UNIVERSIDADE ESTADUAL PAULISTA - UNESP

CAMPUS DE JABOTICABAL

DIETAS COM DIFERENTES RELAÇÕES AMIDO:PROTEÍNA NA COMPOSIÇÃO CORPORAL, METABOLISMO ENERGÉTICO, SACIEDADE, TURNOVER HÍDRICO E ATIVIDADE FÍSICA DE GATOS EM LABORATÓRIO OU EM DOMICÍLIO

CAMILA GOLONI

Médica Veterinária

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Camila Goloni

Orientador: Prof. Dr. Aulus Cavalieri Carciofi

Tese apresentada à Faculdade de Ciências Agrárias e Veterinárias – Unesp, Câmpus de Jaboticabal, como parte das exigências para a obtenção do título de Doutora em Medicina Veterinária (Clínica Médica)

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TÍTULO DA TESE: DIETAS COM DIFERENTES RELAÇÕES AMIDO:PROTEÍNA NA COMPOSIÇÃO CORPORAL, METABOLISMO ENERGÉTICO, TURNOVER HÍDRICO E ATIVIDADE FÍSICA DE GATOS EM LABORATÓRIO OU EM DOMICÍLIO

AUTORA: CAMILA GOLONI
ORIENTADOR: AULUS CAVALIERI CARCIOFI

Aprovada como parte das exigências para obtenção do Título de Doutora em MEDICINA VETERINÁRIA, área: Clínica Médica Veterinária pela Comissão Examinadora:

Prof. Dr. AULUS CAVALIERI CARCIOFI (Participaçao Virtual)
Departamento de Clinica e Cirurgia Veterinaria / FCAV UNESP Jaboticabal

Prof. Dr. RICARDO SOUZA VASCONCELLOS (Participaçao Virtual) p/
Universidade Estadual de Maringá / UEM - Maringá/PR

Prof. Dr. MÁRCIO ANTÔNIO BRUNETTO (Participaçao Virtual) p/
Departamento de Nutrição e Produção Animal / FMVZ / USP - Pirassununga/SP

Profa. Dra. KÊNIA CARDOSO BÍCEGO (Participaçao Virtual) p/
Departamento de Morfologia e Fisiologia Animal / FCAV / UNESP - Jaboticabal

Profa. Dra. ANNELISE CARLA CAMPLESI DOS SANTOS (Participaçao Virtual) p/
Departamento de Clínica e Cirurgia Veterinária / FCAV / UNESP - Jaboticabal

Jaboticabal, 03 de dezembro de 2021

DADOS CURRICULARES DA AUTORA

Camila Goloni nasceu em Camaçari-BA no ano de 1989. Iniciou os estudos no Colégio São Geraldo em Paraíso do Tocantins – TO, concluindo o ensino médio no ano de 2006. Em 2007 iniciou curso preparatório para vestibular no Colégio MEGA em Goiânia – GO. No ano seguinte ingressou no curso de Graduação em Medicina Veterinária na Universidade Federal do Tocantins (UFT). Durante a graduação foi aluna de projeto de extensão e de iniciação científica, monitora na disciplina de Bioquímica e Biofísica e, realizou intercâmbio acadêmico na Universidade de Aveiro em Portugal. Concluiu a graduação em fevereiro de 2014. 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 2014 a 2016. Se tornou Mestra pelo Programa de Pós-Graduação em Medicina Veterinária na área de Clínica Médica Veterinária com ênfase em Nutrição de Cães e Gatos. O estudo desenvolvido durante o mestrado premiou a pós-graduanda com o melhor trabalho científico no CBNA Pet em 2017 e 2018. Atualmente é doutoranda pelo Programa de Pós – Graduação em Medicina Veterinária área de Clínica Médica, com ênfase em Nutrição de Cães e Gatos.

“Pouco conhecimento faz com que as pessoas se sintam orgulhosas. Muito conhecimento, que se sintam humildes. É assim que as espigas sem grãos erguem desdenhosamente a cabeça para o céu, enquanto as cheias as baixam para a terra, sua mãe.”
(Leonardo da Vinci)

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Com carinho,
Camila.

SUMÁRIO

*Capítulos 2,3 e 4 realizado nas normas da Revista British Journal of Nutrition.

CERTIFICADO DO COMITÊ DE ÉTICA

UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"
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CEUA – COMISSÃO DE ÉTICA NO USO DE ANIMAIS

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Certificamos que o projeto de pesquisa intitulado **"Gasto energético diário, composição corporal e fluxo de água em gatos domiciliados alimentados com rações com diferentes relações proteína: amido"**, protocolo nº 009536/18, 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 16 de maio de 2019.

Vigência do Projeto	10/08/2018 a 28/02/2022
Espécie / Linhagem	Gatos domésticos
Nº de animais	48
Peso / Idade	Peso até 10 kg e idade de 1 a 12 anos
Sexo	Ambos os sexos
Origem	Animais domiciliados

Vigência do Projeto	10/08/2018 a 28/02/2022
Espécie / Linhagem	Gatos domésticos
Nº de animais	16
Peso / Idade	Peso até 5 kg e idade de 1 a 12 anos
Sexo	Ambos os sexos
Origem	Animais do Laboratório de Pesquisa em Nutrição e Doenças Nutricionais de Cães e Gatos "Prof Dr. Flávio Prada", FCAV/ UNESP, campus de Jaboticabal.

Jaboticabal, 16 de maio de 2019.

Fabiana Pilarski

Prof.ª Dr.ª Fabiana Pilarski
Coordenadora – CEUA

Faculdade de Ciências Agrárias e Veterinárias
Via de Acesso Prof. Paulo Donato Castellane, s/n CEP 14884-900 - Jaboticabal/ SP - Brasil
tel 16 3209 7100 www.fcav.unesp.br

RESUMO

Há escassez de informações sobre metabolismo energético de gatos em domicílio, principalmente sobre o efeito do sexo, teor proteico da dieta, obesidade, atividade física e condição reprodutiva. Este estudo comparou o gasto energético (GE), composição corporal (CC), turnover de água (TA) e atividade física de gatos de laboratório e em domicílio, alimentados com rações com diferentes relações amido:proteína, em três experimentos distintos. O primeiro experimento avaliou dois grupos em laboratório, não-sobrepeso (NS) e sobrepeso (SB), o segundo avaliou 4 grupos castrados em domicílio, fêmeas (F) não-obesas (NO), fêmeas obesas (OB), machos (M) não-obesos, machos obesos e, o terceiro avaliou dois grupos em domicílio: machos castrados (MC) e machos inteiros (MI). Os gatos em laboratório foram alimentados com quantidade controlada e os gatos em domicílio *ad libitum* com duas dietas experimentais (Alto amido, AA: Amido 40%, PB 38% na MS; Alta proteína, AP: Amido 20%, PB de 55%). Os estudos seguiram delineamento *crossover,* nos gatos de laboratório se adotou arranjo fatorial 2x2 com duas dietas e duas condições corporais, nos gatos domiciliados castrados arranjo 2x2x2 com duas dietas, duas composições corporais e dois sexos, sendo adotado no estudo com gatos inteiros arranjo 2x2, com duas dietas e dois grupos de machos um inteiro outro castrado ($P \leq 0,05$ significativo; $P \leq 0,10$ tendência). Gatos de laboratório SB tiveram menor GE que gatos NSB ($P<0,05$) e menor atividade física ($P<0,05$). O GE não diferiu entre dietas, mas AP induziu maior TA ($P<0,01$). Ao teste de saciedade, gatos NSB e gatos alimentados com AP, independente da composição corporal, tiveram maior consumo alimentar ($P<0,01$), sugerindo efeito de saciedade para o amido. Como a idade de gatos castrados foi diferente no estudo em domicílio ($P<0,05$), esta foi incluída como covariável. O GE tendeu a ser maior em M ($P=0,06$) e semelhante entre NO e OB ($P=0,62$). Quando alimentados com dieta AA não houve alteração do peso corporal (PC), mas diminuiu a massa gorda (MG) em M do grupo NO ($P=0,04$) e aumentou a massa magra (MM) em F do grupo NO ($P<0,01$). O consumo da dieta AP aumentou PC e MM em todos os grupos ($P<0,05$), tendo aumentado a MG de F NO ($P=0,04$). Efeito de dieta, CC e sexo foi observado em TA, maior em M NO alimentados com AP ($P=0,03$). Maior movimento foi observado em gatos com dieta AP ($P=0,05$). Gatos MI apresentaram maior GE, TA e movimento ($P<0,01$) em comparação aos castrados. A dieta AP aumentou o GE nos dois grupos ($P=0,05$). Como conclusão, dieta com alta proteína pode elevar o movimento e consumo hídrico, mas elevado amido parece induzir saciedade e melhor controle de peso em gatos castrados alimentados *ad libitum*.

Palavras-chave: sexo; massa gorda; energia; atividade física; felinos

ABSTRACT

There is little information about energy metabolism of cats living in homes, especially regarding the effects of sex, dietary protein content, obesity, physical activity and reproductive condition. This study compared the energy expenditure (EE), body composition (BC), water turnover (WT) and physical activity of cats living in laboratory and homes, fed diets with different protein:starch ratios, in three separate experiments. The first study evaluated two groups of laboratory cats, non-overweight (NO) and overweight (OV), the second evaluated 4 groups of neutered cats living in homes, females (F) non-obese (NO), females obese (OB), males (M) non-obese, obese males and the third study evaluated two groups of cats, neutered males (NM) and intact males (IM). Cats were fed ad libitum with two experimental diets (High Starch, HS: 40% Starch, 38% CP in DM; High Protein, HP: 20% Starch, 55% CP) in a crossover design. The laboratory cats' study followed a 2x2 factorial arrangement with two diets and two body conditions. The house living neutered cats study a 2x2x2 arrangement with two diets, two body compositions and two sex. The study with IM followed a 2x2 arrangement, with two diets and two reproductive conditions (intact and neutered) ($P \leq 0.05$ significant; $P \leq 0.10$ trend). The OV laboratory cats had lower EE than NO cats ($P<0.05$) and lower physical activity ($P<0.05$). NOV cats had higher WT ($P<0.01$). The intake of the HP diet indued a higher WT ($P<0.01$), but did not change the cat's EE. At the satiety test, NO cats and cats fed HP diets, regardless of body composition presented higher food intake ($P<0.01$), suggesting a satiating effect of starch. As the age of neutered cats was different in the home study ($P<0.05$), this was included as a covariate. The EE tended to be higher in M ($P=0.06$) and similar between NO and OB cats ($P=0.62$). When cats were fed with the HS diet, no changes in body weight (BW) was observed but decreases in fat mass (FM) was observed in NO M cats ($P=0.04$) and increases in lean mass (LM) in NO F cats ($P<0.01$). The intake of the HP diet increased BW and LM in all groups ($P<0.05$), while FM increased in NO F cats ($P=0.04$). Effect of diet, BC and sex was observed in WT, that was higher in NO M fed HP diet ($P=0.03$). Higher movement was observed in cats fed HP diet, regardless of sex and BC ($P=0.05$). The IM cats showed higher EE, WT and movement ($P<0.01$) than neutered males. The HP diet increased EE in both groups of cats ($P=0.05$). As conclusion, high protein diets may increase WT and movement, but high starch intake may induce satiety in cats favoring BW control in neutered cats fed ad libitum.

Keywords: sex; fat mass; energy; physical activity; felines

CAPÍTULO 1- Considerações gerais

1.1 Introdução

Gatos são animais carnívoros com alta exigência de proteína quando comparado a cães, pelo alto consumo de nitrogênio e aminoácidos (Russel et al., 2002). Amido tem alta digestibilidade para espécie sem comprometer as respostas de insulina e glicose (De-Oliveira et al., 2008). Não se conhece a melhor composição de macronutrientes da dieta que promova longevidade e qualidade de vida (Villaverde e Fascetti, 2014), bem como as implicações do consumo de amido ou proteína sobre a composição corporal dos gatos (Vasconcellos et al., 2009), seus efeitos sobre o sexo e são imprecisas as estimativas das necessidades de energia (Bermingham et al., 2010). A preferência alimentar de gatos é por teores altos de proteína e gordura, sendo limitado o consumo em dietas com alto carboidrato, consumindo menor energia, denominado em inglês “ceiling” de carboidrato (Hewson-Hughes et al., 2011), podendo apresentar efeitos interessantes na saciedade. O alto teor de proteína na dieta aumenta a excreção de água em gatos (Garcia et al., 2020), mas são conflitantes quanto ao seu efeito na composição corporal (CC) e metabolismo energético em mantença (Villaverde e Fascetti, 2014), prevenção de obesidade e pouco se sabe sobre sua influência na atividade física.

São hipóteses deste estudo que dieta com alta proteína aumente a massa magra, alterando a composição corporal de gatos e, elevado teor de amido limite a ingestão alimentar promovendo maior saciedade, mantendo a composição corporal e a necessidade energética. Em relação ao fluxo de água, a dieta com alta proteína proporcionará aumento da excreção renal de água, com isso, aumento da diurese. O teor elevado de proteína na dieta irá aumentar a atividade física de gatos pelo aumento de massa magra e maior turnover hídrico provocado pela maior excreção renal de água.

O objetivo deste estudo foi comparar o gasto energético (GE), composição corporal (CC), fluxo de água (FA) e atividade física de gatos castrados de laboratório em sobrepeso e não-sobrepeso e de gatos domiciliados em diferentes condições fisiológicas (machos, fêmeas, obesos ou não obesos, inteiros ou castrados), alimentados com rações secas extrusadas com diferentes proporções amido:proteína.

1.2 Revisão de literatura

Energia do alimento e metabolismo energético

Energia é a propriedade conferida pela oxidação dos macronutrientes proteína, amido e gordura da dieta no processo de catabolismo (Nelson e Cox, 2014), expressa em kcal ou kJ (4,184 kcal). A energia advinda do alimento é definida por etapas: energia bruta (EB) é a energia química proveniente da combustão completa do alimento em bomba calorimétrica; energia digestível (ED) é considerada quando retirada a energia das fezes; quando descontado energia da urina e da fermentação dos gases, é definida como energia metabolizável; e energia líquida (EL) é a eficiência da utilização energética, sendo descontado o incremento calórico ou termogênese do alimento no processo digestivo dos macronutrientes. Os processos de absorção, transporte, transformação e armazenamento dos nutrientes pode corresponder a 10% da taxa metabólica basal (NRC, 2006). Loureiro et al., (2018), ao avaliarem dietas com diferentes teores de proteína (28, 39, 52 e 64 %) consumida por gatas adultas em câmaras de respirometria, encontrou contribuição média do incremento calórica de 6,5 %. Como o incremento calórico é dependente do perfil de macronutrientes da dieta, amplamente variável, oneroso e pouco prático em sua estimativa, este é desconsiderado na avaliação energética da dieta, sendo utilizado a EM (NRC, 2006). A EM de alimentos comerciais pode ser estimada de duas formas: pelo fator modificado de Atwater, que considera a digestibilidade de mais de 90% dos macronutrientes com contribuição de 3,5 kcal/g de proteína, 8,5 kcal/g de gordura e 3,5 kcal/g de amido, mas pode subestimar ou superestimar a energia; ou equações preditivas que utilizam a EB e ED das dietas, sendo descontada a fibra bruta da dieta que influencia negativamente a digestibilidade do alimento e a perda de energia pela urina. Portanto, equações preditivas são consideradas mais assertivas na estimativa de energia metabolizável dos alimentos comerciais para cães e gatos (NRC, 2006; FEDIAF, 2021).

A necessidade energética (NE) de cães e gatos é amplamente estudada a fim de se definir o melhor consumo energético para manutenção da massa corporal. As contribuições energéticas para NE apresentam quatro componentes:

1)Taxa metabólica de repouso: zona termoneutra, sem alimento, em jejum, gasto energético referente ao metabolismo de repouso, representa 60% a 75% do gasto diário total. Inclui a energia gasta pelo organismo para manter suas funções vitais, entre elas o funcionamento dos sistemas cardiovascular, respiratório, gradientes elétricos de membranas. É afetado pelo status hormonal, sistema nervoso autônomo, composição e superfície corporal, status nutricional e idade;

2) Atividade Muscular Voluntária: pode incluir até 30% do gasto diário, varia com o peso e tamanho do animal, grau, duração e intensidade do exercício físico;

3) Incremento calórico ou termogênese induzida pelo alimento: representa aproximadamente 10% do gasto energético, correspondente ao efeito térmico dos alimentos, representando a energia perdida na digestão, absorção, transporte, transformação, assimilação e/ou armazenamento dos nutrientes. Esta varia de acordo com o substrato consumido (composição de macronutrientes da dieta);

4) Termogênese adaptativa: inclui perdas adicionais não obrigatórias decorrentes de mudanças ambientais, alteração no consumo de alimentos e decorrentes de stress.

Necessidade energética e consumo de nutrientes

A população de gatos é muito heterogênea em relação à necessidade energética de manutenção, sendo a necessidade energética influenciada por fatores como sexo, composição corporal, idade, condição reprodutiva, ambiente em que vivem, nível de atividade e dieta (Backus e Wara, 2016; Larsen, 2017; Laflamme, 2020). Estimativas de gasto energético (GE) para gatos adultos para manutenção do peso corporal são propostas por equações no National Resource Council (NRC, 2006) de acordo com a composição corporal, sendo para gatos magros 100 kcal/kg^{0,67}/dia e para gatos obesos 130 kcal/kg^{0,4}/dia. Já o FEDIAF (2021) propõe equações de acordo com a atividade física e condição reprodutiva, sendo para gatos pouco ativos e castrados 75 kcal/kg^{0,67}/dia e gatos ativos 100 kcal/kg^{0,67}/dia. Bermingham et al., (2010) realizaram estudo de meta-análise recolhendo informações de 42 estudos sobre o GE de gatos adultos desde a década de 30 até 2009, divididos em 41 grupos com 1933 animais. O compilado de trabalhos avaliou diferentes métodos para estimativa do gasto energético, sendo estes: método do consumo alimentar, câmara de respirometria e água duplamente marcada. O uso da câmara de

respirometria é o método mais tradicional e difundido para taxa metabólica de repouso (Ligthon, 2008), no entanto, a energia gasta para livre atividade voluntária dos gatos não é incluida, sendo os valores encontrados nesta meta-análise menores quando comparados com os métodos de consumo do alimento e água duplamente marcada. Limitação importante encontrada neste estudo é a classificação utilizada para animais magros, peso normal e obesos. Como este estudo incluiu trabalhos ao longo do século XX, ferramentas para estimativa de composição corporal que demandam maior nível tecnológico não eram aplicadas em trabalhos mais antigos, sendo utilizado para esta classificação apenas o peso corporal dos animais.

Apesar dos pontos considerados, este estudo mostra-se interessante para identificação da variação encontrada no metabolismo energético de gatos adultos em diferentes situações. A média geral do GE em todos os grupos de animais classificados pelo estudo foi de 86,4 ± 2,0 kcal/kg^{0,67}/dia, variando de 47-156,0 kcal/kg^{0,67}/dia. Como pode ser notado, esta variação das calorias ingeridas no estudo abrange diferença de mais de 100 kcal/kg^{0,67}/dia. Ao avaliar o GE nos diferentes grupos de gatos, o sexo não diferiu numericamente, fêmeas castradas apresentaram GE de 86,7 ± 3,9 kcal/kg^{0,67}/dia, com intervalo de 55,5-116,0 kcal/kg^{0,67}/dia, e machos castrados 89,2 ± 6,2 kcal/ kg^{0,67}dia (49-138 kcal/kg^{0,67}/dia). Outros autores encontraram resultados não condizentes com estes apresentados, pois avaliaram o GE com populações homogêneas de machos ou fêmeas em mesmo ambiente e método de avaliação, mostrando que o GE de fêmeas foi 5–16% menor comparado com machos (Fettman et al., 1997; Nguyen et al., 2004[a]; Kienzle et al., 2006; Vasconcellos et al., 2019; Goloni et al., 2020). Gatos considerados com peso corporal ideal apresentaram GE de 88,5 ± 1,2 kcal/kg^{0,67}/dia (48-158,5 kcal/kg^{0,67}/dia) e gatos obesos valores menores com 78,2 ± 1,7 kcal/kg^{0,67}/dia (52-102 kcal/kg^{0,67}/dia). Estudos que compararam a ingestão calórica entre animais com escore de condição corporal ideal e com sobrepeso e obesos encontraram a mesma diferença (Kienzle et al., 2006; Vasconcellos et al., 2009). Hoenig et al., (2007) encontraram valores de ingestão calórica 18% menores em obesos quando comparado com gatos em peso ideal.

Gatos com sobrepeso ou obesos correspondem a 63% da população, identificada como a doença multissistêmica mais prevalente nos dias de hoje (German, 2016; Wall et al., 2019; Cline et al., 2021). A obesidade acontece pelo balanço energético positivo em que há maior consumo de calorias e menor gasto energético na atividade diária de gatos (Zoran, 2010; Clark e Hoenig, 2016), sendo fator predisponente para o desenvolvimento de

doenças endócrinas, osteoarticulares, cardiovasculares, alterações cutâneas, entre outras, que diminuem expectativa e qualidade de vida dos animais de companhia (Laflamme, 2006; Brooks et al., 2014; German, 2016). A deposição de tecido adiposo em excesso promove alteração endócrina e metabólica com estado inflamatório de baixo grau devido à liberação de citocinas pró-inflamatórias que desregulam a homeostase do organismo levando à resistência de insulina e de hormônios da saciedade (Zoran, 2010; Hamper, 2016). Com a maioria dos gatos apresentando esta alteração metabólica, definir a ingestão calórica para evitar esta problemática é imprescindível, sendo tema principal da maioria das pesquisas realizadas atualmente em felinos. Outro aspecto que acompanha a obesidade é a meia idade de gatos, apontada como fator predisponente para maior deposição de gordura corporal. Conforme os gatos chegam à idade de 5 anos a incidência da obesidade torna-se mais frequente (Lund et al., 2005).

Mizorogi et al., (2020) estudaram o perfil metabólico sérico e energético da população de 70 gatos em diferentes idades. Além de apresentarem ganho de peso corporal e aumento do escore de condição corporal com o envelhecimento, resultados do estudo mostraram maior valor de glicose sérica para gatos com 7 a 11 anos e em gatos obesos com 15 anos e, aumento de triglicérides em gatos com 11 a 14 anos. Os marcadores metabólico energéticos empregado no estudo foram o malato desidrogenase e o lactato desidrogenase, e sua razão utilizada como indicador energético pela via glicolítica para produção de ATP. Conforme o avanço da idade esta relação foi numericamente menor para os animais dos grupos de 7 a 14 anos, mostrando menor metabolismo energético por disfunção mitocondrial. Na revisão de meta-análise de Bermingham et al., (2010), gatos de 6 meses a 2 anos apresentaram 91,9 ± 4,3 kcal/kg^{0,67}/dia (50-153 kcal/kg^{0,67}/dia), gatos com > 2 a 7 anos 76,2 ± 3,8 kcal/kg^{0,67}/dia (46-121 kcal/kg^{0,67}/dia) e gatos > 7 anos 78,3 ± 7,5 kcal/kg^{0,67}/dia (54-152 kcal/kg^{0,67}/dia). Em estudo que comparou gatos com 9 e 3 anos de idade, Bermingham et al., (2013) verificou que gatos mais jovens apresentaram consumo de energia metabolizável 5% maior do que gatos mais velhos, no entanto, não foi descrito o escore de condição corporal dos animais.

A condição reprodutiva é fator determinante para o metabolismo energético de gatos (Allaway et al., 2016; Larsen, 2017; Wall et al., 2019). Animais castrados apresentam predisposição ao desenvolvimento de obesidade pelo aumento do consumo de alimento em manejo *ad libitum* e diminuição da taxa metabólica basal pós castração (Fettman et a., 1997; Hoenig et al., 2002; Kanchuk et al., 2003; Mitsuhashi et al., 2011; Wei et al., 2014),

com elevados níveis de glicose e leptina sérica (Fettman et al., 1997; Kanchuk et a., 2003; Vester et al., 2009; Alexander et al., 2011). Backus (2011) demonstrou haver diminuição da concentração de estrógeno endógeno como estradiol em gatos machos após castração. Estrógeno em machos inteiros é produzido pelos testículos e por aromatases da testosterona, que regulam o consumo do alimento e o metabolismo de gorduras (Cooke e Naaz, 2004; Cave et al., 2007; Zoran, 2010), no entanto, os mecanismos que levam a estas alterações metabólicas pós castração não estão totalmente elucidadas. Como visto, o metabolismo energético com a castração é alterado, o que predispõe ao maior acúmulo de tecido adiposo, havendo a necessidade do controle de alimento com manejo alimentar adequado. A necessidade energética de gato machos inteiros foi maior (99,4 ± 6,5; 60-142 kcal/kg^{0,67}/dia) quando comparado com gatos machos castrados (89,2 ± 6,2; 49-138 kcal/kg^{0,67}/dia) no estudo de Bermingham et al., (2010), resultados semelhantes foram também observados em outros estudos (Fettman et al., 1997; Hoenig et al., 2002; Kanchuk et al., 2003). O maior gasto energético de gatos inteiros pode estar associado a maior deposição de massa magra e maior atividade física quando comparado com gatos castrados, uma vez que este último aumenta a deposição de massa gorda. Cline et al., (2018) demonstraram que gatos inteiros em ambiente *outdoor* apresentaram menor massa gorda que gatos castrados em peso ideal em ambiente *indoor*. De-Godoy e Shoveller (2017) em estudo que avaliou atividade diurna e noturna em espaço indoor/outdoor em gatos adultos mostraram que obesos apresentam menor movimento voluntário que gatos em peso ideal. Como sabemos, a atividade física voluntária de gatos contribui de forma marcante para o gasto energético diário (NRC, 2006; Wichert et al., 2007), mas para que haja estímulo ao movimento, a qualidade do ambiente, assim como a definição do espaço em que o animal circula, seja ele *indoor* ou *outdoor* são fundamentais (Health, 2020).

Outro fator que vem sendo estudo com a atividade física de gatos é o consumo de água, seja por maior ingestão de água por alimento úmido ou acréscimo em dieta seca extrusada (Cameron et al., 2010; Alexander et al., 2014; Deng et al., 2014; Thomas et al., 2018). Cameron et al., (2011) em estudo que forneceu *ad libitum* dieta seca com acréscimo de 0, 20 e 40% de água com o intuito de reduzir a densidade energética do alimento verificou maior atividade física em gatos alimentados com dieta com 40% de água. Deng et al., (2014) avaliou a atividade física dos gatos de acordo com a quantidade de refeições fornecidas e o acréscimo de 70% de água em alimento seco extrusado. Os resultados mostraram que houve maior atividade física dos gatos quando alimentados com 4 refeições

comparado com apenas uma refeição em período diurno e, que o maior teor de água na dieta teve tendência a apresentar maior atividade voluntária quando comparado com a dieta sem acréscimo de água no período noturno. Alexander et al., (2014) forneceu dieta seca extrusada com 0, 40 e 80% de acréscimo de água na dieta de gatos adultos com quantidade 200% a mais de sua necessidade energética, resultando em ganho de peso em gatos alimentados com dieta sem adição de água, controle do peso para dietas com acréscimo de água e maior atividade física voluntária para gatos consumindo dieta com 80% de acréscimo de água. Os resultados destes três estudos apresentados mostram que a diluição energética do alimento através do acréscimo de água é interessante no manejo do controle de peso em gatos adultos. No entanto, não é totalmente claro como o aumento da ingestão de água pode influenciar na atividade física de gatos, se estes apresentam maior frequência de micção ou se maior turnover de água influencia na manutenção do peso corporal e no movimento.

