	11.0±2.3	13.3±1.7			
	20% BW loss	24.0±3.3	22.3±2.6	23.2±2.2			
	Mean	19.8±2.1	16.6±1.7				
Total body weight loss (%)	**Initial**	-	-	-	0.754	0.113	0.217
	10% BW loss	10.7±0.4	10.2±0.4	10.5±0.3			
	20% BW loss	10.9±0.5	11.6±0.4	11.2±0.4			
	Mean	10.8±0.3	10.9±0.3				

Weight loss rate (% per week)	**Initial**	-	-	-			
	10% BW loss	0.78±0.10	1.05±0.09	0.91±0.07	0.083	0.004	0.563
	20% BW loss	0.48±0.13	0.62±0.10	0.55±0.08			
	Mean	0.63±0.08	0.83±0.07				
Energy consumption (Kcal/Kg actual $BW^{0.75}$/Day)	**Initial**	57.8±1.4	56.2±1.3	57.0±1.0			
	10% BW loss	57.9±1.4	57.0±1.3	57.5±1.0	0.682	0.842	0.653
	20% BW loss	56.2±1.8	57.1±1.4	56.6±1.2			
	Mean	57.3±1.0	56.7±0.9				
Body fat (kg)	**Initial**	11.1±1.6	8.6±1.3	9.9±1.0			
	10% BW loss	7.7±1.6	6.1±1.3	6.9±1.0	0.343	<0.001	0.782
	20% BW loss	6.0±1.6	4.3±1.2	5.2±1.0			
	Mean	8.3±1.5	6.3±1.1				
Lean mass (kg)	**Initial**	14.3±2.5	11.9±2.0	13.1±1.6			
	10% BW loss	14.7±2.5	12.3±2.0	13.5±1.6	0.536	0.342	0.708
	20% BW loss	13.6±2.5	11.9±2.0	12.8±1.6			
	Mean	14.2±2.5	12.1±1.9				
Body fat (%)	**Initial**	41.8±2.5	40.6±2.0	41.2±1.6			
	10% BW loss	34.7±2.5	33.0±2.0	33.8±1.6	0.314	<0.001	0.684
	20% BW loss	31.9±2.5	27.4±2.0	29.7±1.6			
	Mean	36.2±1.8	33.7±1.4				
Lean mass (%)	**Initial**	58.1±2.5	59.4±2.0	58.8±1.6	0.314	<0.001	0.684

	10% BW loss	65.3±2.5	67.0±2.0	66.2±1.6			
	20% BW loss	68.1±2.5	72.6±2.0	70.3±1.6			
	Mean	63.8±1.8	66.3±1.4				
Fat mass loss (initial – final) (kg)	**T0-T1**	2.5±1.3	2.5±1.0	2.5±0.8			
	T0-T2	4.2±1.3	4.3±1.0	4.2±0.8	0.990	0.021	0.904
	Mean	3.4±1.2	3.4±0.9				
Lean mass loss (initial – final) (kg)	**T1-T0**	0.1±0.8	0.4±0.7	0.3±0.5			
	T2-T0	-0.9±0.8	0.0±0.7	-0.4±0.5	0.531	0.213	0.545
	Mean	-0.4±0.7	0.2±0.6				

[1] Body condition score (BCS) based on 9 points scale (Laflamme, 1997).
[2] Time 1: up to 10% body weight loss (control n=10; amino acid n=11). Time 2: from 10% to 20% body weight loss (control n=7 amino acid n=10).

Nutrient intake during weight loss was calculated based on diets chemical composition and intake data. As diets had similar crude protein content and similar energy intake was observed, crude protein consumption was similar among foods (P=0.944). The supplemented amino acids methionine, methionine plus cystine, tryptophan, and threonine, however, presented higher intake on dogs fed the Amino acid than the Control diet (P<0.05; Table 5). It was noted that the calculated intake of methionine and tryptophan on the Control group mas close to proposed adequate intake, and methionine plus cystine intake was approximately 24% lower on the Control group when compared to the recommendations to dog maintenance (FEDIAF, 2020). All amino acids intake was above the recommended for maintenance on the Amino acid diet fed group.

