UNIVERSIDADE ESTADUAL PAULISTA - UNESP
CÂMPUS DE JABOTICABAL

GRANULOMETRIA DA MATÉRIA PRIMA E CONFIGURAÇÃO DA EXTRUSORA SOBRE OS PARÂMETROS DE PROCESSO, CARACTERÍSTICAS DO EXTRUSADO E DIGESTIBILIDADE DE RAÇÕES PARA GATOS

Karina Nogueira Venturelli Gonçalves
Médica Veterinária

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

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TÍTULO DA TESE: GLANULOMETRIA DA MATÉRIA PRIMA E CONFIGURAÇÃO DA EXTRUSORA SOBRE OS PARÂMETROS DE PROCESSO, CARACTERÍSTICAS DO EXTRUSADO E DIGESTIBILIDADE DE RAÇÕES PARA GATOS

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Jaboticabal, 03 de agosto de 2016

DADOS CURRICULARES DO AUTOR

KARINA NOGUEIRA VENTURELLI GONÇALVES, nascida em 04 de julho de 1979, em Santos, estado de São Paulo, filha de Ricardo Venturelli Gonçalves e Maria da Conceição Nogueira Venturelli Gonçalves, graduada em Medicina Veterinária pela Faculdade de Ciências Agrárias e Veterinárias da Universidade Estadual Paulista “Júlio de Mesquita Filho” em dezembro de 2003. Durante o curso de graduação, fez iniciação científica e ao final do curso optou em realizar um trabalho de graduação na área de Nutrição e Nutrição Clínica sob a orientação do Prof. Dr. Aulus Cavalieri Carciofi. Obteve o título de mestre em Medicina Veterinária - Clínica Médica com ênfase em Nutrição de Cães e Gatos na Universidade Estadual Paulista “Júlio de Mesquita Filho” em 2006 sob a orientação do Prof. Dr. Flávio Prada e prof. Dr. Aulus Cavalieri Carciofi. Iniciou o curso de Doutorado em Medicina Veterinária - Clínica Médica na mesma instituição em março de 2012, onde atuou na área de Nutrição de Cães e Gatos, sob a orientação do Prof. Dr. Aulus Cavalieri Carciofi. Em novembro de 2005 começou a atuar no mercado profissional de alimentos para cães e gatos na empresa Guabi, no departamento técnico. Desde abril de 2013 é gerente técnica na Farmina Pet Foods Brasil e responsável pelas áreas de Pesquisa e Desenvolvimento, Garantia de Qualidade, Serviço de Atendimento ao Consumidor e Assuntos Regulatórios.

Dedico

Aos meus amados e queridos pais, Ricardo e Conceição e irmãos Terezinha e Ricardo, por serem meu porto seguro e por quem tenho um imenso orgulho.

Ofereço

À Deus, pelo dom da vida, por tudo que meu deu e por ser o Senhor da minha vida!

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Agradeço em primeiro lugar à Deus por tudo o que já fez, faz e fará em minha vida. Em especial por diversas situações que ocorreram na minha vida pessoal e profissional ao longo do curso desse Doutorado. De início não pude compreender e aceitar, mas ao longo do tempo amadureci, cresci e me tornei mais forte. Tenho a certeza plena que foi o melhor que poderia ter acontecido, tudo foi conduzido de forma perfeita e com um propósito. Te louvo e dou graças meu Pai.

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Aos meus amados irmãos, Terezinha e Ricardo, pelo amor, alegria, companheirismo e paciência. Ter irmãos é um presente divino.

À toda minha família, avós, tios, tias, primos e primas e cunhado que mesmo distante fisicamente, estão dentro do meu coração e sempre presentes em minha memória e orações.

Às minhas queridas amigas e companheiras de Campinas, Maria Paula Leis e Bruna Fernandes. Pelo companheirismo, motivação, alegrias e gargalhadas e claro algumas vezes choro.