Considerando o estudo de meta-análise em todas as populações, e os demais estudos científicos em populações distintas de gatos, mas com padronizações mais homogêneas, verifica-se superestimação das equações propostas para estimativa da necessidade energética de manutenção de gatos adultos pelo NRC (2006). No entanto, os valores se assemelham ao recomendado pelo FEDIAF (2021), mas até o momento não existem equações que considerem em conjunto os efeitos de idade (envelhecimento), obesidade, sexo e condição reprodutiva. A formulação de alimentos para gatos, deve considerar o metabolismo energético nestas diferentes populações afim de minimizar o impacto significativo na alimentação que pode levar à obesidade e doenças relacionadas. Além dessa problemática atual na população de gatos, uma menor ingestão de alimentos por gatos domiciliados pode ocasionar reduzida ingestão de matéria seca e demais nutrientes, o que precisa ser corrigido aumentando-se a concentração de proteína, de minerais e de vitaminas da dieta. Mendes et al., (2018), realizou estudo com diferentes proporções de proteína na dieta de gatas castradas. Estas foram alimentadas para manutenção do peso corporal, sendo avaliado o gasto energético, composição corporal e metabolismo de proteína. Os animais alimentados com a dieta com menor teor proteico (28%) apresentaram perda de massa magra, o consumo de proteína bruta neste tratamento foi 12% menor e o de fenilalanina + tirosina foram 30% inferiores ao recomendado pelo NRC (2006). Isto ocorreu porque apesar das formulações atenderem às proposições nutricionais do NRC (2006), com teor de proteína e aminoácidos de aproximadamente 30%

superior ao mínimo recomendado, o GE das gatas foi muito baixo, de aproximadamente 59 kcal/kg^{0,67}/dia, bem menor em relação ao proposto pelo NRC (2006) e FEDIAF (2021). Este baixo consumo de calorias resultou em reduzida ingestão de matéria seca e demais nutrientes presentes no alimento.

O FEDIAF, atualizado anualmente, propõe teores de nutrientes de acordo com a caloria ingerida. Animais que consomem menor energia devem ter seus nutrientes mais concentrados na dieta, como exemplo, para gatos pouco ativo que consomem 75 kcal/kg^{0,67}/dia devem ter no mínimo 33,3 g/100g de matéria seca, enquanto gatos ativos que consomem 100 kcal/kg^{0,67}/dia devem apresentar na dieta 25 g/100g. Este é ponto relevante, que para ser convenientemente estudado necessita de estudos com maior número de animais e principalmente de estudos conduzidos em domicílio, quando os gatos expressam suas atividades normais proporcionando resultados de GE mais confiáveis de acordo com a população, que podem inclusive, apresentar valores de energia ingerida menores do que o classificado nos guias nutricionais. Grant et al., (2020) avaliou o consumo dos nutrientes em dieta seca extrusada formulada para perda de peso, com 36% de proteína bruta, 12,3% de gordura e 40,3% de extrativo não nitrogenado, em 30 gatos (machos ou fêmeas) castrados, domiciliados e com idade entre 2 a 7 anos. Os resultados foram comparados com o recomendado pelo NRC (2006). Os animais foram separados em gatos com peso ideal (escore de condição corporal, ECC = 4 e 5) e gatos obesos (ECC = 8 e 9) no período de manutenção do peso corporal (4 semanas) e no período de perda de peso (10 semanas) nos gatos obesos. Para o período de manutenção do peso corporal, os tutores foram orientados a fornecer a quantidade pesada do alimento indicada pelos autores, que utilizaram as equações recomendadas pelo NRC para gatos em peso ideal (100 kcal/kg^{0,67}/dia) e gatos obesos (130 kcal/kg^{0,4}/dia). Para o período de restrição energética para perda de peso dos gatos obesos, foi calculado o consumo de energia pela fórmula: 0.6 x (130 kcal x kg peso corporal ideal^{0,4}). Peso corporal ideal foi calculado de acordo com o ECC e o peso atual do gato. Nos animais com o ECC=9 foi realizado morfometria e estimado a porcentagem de gordura corporal para então ser calculado o peso ideal. O consumo de nutrientes nos grupos e nos dois períodos de avaliação foi estimado a partir dos consumos mínimo, máximo e média de dias do período. Gatos obesos no período de restrição energética para perda de peso apresentaram perda de peso semanal de 0,96%. Este estudo demonstrou consumo insuficiente de arginina com 21% a menos e, colina com 10% a menos do que o recomendado durante o período de restrição calórica de

gatos obesos. O inconveniente deste estudo foi não descrever a média do peso corporal de cada grupo de gatos, apresentando apenas a caloria diária: 272,3 ± 46,5 kcal/dia para gatos em peso ideal, 221,5 ± 24,4 kcal/dia para gatos obesos no período de manutenção e 138,2 ± 10,2 kcal/dia para gatos obesos no período de perda de peso.

Exceto este último estudo apresentado, a maioria dos estudos de metabolismo energético de gatos têm sido realizados com animais alojados em laboratório, em alguns deles não foram descritos ou controlados todos os fatores que podem interferir no gasto energético destes animais, como efeito da idade, dieta, sexo, ambiente que vivem, atividade muscular voluntária, condição reprodutiva e composição corporal. Segundo a última versão do Nutrient Requirements of Dogs and Cats (2006), o conhecimento das necessidades energéticas de gatos ainda é bastante deficiente. Faltam informações precisas sobre o gasto energético nestes diferentes grupos de animais. Conduzir estudos em animais em condição normais de vida, em seu próprio domicílio e sem interferir em sua rotina é importante para se evitar alterações no gasto energético decorrente da vida em laboratórios de pesquisa, que geram informações que podem não ser representativas para gatos em domicílio. Estas informações sobre a necessidade energética de gatos nas suas diferentes particularidades serão importantes para se estabelecer melhores condições de alimentação, com melhor proporção dos macronutrientes energéticos, com vistas a prevenção da obesidade e possíveis deficiências nutricionais.

Efeito dos macronutrientes energéticos na alimentação de gatos

Os gatos apresentam metabolismo energético que inclui a conversão de aminoácidos em glicose para atender sua demanda energética, sendo denominados animais gliconeogênicos (Sturgess e Hurley, 2005; Case et al., 2011). Esta maior exigência proteica na dieta está relacionada também com regulação de determinadas transaminases no metabolismo de nitrogênio, ciclo da ureia e na metabolização de compostos sulfurados, com limitada síntese de dois aminoácidos essenciais, arginina e taurina (Morris, 2002). A proteína, além de atender ao teor de nitrogênio requerido e fornecer os aminoácidos essenciais em quantidades adequadas para suporte da síntese de proteína, é também macronutriente da dieta que se apresenta como substrato energético para estes animais (Villaverde e Fascetti, 2014).

Gatos domésticos evoluíram a partir do gato selvagem africano *Felis silvestres lybica* há 4000 anos (O'brien e Yuhki, 1999) que apresentam como dieta principal outros animais, havendo presas disponíveis para caça na natureza, garantem adequada alimentação (Morris, 2002). A composição energética das presas (pequenos roedores e pássaros) apresenta 52 a 63% de proteína, 25 a 46% de gordura e 2 a 12% de carboidrato (Plantinga et al., 2011; Kremen et al., 2013). Dietas comerciais secas extrusadas para gatos domésticos apresentam, em média 32%, 37% e 31% de sua energia metabolizável advinda da proteína, amido e gordura, dietas úmidas comerciais 41, 45 e 14%, respectivamente (Laflamme, 2020). Frente ao ancestral evolutivo de gatos domésticos, estudos de preferência alimentar dos macronutrientes foram conduzidos. Foi demonstrado que gatos preferem ou selecionam dietas com alta proteína e gordura quando comparados com dieta de alto amido (Hewson-Hughes et al., 2011). Hewson-Hughes et al., (2011) em técnicas geométricas de combinações triangulares alimentares de proteína, gordura e amido encontraram equilíbrio na ingestão dos macronutrientes em gatos, com limite máximo de ingestão de carboidratos, termo denominado em inglês de "ceilling" de carboidrato que limita a ingestão de alimento. Esta perspectiva se torna interessante em alimentação fornecida *ad libitum* e para animais com sobrepeso e obesos em restrição calórica para perda de perda, controlando a ingestão de alimento, mas com fornecimento mínimo necessário de proteína e aminoácidos (Green et al., 2008).

No entanto, gatos que apresentam metabolismo de glicose e insulina alterados não se beneficiam desta dieta devido a assimilação de maior teor de carboidrato em vigência de alterações no metabolismo endócrino (Zoran e Rand, 2013). Os teores de macronutrientes recomendados para gatos pelo NRC (2006) são 4,95 g/kg^{0,67}/dia de proteína e 2,2 g/kg^{0,67}/dia de gordura. Carboidratos não são considerados como nutriente essencial para gatos apesar do amido contribuir energeticamente em seu metabolismo, além de facilitar o processo de extrusão em dietas comerciais secas. O FEDIAF (2021) ajustou o valor recomendado de proteína para nível mais alto, de 6,25 g/kg^{0,67}/dia devido a digestibilidade aparente de gatos para proteína de 80% quando na fase de senilidade.

Vários estudos foram desenvolvidos ao longo das últimas duas décadas que avaliaram o perfil de macronutrientes das dietas para gatos com diferentes teores de proteína e seu impacto no gasto energético e composição corporal (Russel et al., 2002; Riond et al., 2003; Nguyen et al., 2004[a]; Nguyen et al., 2004[b]; Laflamme e Hannah, 2005; Backus, 2007; Hoenig et al., 2007; Green et al., 2008; Vasconcellos et al, 2009; Vester et

al., 2009; Wei et al., 2011; Coradini et al., 2014; Courtis et al., 2015; Keller et al., 2017; Vasconcellos et al., 2019). A maioria destes estudos praticaram restrição energética para perda de peso e poucos avaliaram a alimentação para manutenção da massa corporal (Vasconcellos et al., 2009; Villaverde e Fascetti, 2014). É bem descrito na literatura o efeito de baixa densidade energética e alta proteína na dieta de gatos obesos em programa de perda de peso em restrição do consumo energético, com preservação da massa magra. Laflamme e Hannah (2005) em estudo para perda de peso semanal de 1% em gatos com sobrepeso, demonstraram manutenção da massa magra e perda de massa gorda quando alimentados com dieta com 40% de matéria seca de proteína comparado com dieta com 30%, sem distinção do gasto energético. Vasconcellos et al., (2009), encontrou resultados semelhantes ao estudo anterior para perda de peso em gatos obesos utilizando dieta com 28 e 21 g de proteína bruta/MJ de energia metabolizável. Após perda de 20% de peso corporal (0,92% de perda semanal), os gatos foram mantidos nas duas dietas por 120 dias para manutenção do peso corporal. Nesta fase de manutenção, houve maior consumo calórico gradual nas duas dietas para manutenção do peso corporal havendo maior necessidade energética após a fase de perda de peso e, a partir do dia 80 de consumo, a dieta com maior teor de proteína apresentou maior consumo calórico para manutenção do peso corporal do que a dieta controle, mesmo os dois grupos apresentando semelhante massa magra e massa gorda. Os autores desse estudo sugerem que maior demanda proteica na fase de estabilização do peso corporal após programa de perda de peso possa influenciar em sua estabilização a longo prazo. Russel et al., (2002) verificaram maior consumo calórico em dieta com 52% de energia metabolizável advinda da proteína, do que em dieta com 35% em 50 dias de consumo *ad libitum*, mas não descrevem a composição corporal dos gatos no período.

Courtis et al., (2015), também avaliaram dois teores diferentes de proteína na dieta em gatos obesos, uma com teor moderado de 31,5% da energia metabolizável e outra com teor alto de proteína de 54,2%, fornecendo estas aos animais na quantidade de 70% de sua necessidade energética de manutenção por 8 semanas. Os autores verificaram que apesar dos animais perderem peso com as duas dietas, não houve perda de massa magra com a dieta com alto teor proteico, diferentemente da dieta com proteína moderada, porém ocasionou redução da necessidade energética de repouso destes animais diferentemente encontrado por Vasconcellos et al., (2009). Em estudo concluído por nosso grupo de pesquisa, dietas com mais de 38% de proteína demonstraram expansão da massa magra

em gatas castradas em consumo para manutenção do peso corporal, sem alteração do gasto energético (Mendes et al., 2018). Nguyen et al., (2004ª) em estudo com gatos em peso ideal avaliou a composição corporal e o gasto energético, em consumo alimentar por 6 meses (média de alimento fornecido 72 kcal/kg^{0,67}/dia) em delineamento *crossover*, de duas dietas com diferente teor de proteína, alta proteína com 52% e moderada proteína com 30%. Ao final de cada período, os autores identificaram que a dieta alta proteína manteve o peso corporal dos animais, mas aumentou em 4,2% a massa magra sem haver alteração do gasto energético. A dieta com moderada proteína, por sua vez, diminuiu o peso corporal em 8,7% sem alterar a massa magra, mas numericamente houve diminuição da massa gorda em 5,4%. Não houve diferença no consumo das dietas com diferente teor de proteína. Portanto, parece haver relação de ganho de massa magra em gatos alimentados com dieta com teor mais elevado de proteína quando comparado com o recomendado pelo NRC (2006), mas não há conclusão clara entre os autores sobre seu efeito no metabolismo energético. Estes estudos avaliaram a modulação dos macronutrientes da dieta em gatos adultos de laboratório em peso ideal, sobrepeso, obeso, machos ou fêmeas não avaliando estas populações com necessidade energética distintas de mantença e, em condições de vida em residência de proprietários.

Além do efeito energético da proteína, é discutido seu efeito no controle do apetite e no metabolismo de água em gatos. Em seres humanos, o consumo de proteína está relacionado com a maior sensação de saciação e saciedade (Du et al., 2017). O efeito da satisfação do consumo de alimento classificado como saciação é um mecanismo complexo de resposta a curto prazo, no momento da alimentação, determinando o tamanho da refeição com influência sensorial, cognitiva, gástrica, hormonal e neural. Os hormônios envolvidos nesta fase são a colecistoquinina (CCK), peptídeo semelhante a glucagon-1 (GLP-1), peptídeo YY (PYY) e grelina. O efeito de saciedade é mais prolongado, após o final da alimentação, interferindo na duração do período pós prandial seguindo-se até a refeição seguinte. Os processos metabólicos e as substâncias envolvidas neste processo são: oxidação dos substratos nutricionais, insulina, glucagon, aminoácidos e leptina (Tremblay e Bellisle, 2015). Devido ao efeito termogênico na oxidação de proteína e possível aumento de aminoácidos na circulação sanguínea de gatos no período pós prandial, associou-se maior saciedade em dietas com elevado teor de proteína (Westerterp-Plantenga et al., 1999; Laflamme e Hannah, 2005), devido a maior glicose e insulina pós-prandial com possível interferência na leptina. No entanto, quando estudos avaliaram a

sensibilidade de insulina e glicose e concentração de leptina, em período de mantença após o emagrecimento de gatos castrados consumindo dieta com diferente teor de proteína, não foi verificado efeito de dietas. Diferenças foram vistas apenas quando comparados em período de obesidade e após emagrecimento, insulina e glicose com valores menores e leptina valor maior em obesos que em gatos em peso ideal (Hoenig et al., 2007). Estes resultados são semelhantes ao encontrado por Vasconcellos et al., (2009), em estudo similar, em que a dieta alta proteína não apresentou efeito nos valores de insulina e leptina, a concentração sérica de leptina apresentou estreita correlação com a massa gorda, mas sem efeito na saciedade de gatos obesos devido à possível resistência de sua atuação nos tecidos orgânicos.

Estudos de preferência alimentar com diferentes teores dos macronutrientes em manejo *ad libitum* foram conduzidos, verificando-se maior consumo e ganho de peso corporal em gatos alimentados com dietas com alta proteína (Hewson-Hughes et al., 2011; Coradini et al., 2014). Pouco se sabe sobre o consumo elevado de amido na saciedade e em seu efeito limitante no consumo alimentar de gatos. Estudos demonstraram que seu consumo, mantendo níveis adequados de proteína, mantiveram o peso corporal de gatos sem interferência na massa magra (Nguyen et al., 2004ª; Vasconcellos et al., 2009; Vasconcellos et al., 2019). Asaro et al., (2018) avaliaram a influência do consumo *ad libitum* por 60 minutos, uma vez ao dia, de três dietas com diferentes teores de amido (36,8; 30,7; 23,6%) na resposta glicêmica de gatos em peso ideal. A concentração de glicose em jejum, pós prandial e o pico não foi diferente entre as dietas, porém a concentração de insulina pós prandial foi maior na dieta com alto teor de amido comparada com as outras duas dietas. O consumo de alimento foi maior com a dieta alto amido, assim como as concentrações de glicose e insulina foram maiores, mas quando avaliado o gasto energético em câmara de respirometria não houve diferença entre as dietas no período em jejum, mas o coeficiente respiratório em jejum foi maior para dieta com alto amido. No período pós prandial, o coeficiente respiratório e o gasto energético foram maiores para dieta com alto amido, o que não era esperado pelos autores, mas justificam possível aumento da lipogênese em resposta ao maior consumo de amido, uma vez que a dieta alta em amido apresentou consumo energético maior que as demais dietas. Os autores também discutem sobre a influência do teor de fibra no controle glicêmico, uma vez que a dieta alta em amido apresentava menor quantidade que as demais dietas (1,17; 1,78; 2,58 % de fibra bruta). Estudos que avaliem o efeito de proteína, amido e teor e tipo de fibra (solúvel/insolúvel) na dieta para avaliação da saciedade nos parâmetros hormonais, são

fundamentais para o melhor entendimento do perfil nutricional e possível regulação da ingestão calórica de gatos domésticos.

Em relação ao metabolismo de água em gatos, uma vez que esta espécie teve origem desértica, a ingestão de água torna-se preocupação dos tutores e médicos veterinários frente a possibilidade do aparecimento de doenças do trato urinário inferior (Bartges e Callens, 2015). Segundo Garcia et al., (2020), aumento de quase 70% na rotatividade de água corporal foi verificado ao elevar-se o teor proteico e de sódio da ração, com aumento linear na produção de urina. Atribui-se a alimentos secos baixo consumo de água e produção de urina, com potencial influência na formação de urólitos (Wellman et al., 2012). É possível, assim, que tanto a elevação do gasto energético com maior produção de água metabólica, como a necessidade de eliminação renal da ureia advinda do catabolismo proteico possam resultar em diurese e maior fluxo de água em felinos alimentados com elevada proteína (Hashimoto et al., 1995; Mendonça et al., 2018).

Como se vê pela presente revisão, não se compreende completamente, ainda, os efeitos a longo prazo dos consumos de proteína e amido na composição corporal, gasto energético, saciedade, turnover de água e atividade física de gatos. Também, não se conhece como a atividade física é influenciada pela composição da dieta e influencia, por sua vez, o metabolismo energético e necessidades nutricionais de gatos em domicílio. Estas respostas fisiológicas, apesar de muito importantes, não foram avaliadas ainda devido aos modelos de estudos serem incompletos e restritos a gatos alojados em condições de laboratório. Explorar a proteína e o amido como indutores de ações fisiológicas, suas implicações à saciedade e ingestão voluntária de alimentos, manutenção de peso e composição corporal, bem como sua possível interferência no metabolismo energético é relevante para o melhor entendimento da nutrição de gatos vivendo em domicílios. Estas influências foram avaliadas no presente estudo, que substanciará melhor compreensão dos efeitos dos consumos de proteína e amido na fisiologia de felinos. Assim, dois aspectos relevantes somam-se na proposta da presente pesquisa, a determinação correta e precisa do gasto energético de gatos domiciliados, permitindo-se prever corretamente o consumo de alimento e nutrientes, evitando-se reduzida ingestão de proteínas por gatos que apresentem baixa necessidade calórica, bem como se explorar as implicações da ingestão de proteína e amido na massa magra, gasto energético, turnover hídrico, atividade física e manutenção do peso de gatos alimentados *ad libitum*, o que permitirá decisões mais informadas quanto à melhor composição de macronutrientes de rações para felinos.

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CAPÍTULO 2 – **Artigo 1**

Diets with different starch:protein ratios on body composition, energy metabolism, water turnover, physical activity, and satiety in overweight and non-overweight laboratory cats

Camila Goloni[1], Letícia G. Pacheco[1], Letícia W. Luís, Stephanie S. Theodoro[1], Lucas B. Scarpim[1], Daniela Dalpubel[2], Meire Gallo Rosenburg[2], Celina Torres[3], Gener T. Pereira[1], Aulus C. Carciofi[1*]

[1] Universidade Estadual Paulista (Unesp), Faculdade de Ciências Agrárias e Veterinárias, Jaboticabal, SP, 14884-900, Brazil.

[2] Universidade de São Paulo (USP), Faculdade de Medicina, Ribeirão Preto, SP, Brazil.

[3] Affinity Petcare, Barcelona, Spain.

[*] Correspondência do autor: aulus.carciofi@gmail.com. Número de telefone + 55 16 3209-2626; fax número +55 16 3203-1226. Faculdade de Ciências Agrárias e Veterinárias, UNESP – Univ Estadual Paulista, Via de acesso Prof. Paulo Donato Castellane, s/n, 14884-900, Jaboticabal-São Paulo, Brasil.

Título breve: Energy metabolism in cats fed different starch:protein ratio diets

2.1 Abstract

Considering that cats prefer or select protein and fat intake as an energy substrate over starch, the intake of diets with different starch:protein ratios was compared in overweight (OW) and non-overweight (NOW) laboratory cats. Cats were fed for 33 days a high starch (HS: 40% starch, 38% CP on DM basis) or a high protein (HP: 20% starch, 55% CP) diet, in a cross-over design. Body composition (BC), energy expenditure (EE), water turnover (WT), physical activity and satiety exposing cats to a challenge meal, were evaluated. Results were compared in a 2 (diets) x 2 (BC) arrangement, adding up 4 treatments ($P<0•05$). The OW cats presented lower energy expenditure (EE: 64 SE 6 kcal/kg$^{0.67}$/d), lower water turnover (WT: 46 SE 3 mL/kg$^{0.67}$/d) and lower physical activity ($P<0•05$) than NOW animals (75 SE 6 kcal/kg$^{0.67}$/d; 56 SE 2 mL/kg$^{0.67}$/d; respectively, $P<0•05$). The intake of the HP diet resulted in higher WT (HP: 55 SE 2 mL/kg$^{0.67}$/d; HS: 48 SE 2 mL/kg$^{0.67}$/d; $P<0•01$), not changing EE or physical activity. At the satiety test cats fed the HP diet presented higher later food intake than cats fed the HS food ($P<0•01$). High starch diet limited the

daily voluntary food intake, which may have an influence on energy balance and body weight in the long term.

Key-words: accelerometer: deuterium: lean mass: fatty mass: movement: obesity

2.2 Introduction

Cats are well known true carnivores with elevated protein requirement to supply gluconeogenic amino acids for energy metabolism ([1]RUSSEL et al, 2002). It has been established that cats prefer or select protein and fat as energy substrates over starch, what may be explained by their ancestral diet ([2]HEWSON-HUGHES et al., 2011). These concepts have driven debates about the better diet macronutrient composition to cats, especially considering that dry diets may be high in starch and are routinely fed ad libitum, exposing cat to an excessive energy intake ([3]VILAVERDE & FASCETTI, 2014).

Obesity is a multisystemic condition that is becoming more prevalent over the decades in all the world ([4]KUCZMARSKI et al., 1994; [5]GERMAN, 2016), with estimates of 63% of overweight in the cat population ([6]WALL et al., 2019). The adipose tissue gain in cat's is a problem on different perspectives, as it predisposes to diabetes mellitus, osteoarthritis and result in higher morbidity and mortality rates ([7]BROOKS et al., 2014; [8]CLINE et al., 2021). Described risk factors associated with this condition is neutering, gender, age, quality of environment, activity (indoor or outdoor), feeding style (meal feeding or free choice) and diet type and composition ([9]HARPER et al., 2001; [10]COLLIARD et al., 2009; [11]ZORAN & BUFFINGTON, 2011; [12]ROWE et al., 2015; [5]GERMAN, 2016; [13]De-GODOY & SHOVELLER, 2017; [6]WALL et al., 2019). Although not yet fully understood, the proportion of dietary macronutrients may influence food intake pattern, satiety, energy metabolism, physical activity and ultimately the body composition of cats ([3]VILAVERDE & FASCETTI, 2014; [14]CLARK & LAFLAMME, 2016).

High fat diets are known to increase energy intake and body fat mass deposition, inducing obesity in cats ([15]NGUYEN et al., 2004a; [16]BACKUS, 2007). This means that fat should not be the primary source of energy, especially considering that most cats are neutered, indoor, and fed unrestricted amounts of food, conditions that predisposes obesity development ([3]VILAVERDE & FASCETTI, 2014; [17]LAFLAMME, 2020). However, cats fed high protein diets may also increase their body mass and may become obese ([2]HEWSON-HUGHES et al., 2011; [18]CORADINI et al.,

2014), does not supporting the concept that high protein diets may promotes better satiety and energy intake control ([19]DU et al., 2017). The effect of dietary protein on intake behavior, however, is still controversial as [20]Nguyen et al. (2004b) verified an increase in lean mass without change the energy expenditure nor inducing body weight gain in cats fed high protein diets. These studies, however, was conducted in laboratory cats usually fed controlled amounts of food ([21]COURTIS et al., 2015; [22]VASCONCELLOS et al., 2019), and is difficult to translate the results to home living cats fed *ad libitum*.

Despite the apparent controversy about starch intake, when properly processed cats present high digestibility of starch (> 95%) that can be considered and adequate source of energy ([23]De-OLIVEIRA, et al., 2008; [24]ALVARENGA & ALDRICH, 2020). Additionally, starch intake does not induce noticeable alterations on post-prandial glucose and insulin responses in non-obese cat fed dry foods ([23]De-OLIVEIRA, et al., 2008; [25]HOENIG et al., 2011). Different carbohydrate to protein ratios were evaluated on several studies with obese and not obese cats, which concluded that starch intake does not induce significant changes on hormonal responses or glucose metabolism ([26]HOENIG, et al., 2007; [25]HOENIG, et al., 2011), not supporting concerns about starch consumption in well balanced and complete formulations. It was suggested, additionally, that a high starch diet (considering it is adequate on protein and other nutrients) would limit voluntary food intake, concept termed "carbohydrate ceiling" by [2]HEWSON-HUGHES et al. (2011). This allows to hypothesize that cats would limit starch and food intake in high starch formulations, helping control the amount of food eaten, been starch a possible tool to help the maintenance of a stable body weight in *ad libitum* feeding systems.