Table 5. Protein and amino acid intake (g/kg$^{0.75}$/day) of obese dogs fed with the control or amino acid diets during the weight loss program, and recommendations of NRC (2006) and FEDIAF (2020) for these nutrients' intake.

Item	Consumption		P value	Recommendations	
	Control diet	Amino acid diet		NRC, 2006	FEDIAF, 2020
Observed mean energy intake (kcal/kg^{0,75}/day)[1]	57.7±3.35	57.6±2.95	0.945	-	-
Crude protein	6.08±0.35	6.11±0.31	0.870	3.28	4.95
Arginine	0.38±0.02	0.38±0.02	0.994	0.11	0.14
Histidine	0.15±0.01	0.15±0.01	0.616	0.06	0.06
Isoleucine	0.24±0.01	0.24±0.01	0.741	0.12	0.13
Leucine	0.51±0.03	0.53±0.03	0.161	0.22	0.23
Lysine	0.35±0.02	0.36±0.02	0.215	0.11	0.12
Methionine	0.12±0.01	0.18±0.01	<0.001	0.11	0.11
Phenylalanine	0.26±0.01	0.26±0.01	0.474	0.15	0.15
Tryptophan	0.07±0.01	0.09±0.01	<0.001	0.05	0.05
Threonine	0.25±0.01	0.29±0.01	<0.001	0.14	0.14
Valine	0.29±0.02	0.31±0.02	0.118	0.16	0.16
Methionine + cystine	0.16±0.01	0.23±0.01	<0.001	0.21	0.21
Phenyl + tyrosine	0.44±0,03	0.45±0.02	0.269	0.24	0.24

[1] Mean value of energy consumption on body weight loss phase.

4. DISCUSSION

The data presented include information regarding the digestibility test of the experimental diets, data on the weight loss of dogs, such as their loss of lean mass, time taken to reach the expected weight target and the estimated caloric intake based on prescriptions and isotopic analyzes of body composition. Energy expenditure data referring to the doubly-labeled water method, as well as data on the protein metabolism and plasma concentration of amino acids were not possible to be obtained until the present day due to delays in the analysis as consequence of COVID-19 pandemic.

The study was conducted with the hypotheses that obese client-owned dogs, in the static phase of obesity, have lower energy maintenance requirements than non-obese dogs, and that the supplementation of the amino acids methionine, tryptophan, threonine and valine might result in greater maintenance of lean mass during weight loss compared to a diet similar in composition, but without this supplementation. Both diets presented nutrient values above those recommended for adult dogs maintenance by the NRC (2006) and FEDIAF (2020), being formulated for dogs in caloric restriction.

Regarding the acceptability of the diet, for the group of non-obese dogs initially was reluctant, but it was possible to make all animals accept the diet and ingest the necessary amount, resulting in no changes in weight during phase 1, as intended. A study performed by Hours et al. (2016), evaluated the palatability of diets designed for weight loss in non-obese dogs and cats and found less acceptance of this type of food attributed to its low fat and high fiber content. This behavior is often not observed in dogs during the weight loss program, as energy restriction increases hunger in these animals (German et al., 2007; Hours et al., 2016).

The hypothesis that obese dogs have lower energy requirement for maintenance in the static phase of obesity was confirmed estimating the energy intake of dogs. Thes et al. (2016), in a retrospective study to determine the energy requirement of adult domesticated dogs, also found that overweight dogs consume significantly less energy for maintenance than dogs within ideal body weight. Pedrinelli et al. (2019) found a decrease of 9.8 kcal/$BW^{0,75}$ on

maintenance requirement of dogs for each increase point in 9-point scale of BCS. Ponteau et al. (2000) found maintenance energy requirements 13% lower in overweight dogs when compared to ideal body weight dogs. If the energy expenditure analysis confirms the initial hypothesis that obese dogs in fact have lower maintenance needs, it can be inferred from the results that the owners that participated in the study did not follow the dietary recommendations correctly, omitting information concerning what they provided to the dogs and demonstrating lack of commitment to treatment, a fact that has been observed in several studies on obesity in dogs and cats (Carciofi et al., 2005; German et al., 2007; 2015) .