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À Farmina Pet Foods Brasil, em especial a figura do meu gestor, Sr. Marcelo Basso Garcia, por ter me aceitado na condição de aluna de doutorado, ter acreditado no meu desempenho profissional e pela doação de ingredientes. À toda equipe técnica da Farmina pelo apoio e companheirismo. E ao meu amigo Vilmar Alberti que possui um conhecimento fantástico sobre extrusão.

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À Universidade Estadual Paulista, Campus de Jaboticabal e ao Programa de Pós Graduação, berço de toda minha formação acadêmica. Tenho um orgulho imenso em ter estudado em uma instituição pública, séria e que incentiva e promove pesquisas científicas relevantes.

A todos os funcionários do laboratório de nutrição e da FCAV UNESP Jaboticabal, em especial a Cláudia Nogueira.

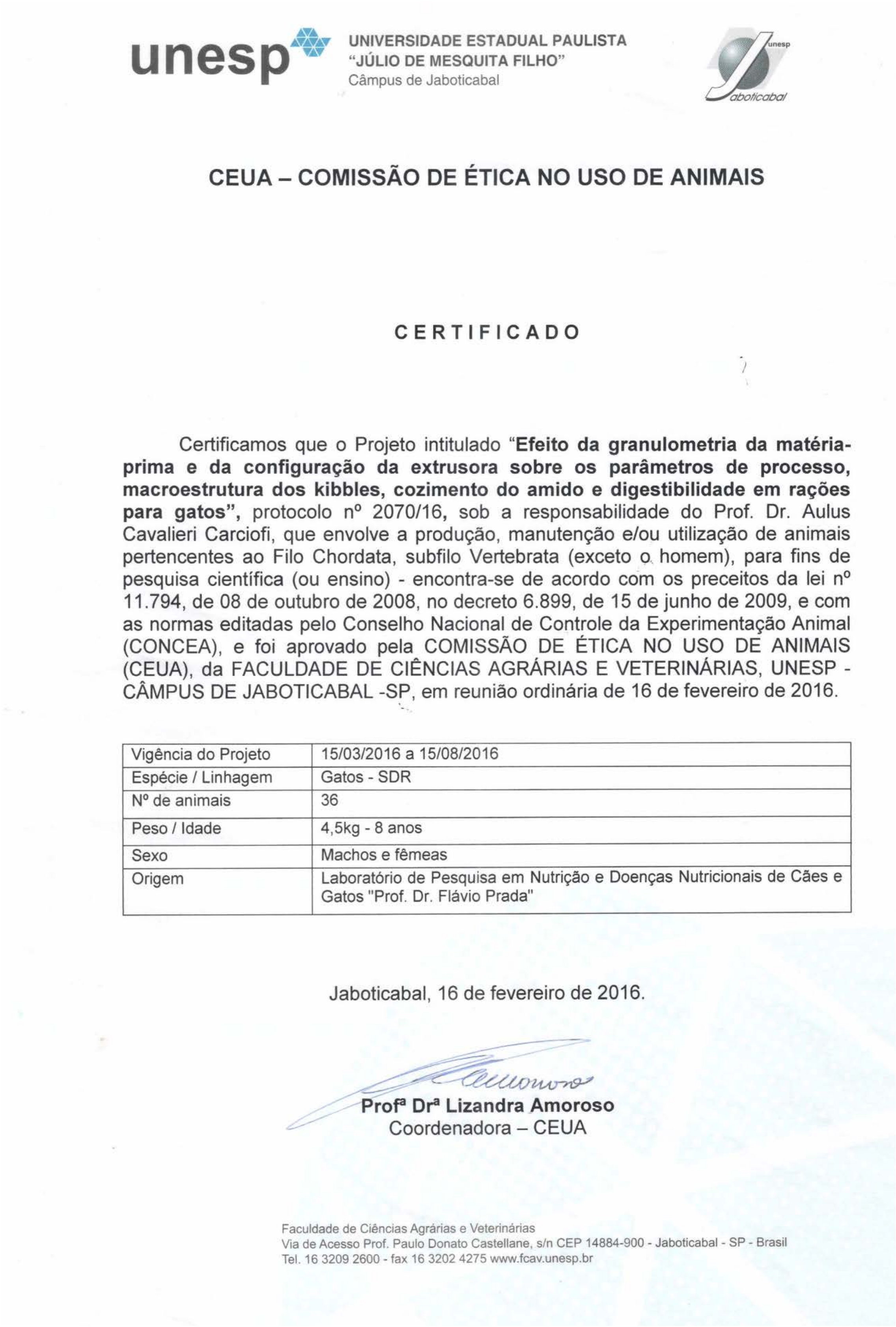
À Guabi Pet Care pelo apoio ao Laboratório de Pesquisa em Nutrição e Doenças Nutricionais de Cães e Gatos da FCAV/UNESP. E a figura do Sr. Sávio Ambrozini, meu gestor por muitos anos, que favoreceu meu ingresso no Doutorado e a condução do mesmo durante o meu primeiro ano de curso.