The physical activity is another important compound of the energy requirement ([27]NRC, 2006; [28]BERMINGHAM et al., 2013), been also related to obesity development ([15]NGUYEN et al., 2004a; [29]KIENZLE et al, 2006; [13]De-GODOY & SHOVELLER, 2017; [24]VASCONCELLOS et al., 2019). The fat mass presents lower metabolic activity and higher muscle mass is associated with higher physical activity ([30]PEARSON, 1990; [31]LANDI et al., 2014; [32]JOANISSE et al., 2020), altogether increasing energy expenditure. Physical activity may be influenced by diet composition and water intake, as several studies had shown increased movements in cats fed high moisture foods ([33]ALEXANDER et al., 2014; [34]THOMAS et al., 2018). The water turnover of cats can also be increased by high protein low starch foods ([35]GARCIA et al., 2020), allowing to hypothesize that high protein intake may influence physical activity in cats, with possible increase in energy expenditure.

Considering the above, are hypothesis of the present study that a high protein diet intake may induces increases on energy expenditure, lean mass content and water turnover, and that a high starch intake my favor satiety reducing the voluntary food intake of cats. To evaluate this, the objective of the present study was to compare the body composition, energy expenditure, water balance, voluntary physical activity and the satiety response of overweight and non-overweight laboratory cats fed two extruded diets, one high in protein and another high in starch.

2.3 Material and Methods

Animals and experimental design

The experiment was conducted in the Laboratory of Research in Nutrition and Nutritional Diseases of Dogs and Cats "Prof. Dr. Flávio Prada". All procedures with animals followed the ethical principles adopted by the Brazilian College of Animal Experimentation and were previously approved by the Ethics Committee on the Use of Animals of São Paulo State University (protocol no. 9536/18).

Sixteen mixed breed and neutered cats were used in the experiment, eight non-overweight (NOW; 4.13 (SD 0.99) years; 3.96 (SD 0.81) kg; 5.00 (SD 0.00) out of 9 body condition score, BCS) and eight overweight (OW), which overweight condition were developed naturally over the years (5.88 (SD 1.25 years; 4.61 (SD 0.81) kg; 7.13 (SD 0.96) BCS). The animals were considered healthy after a physical examination, complete blood count, serum biochemistry (alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, albumin, urea and creatinine) and urine analysis. During the study, the cats were housed for 16 h (from 4:00 p.m. to 8:00 a.m.) in metabolic cages (all cats were previously adapted to the procedure; 0.65 x 0.85 x 0.65 cm), with water and food available, and released into a collective cattery for 8 h (from 8:00 a.m. to 4:00 a.m.) with access to water but not food. For voluntary exercise and social interaction, the cattery presented 65 m^2 and included scratching posts, toys, climbing apparatus and beds with pillows to rest. Cats interacted with people at least three times per day: to be housed in the cages and fed; to be released of the cage and to be brushed for 15 minutes each cat. They stayed on the 12:00 light–12:00 dark cycle and in a normal acclimated place (mean temperature of 28.4 (SD 0.6) °C; Interna Incoterm®).

The study was organized in a factorial arrangement, with 2 diets x 2 body conditions, totalizing 4 experimental treatments. The study followed a randomized cross-over design, and all cats were fed the two diets in alternate periods. Each period of the cross-over lasted 33 d: 14 d for diet adaptation and 19 d for the experimental period. A washout between cross-over periods of 15 days was adopted.

Body composition was evaluated on the 15th day. Energy expenditure, water turnover (by the double labeled water method), and physical activity (with a 3-axyal accelerometer) was evaluated from the 19th to 27th days. The kinetics of food intake was evaluated on the 30th day and a satiety test was conducted on days 31st, 32nd and 33rd.

Experimental diets, nutrient digestibility, water balance and nitrogen balance

During the study, the cats were fed with two balanced diets for cat maintenance ([36]FEDIAF, 2018): High Starch (HS) with 35% crude protein and 38% of starch; High Protein (HP) with 52% of crude protein and 18% of starch, on as fed basis. The increase on protein content was obtained through a reduction of the starch content, thus altering the protein:starch ratio of the diets (Table 1). The fiber and fat contents remained similar. The food was produced in the Extrusion Laboratory of the Faculdade de Ciências Agrárias e Veterinária da UNESP, Jaboticabal, Brazil.

Cats were fed to maintain a constant body weight along the study. To these animals were initially fed with their usual amount of maintenance energy requirement, considering the animal's intake record and the metabolizable energy content of each food. After, cats were weekly weighed (INMETRO: certified weights from 1 to 10 kg OIML E1/2004), and their food amount adjusted to achieve a constant body weight throughout the period.

Table 1. Ingredient composition of the experimental diets with different protein:starch ratios for cats.

Item	Diets	
	High Starch	High Protein
Ingredients		
Poultry by-product meal	35.00	35.00
Corn grain	26.70	0.00
Broken rice	20.00	20.00
Isolated pea protein	0.00	21.07
Isolated swine protein	7.02	10.00
Poultry fat	6.70	6.80
Beet pulp	2.50	2.50
* Palatant enhancer	1.50	1.50
Potassium chlorine	0.65	0.65
Common salt	0.60	0.60
Choline chloride	0.45	0.45
† Vitamin-mineral premix	0.70	0.70
‡ Urine acidifier	0.40	0.40
Taurine	0.20	0.20

Calcium proprionate	0.04	0.04
Potassium sorbate	0.02	0.02
[§] Antioxidant	0.07	0.07

[*] Liquid palatant: SPF Brasil, Descalvado, SP, Brazil).
[†] Vitamin-mineral premix: Rovimix, DSM Produtos Nutricionais Brasil S.A., Jaguaré, Brazil.
[‡] Urine acidifier: Equilibrium, Diana Pet food, Descalvado, Brazil.
[§] Antioxidant: Butylated hydroxyanisole, butylated hydroxytoluene, propyl gallate and calcium carbonate (Alltech do Brazil Agroindustrial Ltda, Araucária, Paraná, Brazil).

The coefficients of the total tract apparent digestibility (CTTAD) of nutrients and the food metabolizable energy content were assessed by the total collection of faeces and urine method. Seven cats were used for each diet, individually housed for 8 consecutive days in metabolic cages with an apparatus to separate the faeces and urine for collection. The offered amounts of food and leftovers were weighted daily to calculate the intake. Faeces was quantitatively collected at least twice a day for 8 consecutive days, weighted and stored at −20°C until analysis. During the collection, the faecal samples were scored according to the following system (De-Oliveira et al., 2008): 1 = watery-liquid that can be poured; 2 = soft, unformed-stool assumes shape of container; 3 = soft, formed, moist-softer stool that retains shape; 4 = hard, formed, dry stool-remains firm and soft; and 5 = hard, dry pellets-small, hard mass. During the same day of faeces collection urine was quantitatively collected in plastic bottles. During the first 5 days collection bottles received 2 ml of 6N HCl to preserve N content. During the last 3 days 100 mg of thymol was added as a preservative, and this urine was used to evaluate pH, and density.

The faeces were thawed, homogenized, pooled per individual cat and dried in a forced-air oven (Fanem, São Paulo, Brazil) at 55°C for 72 hr. The faeces and diets were then ground (MOD 340; ART LAB, São Paulo, Brazil) and analysed according to the [38]AOAC (2010) for dry matter (DM) by oven drying the sample (934.01), crude protein (CP) using the Kjeldahl method, total fat by the acid-hydrolysed fat method (954.02), ash (942.05) and crude fiber (962.09). Starch was determined according to the method of [39]Hendrix (1993). The amino acid content of the diets was analyzed with the methods of [40]White et al., (1986) and [41]Hagen et al., (1989) by high-performance liquid chromatography and tryptophan was analyzed according to [42]Lucas & Sotelo (1980) by and enzymatic method. Gross energy was evaluated on food, faeces and urine samples by a bomb calorimeter (IKA calorimeter, C200; IKA-Werke GmbH & Co.KG, Staufen, Germany). All samples were analysed in duplicate, and the analysis was repeated when the variation between replicates was greater than 5%.

The water balance also was assessed by the total collection of faeces and urine method and was conducted together with the CTTAD evaluation. The offered amounts of water and leftovers were weighted daily to calculate the intake. A blank was used to estimate the water loss by evaporation from the drinking bowl. To this the same volume of water was placed in a similar drinking bowl as that of the cats and was exposed to the environment, and the difference in weight at the start and end was considered the evaporated water, which was subtracted from the water leftover to calculate the water intake. Urine was collected twice a day in plastic bottles with 100 mg thymol as a preservative and was stored under refrigeration (4°C). Each 24 h pooled-urine sample of individual cats was homogenized, the volume, density and pH were determined. The water balance calculations included the water intake via a drinking bowl, the water intake via food, the calculated metabolic water, the total water intake (sum of the first three parameters), the water excretion via faeces, the water excretion via urine, the insensible water loss and the total water excretion (sum of the three previous parameters). The metabolic water that was generated was calculated by multiplying the intake amount (in grams) of digestible starch by 0.566 ml, digestible protein by 0.396 ml and digestible fat by 1.071 ml ([43]BUFFINGTON & CHEW, 1998). The insensible water loss consisted of the sum of the breath, cutaneous and salivary losses of water, which were calculated indirectly by the difference between the total water intake and the sum of faeces and urine water excretion.

The nitrogen balance was also determined, but in this case the urine was collected acidified (1 ml 6 N HCl was added for each 50 ml of urine) and stored at −20°C for analysis. The nitrogen in food, faeces and urine samples was determined ([38]AOAC, 2010; Kjeldahl method). To quantify the nitrogen in urine, the samples were thawed, homogenized and centrifuged at 3500 rpm for 15 minutes. The nitrogen balance (NB) was calculated by the difference between nitrogen intake (Nintake) and excreted in faeces (Nfaeces) and urine (Nurine), by following equation:

$$NB = Nintake – (Nfaeces + Nurine)$$

Isotope injection, analysis and calculation procedure

The body composition, energy expenditure and water turnover were evaluated by the doubly labeled water method (DLW). The isotopes (Sercon Limited, United Kingdom) were administrated in the approximate doses of 0.12 g of ^{2}H at 99.9 atm% and 2 g of ^{18}O at 10 atm% per kg of body water ([44]FERRIOLLI et al., 2008). Then, a 6:100 (v/v) solution of ^{2}H at 99.9 atm% and ^{18}O at 10 atm%, respectively, was prepared and injected in each cat ([44]FERRIOLLI et al., 2008). The solution

was subcutaneously applied between the scapula after 12 h of fasting and 2 h without water. A solution with 0.3 ml of a 20 % NaCl was mixed and infused together to increase the isotope solution osmolality close to the interstice to not to cause discomfort for the cats ([45]GOLONI et al., 2020). To increase the accuracy of the injection, the syringe was weighed empty, with the 20 % NaCl solution, with the isotope solution and again after solution injection, according to the recommendation ([44]FERRIOLLI et al., 2008). Saliva of the cats was used to evaluate the enrichment of body water ([45]GOLONI et al., 2020). Samples were collected before solution injection, after 2 hours to measure the enrichment, and after 4 to 12 days to evaluate isotope elimination (at least 3 days of isotope elimination were observed for each cat). The saliva samples were collected with hydrophilic cotton externally and adjacent to the mouth, next to the animal's lips. To enable this, salivation was stimulated by dropping one drop of sodium dipyrone (500 mg/ml, EMS Pharma, São Bernardo do Campo, Brazil) on the cat's mouth, equivalent to 25 mg of dipyrone per cat (all animals were adapted for the procedure), as previously described ([45]GOLONI et al., 2020). After dipyrone administration, a first piece of cotton was used and discarded and a second piece of cotton was used for a maximum of 5 min to collect the saliva sample. The cotton was then placed in a 20 ml syringe, squeezed, and a minimum of 1.5 ml of saliva per cat was stored in a cryotube with a screw cap sealed with paraffin at −20°C until analysis. Caution was made to cotton not absorb moisture from the air before use, keeping it in sealed bags.

The isotope concentration in saliva was evaluated at the Mass Spectrometry Laboratory of the Ribeirão Preto Medical School, São Paulo, Brazil. The isotopes were analysed by isotope ratio mass spectrometry (ANCA 20-20; Europe Scientific, United Kingdom). Samples for ^{2}H were processed in duplicate (200 µl per replicate) with platinum in vacutainers, after 6 h of resting. Samples for ^{18}O were processed in duplicate (200 µl per replicate) by filling the tubes with CO_2 after 24 h of resting ([44]FERRIOLLI et al., 2008).

The pool size for ^{2}H or ^{18}O for the total of body water was calculated as follows (considering a linear elimination response) ([46]SCHOELLER, 1996):

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

where N is the pool size of body water; W is the amount of water used to dilute the labelled water dose; A is the weight of labelled water administration (g); a is the diluted dose for analysis; δ is the enrichment of dose (a), dilution water (t), post-dose sample (s) and pre-dose baseline (p) samples. For ^{18}O, considering the non-aqueous exchange routes are small no correction was made. To calculate total body

water with ^{2}H, a correction factor was used due to the isotope incorporation in non-aqueous organic molecules during biosynthesis ([47]RACETTE et al., 1994) using the following formula ([48]ELLIS & WONG, 1998)

$$\mathrm{TBW} = \left(\frac{Nd}{f}\right)$$

where TBW is the total body water; Nd is the pool size of body water with ^{2}H; f is the correction factor according to Nd:No (No = pool size of body water estimated with ^{18}O) ratio in each evaluated body fluid.

The lean body mass (LM) of the cats was calculated considering the hydration constant of 73·2 % for mammals by the following equation: lean body mass (kg) = body water (kg)/0·732. The fatty body mass (kg) was estimated as follows: total body mass (kg) – LM (kg) of the animal ([49]PACE & RATHBUM, 1945). The cats were considering overweight when their fat mass was ≥ 25% ([8]CLINE et al., 2021).

The two points formula was used to calculate ^{2}H and ^{18}O elimination ([50]SCHOELLER, 1986):

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

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

The amount of CO_2 produced was established using the following formula ([51]LIFSON & MCCLINTOCK, 1966):

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

where rCO_2 is the CO_2 production; N is the dilution space for ^{18}O; Ko is the rate constant of ^{18}O in body water and Kd is the rate constant of ^{2}H in body water.

Lastly, the energy expenditure (EE) of the cats was calculated as follows ([52]ELIA & LIVESEY, 1992):

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

where EE is the energy expenditure; rCO_2 is the CO_2 production and FQ is the food quotient. The food coefficient was calculated considering the digestible nutrient content of the food, determined *in vivo* with seven cats using the total collection of faeces method ([36]FEDIAF, 2018), as previously shown. The following formula was used ([53]BLACK et al., 1986):

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

where FQ is the food quotient; P is the digestible protein; G is the digestible fat and A is the digestible starch.

The body water turnover rate (WT) was calculated ([54]HENDRIKS et al, 1999), assuming little to no water is lost via evaporative routes that are subject to isotope fractionation:

$$\mathrm{WT\ (mL/day)} = \mathrm{Nd} * \mathrm{Kd} * 18.02$$

where WT is the water turnover rate; Nd is the ^2H body water and Kd is the ^2H rate constant. The EE of the cats was also computed by the food intake method. For this purpose, data on food intake to constant body weight were multiplied by the metabolizable energy content of the diet, as determined in vivo with cats using the total collection of faeces and urine method.

Voluntary physical activity with 3-axyal accelerometer

Voluntary physical activity was evaluated using a 3-axyal accelerometer device (AC; Axy-4, TechnoSmart, Italy) attached to a chest collar placed on the cats. The cats were adapted to use chest collar for 3 to 7 days, and then the AC was fixed in the region of the neck, closer to scapulas, for 3 consecutive days. The position of the AC in the chest collar followed the instructions of the manufactures (Figure 1), close in the centre of mass of the animals ([55]HALSEY et al., 2011). The use of devices to estimate physical activity in cats with the chest collars have been validated by [56]Lascelles et al. (2008) allowed to objectively measure the physical activity without human interference.

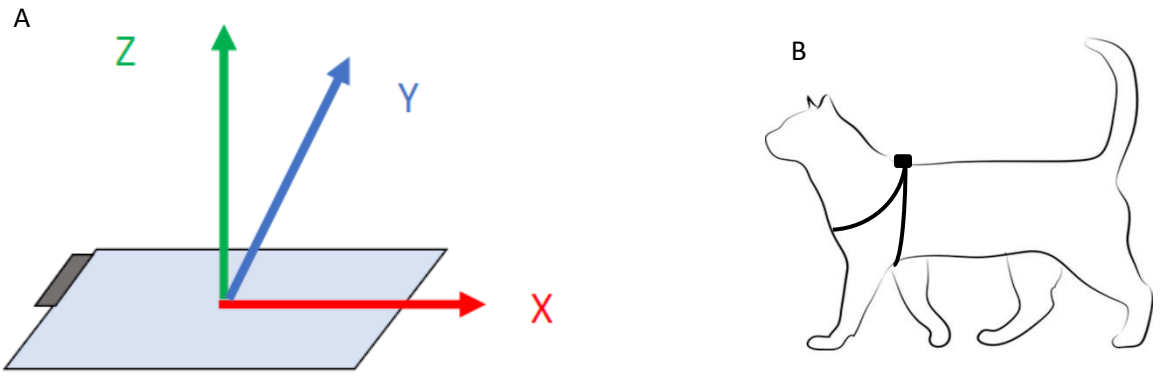

Figure 1. The position of the 3-axyal accelerometer. (A), X = Surge (walking front and back); Z = heave (movement to up and down); Y = sway (movement to right and left). (B) Illustration of accelerometer positioning in the cat.

The AC was configured at a frequency of 10 Hz and 16 G gravitational and 10bit resolution. X, Y and Z axis data were smoothed for each second and subtracted from the corresponding unsmoothed data to generate dynamic acceleration. The smoothed values on the three axes were converted into absolute values and added together to generate an overall dynamic body acceleration (ODBA) ([57]WILSON et al, 2006).

Determination of food intake pattern and the satiety response of the animals

This analysis aimed to know the possible influence of protein and starch consumption on the pattern of food intake and the voluntary consumption of a challenge food, thus indirectly accessing the satiety of the cats. To study food intake pattern, voluntary diet intake was measured every two-hour interval. For this, from 4:00 p.m. to 8:00 a.m., the quantity offered and refused food were weighed and recorded at each two hours interval. The goal was to verify possible influences of the composition of the food on the size of the meals, moments of consumption and total intake of food and energy.

The satiety evaluation was conducted on the last three days of the period. On these days, in addition to the experimental food, the cats were exposed to a commercial food of good palatability (Metabolizable Energy: 4.3 kcal/g; Crude protein: 41%; Crude Fat: 19%, in dry-matter basis; Guabi Natural Gatos, Affinity Pet Care, Campinas, Brazil) to verify their consumption. The food that was used is familiar to the animals of the cattery, fed when they were not in tests, and was selected to

avoid the behaviors of neophobia and neophilia. On these three days the cats received a half of the daily amount of the experimental diet at 4:00 p.m., which was available until 8:00 p.m. At 8:00 p.m they received an *ad libitum* amount of the challenge diet, which was available for one hour (until 9:00 p.m). At 9:00 p.m. the leftovers of the challenge food were collected and weighted, and the second half of the experimental diet was offered, which remained available until 8:00 a.m. of the next day. The quantities of the experimental diets and the challenge diet were weighed, as well as the leftovers, and the consumption was calculated. The intention was to verify if the composition of the food interferes in the consumption of a second meal, limiting or even stimulating it intake by the cats. The greater or lesser consumption of the challenge diet was used to indirectly interpret the satiety sensation of the cats ([58]LOUREIRO et al., 2017). For results analysis, the mean intake of the three days of the test was used.

Statistical analysis

The experimental unit was considered one cat. The sampling size was established based on the results of ANOVA for EE, considering the factorial arrangement of treatments. The test power was set at 0·8 (procedure Opdoe of the R software), $\alpha = 0.05$, standard deviation = 18.7 and a standard error of approximately 27. This analysis was performed with the sample size procedure of the R software, and a sample size of 7 cats per body composition was obtained, which assured adequate comparison of the main outcome (EE) for each treatment. The study was organized in a factorial arrangement, with 2 diets x 2 body conditions, totalizing 4 experimental treatments, and followed a randomized cross-over design. The effects were diet and body condition and the interaction diet * body condition. For the results of physical activity, the area under the curve was calculated by the integration trapezoidal method (Prism GraphPad, 2005) and ODBA total area was used to compare treatments. Additionally, total area of peaks of ODBA was calculated using data above 1 G gravitational to compare treatments. To evaluate food intake pattern, the time was included as a subplot in the factorial arrangement. Results were submitted to analysis of variance, considering the main effects and their interactions. When differences were verified on F test, means was compared by the Tukey test. The digestibility trial, water balance and nitrogen balance were evaluated in a completely randomized design and submitted to analysis of variance. The variables body condition score and faecal score were evaluated by the non-parametric Wilcoxon test. When of interest, the relationship among the body composition variables, EE, WT or movement parameters were accessed by a Person Correlation analysis after adjusting the factorial model on the residuals of the variables.

Values of P<0•05 were considered significant. All data met the assumptions of the analysis of variance and the analyzes were performed using the Proc MIXED to the SAS statistical software (SAS Institute, Cary, NC, EUA, 2003) or the R software version 3.6.3.

2.4 Results

Chemical composition of the experimental diets

The experimental diets presented the expected chemical composition (Table 2); the crude protein content differed in almost 18 points of percentage and the starch amount was a double in the HS food. The protein and amino acid content of the HS food was higher than the minimum recommendation for cat maintenance ([59]FEDIAF, 2021), so this diet can be considered adequate for these nutrients. The calculated energy contribution, in % of the estimated food metabolizable energy content was of 33.3% from protein, 35.4% from starch and 31.3 % from fat in the HS diet, and 49% from protein, 18.6% from starch and 32.4% from fat to HP diet.

Table 2. Analyzed chemical composition of the experimental diets. Values on dry matter basis.

Item	Diets	
	High Starch	High Protein
Moisture (%)	5.62	6.20
Crude protein (%)	37.91	55.87
Total aminoacids	34.49	51.28
Essential aminoacids		
Arginine	2.48	3.91
Histidine	0.81	1.20
Isoleucine	1.36	2.12
Leucine	2.64	3.81
Lysine	2.12	3.68
Methionine	0.72	0.85
Methionine+cystine	1.13	1.49
Phenilalanine	1.44	2.24
Phenilalanie+Tyrosine	2.57	3.90
Threonine	1.44	1.97
Tryptophan	0.32	0.50
Valine	1.68	2.48
Taurine	0.57	0.54
Non-essential aminoacids		
Glycine	3.61	4.46

Alanine	2.37	3.16
Proline	2.64	3.22
Tyrosine	1.13	1.66
Cystine	0.41	0.63
Aspartic acid	2.62	4.93
Glutamic acid	4.49	7.39
Serine	1.65	2.53
Acid-hydrolysed fat (%)	14.69	15.66
Starch (%)	40.28	20.56
Crude fibre (%)	1.81	1.31
Ash (%)	6.29	7.25
*Starch gelatinazation degree (%)	95.38	96.91

*Used do access the quality of the extrusion processing ([60]SA et al., 2013).

Dry matter intake during digestibility study did not differ (Table 3), but protein and starch consumption was different, according to each diet composition (P<0•01). The CTTAD and food metabolizable energy content was similar between diets (P>0•05). Nitrogen intake, faecal excretion and urinary excretion was higher for cats fed the HP food (P<0•05), but N retention was similar among diets (P=0•37). The cats fed the HP diet presented urine production almost 40% higher than cats the HS food (P<0•01). Other parameters of water balance, urine pH and density were similar between diets (P>0•05).

Table 03. Nutrient intake during the digestibility test, coefficients of total tract apparent digestibility of nutrients, metabolizable energy content, nitrogen balance and water balance of cats fed the experimental diets with different starch:protein ratios.

Item	Diets		* SEM	P value
	High Starch	High Protein		
Food Intake (g/kg$^{0.67}$/day)				
Dry Matter	19.54	19.99	1.71	0•39
Organic Matter	18.31	18.50	1.59	0•70
Crude Protein	7.59	11.42	0.94	<0•01
Acid-hydrolysed fat	2.98	3.14	0.27	0•06
Starch	6.48	3.74	0.56	<0•01
Apparent digestibility values (%)				
Dry matter	88.01	88.24	7.73	0•83
Organic matter	90.51	90.72	7.96	0•82
Crude Protein	91.87	92.55	8.10	0•30
Acid-hydrolysed fat	93.49	92.71	8.17	0•18

Starch	99.95	99.97	8.87	0•41
† Metabolizable energy (kcal/g)	3.97	4.09	3.55	0•06
Nitrogen Balance (mg/kg/day)				
Intake	694.05	969.41	42.64	<0•01
Excreted in faeces	62.19	80.95	4.46	0•03
Excreted in urine	558.01	788.76	42.09	<0•01
Retention	73.80	99.70	17.56	0•37
Water Balance (mL/kg$^{0.67}$/day)				
Water Intake via drinking bowl	29.80	33.26	1.25	0•17
Water Intake via food	1.25	1.27	0.02	0•08
Metabolic Water	9.28	9.36	0.12	0•76
Total Water Intake	40.32	43.91	1.24	0•15
Urine Production	18.15	25.55	1.34	<0•01
Faecal Excretion of water	2.40	2.87	0.26	0•30
Insensible Water losses	19.37	15.48	1.36	0•16
Urine pH	6.31	6.32	0.03	0•90
Urine density	1.06	1.06	0.00	0•70
Faecal score	3.66	3.49	0.23	0•36

* SEM: standard error mean (n=7 cats for each diet).

† Evaluated by total collection of faeces and urine.

The feeding management and procedures adopted to cats maintain a constant body weight along the two periods of the cross-over was effective, and the initial and final body weight was similar for cats fed the two diets (data not shown; P=0•25 to HS and P=0•19 to HP diet). After body composition results, it was observed that one male cat of the OW group had a fat mass lower than 25%, so this cat was relocated in the NOW group. One OW female cat could not complete the experimental period, for reasons not related to the diet or experimental procedures. Therefore, nine cats finished in the NOW group and six cats finished the OW group (Table 4). As expected, the OW cats had higher body weight and fat mass (P<0•01), with similar lean mass in kg but lower lean mass % than the NOW group (P<0•01). As a cross-over design was used and all cats were fed both diets, no diet effect was observed on body weight and the body composition of cats.