It was also hypothesized that supplemented amino acids might help to preserve lean mass, but results obtained so far do not corroborate this, considering that there was no statistical difference in body composition assessed by the deuterium labelled water method between animals ingesting the different diets. Note, however, that the group that received the supplemented experimental diet did not show loss of lean mass after weight loss, while the control group lost an average of approximately 900 grams. In addition, the supplemented group had a faster weight loss, which could have predisposed these animals to greater loss of lean mass compared to the control group, as the rapid weight loss is associated with loss of muscle mass (Burkholder & Toll, 2000).

Several studies performed with dogs undergoing weight loss confirm that energy restriction improves metabolic efficiency by reducing the energy requirement for maintenance (Ramsey & Hagopian, 2006; German et al., 2015). In the present study, there was a significant difference between the duration of each weight loss stage, being the loss up to 10% faster and with higher weight loss rate per week, even with a more stable energy consumption between the groups throughout the period. This corroborates with previously mentioned studies, suggesting metabolic adaptations and lower energy requirements over time. Another possibility for this finding could be the lack of commitment from the owners, who could be providing the dogs with extra calories (Yaissle, Holloway & Buffington, 2004; German et al., 2007; Hours et al., 2016). This might be confirmed with energy expenditure data from the doubly-labeled water method.

Although there was no statistical difference between groups in weight loss rate per week, there was a tendency to a difference, with the mean loss of the supplemented group being higher than the control group. However, without the concise data from the energy expenditure analysis, it is not yet possible to confirm that animals on the supplemented diet could be losing weight faster with the same energy intake.

Limitations of the study also need to be considered in addition to the analysis delay. The major point of difficulty was the lack of collaboration from the owners. Many quit the study throughout the weight loss program, making it necessary to start the program with new animals and delaying the progress of data collection. In addition, the lack of commitment of most of them made it very difficult to finish out the study as well as delayed the weight loss. Another important limitation was that the experimental part coincided with the covid-19 pandemic, also compromising the collaboration of the owners and affecting negatively the weight loss of the dogs, since owners at home tend to offer more extra food to their pets. One last limitation was the non-assessment of activity level of the dogs, knowing that exercise plays a role in the preservation of muscle mass during weight loss (German et al., 2007; Vitger, Stallknecht, Nielsen & Bjornvad, 2016), this factor may have interfered with assessments.

As conclusion, obese dogs presented lower energy requirement to maintain a stable body weight than non-obese animals. This lower energy expenditure imposes an elevated food and energy restriction to induce weight loss and fat mass reduction. Under this situation careful attention is necessary to amino acid intake, with special emphasis on methionine, methionine plus cystine, and tryptophan. The correction of this amino acids in a diet for weight loss might induce faster body weight reduction and may help prevent lean body mass loss. Future studies on better amino acid formulation and protein metabolism during negative energy balance are required to dogs.