À Folem, na figura de Lendro e Edegar; à Tectron, por intermédio de Dimas e à Marcelo Beraldo da AFB Brasil pela doação de alguns ingredientes utilizados na formulação das dietas.

À Manzoni Industrial pela doação da extrusora à FCAV UNESP Jaboticabal, equipamento utilizado para a produção das dietas e objeto de estudo da presente tese.

À SPF Brasil e ao Panellis, pela condução dos testes de palatabilidade do presente estudo.

SUMÁRIO



UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"
Câmpus de Jaboticabal

CEUA – COMISSÃO DE ÉTICA NO USO DE ANIMAIS

CERTIFICADO

Certificamos que o Projeto intitulado "**Efeito da granulometria da matéria-prima e da configuração da extrusora sobre os parâmetros de processo, macroestrutura dos kibbles, cozimento do amido e digestibilidade em rações para gatos",** protocolo nº 2070/16**,** 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 junho de 2009, e com as normas editadas pelo Conselho Nacional de Controle da 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 fevereiro de 2016.

Vigência do Projeto	15/03/2016 a 15/08/2016
Espécie / Linhagem	Gatos - SDR
Nº de animais	36
Peso / Idade	4,5kg - 8 anos
Sexo	Machos e fêmeas
Origem	Laboratório de Pesquisa em Nutrição e Doenças Nutricionais de Cães e Gatos "Prof. Dr. Flávio Prada"

Jaboticabal, 16 de fevereiro de 2016.

Profª Drª Lizandra Amoroso
Coordenadora – CEUA

Granulometria da matéria prima e configuração da extrusora sobre os parâmetros de processo, características do extrusado e digestibilidade de rações para gatos