Table 4. Body weight and body composition of overweight and non-overweight laboratory cats fed the experimental diets with different starch:protein ratios.

Item	Diet		Mean	* SEM	† P value	
	High Starch	High Protein			Diet	‡ BC
Body Weight (kg)						

Non-overweight	3.93	3.96	3.93	0.27		
Overweight	4.90	4.92	4.91	0.33		
Mean	4.40	4.44		0.21	0•85	<0•01
Fat Body Mass (kg)						
Non-overweight	0.66	0.64	0.65	0.10		
Overweight	1.41	1.32	1.36	0.13		
Mean	1.04	0.98		0.08	0•49	<0•01
Lean Body Mass (kg)						
Non-overweight	3.23	3.31	3.27	0.21		
Overweight	3.50	3.58	3.54	0.26		
Mean	3.36	3.44		0.17	0•64	0•14
Fat Body Mass (%)						
Non-overweight	16.84	15.95	16.40	1.65		
Overweight	28.28	26.42	27.35	2.02	0•30	<0•01
Mean	22.56	21.19		1.30		
Lean Body Mass (%)						
Non-overweight	83.15	84.04	83.59	1.65		
Overweight	71.71	73.57	72.64	2.02		
Mean	77.43	78.80		1.30	0•30	<0•01

* SEM: standard error mean (n=6 for overweight; n=9 for non-overweight).
† P-value: Interaction not found for diet * body composition (P>0•05).
‡ BC: Body composition.

The mean food intake along the 33 days of each cross-over period was higher for NOW (18.15 SE 0.75 g/kg$^{0.67}$/day; P<0•01) compared to OW cats (14.85 SE 0.92 g/kg$^{0.67}$/day; Table 05). The EE, evaluated by DLW was also lower for OW (64.85 SE 6.87 kcal/kg$^{0.67}$/day) than NOW cats (75.57 SE 6.40; P=0•03). These values, however, was similar when calculated in a lean mass basis (P=0•38). A negative correlation was observed among EE and cats' fat mass content (EE (kcal/kg$^{0.67}$/day = -0.6418*(% Fat mass) + 84.011; R^2: 0.12; P=0•05; n=32; Figure 2).

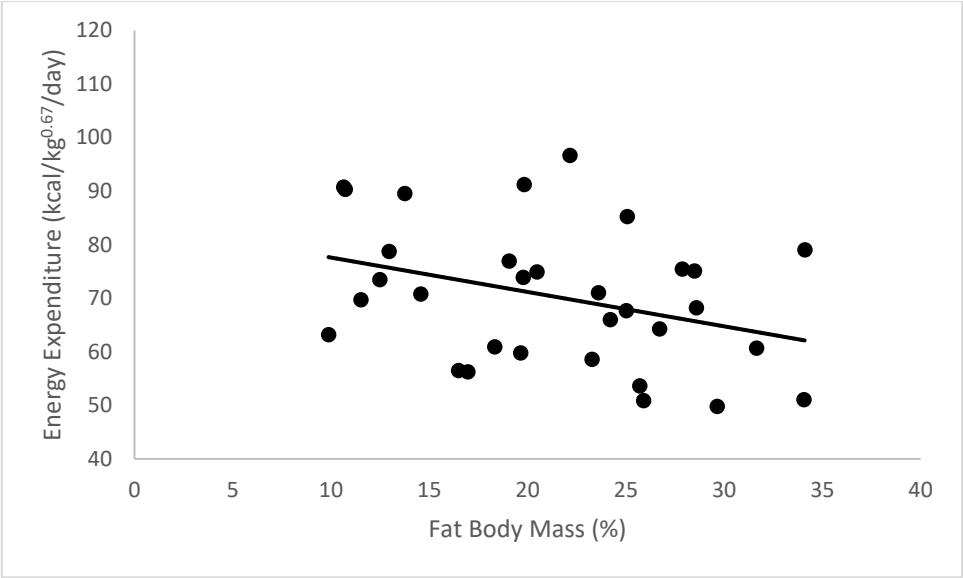

Figure 02. Pearson Correlation between energy expenditure vs. fat body mass of non-overweight and overweight laboratory cats fed diets with different starch:protein ratios (Linear regression; y= = -0.6418x + 84.011; R^2: 0.12; P=0•05; n=32).

Diet effect was not observed for food intake or EE (P>0•05), but the WT was approximately 15% higher in cats fed the HP food and 22% higher for the NOW animals than the HS diet and OW cats, respectively (P<0•01). A negative correlation was observed among WT and cats' fat mass content (WT (mL/$kg^{0.67}$/day) = -0.8454*(% Fat mass) + 69.98; R^2: 0.49; P<0•01; n=32; Figure 3).

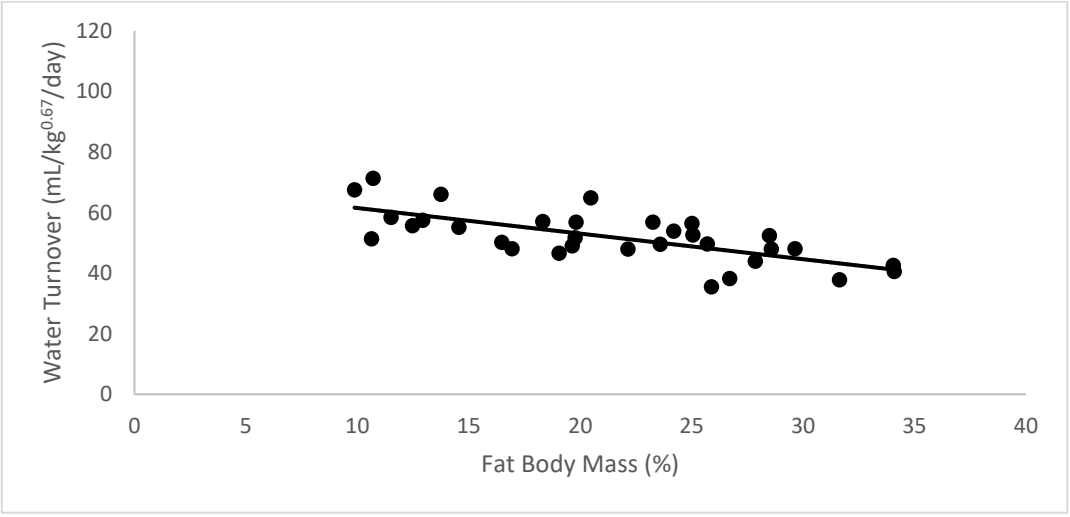

Figure 03. Pearson Correlation between water turnover vs. fat body mass of non-overweight and overweight laboratory cats fed diets with different starch:protein ratios (Linear regression; y= -0.8454x + 69.98; R^2: 0.49; P<0•01; n=32).

Additionally, EE was positively correlated with WT (WT (mL/kg$^{0.67}$/day) = 0.2518*(EE kcal/kg$^{0.67}$/day) + 34.23; R^2: 0.15; P=0•02; n=32; Figure 4).

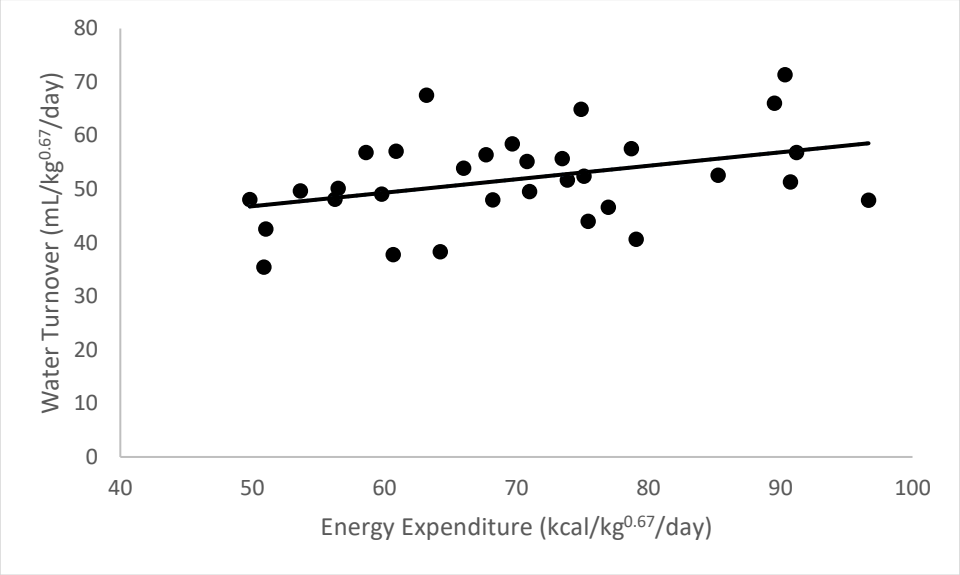

Figure 04: Pearson Correlation between energy expenditure vs. water turnover of non-overweight and overweight laboratory cats fed diets with different starch:protein ratios (Linear regression; 0.2518x + 34.23; R^2: 0.15; P=0•02; n=32).

Table 05. Food intake, energy expenditure, and water turnover of overweight and non-overweight laboratory cats fed diets with different starch:protein ratios to maintain a constant body weight.

Item	Diet		Mean	* SEM	† P value	
	High Starch	High Protein			Diet	‡ BC
Food Intake (g/kg$^{0.67}$/day)						
Non-overweight	18.08	18.22	18.15	0.75		
Overweight	15.01	14.67	14.84	0.92		
Mean	16.54	16.45		0.59	0•87	<0•01
Energy Expenditure (kcal/kg$^{0.67}$/day)						
Non-overweight	79.58	71.57	75.57	6.40		
Overweight	63.69	66.02	64.85	6.87		
Mean	71.63	68.79		4.50	0•53	0•03
Energy Expenditure (kcal/kg lean mass$^{0.67}$/day)						
Non-overweight	89.89	80.55	85.22	7.09		
Overweight	79.11	81.26	80.19	8.41		
Mean	84.50	80.90		5.50	0•52	0•38
Water turnover (mL/kg$^{0.67}$/day)						

Non-overweight	51.78	61.30	56.54	2.54		
Overweight	44.21	48.67	46.44	3.12		
Mean	47.99	54.99		2.01	<0•01	<0•01

* SEM: standard error mean (n=6 for overweight; n=9 for non-overweight).
† P-value: Interaction not found for diet * body composition (P>0•05).
‡ BC: Body condition.

The physical activity measured by the accelerometers was higher for NOW than OW cats (P<0•05; Table 06), although the ODBA number of peaks was similar. A negative correlation was observed among total ODBA area and cats' fat mass (Total ODBA area (G) = -28.188*(% Fat mass) + 4951.6; R^2: 0.39; P<0•01; n=25; Figure 5).

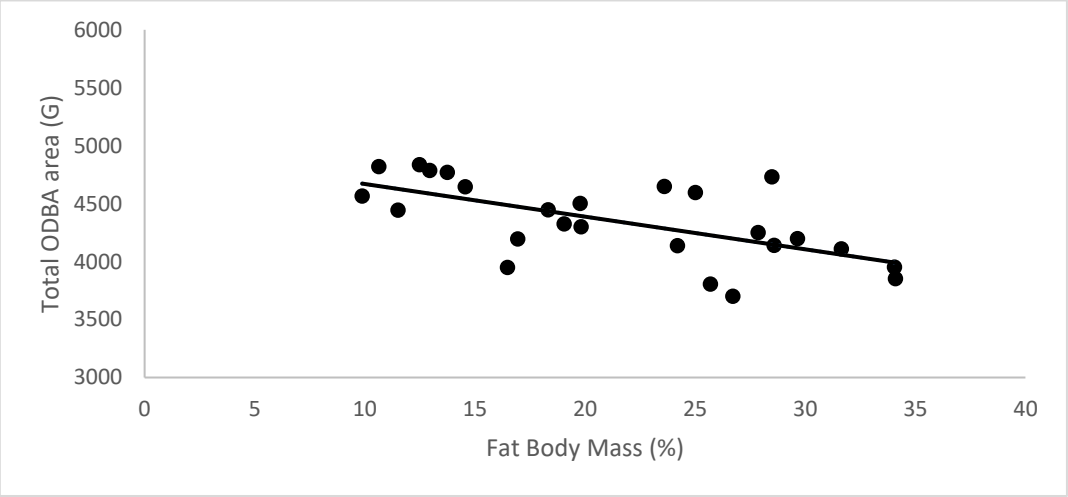

Figure 05. Pearson Correlation between fat body mass vs. total overall dynamic body acceleration (ODBA) of non-overweight and overweight laboratory cats fed diets with different starch:protein ratios (Linear regression; -28.188x + 4951.6; R^2: 0.39; P<0•01; n=25).

No diet effect was observed for physical activity parameters (P>0•05). Interesting, a positive correlation was observed among EE and the total ODBA area (EE (kcal/$kg^{0.67}$/day) = 0.026*(total ODBA area, G) - 43.888; R^2: 0.49; P<0•01; n=25; Figure 6).

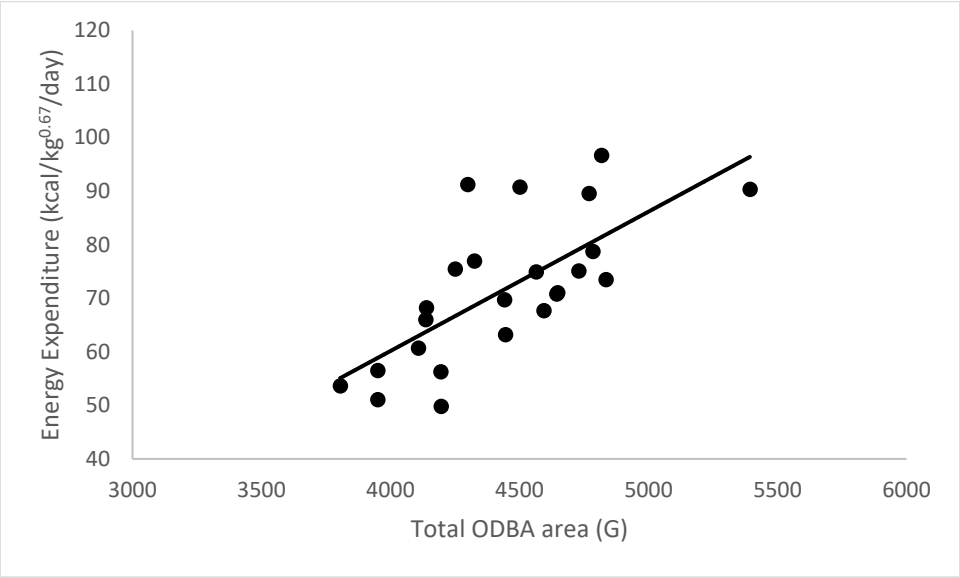

Figure 06. Pearson Correlation between total overall dynamic body acceleration (ODBA) vs. energy expenditure of non-overweight and overweight laboratory cats fed diets with different starch:protein ratios (Linear regression; y= 0.026x - 43.888; R^2: 0.49; P<0•01; n=25).

Table 06. Physical activity measured by 3-axyal accelerometer of overweight and non-overweight laboratory cats fed diets with different starch:protein ratios to maintain a constant body weight.

Item	Diets			* SEM	† P value	
	High Starch	High Protein	*Mean*		Diet	‡ BC
§ **ODBA total area (G)**						
Non-overweight	4665.41	4683.46	4674.44	183.85		
Overweight	4186.46	4036.80	4111.63	229.73		
Mean	4425.93	4360.13		147.00	0•65	<0•01
§ **ODBA total area of peaks (G)**						
Non-overweight	1734.84	1798.65	1766.74	289.57		
Overweight	1184.37	1150.82	1167.59	361.88		
Mean	1459.60	1474.73		231.84	0•94	0•02

* SEM: standard error mean (n=6 for overweight; n=9 for non-overweight).
† P value: Interaction not found for diet * body composition (P>0•05).
‡ BC: body condition.
§ ODBA: overall dynamic body acceleration.

The intake kinetics of the animals was determined after 30 days of consumption of the diets in each experimental period, to observe possible differences in response to the different starch:protein ratios or the body condition of the cats. The food intake was influenced by the mealtime (P<0•01), body composition (P<0•01) and a time * diet interaction was also observed (P<0•01), as can be seen

in Figure 07. Probably due to the fasting period of 8 h prior to the test, or the anticipation promoted by the expectation of meal availability, the food intake was significantly higher in the first 2h interval (from 4 pm to 6 pm), when the cats ingested a mean of 8.17 SE 0.27 kcal/kg$^{0.67}$ (P<0•01). A diet * time interaction was observed in this period (P<0•01), and the NOW cats fed the HP diet showed a higher intake on this first 2h period than the other groups (P<0•01), which presented similar intake amounts. This initial food intake was also higher for cats fed the HP than the HS food (HP: 9.67 SE 0.37 g/kg$^{0.67}$; HS: 6.6 SE 0.40 g/kg$^{0.67}$; P<0•01). For the remaining periods, cats showed a mean intake of 0.92 SE 0.24 kcal/kg$^{0.67}$ at each 2h period, without effect of diet or body composition.

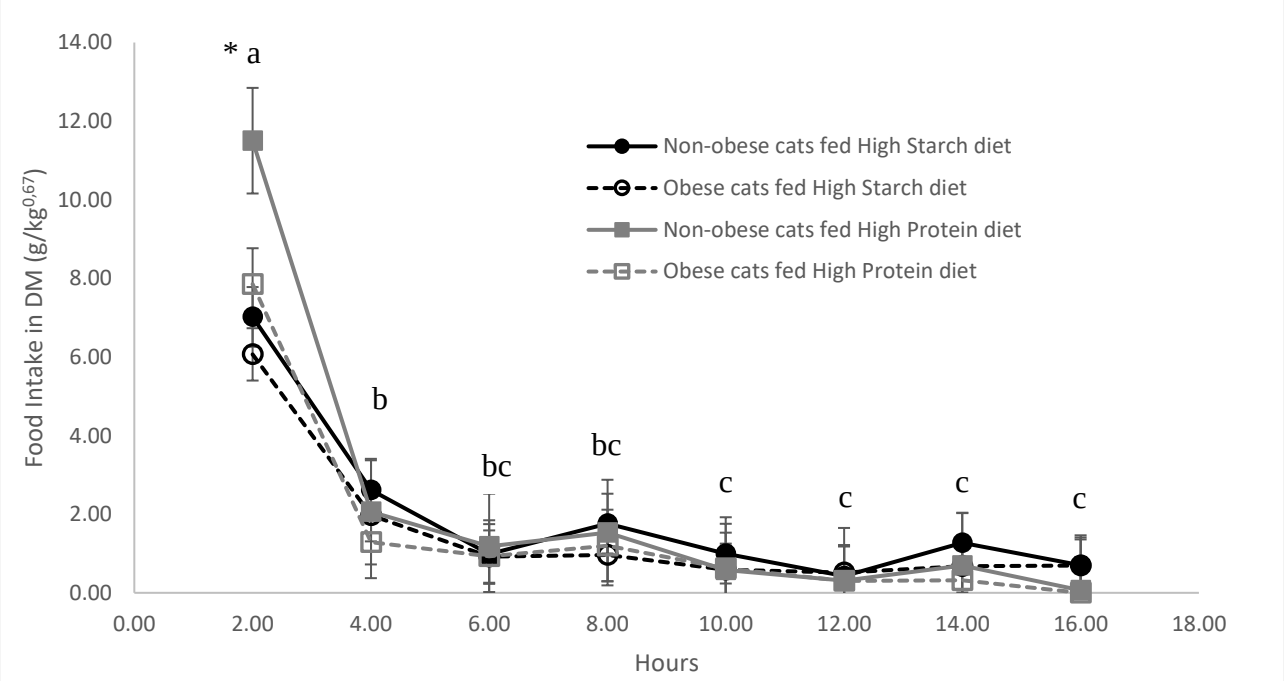

Figure 07. Kinetics of food consumption (g of DM/kg$^{0.67}$ every two hours) of overweight (n-6) and non-overweight (n=9) laboratory cats fed diets with different starch:protein ratios to maintain a constant body weight. [a, b, c] - Periods without a common lowercase letter presented different food intake (P<0•05). * - higher intake for non-overweight cats fed the high protein diet than the other groups (P<0•05).

The mean results of the three days of the satiety test were presented in Table 07. The food intake in the first 4 hours of offering (4:00 to 8:00 p.m.), the challenge food intake, and the accumulate intake of the first offering of the experimental food plus the challenge food was similar among the HS and HP foods (P>0•05). However, in the second exposition to the experimental diets cats fed the HP diet showed a food intake 28% higher than cats fed the HS diets (P<0•01), resulting in higher total food intake along the day (P=0•03). The NOW cats had higher experimental food intake in both

periods, as well of the challenge food, resulting the higher total food intake in comparison with the OW cats (P<0•01).

Table 07. Mean results of the three consecutive days of the satiety test in overweight and non-overweight laboratory cats after 30 days fed with diets with different starch:protein ratios to maintain a constant body weight.

Food Intake	Diets			* SEM	† P value	
	High Starch	High Protein	*Mean*		Diet	‡ BC
Dry matter (g/kg$^{0.67}$). Mean value of 3 days of observation						
§ Experimental diet intake from 4:00 to 8:00 p.m.						
Non-overweight	7.73	8.22	7.98	0.41		
Overweight	6.95	7.31	7.13	0.48		
Mean	7.34	7.77		0.31	0•18	0•01
‖ Challenge food intake from 8:00 to 9:00 p.m.						
Non-overweight	7.84	7.22	7.84	0.72		
Overweight	6.39	6.39	6.12	0.86		
Mean	6.81	7.15		0.56	0•54	<0•01
Experimental diet + challenge food accumulated intake						
Non-overweight	15.77	16.59	15.98	0.97		
Overweight	13.20	13.23	12.35	1.16		
Mean	14.06	14.91		0.75	0•26	<0•01
¶ Experimental diet intake from 9:00 p.m. to 8:00 a.m.						
Non-overweight	4.74	6.07	5.41	0.48		
Overweight	4.11	5.27	4.69	0.58		
Mean	4.42	5.67		0.38	<0•01	0•07
Total food intake on the day						
Non-overweight	19.72	22.68	21.20	1.07		
Overweight	17.50	18.71	18.10	1.27		
Mean	18.61	20.69		0.82	0•01	<0•01

* SEM: standard error mean (n=6 for overweight; n=9 for non-overweight).
† P value: Interaction not found for diet * body composition (P>0•05).
‡ BC: body composition.
§ First offering of the experimental diets, amount equivalent to 50% of the average daily consumption verified throughout the study.
‖ Challenge diet (Guabi Natural Filhotes, Affinity Pet Care, Campinas, Brazil) offered in an ad libitum amount.
¶ Second offering of the experimental diets, amount equivalent to 50% of the average daily consumption verified throughout the study.

2.5 Discussion

The high starch and high protein diets were great accepted by cats. The apparent digestibility of crude protein were >90% and starch were 99% in both diets. The isolate pea protein (lower in sodium) used in the high protein diet was great as source of protein to the digestibility of cats and the

high content of starch didn't compromise the digestibility of cats as seen by [23]De-OLIVEIRA et al., (2008). It is known that the nitrogen balance sub estimates at least 3 times the optimum protein intake to maintain lean body mass on cats ([61]LAFLAMME & HANNAH, 2013; [17]LAFLAMME, 2020), but the high protein content in the diet was capable to promote higher intake of nitrogen and excretion by faeces and urine on the study. Besides, nitrogen retention wasn't different between diets, but the high protein diet had higher numerically value. With higher excretion of nitrogen by urine, was possible to show higher urine production on water balance and water turnover, which corroborate with other authors ([62]WELLMAN, 2012; [35]GARCIA et al., 2020). The amount of protein intake (52%) proved to promote renal water excretion and possibly can be used to reducing the concentration of calculogenic substances as dietary strategy to urolithiases in cats.

The body composition of overweight cats had higher body fat mass and lower percent of body lean mass than non-overweight cats, as has been seen in many studies ([63]FETTMAN et al., 1997; [64]KANCHUCK et al., 2003; [65]GERMAN, 2008) and their implications on energy metabolism ([66]BERMINGHAM et al., 2010; [29]KIENZLE et al., 2006). The overweight cats had lower energy intake (64 kcal/$kg^{0.67}$/day) than non-overweight cats in the study, lower than recommended by [59]FEDIAF (2021), 75 kcal/$kg^{0.67}$/day, but without nutrient deficiency. When energy expenditure was expressed by kg of lean body $mass^{0.67}$ difference between body condition wasn't find, as presented by other authors compared lean and obese cats ([63]FETTMAN et al., 1997; [64]KANCHUK et al., 2003; [29]KIENZLE et al., 2006). In some situations, on cats living in homes, the energy intake can be lower and promote nutrient deficient as found by [67]Grant et al., (2020), which showed arginine and choline deficient on cats fed commercial diets to weight control. [66]Bermingham et al., (2010) shown a great difference of energy intake between different populations of cats. In populations which the energy intake is lower than recommended, diets must have more concentration of nutrients.

Another difference found between overweight and non-overweight cats in the study was the water turnover and physical activity. Overweight cats had lower water turnover than non-overweight cats. This study is the first one that compared the water turnover in different body composition of cats. One of possible explanation of the overweight cats had lower water turnover is the presence of more deposition of fat body mass (R^2: 0.49) than non-overweight cats. The adipose tissue stored energy in the form of triglyceride and have an important endocrine function ([68]ZORAN, 2010), but the metabolic activity of lean body mass is greater ([52]ELIA, 1992; [27]NRC, 2006) demanding 73% of water functions in mammals ([49]PACE & RATHBUM, 1945; [69]SERRA-PRAT et al., 2019), while in adipose tissue is 10% ([70]ROUMELIOTI et al., 2018; [69]SERRA-PRAT et al., 2019).

The voluntary movement was higher on non-overweight than overweight cats, as verified by [13]De-Godoy & Shoveller, (2017). Comparison between energy expenditure and voluntary physical

activity had good correlation on positive linear regression (R^2: 0.49). The voluntary physical activity contributed to energy requirement of cats as seen on [27]NRC (2006), [71]Wichert et al., (2007) and [59]FEDIAF (2021).