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APÊNDICES

1. Descrição dos cães não-obesos que participaram do estudo.

Animal	Raça	Idade (anos)	Sexo	Condição sexual	Peso (kg)	*ECC
CO						
1	Poodle	7	Fêmea	Castrado	5,0	5
2	Bulldog francês	4	Macho	Inteiro	19,9	5
3	SRD	3	Fêmea	Castrado	16,4	5
4	SRD	2	Fêmea	Castrado	16,5	4
5	SRD	6	Fêmea	Castrado	11,7	5
6	SRD	4	Fêmea	Castrado	10,9	4
7	Poodle	3	Fêmea	Castrado	5,4	5
8	SRD	5	Fêmea	Castrado	18,3	5
9	SRD	7	Fêmea	Castrado	8,9	5
10	SRD	6	Fêmea	Castrado	7,4	4
AA						
11	SRD	5	Fêmea	Inteiro	9,8	5
12	SRD	3	Fêmea	Castrado	20,7	5
13	SRD	3	Fêmea	Castrado	19,4	5
14	SRD	3	Fêmea	Castrado	16,4	4
15	Shih-tzu	7	Fêmea	Inteiro	3,9	4
16	Shih-tzu	7	Fêmea	Inteiro	5,1	4
17	SRD	4	Fêmea	Castrado	14,8	5
18	Daschund	3	Macho	Castrado	6,9	5
19	SRD	6	Fêmea	Castrado	11,5	4
20	Pastor de malinois	6	Fêmea	Castrado	34,9	4

* Escore de condição corporal (ECC) baseado em escala de 9 pontos de acordo com Laflamme (1997).

2. Descrição dos cães obesos que participaram do estudo.

Animal	Raça	Idade (anos)	Sexo	Condição sexual	Peso inicial (kg)	*ECC inicial	Exames endócrinos*			
							T4 livre ng/dL	TSH (ng/dL)	Cortisol basal (mcg/dL)	Cortisol pós-supressão* (mcg/dL)
CO										
1	Labrador	6	Fêmea	Castrado	41,0	9	0,83	0,28	4,26	<1
2	Blue heeler	2	Fêmea	Castrado	26,9	8	1,32	0,18	4,60	<1
3	Poodle	8	Fêmea	Castrado	8,5	9	0,92	0,27	5,50	<1
4	SRD	5	Macho	Inteiro	25,5	8	1,32	0,20	6,30	<1
5	SRD	5	Fêmea	Castrado	39,3	9	0,49	0,08	6,90	1,25
6	Golden retriever	7	Fêmea	Castrado	40,2	8	0,94	0,05	1,33	<1
7	SRD	6	Fêmea	Castrado	17,3	7	0,56	0,17	4,10	<1
8	Spitz alemão	4	Fêmea	Castrado	7,4	8	1,13	0,08	4,04	<1
9	Spitz alemão	4	Fêmea	Castrado	7,7	8	1,10	0,10	1,26	<1
10	Golden Retriever	6	Fêmea	Castrado	55,7	9	0,81	0,44	5,00	<1
AA										
11	Beagle	4	Fêmea	Inteiro	17,6	9	0,97	0,43	7,20	<1
12	Border collie	2	Fêmea	Inteiro	29,1	8	1,20	0,36	6,10	<1
13	SRD	5	Fêmea	Castrado	18,6	8	2,03	0,22	4,44	<1
14	Border collie	4	Fêmea	Castrado	21,4	8	1,82	0,23	3,48	<1
15	Blue heeler	6	Macho	Castrado	32,1	9	1,85	0,16	2,10	<1
16	Border collie	4	Fêmea	Castrado	29,8	8	1,91	0,18	6,20	<1
17	Blue heeler	8	Fêmea	Castrado	26,3	8	1,15	0,18	7,20	1,32
18	SRD	5	Fêmea	Castrado	7,5	8	2,20	0,09	13,4	<1
19	Shih-tzu	7	Fêmea	Castrado	6,1	7	1,16	0,05	7,50	<1
20	Boxer	7	Fêmea	Inteiro	49,2	9	0,92	0,08	1,12	<1
21	Pug	6	Fêmea	Castrado	12	8	1,74	0,10	2,08	<1

*Valores de referência dos exames endócrinos: T4 livre: 0,39-1,69 ng/dL; TSH: 0,05-0,50 ng/dL; cortisol basal: 0,5 a 5,5 mcg/dL e cortisol pós-droga: Inferior ao basal.