RESUMO - A maior parte dos alimentos industrializados destinados à gatos são secos e extrusados. A aplicação de energia termomecânica durante a extrusão promove mudanças físico-químicas e nutricionais nos ingredientes com eficiência e baixo custo relativo. Por outro lado, ela também pode promover efeitos indesejáveis, incluindo destruição de vitaminas, oxidação de lipídeos, destruição e redução na disponibilidade da proteína e aminoácidos, principalmente a lisina. Não foram encontradas na literatura as implicações do tamanho da partícula da matéria prima e o efeito de diferentes configurações de extrusora em alimentos para gatos sobre a formação dos “kibbles” e aspectos nutricionais. O presente estudo teve como objetivo estudar as implicações nutricionais, à macroestrutura de extrusados e aos parâmetros de processo de alimentos para gatos produzidos a partir de diferentes graus de moagem da matéria prima e configurações de extrusora. Para tanto foram conduzidos dois experimentos. O primeiro avaliou o efeito do tamanho da partícula da matéria-prima e área aberta da matriz da extrusora sobre os parâmetros de processamento, gelatinização do amido (GA), digestibilidade *in vitro* da matéria orgânica, macroestrutura dos “kibbles”, digestibilidade *in vivo* dos nutrientes (DIV) e teor de lisina. O experimento seguiu um arranjo fatorial 6 x 2 (6 áreas abertas e 2 tamanhos de partículas), totalizando 12 tratamentos. Uma dieta para gatos adultos foi formulada e moída em moinho de martelo em duas aberturas de peneira (0,5 e 1,2mm), as quais geraram diâmetro geométrico médio (DGM) de 195 e 254 μm respectivamente. O material foi extrusado a partir das seguintes áreas abertas (AA) alvo: 59, 118, 177, 236, 294, 353 mm2/ton/h. Todos os outros parâmetros de software e de hardware da extrusora não foram alterados. A DIV feita pelo método de coleta total de fezes, nas dietas com 195 μm e 59 mm^2/ton/ h, 195 μm e 353 mm^2/ton/h, 254 μm e 59 mm^2/ton/h, 254 μm e 353 mm^2/ton/h, usando 6 gatos por dieta. A lisina total, ligada e reativa foi analisada em 5 tratamentos: mistura dos ingredientes não extrusada e nos 4 tratamentos escolhidos para a DIV. Os dados foram submetidos à análise de variância considerando os efeitos de DGM e AA e suas interações e as médias foram comparadas por contrastes polinomiais ($P<0,05$). Para a DIV interação foi considerada significativa quando $P<0,15$. Os resultados de lisina foram comparados pelo teste de Dunnet ($P<0,05$), sendo a mistura não extrusada considerado como referência. Com relação aos parâmetros de processo interação entre DGM e AA foi observada na pressão e na temperatura da última camisa da extrusora, sendo que os menores valores foram observados no menor DGM. Os valores de flash off foram semelhantes entre ambos DGM e diminuíram linearmente com aumento da AA. A energia mecânica específica (EME) e a energia térmica específica (ETE) foram influenciadas pela interação entre DGM e AA. Em ambos DGM houve redução quadrático na EME e aumento ETE com o aumento da AA. Todos os parâmetros da macroestrutura dos “kibbles” foram influenciados pela interação. As dietas com maiores DGM apresentaram maior densidade aparente e específica, comprimento específico e menor expansão radial. A GA e digestibilidade *in vitro* da matéria orgânica não foram influenciados pela AA, apenas pelo DGM. Foi verificada uma interação ($P<0,15$) para a digestibilidade da proteína bruta e da matéria seca e orgânica. Os menores resultados foram vistos no menor DGM e área mais restrita. Houve aumento da lisina total e reativa em todas as dietas extrusadas, mas não na lisina ligada. O efeito conjunto do DGM e da AA influenciaram fortemente os parâmetros do processo, a transferência de EM e as características dos “kibbles”. O segundo estudo avaliou o efeito de diferentes configurações de extrusão sobre os parâmetros de processamento, GA, digestibilidade *in vitro* da matéria orgânica, macroestrutura dos “kibbles” e DIV. O experimento seguiu um arranjo fatorial 4 x 2 (4 áreas abertas e 2 restrições após rosca da extrusora), totalizando 8 tratamentos. A mesma dieta usada no primeiro experimento foi extrusada com uma combinação de 60 ou 90% de restrição após a rosca da extrusora (RAR) e quatro AA alvo: 59, 118, 236 e 353 mm^2/ton/h). Todos os outros parâmetros de software e de hardware da extrusora não foram alterados. A DIV foi feita pelo método de coleta total de fezes, nas dietas com 90% de restrição e 353 mm^2/ton/h e restrição de 60% e 59 mm^2/ton/h, usando 6 gatos por dieta. Os dados foram submetidos à análise de variância, considerando os fatores RAR e AA e suas interações. As médias foram comparadas por contrastes polinomiais ($P<0,05$). A amperagem do motor foi influenciada pela interação, sendo que as maiores médias foram observadas nas dietas com 90% de RAR e foi notado redução com aumento AA. A umidade no canhão da extrusora foi semelhante entre os tratamentos. A pressão da massa medida antes da matriz alterou somente com AA. Houve redução no flash off apenas nas dietas com 90% de RAR e diminuiu com aumento AA. Houve redução na temperatura média da quarta camisa com aumento da AA. A EME não sofreu influência da interação e foi maior para as dietas com 90% RAR. A ETE foi semelhante entre os tratamentos e a energia total aplicada foi semelhante para todas AA nas dietas com 60% de RAR, mas reduziu com o aumento da AA nas extrusadas com 90% de RAR. Foi observado efeito ($P<0,05$) de AA para todas as características dos “kibbles”. Houve interação para a densidade aparente e específica e aumento nas maiores AA. A expansão mais elevada foi verificada nas dietas com 90% RAR. Gelatinização do amido e digestibilidade *in vitro* não foram influenciadas pela interação. A digestibilidade *in vitro* foi alterada de acordo com AA, mas a DIV não mudou ($P>0,05$) entre os tratamentos. A RAR e AA exercem forte impacto sobre os parâmetros de processos, transferência de EME e formação dos “kibbles”. A DIV não foi influenciada pela expansão dos “kibbles”.