The food intake and energy expenditure weren't different between diets on fed control meal, as found by [21]Courtis et al., (2015). However, non-overweight cats fed high protein diet had higher food intake in the first two hours on food intake pattern. In the satiety test, non-overweight and overweight cats fed HP diet had higher intake on second part of experimental diet and total food amount. These results showed high protein content in diet doesn't promoted satiety, as according to [2]Hewson-Hugher et al., (2011) which showed cats prefer diets with high content of protein. On some moment, had been speculated higher protein intake could promote satiety in cats because of thermogenic effect of protein oxidation and possible increase of amino acids in the blood circulation in the postprandial period ([72]WESTERTERP-PLANTENGA et al., 1999; [61]LAFLAMME & HANNAH, 2005). However, when studies evaluated the sensitivity of insulin and glucose and leptin concentration in neutered cats consuming a diet with different protein content, no effect of diets was verified ([26]HOENIG et al., 2007; [73]VASCONCELLOS et al., 2009). Diets high in starch improved satiety than diets high in protein content, limiting the energy intake of cats, but more studies are interesting to understanding, at hormones levels, how starch promote satiety on cats. Nevertheless, the food intake limitation by high starch diet can be a problem if the concentration of nutrients is not adequate. Although, if the cats have adequate caloric intake, the limitation of food can collaborate for the body weight control on an *ad libitum* energy system for cats.

2.6 Conclusion

The consumption of the high protein diet increased the water turnover, urine volume and the voluntary food consumption of laboratory cats but did not alter the body composition, energy expenditure and physical activity in a short time. The diet with high starch limited the late food intake and, therefore, reduced the voluntary daily intake of food, which may have an influence on energy balance and body weight in the long term, aspects that deserve further studies. The overweight cats presented lower energy expenditure, voluntary activity and water turnover than the non-overweight cats hosed in laboratory. Additionally, it was observed that higher fat body mass was correlated with lower energy expenditure, water turnover, and physical activity, and that higher energy expenditure was correlated with increased water turnover and physical activity.

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CAPÍTULO 3 – Artigo 2

Different starch to protein ratio in kibble diets fed ad libitum influence body weight control, body composition, water turnover and physical activity in neutered cats living in homes

Camila Goloni[1], Letícia G. Pacheco[1], Letícia W. Luís, Stephanie S. Theodoro[1], Lucas B. Scarpim[1], Celina Torres[2], Gener T. Pereira[1], Aulus C. Carciofi[1*]

[1] Universidade Estadual Paulista (Unesp), Faculdade de Ciências Agrárias e Veterinárias, Jaboticabal, SP, 14884-900, Brazil.

[2] Affinity Petcare, Barcelona, Spain.

[*] Correspondência do autor: aulus.carciofi@gmail.com. Número de telefone + 55 16 3209-2626; fax número +55 16 3203-1226

Brief title: Starch to protein ratio in kibble diets to cats fed ad libitum

3.1 Abstract

The intake of diets with different starch:protein ratios was compared in neutered cats housed in homes. Male (M) and female (F), obese (OB) and non-obese (NO) cats were fed ad libitum for four months kibble diets high in starch (HS: Starch 40%, protein 38%; DM-basis) or high in protein (HP: Starch 20%, protein 55%), in a cross-over design. Physical activity was evaluated with an accelerometer, and body composition (BC), energy expenditure (EE) and water turnover (WT) by the doubly labeled water method. Results were compared in 2 diets x 2 sex x 2 body conditions factorial arrangement, totally 8 treatments. Cats fed HS diet maintain a constant body weight (BW), but lean mass (LM) tended to reduce in F-OB group (P=0•07). The intake of HP diet induced an increase on cats BW and LM (P<0•05), without altering body composition. The EE tended to be high in M (84 SE 2 kcal/kg$^{0.67}$/day) than F (79 SE 2; P=0•06), was not affected by diet or BC, and reduced as the age of the cats increase (R^2=0.44; P<0•01). The EE increases as the physical activity increase (R^2 0.58; P<0•01). The WT was higher for HP diet (P<0•01) and increased as the EE increases (R^2 0.65; P<0•01; 0.61 SE 0.01 mL/kcal of EE). The HS diet favored BW control in ad libitum feeding. Caution

is necessary to balance protein on diets to F-OB with more than 4 years old, as they may have low energy and food intake.

Key-words: accelerometer: deuterium: lean mass: obesity: sex

3.2 Introduction

The best composition of the dietary macronutrients that promotes longevity and quality of life in cats is not known ([1]VILLAVERDE & FASCETTI, 2014). Specially, the metabolic consequences of starch or protein intake on the body composition, energy intake, carbohydrate metabolism ([2]HOENIG & FERGUSON DC, 2002; [3]VASCONCELLOS et al., 2009), water turnover ([4]GARCIA et al., 2020) and physical activity are not clear. Considering the elevated protein requirement of cats to supply gluconeogenic amino acids for energy metabolism ([5]RUSSEL et al, 2002), and their preference selecting protein and fat as energy substrates over starch ([6]HEWSON-HUGHES et al., 2011), studies about the implications of starch intake specially in cats living in homes and fed dry diets in *ad libtum* systems are necessary.

Some studies suggested that high protein diets may increase the lean body mass of cats ([7]NGUYEN et al., 2004; [8]WEI et al., 2010) and induce higher heat increment, thus might increasing their daily energy expenditure in comparison to a high starch food ([9]GREEN et al., 2008). If this is really true, high protein diets might favor body weight maintenance and a better body composition in cats with unrestricted access to kibble diets. In this situation, the lower starch intake would also favor carbohydrate metabolism, reducing adiposity ([10]KELLER et al., 2017). However, some studies reported increase on body mass in cats fed high protein diets ([6]HEWSON-HUGHES et al., 2011; [11]CORADINI et al., 2014), pointing that the concept that high protein diets may promotes better satiety and energy intake control may be not valid ([12]DU et al., 2017).

On the other hand, it had been demonstrated that high starch diets didn't induce obesity, but high fat diets ([13]BACKUS et al., 2007). Starch on extruded diets present very high digestibility, been an adequate energy source ([14]De-OLIVEIRA, et al., 2008; [15]ALVARENGA & ALDRICH, 2020). Starch intake also did not induce evident alterations on post-prandial glucose and insulin responses in cat fed dry foods ([14]De-OLIVEIRA, et al., 2008; [16]HOENIG et al., 2011). Even in obese cats, different carbohydrate to protein ratios on diets did not induce significant changes on hormonal responses or glucose metabolism ([17]HOENIG, et al., 2007; [16]HOENIG, et al., 2011), suggesting that in a properly balanced food, starch intake is safe to cats. One interesting theory applicated to cats is

the “carbohydrate ceiling”, which suggests that cats may limit the starch intake by some amount and present lower intake of high starch foods. The authors that proposed this theory observed that cats prefer to eat protein as an energy substrate and may eat higher amounts of diets with high protein content, consequently increasing their body mass ([6]HEWSON-HUGHES et al., 2011). So, it is possible that a high starch diet, but properly balanced on amino acids and other nutrients, would limit voluntary food intake helping the maintenance a stable body weight of cats in *ad libitum* feeding systems ([8]WEI et al., 2010; [11]CORADINI et al., 2014).

For cats living in homes, studies about maintenance energy requirements are still needed, as most publications used laboratory cats that may have different living condition and physiological status ([18]BERMINGHAM et al., 2010). The available equations also did not account for the possible influenced of sex, sexual condition, body composition, age and physical activity ([19]KIENZLE et al., 2006; [18]BERMINGHAM et al., 2010; [20]BACKUS & WARA, 2016). Higher lean mass and energy expenditure (EE) on males, in comparison to females have been reported ([21]FETTMAN et al., 1997;[24]VASCONCELLOS et al., 2019; [22]GOLONI et al., 2020). The increase on body fat mass content, in overweight and obese cats is also associated with a reduced EE for maintenance, limiting the amount of food intake by these cats and increasing their chance to gain weight in *ad libitum* feeding systems ([23]KANCHUK et al., 2003; [17]HOENIG et al., 2007; [24]VASCONCELLOS et al., 2019). The influence of physical activity on EE is also little studied in cats ([1]VILLAVERDE & FASCETTI, 2014), but it importance can be observed by the effect of the environment (indoor or outdoor living stile) in the level of physical activity and the energy balance of cats ([25]BERMINGHAM et al., 2013; [26]PALLOTTO et al., 2018). This is, may be, the most important differences of cats living in homes or in laboratory conditions that reinforce the necessity of studies about energy metabolism, physical activity, and voluntary food intake in cats living in homes.

The elevated incidence of obesity in domiciliated cats ([27]GERMAN, 2016; [28]WALL et al., 2019) justify the importance of studies about the implications of diet macronutrient composition on energy embolism, physical activity, water turnover, and body composition. Obesity compromises the lifespan and quality of life ([29]BROOKS et al., 2014; [30]CLINE et al., 2021), may increasing inflammation and reducing energy metabolism in liver and kidneys ([31]MIZOROGI et al., 2020). It has been associated with age, neutering, housing condition, owner conscientiousness, feeding method and the energy density of the food ([1]VILLAVERDE & FASCETTI, 2014; [28]WALL et al., 2019). It was observed that diet composition and water intake may affect physical activity, as observed in cats fed high moisture foods ([32]ALEXANDER et al., 2014; [33]THOMAS et al., 2018). Considering that

high protein low starch foods can also increase water turnover of cats ([4]GARCIA et al., 2020), it is possible that high protein diets might also affect physical activity and energy expenditure.

Taken this into consideration, in the present study it was hypothesized that cats living in homes in *ad libitum* feeding system fed with a high protein diet may present an increase in lean body mass, energy expenditure, water turnover, and physical activity, and that a high starch intake my favor satiety reducing the voluntary intake of food of the cats. Thus, the objective of the present study was to compare the body weight and composition, energy expenditure, water balance, and voluntary physical activity of neutered male or female cats, obese or non-obese living in homes, fed unrestricted amounts of two extruded diets, one high in protein and another high in starch.

3.3 Material and Methods

Animals and experimental design

The experiment was conducted with client-owned cats at their homes, and in the Clinical Nutrition Service of Cats and Dogs of UNESP, campus of Jaboticabal, São Paulo, Brazil. All procedures with animals followed the ethical principles adopted by the Brazilian College of Animal Experimentation and were previously approved by the Ethics Committee on the Use of Animals of São Paulo State University (protocol no. 9536/18). Before engagement on study, owners were informed about all the procedures, and all signed a free consent of participation.

Client-owned cats were recruited between April/2019 until June/2020. The criteria to participate on the study included: cats must be indoor, with access to backyard allowed but not to streets; age between 1 to 7 years old; confirmed healthy along all the study; neutered at least 6 months before the engagement; commercial dry kibble food comprises >90% of daily energy intake; be fed *ad libitum* by an owner decision; the owner commit to fed only the experimental diet along the study, not giving any other food or snack. After the first contact with the owners to present the study and get the signed authorization of participation, the cats were accessed by a veterinarian that evaluated their health by physical examination, complete blood count, serum biochemistry (alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, albumin, urea and creatinine) and urine analysis.

The selected cats were divided in 4 groups according to body condition score (BCS; [34]LAFLAMME, 1997) and sex: female non-obese cats (F-NO; BCS between 5 and 6), male non-obese cats (M-NO; BCS between 5 and 6), female obese cats (F-OB; BCS of 7, 8 or 9) and male obese cats (M-OB; BCS of 7, 8 or 9). The obese cats must to be naturally overweight/obese for more than one year, based on the body weight records of the animal.

The treatments were organized in a factorial arrangement, with 2 diets x 2 body conditions x 2 sex, totalizing 8 experimental treatments. The study followed a cross-over design, and all cats were fed the two diets, in a randomized order. Each period of the cross-over lasted for 4 months, and the study lasted 8 months. In each cross-over period, on the first day the cats body weight was recorded (INMETRO: certified weights from 1 to 10 kg OIML E1/2004) and body condition score were documented. Animals body composition in the beginning of the study was evaluated by the deuterium oxide method. Cats, then, received only their designed experimental food and water for 4 mounts. The experimental food was introduced gradually over a period of 7 days. Only when the experimental food was the sole source of nutrients the experimental period was started. The food management, including the offered amount and feeding times was not altered and the owner continued doing their usual routine with the cats: *ad libitum* feeding, with leftovers present daily in the bowls. When the house had more than one cat, all of them was fed the experimental diet.

When each cat completed four months of experimental food intake the animal was weighted, classified according to BCS, had the physical activity evaluated with a 3-axial accelerometer for 3 consecutive days, and the body composition, energy expenditure and water turnover accessed by the doubly labelled water method (DLW). To complete all these evaluations a period of 15 to 20 days was necessary. Finalized the first period of the cross-over, the cats started the second period, with the other diet sorted.

Experimental diets

Two extruded diets were used, balanced for cat maintenance ([35]FEDIAF, 2018): High Starch (HS) with 38% crude protein and 40% of starch; High Protein (HP) with 55% of crude protein and 20% of starch, on as dry matter basis. The increase on protein content was obtained through a reduction of the starch content, thus altering the protein:starch ratio of the diets. The fiber and fat contents were remained similar. Diets were produced in two different moments, assuring fresh food along the experiment, and manufactured at the Extrusion Laboratory of the Faculdade de Ciências Agrárias e Veterinárias da UNESP, Jaboticabal, Brazil. The calculated energy contribution, in % of the estimated food metabolizable energy content was of 33.3% from protein, 35.4% from starch and 31.3 % from fat for the HS diet, and 49% from protein, 18.6% from starch and 32.4% from fat for the HP diet. The analysed chemical composition of the diets is presented on Table 01.

Table 01. Analyzed chemical composition of the experimental diets. Values on dry matter basis.

Item	Diets			
	[*] High Starch		[†] High Protein	
	Mean	[‡] SD	Mean	[‡] SD
Moisture (%)	5.62	0.44	6.20	0.27
Crude protein (%)	37.91	0.45	55.87	0.56
Total aminoacids	34.49	0.29	51.28	0.60
Essential aminoacids				
Arginine	2.48	0.04	3.91	0.05
Histidine	0.81	0.00	1.20	0.02
Isoleucine	1.36	0.05	2.12	0.00
Leucine	2.64	0.03	3.81	0.01
Lysine	2.12	0.22	3.68	0.02
Methionine	0.72	0.03	0.85	0.01
Methionine+cystine	1.13	0.02	1.49	0.01
Phenilalanine	1.44	0.07	2.24	0.01
Phenilalanie+Tyrosine	2.57	0.15	3.90	0.02
Threonine	1.44	0.07	1.97	0.02
Tryptophan	0.32	0.05	0.50	0.01
Valine	1.68	0.05	2.48	0.02
Taurine	0.57	0.05	0.54	0.01
Non-essential aminoacids				
Glycine	3.61	0.10	4.46	0.26
Alanine	2.37	0.09	3.16	0.09
Proline	2.64	0.19	3.22	0.11
Tyrosine	1.13	0.08	1.66	0.01
Cystine	0.41	0.02	0.63	0.02
Aspartic acid	2.62	0.48	4.93	0.03
Glutamic acid	4.49	0.31	7.39	0.01
Serine	1.65	0.01	2.53	0.02
Acid-hydrolysed fat (%)	14.69	0.78	15.66	0.11
Starch (%)	40.28	0.48	20.56	1.30
[§] Starch gelatinazation degree (%)	95.38	1.15	96.91	0.63
Crude fibre (%)	1.81	0.19	1.31	0.03
Ash (%)	6.29	0.53	7.25	0.31
[‖] Metabolizable energy (kcal/g)	3.97	0.00	4.09	0.00

[*]Ingredient composition of the high starch food: poultry by-product meal 35 %; corn grain 26.7 % (high starch diet; HS), broken rice 20 %; isolated protein pean 21.07 % (high protein diet; HP), isolated swine protein 7 % (HS) and 10 % (HP), poultry fat 6.7 %; beet pulp 2.5%, palatant enhancer 1.5 % (SPF Brasil); potassium chlorate 0.65%, common salt 0.6 %; choline chloride 0.45 %; vitamin–mineral premix 0.7 % (Rovimix, DSM Produtos Nutricionais Brasil S.A.); urine acidifier 0.45 % (Diana Pet food, Descalvado, Brazil), taurine 0.20%; calcium proprionate 0.04 %; potassium sorbate 0.02 %; antioxidant 0.07% (Alltech do Brazil Agroindustrial Ltda).

† Ingredient composition of the high protein food: poultry by-product meal 35 %; corn grain 26.7 % (high starch diet; HS), broken rice 20 %; isolated protein pean 21.07 % (high protein diet; HP), isolated swine protein 7 % (HS) and 10 % (HP), poultry fat 6.7 %; beet pulp 2.5%, palatant enhancer 1.5 % (SPF Brasil); potassium chlorate 0.65%, common salt 0.6 %; choline chloride 0.45 %; vitamin–mineral premix 0.7 % (Rovimix, DSM Produtos Nutricionais Brasil S.A.); urine acidifier 0.45 % (Diana Pet food, Descalvado, Brazil), taurine 0.20%; calcium proprionate 0.04 %; potassium sorbate 0.02 %; antioxidant 0.07% (Alltech do Brazil Agroindustrial Ltda).
‡ SD standard deviation of the two lots of production.
§ Used to access the quality of the extrusion processing ([36]SA et al., 2013).
‖ Evaluated by total collection of faeces and urine of the the diets on first production moment.

Voluntary physical activity with 3-axyal accelerometer

Voluntary physical activity was evaluated using a 3-axyal accelerometer device (AC; Axy-4, TechnoSmart, Italy) attached to a chest collar placed on the cats. The cats were adapted to use chest collar for 3 to 7 days, and then the AC was fixed in the region of the neck, closer to scapulas, for 3 consecutive days. The position of the AC in the chest collar followed the instructions of the manufactures (Figure 1), close in the centre of mass of the animals ([37]HALSEY et al., 2011). The use of devices to estimate physical activity in cats with the chest collars have been validated by [38]Lascelles et al. (2008) allowing to objectively measure the physical activity without human interference.

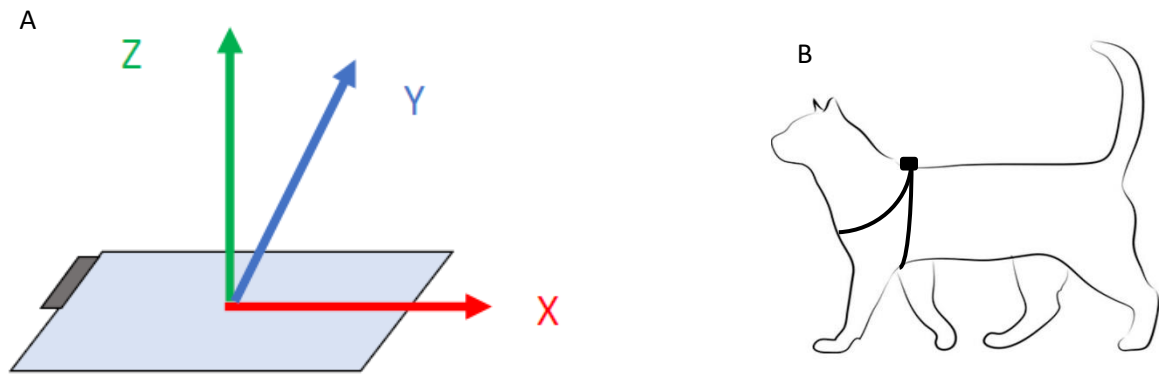

Figure 1. The position of the 3-axyal accelerometer. (A), X = Surge (walking front and back); Z = heave (movement to up and down); Y = sway (movement to right and left). (B) Illustration of accelerometer positioning in the cat.

The movement registration with the AC was performed for at least 3 consecutive days for each cat. Equipment was configured at a frequency of 10 Hz and 16 G gravitational and 10bit resolution. The X, Y and Z axis data were smoothed for each second and subtracted from the

corresponding unsmoothed data to generate dynamic acceleration. The smoothed values on the three axes were converted into absolute values and added together to generate an overall dynamic body acceleration (ODBA) ([39]WILSON et al, 2006). Additional recommendation to cat owners for this period were: they must not hold the cat and maintain their usual routine. The available space of the cat's was variable according to each home, including animals housed in apartments and houses with backyard, with or without environment enrichment. All then, however, were restricted to the houses without access to streets. As all cats ate the two foods, the environmental effect was diluted by the cross-over design.

Isotopes Evaluation

The initial body composition was evaluated with deuterium oxide (D_2O). A 7% (70:930; v/v) solution of ^{2}H at 99.9 atm% (Sercon Limited, United Kingdom) and NaCl 0.9% was prepared and 1 mL per kg of body weight injected in each cat ([40]FERRIOLLI et al., 2008). The solution was subcutaneously applied between the scapula after 12 h of fasting and 2 h without water. A sample of saliva were collected before solution injection and after 2 hours to access isotope enrichment in body water. To increase the accuracy of the injection, the syringe was weighed empty, with the isotope solution and again after solution injection ([40]FERRIOLLI et al., 2008). The saliva samples were collected according to [22]Goloni et al., (2020), with hydrophilic cotton externally and adjacent to the mouth, next to the animal's lips. To enable this, salivation was stimulated by dropping one drop of sodium dipyrone (500 mg/ml, EMS, São Bernardo do Campo, Brazil) on the cat's mouth, equivalent to 25 mg of dipyrone per cat (all animals were adapted previously for the procedure). After dipyrone administration, a first piece of cotton was used and discarded, and a second piece of cotton was used for a maximum of 5 min to collect the saliva sample. The cotton was then placed in a 20 ml syringe, squeezed, and a minimum of 1.5 ml of saliva per cat was stored in a cryotube with a screw cap sealed with paraffin at −20°C until analysis. Caution was made to cotton not absorb moisture from the air before use, keeping it in sealed bags.

The body composition, energy expenditure and water turnover were determined by the DLW method in all cats at the end of the four months period of experimental food intake. The isotopes (Sercon Limited, United Kingdom) were administrated in the approximate doses of 0.12 g of ^{2}H at 99.9 atm% and 2 g of ^{18}O at 10 atm% per kg of body water ([40]FERRIOLLI et al., 2008). A 6:100 (v/v) solution of ^{2}H at 99.9 atm% and ^{18}O at 10 atm%, respectively, was prepared and injected in each cat ([40]FERRIOLLI et al., 2008). The solution was subcutaneously applied between the scapula after 12 h of fasting and 2 h without water. An amount of 0.3 ml of a 20 % NaCl was mixed and infused

together to increase the isotope solution osmolality close to the interstice, avoiding discomfort to the cats ([22]GOLONI et al., 2020). To increase the accuracy of the injection, the syringe was weighed empty, with the 20 % NaCl solution, with the isotope solution and again after injection ([40]FERRIOLLI et al., 2008). Saliva samples were used to evaluate body water enrichment, and collected before solution injection, after 2 hours to access isotopes enrichment, and three times from days 4 to 12 to evaluate isotope elimination rate from body water. Saliva samples were collected and stored as described above.

The isotope concentration in saliva was evaluated at the Mass Spectrometry Laboratory of the Ribeirão Preto Medical School, São Paulo, Brazil. The isotopes were analyzed by isotope ratio mass spectrometry (ANCA 20-20; Europe Scientific, United Kingdom). Samples for ^{2}H were processed in duplicate (200 µl per replicate) with platinum in vacutainers, after 6 h of resting. Samples for ^{18}O were processed in duplicate (200 µl per replicate) by filling the tubes with CO_2 after 24 h of resting ([40]FERRIOLLI et al., 2008).

The pool size for ^{2}H or ^{18}O for the total of body water was calculated as follows (considering a linear elimination response) ([41]SCHOELLER, 1996):

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

where N is the pool size of body water; W is the amount of water used to dilute the labelled water dose; A is the weight of labelled water administration (g); a is the diluted dose for analysis; δ is the enrichment of dose (a), dilution water (t), post-dose sample (s) and pre-dose baseline (p) samples. For ^{18}O, considering the non-aqueous exchange routes are small no correction was made. To calculate total body water with ^{2}H, a correction factor was used due to the isotope incorporation in non-aqueous organic molecules during biosynthesis ([42]RACETTE et al., 1994) using the following formula ([43]ELLIS & WONG, 1998):

$$\text{TBW} = \left(\frac{Nd}{f}\right)$$

where TBW is the total body water; Nd is the pool size of body water with ^{2}H; f is the correction factor according to Nd:No (No = pool size of body water estimated with ^{18}O) ratio in each evaluated body fluid.

The lean body mass (LM) of the cats was calculated considering the hydration constant of 73·2 % for mammals by the following equation: lean body mass (kg) = body water (kg)/0·732. The fatty

body mass (kg) was estimated as follows: total body mass (kg) – LM (kg) of the animal ([44]PACE & RATHBUM, 1945). The cats were considering overweight when their fat mass was ≥ 25% ([30]CLINE et al., 2021).

The two-point formula was used to calculate ^{2}H and ^{18}O elimination ([45]SCHOELLER, 1986):

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

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

The amount of CO_2 produced was established using the following formula ([46]LIFSON & MCCLINTOCK, 1966):

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

where rCO_2 is the CO_2 production; N is the dilution space for ^{18}O; Ko is the rate constant of ^{18}O in body water and Kd is the rate constant of ^{2}H in body water.

Lastly, the energy expenditure (EE) of the cats was calculated as follows ([47]ELIA & LIVESEY, 1992):

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

where EE is the energy expenditure; rCO_2 is the CO_2 production and FQ is the food quotient. The food coefficient was calculated considering the digestible nutrient content of the food, determined *in vivo* with seven cats using the total collection of faeces method (FEDIAF, 2018), as previously shown. The following formula was used ([48]BLACK et al., 1986):

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

where FQ is the food quotient; P is the digestible protein; G is the digestible fat and A is the digestible starch.

The body water turnover rate (WT) was calculated as ([49]HENDRIKS et al, 1999), assuming little to no water is lost via evaporative routes that are subject to isotope fractionation:

$$\text{WT (mL/day)} = \text{Nd} * \text{Kd} * 18.02$$

where WT is the water turnover rate; Nd is the ^{2}H body water and Kd is the ^{2}H rate constant. The EE of the cats was also computed by the food intake method. For this purpose, data on food intake to constant body weight were multiplied by the metabolizable energy content of the diet, as determined in vivo with cats using the total collection of faeces and urine method.

Statistical analysis

The experimental unit was considered one cat. The test power was set at 0.8 and the $\alpha = 0.05$. The information of the variance between treatments (mean square) and within treatments (mean square of residue) were used following the ANOVA procedure (power.ANOVA.test of the R software) to obtain the minimum sample size for EE, resulting in six cats per treatment, which assured adequate comparison of the main outcomes. The study followed a cross-over design and was organized in a factorial arrangement of treatments with 2 diets x 2 sex x 2 body conditions, totalizing 8 experimental treatments. The analysis was organized considering the effects diet, sex and body conditions, and the interactions diet * sex, diet * body condition, sex * body condition and diet * sex * body condition. The age of the cats (years) differed between groups (P≤0•05) and due this age was used as a covariate in the statistical model. When differences were verified on F test, means was compared by the Tukey test for the variables body composition, energy expenditure, water turnover and physical activity. For the results of physical activity, the area under the curve was calculated by the integration trapezoidal method (Prism GraphPad, 2005) and ODBA total area was used to compare treatments. Additionally, total area of peaks of ODBA was calculated using data above 1 G gravitational to compare treatments. Inside each food group, the body weight, fat body mass and lean body mass of cats at the beginning and after the period of food intake was compared by the paired Student t-test. To compare these variables between diets at the beginning and final periods, the Student t-test was used. The variable body condition score was evaluated with the non-parametric Wilcoxon test. When of interest, the relationship among the body composition variables, EE, WT, movement parameters and nutrient intake were accessed by a Person Correlation analysis after adjusting the factorial model on the residuals of the variables. Values of P<0•05 were considered significant. Values of P≤0•05 were considered significant and P≤0•10 as a tendency. All data complied with the presuppositions of the variance analysis. The analyzes were performed using the Proc GLM of the SAS statistical software (SAS Institute, Cary, NC, EUA, 2003) or the R software version 3.6.3.

3.4 Results

The experiment started in November 2019 and finished in January 2021. A total of 108 client-owned cats were recruited. From this, 95 animals were submitted to physical examination, blood work and was interviewed about their possibility to completely follow the study requirements. From this amount 43 animals started the project, but 13 animals were removed, or their owners decided to not complete the study. During the study three cats had the living environment changed in one of the cross-over periods, because the owner moved to another house with a large backyard and that period were not considered on the study. A male obese cat started to had access to the street, due this the animal lost body weight, and that period was not considered too. A female lean cat gained body weight when fed with the HP diet, so in the second period she was moved to the F-OB group to start the HS diet. A male lean cat did not accept the HS diet, so the animal was removed from the study.

A total of 30 cats, belonged to 23 owners finished the project. The cats which complete the study were divided in the following groups: F-NO with 2.9 SD 2.1 years (n=9); F-OB with 4.2 SD 1.6 years (n=7); M-NO with 2.0 SD 1.1 years (n=9); M-OB with 4.5 SD 2.6 years (n=5). The mean age of the groups differed (P<0•05), and then age was used in the statistical analysis as a co-variate.

The cats ate adequately the foods, and according to their owners' foods was well accepted, the adaptations to the new diets was easy, and the faeces produced was adequate. The food management was made only by the owner in 30.4% (n=7) of the houses, and in 69.6% (n=16) the owner and another relative that lived at home also fed the cats. Food was provided *ad libitum* and placed once a day in 65.2% (n=15) of the houses, and twice a day in 34.8% (n=8) of the houses. Six owners admitted supplying a small amount of snacks (less than 10% of the daily calories of the cat), in a frequency of once a week (n=2 owners), once every two weeks (n=1 owner) and once a month (n=3 owners). Ten owners reported that blood collection to health assessment was a stressful situation for their cats along the study, six owners indicated that saliva collection in the DLW method was stressful for their cats, for 2 owners both blood and saliva collection was a stressful situation and 5 owners answered that none of sample collection was stressful for their cats. The chest collar was difficult to be used for 6 cats, and they did not participate on the physical activity evaluation. Nine owners did not change the cat's activity in the period of physical activity evaluation, 7 owners admitted that they changed the cat's routine, specially holding their cats in their arms as they usually did, and 7 owners did not remember if they interfered on the cat's activity or not. For all the owners

(n=23) the greater difficult of the study protocol was to keep cats fasting for 12 hours prior to start the DLW method.

The results of body composition shows that regardless of sex and diet, OB cats had higher fat body mass (FM) (OB: 30.7 SE 1.10 % and 1.81 SE 0.12 kg; NOB: 15.8 SE 0.70 % and 0.66 SE 0.04 kg; P<0•01), lower lean body mass (LM) in percentage (OB: 69.3 SE 0.96 %, NO: 84.7 SE 0.74 %; P<0•01), but higher LM in kg than NO cats (OB: 3.92 SE 0.16 kg; NO: 3.4 SE 0.11 kg; P<0•01). Male cats, regardless of body composition and diet had higher LM in kg than female cats (M: 4.11 SE 0.04 kg; F: 3.22 SE 0.05 kg; P<0•01), but the values were similar in percentage (M: 77.4 SE 0.9 %; F: 75.5 SE 0.8 %; P=0•13). Males and females had the same fat mass in % and in kg (M: 22.69 SE 0.91 %, 1.32 SE 0.12 kg; F: 23.69 SE 0.83 %, 1.16 SE 0.13 kg; P>0•10).

When fed the HS diet the body weight of the cats (Table 02) did not change for any sex and body condition (P>0•05). However, the HP diet intake induced body weight gain in all groups of cats studied (P<0•05). The BCS did not change in any group or diet, and no differences between diets was observed on body weight or the BCS of cats.

Table 02. Initial and final body weight and body condition score of client-owned neutered cats fed ad libitum for four months diets with different starch:protein ratios.

Groups	Body Weight (kg)				Body Condition Score			
	High Starch	High Protein	*SEM	*P value*	High Starch	High Protein	*SEM	*P value*
Non-Obese Female (n=9)								
Initial	3.39	3.41	0.12	*0•92*	5.10	5.00	0.05	*0•99*
Final	3.34	3.59	0.11	*0•26*	5.00	5.10	0.05	*0•99*
P value	*0•57*	*0•01*			*0•99*	*0•99*		
Obese Female (n=7)								
Initial	5.93	5.78	0.35	*0•85*	7.38	7.38	0.20	*1•00*
Final	5.74	6.09	0.37	*0•69*	7.29	7.38	0.24	*0•68*
P value	*0•16*	*<0•01*			*0•78*	*1•00*		
Non-Obese Male (n=9)								
Initial	4.40	4.47	0.15	*0•83*	5.33	5.00	0.08	*0•14*
Final	4.35	4.70	0.15	*0•27*	5.00	5.11	0.05	*0•99*
P value	*0•43*	*0•02*			*0•14*	*0•99*		
Obese Male (n=5)								
Initial	6.09	5.98	0.30	*0•87*	7.40	7.20	0.26	*0•73*
Final	6.05	6.24	0.29	*0•75*	7.20	7.50	0.18	*0•99*
P value	*0•77*	*<0•01*			*0•73*	*0•99*		

* SEM: standard error mean.

When fed the HS diet, the M-NO cats presented a reduction in FM (Table 03), both in kg and % (P=0•04), and the F-NO cats tended to present a reduction on the % of FM (P=0•06). However, when fed the HP food, the kg of FM increased in F-NO (P=0•04) and tended to increase in F-OB (P=0•06). Additionally, the % of FM tented to increase on F-NO (P=0•07). Due this, although at start F-NO cats presented the same body composition for both diets, at the end of the period F-NO cats fed the HP food showed more FM, both in kg and % then when fed with the HS food (P<0•05).

Table 03. Initial and final fat body mass of client-owned neutered cats fed ad libitum for four months diets with different starch:protein ratios.

Groups	Fat Body Mass (kg)				Fat Body Mass (%)			
	High Starch	High Protein	*SEM	*P value*	High Starch	High Protein	*SEM	*P value*
Non-Obese Female (n=9)								
Initial	0.61	0.59	0.04	*0•76*	17.80	17.04	0.83	*0•65*
Final	0.50	0.69	0.04	*0•04*	14.86	19.01	0.94	*0•05*
P value	*0•11*	*0•04*			*0•06*	*0•07*		
Obese Female (n=7)								
Initial	1.77	1.82	0.14	*0.92*	29.92	31.12	1.14	*0•58*
Final	1.73	1.95	0.17	*0.61*	31.53	31.36	1.29	*0•96*
P value	*0•71*	*0•06*			*0•27*	*0•66*		
Non-Obese Male (n=9)								
Initial	0.85	0.76	0.05	*0•31*	19.03	16.65	0.83	*0•19*
Final	0.67	0.71	0.06	*0•91*	15.35	14.74	1.13	*0•81*
P value	*0•04*	*0•34*			*0•04*	*0•24*		
Obese Male (n=5)								
Initial	1.85	1.78	0.12	*0•80*	30.13	29.50	0.58	*0•71*
Final	1.80	1.83	0.13	*0•99*	29.50	28.88	0.77	*0•77*
P value	*0•59*	*0•74*			*0•71*	*0•71*		

* SEM: standard error mean.

The lean body mass (LM) in kg increased in F-NO cats (P<0•01) and tended to increase in M-NO cats fed the HS diet (P=0•08), as observed on Table 04. The LM % also increased on M-NO cats (P=0•04) and tended to increase on F-NO cats fed the HS diet (P=0•06). In the period receiving the HP formulation, all cat groups gained LM in kg (P<0•05), although the LM % tended to reduce in the F-NO cats (P=0•07) and did not change for the other groups. The only group that tended to reduce the LM (kg) along the study was the F-OB cats (P=0•07) fed the HS diet, which LM was 220 g (4.3 %) lower comparing the initial and final values.

Table 04. Initial and final lean body mass of client-owned neutered cats fed ad libitum for four months diets with different starch:protein ratios.

Groups	Lean Body Mass (kg)				Lean Body Mass (%)			
	High Starch	High Protein	*SEM	*P value*	High Starch	High Protein	*SEM	*P value*
Non-Obese Female (n=9)								
Initial	2.78	2.82	0.60	*<0•01*	82.20	82.96	0.83	*0•65*
Final	2.84	2.91	0.65	*0•65*	85.14	80.99	0.94	*0•05*
P value	*<0•01*	*0•02*			*0•06*	*0•07*		
Obese Female (n=7)								
Initial	3.84	3.62	0.25	*0•55*	70.27	68.88	1.14	*0•58*
Final	3.62	3.81	0.21	*0•59*	68.47	68.64	1.29	*0•96*
P value	*0•07*	*<0•01*			*0•27*	*0•67*		
Non-Obese Male (n=9)								
Initial	3.55	3.71	0.12	*0•55*	80.97	83.35	0.83	*0•19*
Final	3.68	3.98	0.12	*0•24*	84.65	85.26	1.13	*0•81*
P value	*0•08*	*<0•01*			*0•04*	*0•24*		
Obese Male (n=5)								
Initial	4.24	4.05	0.21	*0•64*	69.87	70.50	0.58	*0•71*
Final	4.24	4.34	0.17	*0•79*	70.41	71.12	0.77	*0•77*
P value	*0•81*	*0•04*			*0•52*	*0•70*		

* SEM: standard error mean.

The protein and amino acids intake of the cats was calculated considering the EE estimated by the DLW method, and the protein, amino acids and metabolizable energy content (determined in vivo) of each diet. The cats fed with the HS diet had a mean intake of 7.82 SE 0.21 g of CP/kg$^{0.67}$/day, and cats fed the HP food an intake of 11.43 SE 0.33 g of CP/kg$^{0.67}$/day. When CP and amino acid intake of each cat group was verified, values in general greater than the suggested by the [50]FEDIAF (2021) was observed. However, F-OB cats fed the HS diet had numerically lower EE than the other groups, with a mean intake of 6.84 SE 0.38 g of CP/kg$^{0.67}$/day. Although this value of CP consumption was 9% higher than the minimum recommended by FEDIAF (2021), according to this food amino acid composition the mean intake of phenylalanine + tyrosine (0.46 SE 0.06 g/kg$^{0.67}$/day) for this cat group was only 4% higher than the minimum recommended (FEDIAF, 2021). However, two cats of the F-OB group fed the HS diet had low EE values, of only 65 kcal and 53 kcal/kg$^{0.67}$/day. Due this lower EE, their calculated intake of phenylalanine + tyrosine was, respectively, 5% and 20% below than the recommended minimum value, justifying the loss on LM in this group of cats.

A Pearson correlation among the CP and phenylalanine + tyrosine intake (x axe) and the differences in LM at the initial and final evaluation of each period of the cross over (final LM kg – initial LM kg; y axe) are presented on Figure 02. The correlation among CP intake and LM variation observed was described as: LM variation (kg) = 0.0534 * CP intake (g/kg$^{0.67}$/day) – 0.388; R^2 = 0.42; P<0•01; n=55. According to this model, in the conditions of the present study, cats would need approximately 7.26 g of CP/kg$^{0.67}$/day to present a stable LM. Considering phenylalanine + tyrosine intake the equation was: LM variation (kg) = 0.7357 * Phenylalanine + tyrosine intake (g/kg$^{0.67}$/day) – 0.3624; R^2 = 0.42; P<0•01; n=55). To a stable LM, in the conditions of the current study, cats would need to eat 0,49 g of Phenylalanine + tyrosine/kg$^{0.67}$/day.

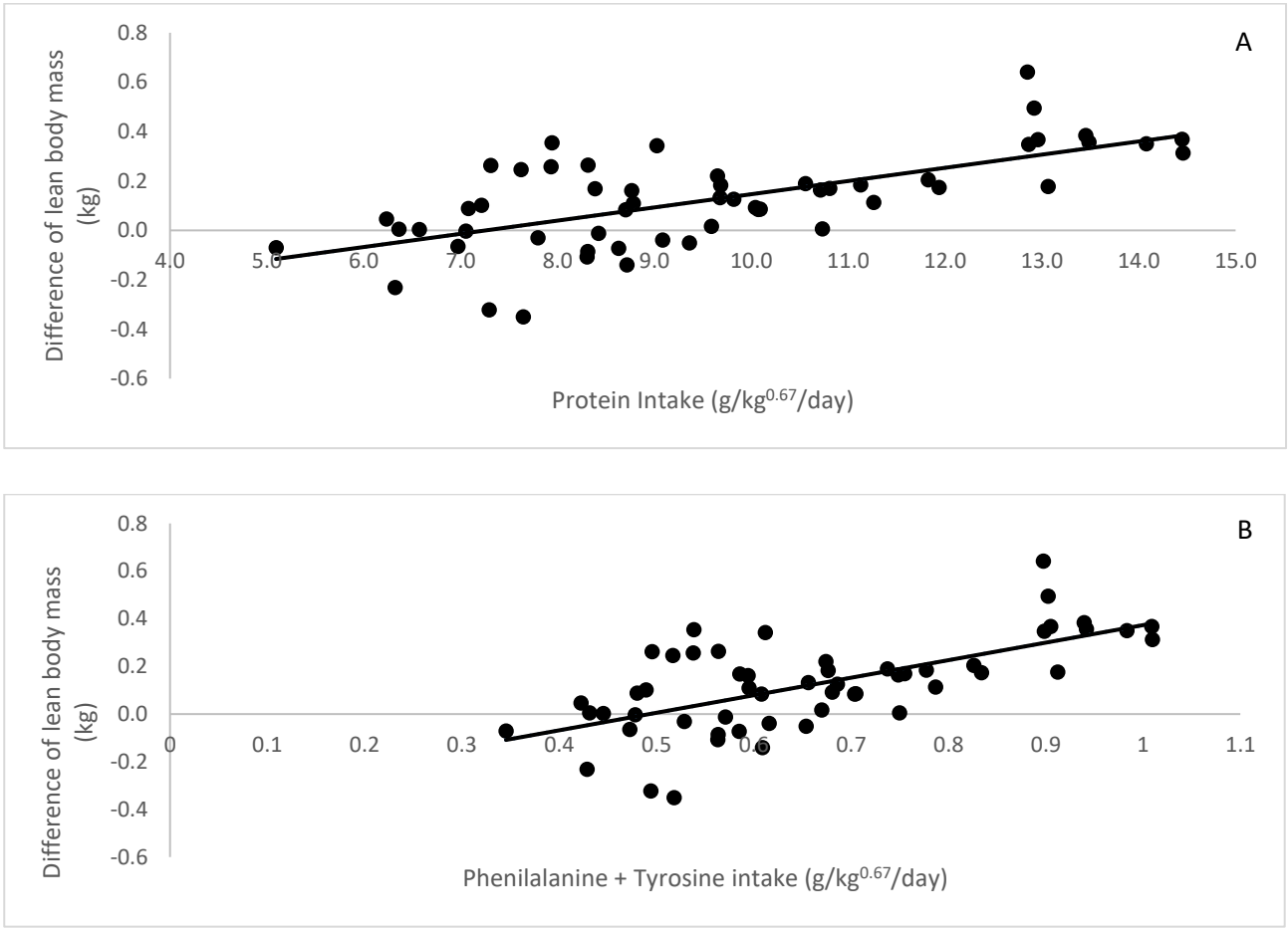

Figure 02. Pearson Correlation between: (A) Protein intake vs. Difference in lean body mass: y = 0.0534x – 0.388; R^2 = 0.42; P<0•01; n=55). (B) Phenylalanine + Tyrosine intake vs. Difference in lean body mass (y = 0.7357x – 0.3624; R^2 = 0.42; P<0•01; n=55).

No diet effect was observed for EE (P=0•62). The mean daily EE (kcal/kg$^{0.67}$/day), regardless of diet and body condition tended to be higher in male than female cats (M: 85.77 SE 1.85; F: 77.05

SE 1.97; P=0•06), as shown on Table 05. When computed in a LM basis, obese cats presented higher EE than non-obese animals (OB: 101.77 SE 2.51; NOB: 93.38 SE 1.85; P<0•01). Age was used as a covariate the statistical model due to its effect on cats EE. The Pearson correlation between EE and age was described as: EE (kcal/kg$^{0.67}$/day) = -4.2964*(Age in years) + 95.918; R^2=0.44; P<0•01; n=64; Figure 03. According to the model, every year of life would result in a reduction of 4.29 kcal/kg$^{0.67}$/day in the cats EE.

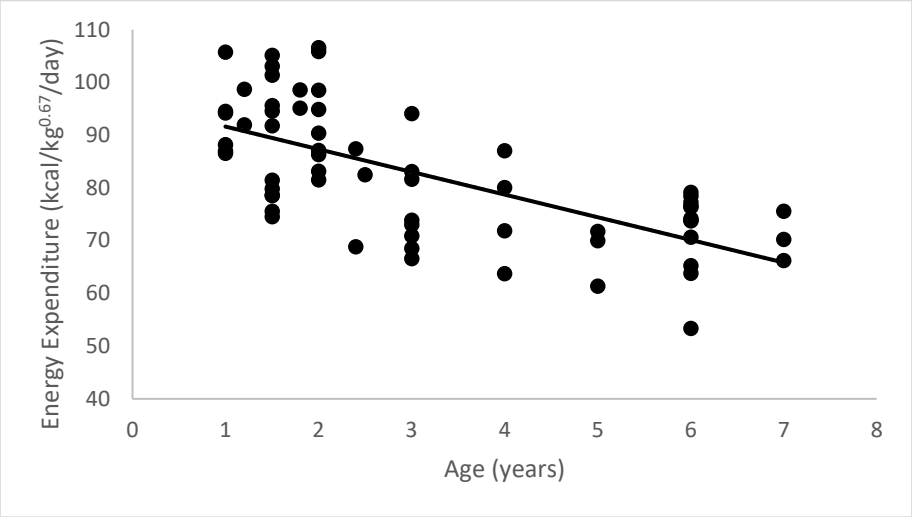

Figure 03. Pearson Correlation between Energy Expenditure vs Age of the cat: y = -4.2964x + 95.918; R^2 0.44; P<0•01; n=64.

Table 05. Energy expenditure and water turnover of client-owned neutered cats fed ad libitum for four months diets with different starch:protein ratios.

Item	Diets		Mean	* SEM	† P value		
	High Starch	High Protein			Diet	‡ BC	Sex
Energy Expenditure (kcal/body weight kg$^{0.67}$/day)							
Female Cats			79.75	1.85			
Non-obese	82.76	78.06	80.41	4.67			
Obese	77.64	80.54	79.09	5.92			
Mean	80.20	79.30		3.77			
Male Cats			84.48	1.97			
Non-obese	83.91	86.37	85.14	4.64			
Obese	81.66	85.97	83.82	6.44			
Mean	82.79	86.17		3.95			
Mean non-obese	83.34	82.21	82.77	2.64			
Mean obese	79.65	83.25	81.45	3.82			
General mean	81.49	82.73		2.33	0•62	0•62	0•06
Energy Expenditure (kcal/lean body mass kg$^{0.67}$/day)							
Female Cats			95.65	2.27			
Non-obese	92.71	89.12	90.92	2.92			

Obese	98.60	102.17	100.39	3.49			
Mean	95.66	95.65		4.63			
Male Cats			99.51	2.47			
Non-obese	94.09	97.61	95.85	2.87			
Obese	101.44	104.88	103.16	4.03			
Mean	97.77	101.25		4.95			
Mean non-obese	93.40	93.37	93.38	1.85			
Mean obese	100.02	103.53	101.77	2.51			
General mean	96.71	98.45		3.14	0•57	0•01	0•21
[§] **Water Turnover (mL/body weight kg$^{0.67}$/day)**							
Female Cats			48.78	1.38			
Non-obese	50.07^{ab}	55.04^{a}	52.56^{AB}	1.83			
Obese	40.14^{b}	49.85^{ab}	44.99^{BC}	2.06			
Mean	45.11	52.45		3.67			
Male Cats			49.87	1.61			
Non-obese	52.95^{ab}	64.07^{a}	58.51^{A}	1.90			
Obese	41.87^{b}	40.59^{b}	41.23^{C}	2.61			
Mean	47.41	52.33		3.90			
Mean non-obese	51.51^{II}	59.56^{I}	55.54	1.44			
Mean obese	41.00^{III}	45.22$^{II, III}$	43.11	1.76			
General mean	46.26	52.39		2.33	<0•01	<0•01	0•62

[*] SEM: standard error mean.
[†] P value: For EE interaction not found for diet * body composition (P>0•05).
[‡] BC: Body condition.
[§] For water turnover: diet * body condition * sex interaction was verified (P=0•03), $^{a, b, c}$ – means inside each sex without a common lowercase letter differs; body condition * sex interaction was verified (P=0•05), $^{A, B, C}$ – means in the column without a common uppercase letter differs; body condition * diet (P=0•05), $^{I, II, III}$ – general means for non-obese or obese without a common Roman number differs.

The WT was higher in cats fed with the HP (52.39 SE 2.33 mL/kg$^{0.67}$/day) than HS diet (46.26 SE 2.33 mL/kg$^{0.67}$/day; P<0•01) and higher for non-obese (55.54 SE 2.33 mL/kg$^{0.67}$/day) than obese cats (43.11 SE 2.33 mL/kg$^{0.67}$/day; P<0•01). A triple interaction diet * body condition * sex (P=0•03) was observed, and regardless of diet, WT was higher in non-obese males and females, intermediary in F-NO, and lower in M-OB animals. Inside each sex, WT was higher for non-obese cats fed the HP diet and lower for obese cats fed the HS food (P<0•05). The WT presented a positive correlation with EE: Water turnover (mL/kg$^{0.67}$/day) = 0.8668*Energy expenditure (kcal/kg$^{0.67}$/day) – 20.539; R^2 0.65; P<0•01; y =n=49 (Figure 04). According to this equation, the WT corresponded to 0.61 SE 0.01 mL to each kcal of EE per day.

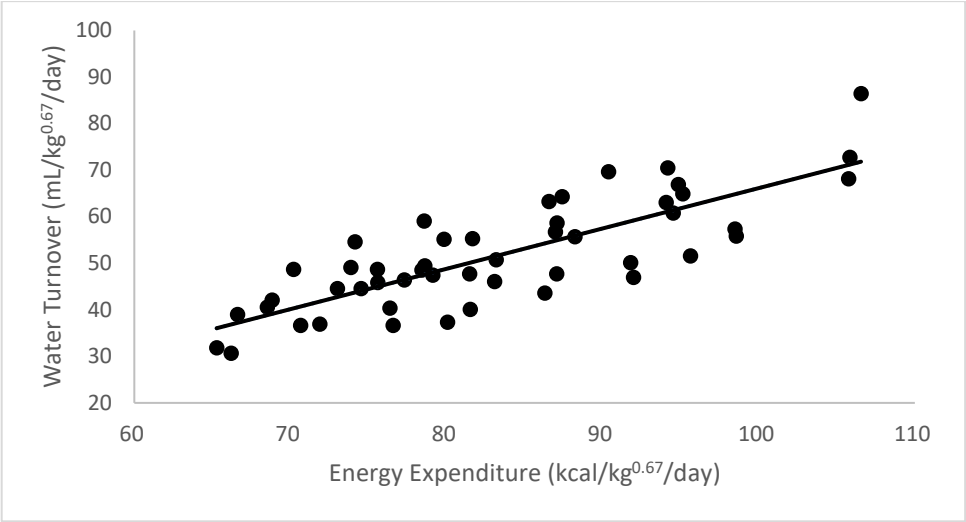

Figure 04. Pearson Correlation between energy expenditure vs. water turnover: y = 0.8668x – 20.539; R^2 0.65; P<0•01; n=49.

The physical activity parameters were not affected by body condition or sex (P>0•05; Table 06). Cats fed the HP diet, however, presented higher ODBA total peak area than cats fed the HS food (P<0•05). A positive correlation among total ODBA area (G/day) and EE was observed: Energy Expenditure (kcal/$kg^{0.67}$/day) = 34.361*(total ODBA area, G) + 2156.2; R^2 0.58; P<0•01; n=44; Figure 05. According to the equation, as the cat's movement detected by the accelerometers increase, higher de EE of the animal.

Table 06. Physical activity parameters of client-owned neutered cats fed ad libitum for four months diets with different starch:protein ratios.

Item	Diets		Mean	* SEM	† P value		
	High Starch	High Protein			Diet	‡ BC	Sex
§ **ODBA total area (G)**							
Female Cats			4885.74	102.09			
Non-obese	4728.59	5100.65	4914.62	126.83			
Obese	4757.84	4955.86	4856.85	160.12			
Mean	4743.22	5028.25		210.16			
Male Cats			4939.86	89.53			
Non-obese	4894.51	5135.82	5015.16	109.45			
Obese	4837.40	4891.72	4864.56	141.65			
Mean	4865.96	5013.77		178.59			
Mean non-obese	4811.55	5118.23	4964.89	49.93			
Mean obese	4797.62	4923.79	4860.71	75.30			

General mean	4804.59	5021.01		92.72	0•11	0•44	0•69
[§] **ODBA total area of peaks (G)**							
Female Cats			2121.84	145.39			
Non-obese	1920.96	2485.24	2203.10	191.83			
Obese	1790.97	2290.18	2040.58	218.51			
Mean	1855.97	2387.71		303.31			
Male Cats			2231.84	131.11			
Non-obese	2108.68	2483.33	2296.01	158.54			
Obese	2149.79	2185.58	2167.68	208.76			
Mean	2129.23	2334.45		261.97			
Mean non-obese	2014.82	2484.28	2249.55	103.83			
Mean obese	1970.38	2237.88	2104.13	129.69			
General mean	1992.60	2361.08		171.68	0•05	0•44	0•56

[*] SEM: standard error mean.
[†] P value: interactions diet, BC or sex not found (P>0•05).
[‡] BC: Body condition.
[§] ODBA: overall dynamic body acceleration.