Palavras-chave: gato, extrusão, moagem, configuração da extrusão

Abreviaturas: AA: área aberta da extrusora, DGM: diâmetro geométrico médio, DIV: digestibilidade *in vivo* dos nutrientes, EME: energia mecânica específica, ETE: energia térmica específica, GA: gelatinização do amido; RAR: restrição após rosca da extrusora.

Raw material particle size and extruder configuration on processing parameters, kibble macrostructure, starch cooking and digestibility of cat diets

ABSTRACT – Most processed foods intended for cats are dried and extruded. Application of thermomechanical energy during extrusion promotes physical-chemical and nutritional changes in the ingredients in an efficient and relatively low cost. Moreover, it can also promote undesirable effects, including the destruction of vitamins, lipid oxidation, destruction and reduction in the availability of protein and amino acids, especially lysine. The author was not able to found scientific literature on the implication of the raw material particle size and extruder configuration on kibble formation and the nutritional aspects of extruded foods for cats. Taken these considerations, this study aimed to evaluate the implications of two raw particle size and changes in extruder configuration on the extrusion traits, kibbles, starch gelatinization (SG) degree and digestibility of cat food formulation. It was conducted two experiments. The first evaluated the effect of raw material particle size and extruder open area (Eoa) on the processing parameters, SG, *in vitro* digestibility of organic matter, kibble macrostructure, in vivo nutrient digestibility (IVND) and content of lysine. The experiment followed a factorial 6 x 2 (6 Eoa and 2 raw particle size), totaling 12 treatments. A diet was formulated for adult cats and ground in a hammer mill in two sieve openings (0.5 and 1.2) which generated mean geometric diameter of raw material (MGD) of 195 and 254 μm respectively. The material was extruded with these Eoa target: 59, 118, 177, 236, 294, 353 mm^2/ton/h. All other software parameters and extruder hardware have not changed. The IVND was conducted by the method of total collection of feces, in the diets of 195 μm and 59 mm^2/ton/h, 195 μm and 353 mm^2/ton/h, 254 μm and 59 mm^2/ton/h, 254 μm and 353 mm^2/ton/h, using 6 cats per diet. The results of total, linked and reactive lysine were measured at 5 treatments: raw material and four treatments chosen for IVND. Data were submitted to analysis of variance considering the MGD and Eoa effects and their interactions, and means were compared by polynomial contrasts ($P < 0.05$). The digestibility interaction was considered significant when $P < 0.15$. Lysine results were compared by the Dunnett test ($P < 0.05$) and raw material was considered as the reference. Regarding the process parameters interaction between MGD and Eoa were observed in die pressure and temperature of the last extruder jacket, and the lowest values were observed in the diets with 195 μm MGD. Flash off values were similar between MGD and decreases linearly with increasing Eoa. Specific mechanical energy (SME) and specific thermal energy (STE) were influenced by the interaction. In both MGD there was a reduction in quadratic SME, and STE increased with increasing Eoa. All macrostructure parameters of kibbles were influenced by interaction. Diets with larger MGD presented higher bulk and piece density, specific length and less radial expansion rate. The SG and *in vitro* digestibility of organic matter were not affected by Eoa, only by MGD. For digestibility a