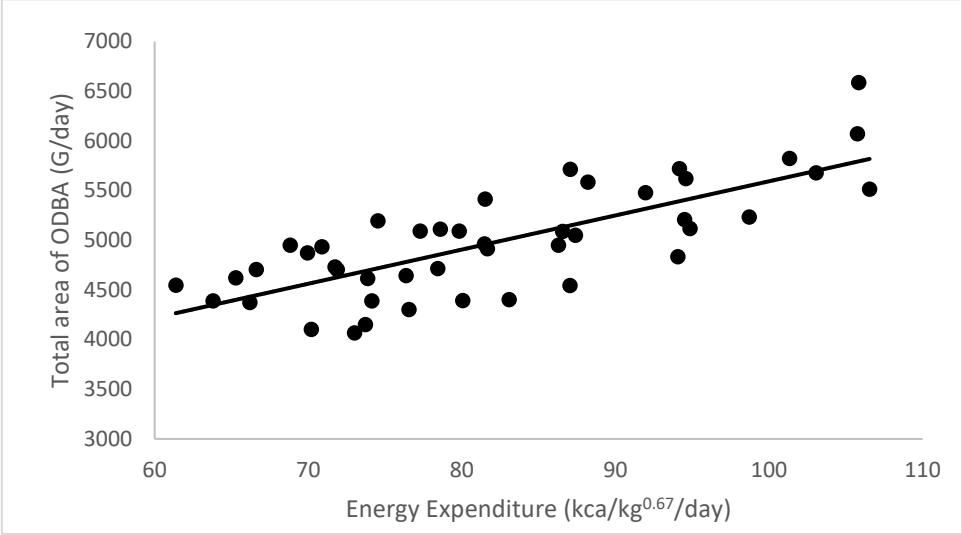

Figure 05. Pearson Correlation between Energy Expenditure vs. Total area of ODBA area: y = 34.361x.3x + 2156.2; R^2 0.58; P<0•01; n=44.

3.5 Discussion

The study is the first one, at moment, that compared different diet starch: protein ration on different physiologic conditions on cats living homes. To owners, the study was not difficult to participate, but the complication of the COVID-19 pandemic situation, which the experimental trial

had be done in the biggest part, was important and delicate moment that followed the correct health recommendations advocated by the World Health Organization (WHO). The contact by the authors and owners were done frequently in these 8 months of experimental trial. In one situation the sample collections of two cats had to be delayed per 20 days because the owner was infected by SARS-CoV-2.

According to results presented in the study, obese cats were heavier with higher fat mass in kg and percentual than non-obese cats, as expected ([17]HOENIG et al., 2007; [3]VASCONCELLOS et al., 2009). The higher body weight promoted higher lean mass in kg which had influenced in the EED in kcal/kg of $LM^{0.67}$/day. In the statistical analysis, age was considered as covariate because groups were different and an accurate comparation to diet and sex were necessary. However, include age in the model to compare obese and non-obese doesn't reflect (on numerically values) the life reality, because it is known that obese cats tend to be aging than non-obese cats ([51]LUND, 2005; [28]WALL et al., 2019; [52]LAFLAMME, 2020). When age was removed as covariate to compare EED of obese and non-obese cats, non-obese presented higher EE (85 SE 2 kcal/$kg^{0.67}$/day) than obese (77 SE 3 kcal/$kg^{0.67}$/day; P<0•01). According to increase of the age the energy expenditure daily became lower for the population used in the study (Figure 03) and the same was seen by [19]KIENZLE et al., 2006. The age of animals was a limitation of the study but shows the standard distribution of obesity in the middle-aged cats ([27]GERMAN et al., 2016).

Male cats had higher lean mass than female cats, but they had same fat body mass in this study. [24]Vasconcellos et al., (2019) shows different results to obese cats, with higher fat mass in females than male. When female was spayed an increase of gain weight and fat mass was seen compared to male after 3 months ([21]FETTMAN et al., 1997). Regardless obesity, in the maintenance phase, male and female didn't have difference to percentual of fat mass, and values were similar with found by the author of the present study.

The high protein diet increased the body weight and the lean body mass in both sex and non-obese and obese cats fed ad libitum. The same was seen by [11]Coradini et al., (2014) and just the increased of lean mass by [6]Nguyen et al. (2004), on free feeding in 2 and 6 months, respectively. The higher lean mass in these cases can be associated with the increase of protein oxidation improved by high protein content in food, consequently, higher intake of catabolic amino acids ([5]RUSSEL et al., 2002). The preservation of lean mass is based on properly amino acids intake and adequate muscular activity ([53]ZORAN & BUFFINGTON, 2011; [52]LAFLAMME, 2020), the increase of muscle mass is associated with type of amino acid can play a role in protein turnover as the branched-chain amino acids, especially leucine ([54]RONDANELLI et al., 2016; [52]LAFLAMME, 2020). The leucine is

capable to reduces proteolysis ([55]BAND et al., 2018) and enhances protein synthesis by triggers insulin release ([56]CURRY et al., 1982; [8]WEI et al., 2010), which promotes protein synthesis ([55]BAND et a., 2018). In this study, cats fed 48 SD 20% higher protein intake in the high protein diet and 45 SD 20% higher of leucine than when they fed high starch diet. The cats fed high protein diet had higher physical activity what can be improve more lean mass. However, the question is: They became more active because of lean body mass increased fed higher protein intake, or the higher protein intake improved lean body mass and the cats became more active?

The body weight of cats fed the high starch diet no changed as found by the study of [11]Coradini et al., 2014, that evaluated the ad libitum food intake per 8 weeks with similar diets of this study, however when they fed the high protein diet their body weight increased and all the animals increased their lean body mass too. The female cats increased their fat body mass fed the high protein diet showing that the high starch diet was great to maintaining their fat body mass. The energy expenditure was non different between diets, but the intake of high starch diet was lower in female obese cats with lower consumption of gram of protein compared to other groups and, consequently the intake of the amino acids phenylalanine + tyrosine was limited. To animals that have the caloric intake lower than 65 kcal/$kg^{0.67}$ is important that the nutrients, especially amino acids, are more concentration in this diet to do not cause nutrient deficiencies. The high starch diet shows be interesting to non-obese male cats, because no changed the body weight and even so transformed the fat body mass to lean body mass in this group of animals, however the high protein diet was interesting too because of increased the lean body mass.

Some important limitations of the study must be considering. The animals had different routine living in their houses and was influenced by different owners. The number of animals in the houses were different and the samples collections, that was realized in the owned house was done in different periods of time. Nevertheless, Brazil is a tropical country and temperature during the experimental trial didn't suffer great changes (24 SD 1.7 ºC) not compromising great changes on the energy metabolism.

3.6 Conclusion

In *ad libitum* feeding system, the intake of a kibble diet with 38% CP and 40% starch favored a better control of the cat's body weight that remained constant, meanwhile cats fed a diet with 55% CP and 20% starch gained body weight. Differences regarding body condition was observed, as the non-obese cats (males and females) reduced body fat mass (%) and increased lean mass (kg), but not

obese animals fed the high starch food. The *ad libitum* feed of the high protein diet, besides to increase body weight, increased lean mass (kg), physical activity, and water turnover in all groups, and the fat mass (kg) in females, but not increased energy expenditure or the % of lean body mass in any group. Daily energy expenditure presented a tendency to be higher for males, that also presented higher lean body mass. It was observed a reduction on energy expenditure as the cats age increased. Energy expenditure correlated positively with water turnover and cats' physical activity. Caution is necessary to balance protein content on diets of obese females with more than 4 years old, as this cat group may have low energy expenditure, limiting food and amino acid intake resulting in lean mass loss.

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CAPÍTULO 4 – Artigo 3

The effect of starch to protein ratio in energy expenditure, body composition, and physical activity are different in neutered and intact, non-obese male cats

Camila Goloni[1], Letícia G. Pacheco[1], Letícia W. Luís, Stephanie S. Theodoro[1], Lucas B. Scarpim[1], Celina Torres[2], Gener T. Pereira[1], Aulus C. Carciofi[1*]

[1] Universidade Estadual Paulista (Unesp), Faculdade de Ciências Agrárias e Veterinárias, Jaboticabal, SP, 14884-900, Brazil.

[2] Affinity Petcare, Barcelona, Spain.

[*] Correspondência do autor: aulus.carciofi@gmail.com. Número de telefone + 55 16 3209-2626; fax número +55 16 3203-1226

Título breve: Energy metabolism in neutered and intact cats fed different diets

4.1 Abstract

Neutering is a risk factor for obesity, altering energy metabolism and body composition of male cats. The intake of diets with different starch to protein ratios was compared in non-obese neutered (NM) and intact (IM) male cats living in homes and fed ad libitum. A kibble diet high in starch (HS: Starch 40%, crude protein [CP] 38%) or high in CP (HP: Starch 20%, 55% CP) was fed for 4 months in a cross-over design. The physical activity, body composition, energy expenditure (EE) and water turnover (WT) were evaluated. Results were compared in a 2 (diets) x 2 (reproductive conditions) arrangement (P≤0•05). The IM presented higher EE (115.58 SE 6.96 kcal/kg$^{0.67}$/d) than NM (91.46 SE 6.72 P<0•01), and presented higher lean mass (%), physical activity and water turnover compared to NM (P<0•05). When NM were fed the HS diet they did not change body weight (BW), but their fatty mass reduced (P=0•04) and lean mass increased (P<0•01). When fed the HP diet, the BW and LM increased in NM (P<0•05). No diet effect was observed for BW or composition in IM. The intake of the HP diet, regardless of neutering, increased EE (P<0•05) and tended to increase the physical activity of the cats (P=0•09). In NM the intake of the HS diet helped to maintain BW and improved body composition.

Key-words: accelerometer; deuterium; lean mass; reproductive condition

4.2 Introduction

Gonadectomy is a common procedure to help control the population of cats (KUSTRIZ, 2007; LARSEN, 2017). Besides societal benefits, gonadectomy improve cats behavior and avoid medical situations (HART & COOPER, 1984; KUSTRIZ, 2014). Alongside these obvious benefits, neutering is an important risk factor for obesity development, especially in male cats (LUND, 2005; ZORAN 2010; GERMAN, 2016; LARSEN, 2017; WALL et al., 2019). Studies in obesity distribution indicate that between 43.6 % of neutered males are obese, but only from 4 % of intact male cats are obese (APOP, 2019).

It was shown that cats after neutering increases food intake on *ad libitum* feeding systems, resulting in the expansion of the body fat mass (FETTMAN et al., 1997; KANCHUK et al., 2003). Other than an altered food intake control several papers documented a decreased in energy requirement after neutering, as cats needed less food and energy to keep a constant body weight (MARTIN ET AL., 2001; HOENIG & BUFFINGTON, 2002), or the measured energy expenditure was lower after gonadectomy (FETTMAN et al., 1997; KANCHUK et al., 2003; CAVE et a., 2007). Gonadectomized cats present lower circulating levels of sexual hormones (BACKUS, 2011), including reductions in estradiol, that interfere in the regulation of fat metabolism and satiety (COOKE & NAAZ, 2004; CAVE et al., 2007; ZORAN, 2010), and testosterone, that is associated with muscle mass development and size (GRIGGS et al., 1989; MORGANTALER & TRAISH, 2018). All this changes in hormone regulation may explain the lower energy requirement and the altered body composition, with proportionally higher fat mass and lower lean mass in neutered compared to intact male cats (NRC, 2006; WICHERT et al., 2007; BERMINGHAM et al., 2010; FEDIAF, 2021). Other aspects in which neutering might interfere are voluntary physical activity (OHLUND ET AL., 2018) and water turnover, that may also influence energy expenditure (EE) or body composition deserving more studies. The authors were unable to find studies on the effect of neutering and water turnover.

Considering that obesity in cat's is a problem on different perspectives, as it predisposes to diabetes mellitus, osteoarthritis and result in higher morbidity and mortality rates (BROOKS et al., 2014; CLINE et al., 2021), nutrition strategies that help avoid body weight gain and obesity development after neutering are of importance to mitigate this detrimental condition occurrence (SPOFFORD et al., 2014).

It is believed that dietary macronutrients may influence satiety, energy metabolism, physical activity and ultimately the body composition of cats (VILAVERDE & FASCETTI, 2014; CLARK & LAFLAMME, 2016). High fat diets may increase energy intake and body fat mass deposition, and due this fat should not be the primary source of energy for neutered cats (NGUYEN et al., 2004a; BACKUS, 2007). Controversy exists, however, about the metabolic implications of starch or protein consumption and its effects on the body composition, energy intake (HOENIG & FERGUSON DC, 2002; VASCONCELLOS et al., 2009) and water turnover of cats (GARCIA et al., 2020).

Cats have high protein requirement to supply gluconeogenic amino acids for energy metabolism (RUSSEL et al, 2002) and prefer protein over starch, selecting protein and fat as energy substrates (HEWSON-HUGHES et al., 2011). May be due this, although some studies suggested that high protein diets may increase the lean body mass (NGUYEN et al., 2004; WEI et al., 2010) and induce higher heat increment increasing EE in comparison to a high starch food (GREEN et al., 2008), studies had shown that cats fed high protein diets may also increase their body mass and may become obese (HEWSON-HUGHES et al., 2011; CORADINI et al., 2014), does not supporting the concept that high protein diets may promotes better satiety and energy intake control (DU et al., 2017).

Starch in extruded diets properly processed present elevated digestibility for cats (De-OLIVEIRA, et al., 2008; ALVARENGA & ALDRICH, 2020). Starch intake also does not induce evident alterations on glucose and insulin post-prandial responses, especially in non-obese cats (De-OLIVEIRA, et al., 2008; HOENIG, et al., 2007; HOENIG et al., 2011). As cats prefer to eat protein over starch, it has also been suggested that an elevated starch content, in well balanced formulations with adequate protein would limit voluntary food intake, a concept called “carbohydrate ceiling” by HEWSON-HUGHES et al. (2011). This allows to hypothesize that in ad libitum feeding systems cats would limit starch intake and consequently the amount of food consumed, may helping to control the amount of energy eaten, helping the maintenance of a stable body weight. All this responses to starch and protein intake, however, might be different in intact or neutered cats (VESTER et al., 2009), justifying the comparison of the diet effect inside each reproductive condition.

Considering the above, in the present study it was hypothesized that neutered and intact male cats living in homes and fed ad libitum will differ in response to diets with different starch to protein ratios. A high protein diet intake will induce increases on energy expenditure, lean mass content and water turnover, and a high starch intake will favor satiety and better control of body weight, but these effects will be more important for neutered than intact males. Thus, the objective of the present study was to compare the body weight and composition, energy expenditure, water balance, and voluntary physical activity of non-obese neutered or intact male cats living in homes, fed unrestricted amounts of two extruded diets, one high in protein and another high in starch.

4.3 Material and Methods

Animals and experimental design

The experiment was conducted with client-owned cats at their homes, and in the Clinical Nutrition Service of Cats and Dogs of UNESP, campus of Jaboticabal, São Paulo, Brazil. All procedures with animals followed the ethical principles adopted by the Brazilian College of Animal Experimentation and were previously approved by the Ethics Committee on the Use of Animals of São Paulo State University (protocol no. 9536/18). Before engagement on study, owners were informed about all the procedures, and all signed a free consent of participation.

Client-owned cats were recruited between April/2019 until September/2020. The criteria to participate on the study included: cats must be indoor, with access to backyard allowed but not to streets; age between 1 to 4 years old; non-obese, with a body condition score (BCS; LAFLAMME, 1997) of 5 or 6; confirmed healthy along all the study; commercial dry kibble food comprises >90% of daily energy intake; be fed *ad libitum* by an owner decision; the owner commit to fed only the experimental diet along the study, not giving any other food or snack. After the first contact with the owners to present the study and get the signed authorization of participation, the cats were accessed by a veterinarian that evaluated their health by physical examination, complete blood count, serum biochemistry (alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, albumin, urea and creatinine) and urine analysis. The selected cats were divided in 2 groups according to reproductive condition: intact male cats (IM) and neutered male cats (NM). The neutered male cats must be neutered at least 6 months before the study.

The treatments were organized in a factorial arrangement, with 2 diets x 2 reproductive conditions, totalizing 4 experimental treatments. The study followed a cross-over design, and all cats were fed the two diets, in a randomized order. Each period of the cross-over lasted 4 months, and the study lasted 8 months. In each cross-over period, on the first day the cats body weight was recorded (INMETRO: certified weights from 1 to 10 kg OIML E1/2004) and body condition score were documented. Animals body composition in the beginning of the study was evaluated by the deuterium oxide method. Cats, then, received only their designed experimental food and water for 4 mounts. The experimental food was introduced gradually over a period of 7 days. Only when the experimental food was the sole source of nutrients the experimental period counting was started. The food management, including the offered amount and feeding times was not altered and the owner continued doing their usual routine with the cats: *ad libitum* feeding, with leftovers present daily in the bowls. When the house had more than one cat, all of them was fed the experimental diet.

When each cat completed four months of experimental food intake the animal was weighted, classified according to BCS, had the physical activity evaluated with a 3-axial accelerometer for 3 consecutive days, and the body composition, energy expenditure and water turnover accessed by the doubly labelled water method (DLW). To complete all these evaluations a period of 15 to 20 days was necessary. Finalized the first period of the cross-over, the cats started the second period, with the other diet sorted.

Experimental diets

Two extruded diets were used, balanced cats for cat maintenance (FEDIAF, 2018): High Starch (HS) with 38% crude protein and 40% of starch; High Protein (HP) with 55% of crude protein and 20% of starch, on as dry matter basis. The increase on protein content was obtained through a reduction of the starch content, thus altering the protein to starch ratio of the diets. The fiber and fat contents were remained similar. Diets were produced in two different moments, assuring fresh food along the experiment, and manufactured at the Extrusion Laboratory of the Faculdade de Ciências Agrárias e Veterinárias da UNESP, Jaboticabal, Brazil. The calculated energy contribution, in % of the estimated food metabolizable energy content was of 33.3% from protein, 35.4% from starch and 31.3 % from fat for the HS diet, and 49% from protein, 18.6% from starch and 32.4% from fat for the HP diet. The analysed chemical composition of the diets are presented on Table 01.

Table 01. Analyzed chemical composition of the experimental diets. Values on dry matter basis.

Item	Diets			
	* High Starch		† High Protein	
	Mean	‡ SD	Mean	‡ SD
Moisture (%)	5.62	0.44	6.20	0.27
Crude protein (%)	37.91	0.45	55.87	0.56
Total aminoacids	34.49	0.29	51.28	0.60
Essential aminoacids				
Arginine	2.48	0.04	3.91	0.05
Histidine	0.81	0.00	1.20	0.02
Isoleucine	1.36	0.05	2.12	0.00
Leucine	2.64	0.03	3.81	0.01
Lysine	2.12	0.22	3.68	0.02
Methionine	0.72	0.03	0.85	0.01
Methionine+cystine	1.13	0.02	1.49	0.01
Phenilalanine	1.44	0.07	2.24	0.01

Phenilalanie+Tyrosine	2.57	0.15	3.90	0.02
Threonine	1.44	0.07	1.97	0.02
Tryptophan	0.32	0.05	0.50	0.01
Valine	1.68	0.05	2.48	0.02
Taurine	0.57	0.05	0.54	0.01
Non-essential aminoacids				
Glycine	3.61	0.10	4.46	0.26
Alanine	2.37	0.09	3.16	0.09
Proline	2.64	0.19	3.22	0.11
Tyrosine	1.13	0.08	1.66	0.01
Cystine	0.41	0.02	0.63	0.02
Aspartic acid	2.62	0.48	4.93	0.03
Glutamic acid	4.49	0.31	7.39	0.01
Serine	1.65	0.01	2.53	0.02
Acid-hydrolysed fat (%)	14.69	0.78	15.66	0.11
Starch (%)	40.28	0.48	20.56	1.30
[§] Starch gelatinazation degree (%)	95.38	1.15	96.91	0.63
Crude fibre (%)	1.81	0.19	1.31	0.03
Ash (%)	6.29	0.53	7.25	0.31
[‖] Metabolizable energy (kcal/g)	3.97	0.00	4.09	0.00

[*]Ingredient composition of the high starch food: poultry by-product meal 35 %; corn grain 26.7 % (high starch diet; HS), broken rice 20 %; isolated protein pean 21.07 % (high protein diet; HP), isolated swine protein 7 % (HS) and 10 % (HP), poultry fat 6.7 %; beet pulp 2.5%, palatant enhancer 1.5 % (SPF Brasil); potassium chlorate 0.65%, common salt 0.6 %; choline chloride 0.45 %; vitamin–mineral premix 0.7 % (Rovimix, DSM Produtos Nutricionais Brasil S.A.); urine acidifier 0.45 % (Diana Pet food, Descalvado, Brazil), taurine 0.20%; calcium proprionate 0.04 %; potassium sorbate 0.02 %; antioxidant 0.07% (Alltech do Brazil Agroindustrial Ltda).

[†] Ingredient composition of the high protein food: poultry by-product meal 35 %; corn grain 26.7 % (high starch diet; HS), broken rice 20 %; isolated protein pean 21.07 % (high protein diet; HP), isolated swine protein 7 % (HS) and 10 % (HP), poultry fat 6.7 %; beet pulp 2.5%, palatant enhancer 1.5 % (SPF Brasil); potassium chlorate 0.65%, common salt 0.6 %; choline chloride 0.45 %; vitamin–mineral premix 0.7 % (Rovimix, DSM Produtos Nutricionais Brasil S.A.); urine acidifier 0.45 % (Diana Pet food, Descalvado, Brazil), taurine 0.20%; calcium proprionate 0.04 %; potassium sorbate 0.02 %; antioxidant 0.07% (Alltech do Brazil Agroindustrial Ltda).

[‡] SD standard deviation of the two lots of production.

[§] Used to access the quality of the extrusion processing ([36]SA et al., 2013).

[‖] Evaluated by total collection of faeces and urine of the the diets on first production moment.

Voluntary physical activity with 3-axyal accelerometer

Voluntary physical activity was evaluated using a 3-axyal accelerometer device (AC; Axy-4, TechnoSmart, Italy) attached to a chest collar placed on the cats. The cats were adapted to use chest collar for 3 to 7 days, and then the AC was fixed in the region of the neck, closer to scapulas, for 3 consecutive days. The position of the AC in the chest collar followed the instructions of the manufactures (Figure 1), close in the centre of mass of the animals (HALSEY et al., 2011). The use of devices to estimate physical activity in cats with the chest collars have been validated by Lascelles et al. (2008) allowed to objectively measure the physical activity without human interference.

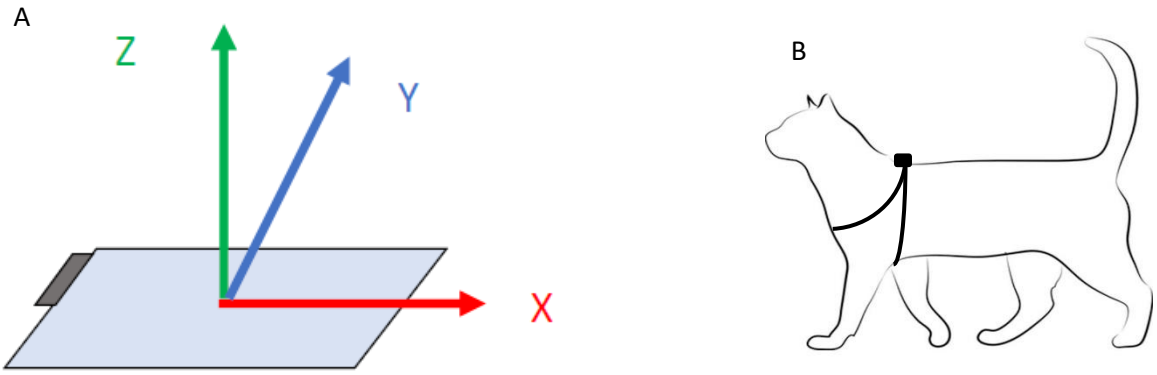

Figure 1. The position of the 3-axyal accelerometer. (A), X = Surge (walking front and back); Z = heave (movement to up and down); Y = sway (movement to right and left). (B) Illustration of accelerometer positioning in the cat.

The movement registration with the AC was performed for at least 3 consecutive days for each cat. Equipment was configured at a frequency of 10 Hz and 16 G gravitational and 10bit resolution. The X, Y and Z axis data were smoothed for each second and subtracted from the corresponding unsmoothed data to generate dynamic acceleration. The smoothed values on the three axes were converted into absolute values and added together to generate an overall dynamic body acceleration (ODBA) (WILSON et al, 2006). Additional recommendation to cat owners for this period were: they must not hold the cat and maintain their usual routine. The available space of the cat's was variable according to each home, including animals housed in apartments and houses with backyard, with or without environment enrichment. All then, however, were restricted to the houses without access to streets. As all cats ate the two foods, the environmental effect was diluted by the cross-over design.

Isotopes Evaluation

The initial body composition was evaluated with deuterium oxide (D_2O). A 7% (70:930; v/v) solution of ^{2}H at 99.9 atm% (Sercon Limited, United Kingdom) and NaCl 0,9% was prepared and 1 mL per kg of body weight injected in each cat (FERRIOLLI et al., 2008). The solution was subcutaneously applied between the scapula after 12 h of fasting and 2 h without water. A sample of saliva were collected before solution injection and after 2 hours to access isotope enrichment in body water. To increase the accuracy of the injection, the syringe was weighed empty, with the isotope solution and again after solution injection (FERRIOLLI et al., 2008). The saliva samples were collected according to Goloni et al., (2020), with hydrophilic cotton externally and adjacent to the mouth, next to the animal's lips. To enable this, salivation was stimulated by dropping one drop of sodium dipyrone (500 mg/ml, EMS, São Bernardo do Campo, Brazil) on the cat's mouth, equivalent to 25 mg of dipyrone per cat (all animals were adapted previously for the procedure). After dipyrone administration, a first piece of cotton was used and discarded, and a second piece of cotton was used for a maximum of 5 min to collect the saliva sample. The cotton was then placed in a 20 ml syringe, squeezed, and a minimum of 1.5 ml of saliva per cat was stored in a cryotube with a screw cap sealed with paraffin at −20°C until analysis. Caution was made to cotton not absorb moisture from the air before use, keeping it in sealed bags.

The body composition, energy expenditure and water turnover were determined by the DLW method in all cats at the end of the four months period of experimental food intake. The isotopes (Sercon Limited, United Kingdom) were administrated in the approximate doses of 0.12 g of ^{2}H at 99.9 atm% and 2 g of ^{18}O at 10 atm% per kg of body water (FERRIOLLI et al., 2008). A 6:100 (v/v) solution of ^{2}H at 99.9 atm% and ^{18}O at 10 atm%, respectively, was prepared and injected in each cat (^{40}FERRIOLLI et al., 2008). The solution was subcutaneously applied between the scapula after 12 h of fasting and 2 h without water. An amount of 0.3 ml of a 20 % NaCl was mixed and infused together to increase the isotope solution osmolality close to the interstice, avoiding discomfort to the cats (GOLONI et al., 2020). To increase the accuracy of the injection, the syringe was weighed empty, with the 20 % NaCl solution, with the isotope solution and again after injection (FERRIOLLI et al., 2008). Saliva samples were used to evaluate body water enrichment, and collected before solution injection, after 2 hours to access isotopes enrichment, and three times from days 4 to 12 to evaluate isotope elimination rate from body water. Saliva samples were collected and stored as described above.

The isotope concentration in saliva was evaluated at the Mass Spectrometry Laboratory of the Ribeirão Preto Medical School, São Paulo, Brazil. The isotopes were analyzed by isotope ratio mass spectrometry (ANCA 20-20; Europe Scientific, United Kingdom). Samples for ^{2}H were processed in

duplicate (200 µl per replicate) with platinum in vacutainers, after 6 h of resting. Samples for ^{18}O were processed in duplicate (200 µl per replicate) by filling the tubes with CO_2 after 24 h of resting (FERRIOLLI et al., 2008).

The pool size for ^{2}H or ^{18}O for the total of body water was calculated as follows (considering a linear elimination response) (SCHOELLER, 1996):

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

where N is the pool size of body water; W is the amount of water used to dilute the labelled water dose; A is the weight of labelled water administration (g); a is the diluted dose for analysis; δ is the enrichment of dose (a), dilution water (t), post-dose sample (s) and pre-dose baseline (p) samples. For ^{18}O, considering the non-aqueous exchange routes are small no correction was made. To calculate total body water with ^{2}H, a correction factor was used due to the isotope incorporation in non-aqueous organic molecules during biosynthesis (RACETTE et al., 1994) using the following formula (ELLIS & WONG, 1998):

$$\text{TBW} = \left(\frac{Nd}{f}\right)$$

where TBW is the total body water; Nd is the pool size of body water with ^{2}H; f is the correction factor according to Nd:No (No = pool size of body water estimated with ^{18}O) ratio in each evaluated body fluid.

The lean body mass (LM) of the cats was calculated considering the hydration constant of 73·2 % for mammals by the following equation: lean body mass (kg) = body water (kg)/0·732. The fatty body mass (kg) was estimated as follows: total body mass (kg) – LM (kg) of the animal (PACE & RATHBUM, 1945).

The two-point formula was used to calculate ^{2}H and ^{18}O elimination (^{45}SCHOELLER, 1986):

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

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

The amount of CO_2 produced was established using the following formula (LIFSON & MCCLINTOCK, 1966):

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

where rCO_2 is the CO_2 production; N is the dilution space for ^{18}O; Ko is the rate constant of ^{18}O in body water and Kd is the rate constant of ^{2}H in body water.

Lastly, the energy expenditure (EE) of the cats was calculated as follows (ELIA & LIVESEY, 1992):

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

where EE is the energy expenditure; rCO_2 is the CO_2 production and FQ is the food quotient. The food coefficient was calculated considering the digestible nutrient content of the food, determined *in vivo* with seven cats using the total collection of faeces method (FEDIAF, 2018), as previously shown. The following formula was used (BLACK et al., 1986):

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

where FQ is the food quotient; P is the digestible protein; G is the digestible fat and A is the digestible starch.

The body WT was calculated as (HENDRIKS et al, 1999), assuming little to no water is lost via evaporative routes that are subject to isotope fractionation:

$$\mathrm{WT\ (mL/day)} = \mathrm{Nd} * \mathrm{Kd} * 18.02$$

where WT is the water turnover rate; Nd is the ^{2}H body water and Kd is the ^{2}H rate constant. The EE of the cats was also computed by the food intake method. For this purpose, data on food intake to constant body weight were multiplied by the metabolizable energy content of the diet, as determined in vivo with cats using the total collection of faeces and urine method.

Statistical analysis

The experimental unit was considered one cat. The test power was set at 0·8 and the $\alpha = 0{\cdot}05$. The information of the variance between treatments (mean square) and within treatments (mean square of residue) were used following the ANOVA procedure (power.ANOVA.test of the R software) to obtain the minimum sample size for EE, resulting in six cats per treatment, which assured adequate comparison of the main outcomes. The study followed a cross-over design and organized in a factorial arrangement 2 diets x 2 reproductive condition, totalizing 4 experimental treatments. The main effects were diet and reproductive condition and the interaction diet * reproductive condition. When differences were verified on F test, means was compared by the Tukey test for the variables body composition, energy expenditure, water turnover and physical activity. For the results of physical activity, the area under the curve was calculated by the integration trapezoidal method (Prism GraphPad, 2005) and ODBA total area was used to compare treatments. Additionally, total area of peaks of ODBA was calculated using data above 1 G gravitational to compare treatments. Inside each food group, the body weight, fat body mass and lean body mass of cats at the beginning and after the period of food intake was compared by the paired Student t-test. To compare these variables between diets at the beginning and final periods, the Student t-test was used. To variable body condition score the non-parametric Wilcoxon test was used. The variable body condition score was evaluated with the non-parametric Wilcoxon test. When of interest, the relationship among the body composition variables, EE, WT, and movement parameters were accessed by a Person Correlation analysis after adjusting the factorial model on the residuals of the variables. Values of $P<0{\bullet}05$ were considered significant. Values of $P\leq0{\bullet}05$ were considered significant and $P\leq0{\bullet}10$ as a tendency. All data complied with the presuppositions of the variance analysis. The analyzes were performed using the procedure MIXED of the SAS software (SAS Institute, Cary, NC, EUA, 2003) and R software version 3.6.3.

4.4 Results

The experiment started in November 2019 and finished in March 2021. A total of 54 client-owned cats were recruited. From this, 45 animals were submitted to physical examination, blood work and was interviewed about their possibility to completely follow the study requirements. From this amount 22 animals started the project, but 3 animals were removed because they have access to street. A total of 19 cats, belonged to 11 owners finished the project. The cats which complete the study were divided in the groups: IM, with 1.6 SD 0.8 years, and a BCS 5.0 SD 0.0 (n=10); NM, with 2.2 SD 1.2 years and a BCS 5.1 SD 0.1 (n=9). The age of the cats (P=0.54) and their BCS (P=0.98) were similar between groups. All cats accepted adequately the two experimental diets, and according to

their owners' faeces and behavior was normal. No health issues were recorded during the study. The analyzed amino acid composition of the two diets showed values adequate and higher than the recommend to cat maintenance by FEDIAF (2021). The protein and amino acids intake of the cats was calculated considering the EE estimated by the DLW method, and the protein, amino acids and metabolizable energy content (determined in vivo) of each diet. The cats fed with the HS diet had a mean intake of 9.28 SE 1.39 g of CP/kg$^{0.67}$/day, and cats fed the HP food an intake of 14.90 SE 2.85 g of CP/kg$^{0.67}$/day, values higher than the minimum requirement suggested by adults (FEDIAF, 2021).

Results of body weight and body composition independent of the experimental diet and period showed lower body weight (BW: 3.9 SE 0.1 kg; P<0•01), fat mass (FM; 0.4 SE 0.1 kg; P<0•01) and lean mass (LM; 3.5 SE 0.1 kg; P<0•01) to IM compared to NM cats (BW: 4.5 SE 0.1 kg; FM 0.7 SE 0.1 kg; 3.8 SE 0.1 kg), however the percentage of LM was higher for intact than neutered cats (IM: 90 %; NM: 84 %; P<0•01). The intake of the HS or HP diets didn't change the body weight and body composition of IM cats (P>0•05), that remained similar between the start and end of the experimental periods (Table 02 and Table 3). When NM cats were fed the HS diet, they maintained the body weight, but presented a reduction in FM, both in kg and % (P=0•04), with a tendency to increase LM in kg and an increase of the LM in % (P=0•04). In the period receiving the HP formulation, the NM cats gained body weight and LM in kg (P<0•01), but did not alter FM and body composition in %.

Table 02. Initial and final body weight and body condition score of client-owned intact or neutered male cats fed ad libitum for four months diets with different starch to protein ratios.

Groups	Body Weight (kg)				Body Condition Score			
	High Starch	High Protein	*SEM	*P value*	High Starch	High Protein	*SEM	*P value*
Neutered Male (n=9)								
Initial	4.40	4.47	0.15	*0•83*	5.33	5.00	0.08	*0•14*
Final	4.35	4.70	0.15	*0•27*	5.00	5.11	0.05	*0•99*
P value	*0•43*	*0•02*			*0•14*	*0•99*		
Intact Male (n=5)								
Initial	3.84	3.91	0.15	*0•73*	5.00	5.00	0.00	*1•00*
Final	3.85	4.03	0.13	*0•42*	5.00	5.00	0.00	*1•00*
P value	*0•92*	*0•33*			*1•00*	*1•00*		

* SEM: standard error mean.

Table 03. Initial and final body fatty body mass and lean body mass of client-owned intact or neutered male cats fed ad libitum for four months diets with different starch to protein ratios.

Groups	Fat Body Mass (kg)				Fat Body Mass (%)			
	High Starch	High Protein	*SEM	*P value*	High Starch	High Protein	*SEM	*P value*
Neutered Male (n=9)								
Initial	0.85	0.76	0.05	*0•31*	19.03	16.65	0.83	*0•19*
Final	0.67	0.71	0.06	*0•91*	15.35	14.74	1.13	*0•81*
P value	*0•04*	*0•34*			*0•04*	*0•24*		
Intact Male (n=10)								
Initial	0.45	0.46	0.04	*0•49*	11.73	11.23	0.65	*0•61*
Final	0.40	0.38	0.04	*0•98*	10.27	9.33	0.81	*0•58*
P value	*0•21*	*0•38*			*0•16*	*0•34*		
Groups	Lean Body Mass (kg)				Lean Body Mass (%)			
	High Starch	High Protein	*SEM	*P value*	High Starch	High Protein	*SEM	*P value*
Neutered Male (n=9)								
Initial	3.55	3.71	0.12	*0•55*	80.97	83.35	0.83	*0•19*
Final	3.68	3.98	0.12	*0•24*	84.65	85.26	1.13	*0•81*
P value	*0•08*	*<0•01*			*0•04*	*0•24*		
Intact Male (n=10)								
Initial	*3.39*	*3.50*	*0.14*	*0•65*	88.27	88.77	0.65	*0•61*
Final	*3.45*	*3.65*	*0.13*	*0•41*	89.73	90.37	0.81	*0•58*
P value	*0•18*	*0•14*			*0•16*	*0•35*		

* SEM: standard error mean.

The IM cats, regardless of diet presented higher EE per unit of body weight and unit of LM (115.58 SE 6.96 kcal/kg$^{0.67}$/d and 123.56 SE 7.39 kcal/kg of LM$^{0.67}$/d, respectively; P<0•01) than the NM cats (91.46 SE 6.95 kcal/kg$^{0.67}$/d and 102.46 SE 6.93 kcal/kg of LM$^{0.67}$/d, respectively; Table 04). The same was observed for WT, which was higher in IM cats (89.69 SE 6.93 mL/kg$^{0.67}$/d; P<0•01) than NM cats (65.94 SE 6.93 mL/kg$^{0.67}$/d), and for physical activity (P<0•01). No diet*sexual condition interaction was observed for any studied parameter. The intake of the HP diet increased the EE, both per unit of body weight and unit of LM (P<0•05), tended to increase the ODBA total area of peaks (P=0•09), but didn't change the WT of the cats.

Table 04. Energy expenditure, water turnover and physical activity parameters of client-owned intact or neutered male cats fed ad libitum for four months diets with different starch to protein ratios.

Item	Diets		Mean	* SEM	† P value	
	High Starch	High Protein			Diet	‡ RC
Energy Expenditure (kcal/body weight kg$^{0.67}$/day)						
Neutered	89.57	93.36	91.46	6.72		
Intact	107.59	123.56	115.58	6.96		

Mean	98.58	108.46		4.84	0•05	<0•01
Energy Expenditure (kcal/lean body mass kg$^{0.67}$/day)						
Neutered	100.47	104.45	102.46	7.39		
Intact	114.95	132.74	123.85	7.66		
Mean	107.71	118.60		5.32	0•05	<0•01
Water Turnover (mL/body weight kg$^{0.67}$/day)						
Neutered	56.68	63.19	65.94	6.39		
Intact	89.70	89.67	89.69	6.39		
Mean	73.19	76.43		4.52	0•48	<0•01
§ **ODBA total area (G)**						
Neutered	5314.50	5535.19	5424.84	200.60		
Intact	5773.79	6014.91	5894.35	192.45		
Mean	5544.14	5775.05		139.38	0•11	<0•01
§ **ODBA total area of peaks (G)**						
Neutered	2616.35	2954.60	2785.48	293.30		
Intact	3278.35	3654.86	3466.61	281.39		
Mean	2947.35	3304.73		203.79	0•09	<0•01

* SEM: standard error mean.
† P value: Interaction not found for diet * sexual condition (P>0•05).
‡ RC: reproductive condition.
§ ODBA: overall dynamic body acceleration.

Positive Pearson Correlations were observed between EE and WT (R^2 = 0.62; P<0•01; n=41), EE and ODBA total peak area (R^2 = 0.42; P<0•01; n=37), and WT and ODBA total peak area (R^2 = 0.46; P<0•01; n=37), as illustrated on Figure 02. According to the equation, the WT corresponded to 0.63 SE 0.01 mL to each kcal of EE per day.

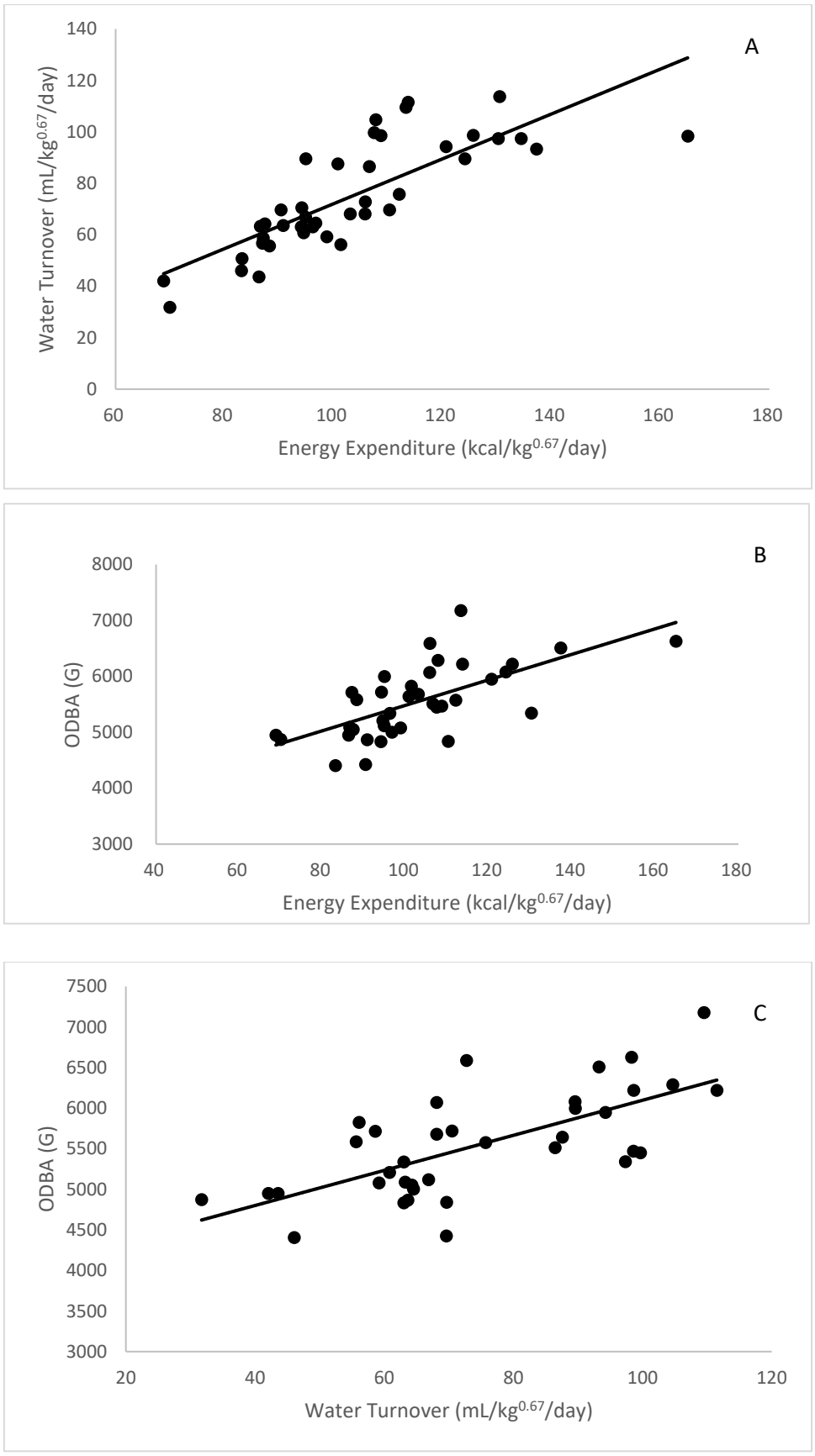

Figure 02. Pearson Correlation between: A - Energy Expenditure vs. Water Turnover (y = 0.87x – 15.17; R^2 0.62; P<0•01; n=41). B - Energy Expenditure vs. Total ODBA (y = 22.779x + 3205; R^2

0.42; P<0•01; n=37). C - Water Turnover vs. Total ODBA (y = 21.61x + 3935.80; R^2 0.46; P<0•01; n=37).

4.5 Discussion

Male intact cats had higher percent of lean body mass and lower fat mass compared with male neutered cats in the study. The same was verified by Martin et al., (2001) comparing male and female cats before and after 6 months of gonadectomy on ad libitum dry food intake, as other authors found higher fat mass after gonadectomy (FETTMAN et al., 1997; HOENIG et al., 2002; KANCHUK et al., 2003; WEI et al., 2014) been 25 to 30% of gain weight post neutering in domestic cats (BACKUS, 2011). Cline et al., (2018), found the same difference compared cats indoor and outdoor, which this last group had more voluntary physical activity. In the study, intact cats had higher voluntary physical activity than neutered male cats, as the energy expenditure in kcal/ $kg^{0.67}$/day and kcal/ kg of lean body $mass^{0.67}$/day, which was 26% and 20% percent higher, respectively. Other authors found 10 a 14% higher energy expenditure in kcal/$kg^{0.67}$/day on intact cats than gonadectomized cats (MARTIN et al., 2001; BERMINGHAM et al., 2010). The value of energy expenditure on intact male cats was 15% higher than indicated by FEDIAF (2021) to intact and active cats, probably because the sex effect is present in this mean value by FEDIAF. The energy expenditure and voluntary physical activity had good correlation and many studies have been discussing the influence of activity improve energy expenditure (NGUYEN et al., 2004; WICHERT et a., 2007; BERMINGHAM et al., 2010; BERMINGHAM et al., 2013).

The water turnover was higher in intact male cats, which probably was accompanied by the higher lean body mass and, the energy expenditure and voluntary movement as showed by Pearson correlation. The lean body mass is metabolic active tissue with higher water content, 70%, than fat mass, 10% (Elia, 1992; SERRA-PRAT et al., 2019). The testosterone and estradiol have important functions on body composition control, energy metabolism and satiety signals to food intake (NUNEZ et al., 1980; COOKE & NAAZ, 2004; CAVE et al., 2007; ZORAN, 2010). The concentration of estradiol (BACKUS, 2011) to regulating lipogenesis and satiety signaling (COOKE & NAAZ, 2004), as testosterone to modulate the muscle mass and protein turnover (GRIGGS et al., 1989; MORGANTALER & TRAISH, 2018) on male cats are lower post gonadectomy, even as higher serum concentration of leptin (FETTMAN et al., 1997; HOENIG et al., 2002; VASCONCELLOS et al., 2009) and non-esterified fatty acids, suggesting decreased insulin sensitivity factor (HOENIG et al., 2002), becomes neutering a risk factor to obesity on cats (BACKUS & WARA, 2016; LARSEN,

2017; WALL et al., 2019). The gonadectomy is a necessary procedure on clinical routine to control birth rate (KUSTRIZ, 2007) and food management as the profile of macronutrients in the diets are great strategy to body weight control.

The neutered male cats suffered the influence of diets in their body composition, except intact male cats in the study. The higher protein content diet increased their lean body mass, as the same results found by Nguyen et al. (2004), and the high starch content diet didn't change their body weight, according to other studies (NGUYEN et al., 2004; BACKUS et al., 2007; CORADINI et al., 2014). However, the high starch diet converted fat mass on lean body mass in this population of cats, besides limited the food intake as seen by Hewson-Hughes et al., (2011). Studies that evaluate the influence of high starch content in the diet of cats, on hormone levels of satiation and satiety will be clarifying these results.

The voluntary movement was trend to be higher to cats fed high protein diet, which has been associated with higher lean body mass in neutering cats as seen by Cline et al., 2018. Intact male cats fed high protein diet increased their energy expenditure using the protein content as source of energy (RUSSELL et al., 2002) and become more actives. To understand the physiologic aspect on intact cats fed high protein diet, future studies with protein oxidation will be interesting.

The limitations of the study were the difference in the routine on differences houses, number of animals in the houses and the samples collections realized in the owned house on different periods of time, because cats started the experimental trial in different times according with process of the animal's selections. Nevertheless, Brazil is a tropical country and temperature during the experimental trial didn't suffer great changes (24 SD 1.7 ºC).

4.6 Conclusion

Intact and neutered non-obese male cats living in homes responded differently to the ad libitum intake of diets with different starch to protein ratios. Although no diet effect was observed for intact males, the neutered males fed the high starch diet maintained a constant body weight, with a decrease in fat mass and an increase in lean body mass, but when fed the high protein diet presented an increase in body weight and lean mass, not altering body composition in percentage. For intact and neutered males, the high protein diet intake increased energy expenditure and physical activity.

Intact male cats had lower fat mass and higher lean body mass in percentage, and higher energy expenditure, water turnover and physical activity than neutered male non-obese cats.

4.7 References

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CAPÍTULO 5 - Considerações finais

A necessidade energética de gatos domésticos é ainda insuficientemente compreendida. Estes apresentam diferenças no metabolismo energético de acordo com as diferentes situações fisiológicas, ambiente em que vivem, nível de atividade física e em função da relação dos macronutrientes proteína, amido e gordura da dieta. Estes parâmetros influenciam não somente as necessidades energéticas, mas também se refletem na composição corporal destes animais. No Brasil, e mesmo no mundo, a castração é fator bastante presente no manejo de gatos domiciliados e estes representam a grande maioria dos animais. Esta, no entanto, se reflete negativamente na composição corporal e reduz a necessidade energética e ingestão de alimentos. Ainda, o manejo alimentar mais tradicionalmente adotado por proprietários de felinos é o *ad libitum*, quando não há controle da oferta de energia, o que favorece o desenvolvimento da obesidade, especialmente em vigência da combinação de falta de exercício fisco (animais restritos às casas) e castração.

Este cenário motivou a proposta da presente pesquisa. Tendo por objetivo a produção de dados práticos e relevantes, duas relações amido:proteína foram avaliadas em gatos castrados domiciliados alimentados *ad libitum* por seus proprietários. Assim, as implicações fisiológicas destas formulações puderam ser estudas em situação relevante e real, contribuindo para a compreensão do fenômeno na população de interesse direto.

O primeiro experimento avaliou o consumo das dietas em gatos de laboratório, alimentados com quantidade controlada para peso constante. O objetivo aqui foi se obter dados em situação controlada para melhor fundamentação da interpretação dos resultados na população domiciliada. Resultado bastante interessante foi o aumento do consumo alimentar voluntário promovido pela ração com alta proteína ao teste de saciedade, e mesmo o maior consumo inicial desta ração na cinética de ingestão. A ração com alto amido, por outro lado, limitou o consumo tardio de alimento e com isto a ingestão energética voluntária total. Neste estudo não houve efeito da composição de macronutrientes no gasto energético e atividade física, mas alta proteína elevou o turnover hídrico dos gatos.

Nos experimentos dois e três estas mesmas rações foram fornecidas em sistema *ad libitum* para gatos domiciliados. No segundo experimento a população de estudo incluiu apenas gatos castrados, organizados de acordo com o sexo (macho ou fêmeas) e condição corporal (obesos ou não obesos). Foi verificado que a dieta alto amido, em sistema *ad*

libitum favoreceu melhor controle do peso corporal pois nenhum grupo de gatos ganhou peso. Ainda, na dieta alto amido gatos não obesos, tanto machos como fêmeas apresentaram melhora da composição corporal com redução da massa gorda e aumento da massa magra, o que pode vir a ter reflexos importantes na saúde à longo prazo que merecem mais estudos. Confirmando o verificado na situação controlada de laboratório, quando alimentos *ad libitum* com a ração alta proteína todos os grupos de gatos ganharam peso corporal. Apesar deste ganho ter sido acompanhado de aumento da massa magra, massa gorda também amentou nas fêmeas (obesas e não obesas) indicando possível diferença entre sexos que precisaria ser melhor estudada. Este efeito de sexo pode ser tanto relativo a diferenças na necessidade de energia e consequente ingestão de nutrientes, ou mesmo decorrente de possível diferença na necessidade de proteína ligada ao sexo dos gatos.

Em gatos machos domiciliados, o efeito da castração foi investigado no terceiro experimento. Relevante efeito de castração foi observado em quase todos os parâmetros observados, ressaltando a importante implicação desta condição no manejo alimentar de gatos. Assim, gatos inteiros mantiveram peso e composição corporal constantes, independentemente da dieta. Já nos machos castrados, enquanto a dieta com elevado amido, à semelhança do estudo dois propiciou manutenção do peso corporal, inclusive com melhora da composição corporal (aumento de massa magra e redução de massa gorda), o consumo da ração com elevada proteína induziu ganho de peso. Numa avaliação independente de dieta, foi confirmado nos gatos castrados menor gasto energético, atividade física e turnover de água do que nos gatos inteiros. A redução de gasto energético após a castração é bem explorada na literatura científica, mas as implicações da redução do turnover de água, que foi 49% menor nos castrados ainda necessita ser estuda quanto a suas possíveis implicações à saúde do trato urinário e urolitíases.

Assim, pôde-se verificar que no sistema *ad libitum* gatos domiciliados castrados têm melhor chance de manterem condição corporal saudável em dieta corretamente balanceada e com quantidade adequada de proteína, mas com 40% de amido. Estudos são necessários para se compreender como esta ingestão de amido afeta o controle endócrino, saciedade e consumo voluntário de alimentos, e mesmo a deposição de massa magra e tecido adiposo nos gatos. Por fim, se entender as implicações a longo prazo de tudo isto, bem como seus reflexos à saúde, longevidade, homeostasia renal e urolitíases é importante.

Outros achados relevantes, transversais aos três estudos foram a redução do gasto energético com o aumento da idade, o menor gasto energético, turnover hídrico e movimento nos animais obesos e a correlação positiva verificada entre gasto enérgico e atividade física, e entre gasto energético e turnover hídrico.