significant interaction ($P < 0.15$) between MGD and Eoa was observed on crude protein and dry and organic matter. The lower results were seen in the smaller MGD and more restricted area (195 μm and 59 mm^2/ton/h). There was increase in total and reactive lysine in all extruded diets, but not on lysine linked. The combined effect of DGM and Eoa strongly influence the process parameters, the transference of SME and kibbles traits. The second experiment evaluated the effect of different extruder configuration on the processing parameters, SG, in vitro digestibility of organic matter, kibble macrostructure, and IVND of extruded cat foods. The experiment followed a 4 x 2 factorial arrangements of treatments, with 4 Eoa and 2 restrictions after extruder screw (RASc), a total of 8 treatments. A single food was formulated for cat maintenance, and processed in a single screen extruder with a combination of 60% or 90% RASc, and 4 Eoa target: 59, 118, 236, 353 mm2/ton/h. All other extruder software and hardware parameters was unchanged during the study. IVND was measured by the total collection of feces method on the diets processed with 90% RASc and 353 mm^2/ton/h, and 60% RASc and 59 mm^2/ton/h, using 6 cats per diet. Data were submitted to analysis of variance considering RASc and Eoa effects, as well their interactions. Means were compared by polynomial contrasts ($P < 0.05$). For motor amperage was verified an interaction between RASc and Eoa, with greater means for the 90% RASc diets and a reduction with increase Eoa. In barrel moisture was similar among treatments. Die pressure did not change according to RASc, only Eoa, it decreased. Flash off was different only in diets with 90% of RASc and decreased with the increase Eoa. There was a reduction in mean temperatures of forth extruder jacket with increase Eoa. SME application did not suffer influence of the interaction between RASc and Eoa, but was higher for 90% RASc diets. STE was similar among treatments. Total specific energy application was similar for all Eoa on 60% RASc, but decreased with the increase on Eoa for 90% RASc. A significant effect of Eoa was observed for all analyzed kibble traits. There was an interaction for bulk and piece density and both item showed an increase with increase on Eoa. Higher expansion were verified for 90% RASc diets. SG and in vitro digestibility were not influenced by the interaction of RASc and EOA. In vitro digestibility changed according to Eoa, but IVND was similar ($P> 0.05$) between treatments and did not suffer influence of bulk density. RARs and Eoa have a strong impact on the process parameters, transference of SME and kibble traits.

Key words: cat, extrusion, grind, extruder configuration.

Abbreviation: Eoa, extruder open area; IVND, *in vivo* nutrient digestibility, MGD, mean geometric size, SG, starch gelatinization, SME, specific mechanical energy; STE, specific thermal energy; RASc, restriction after extrusion screw.

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A	Ampere
AA	Área aberta da matriz
ABINPET	Associação brasileira da indústria de produtos para animais de estimação
AOAC	Association of the official agricultural chemists
CDV	Calorimetria diferencial de varredura
cm	Centímetro
cm^3	Centímetro cúbico
DGM	Diâmetro geométrico médio
DIV	Digestibilidade in vivo dos nutrientes
DPG	Diâmetro padrão geométrico
EET	Energia específica total
EME	Energia mecânica específica
Eoa	Extruder open area
ETE	Energia total específica
EPM	Erro padrão da média
FEDIAF	Federação Europeia das Indústrias de Pet Food (The European Pet Food Industry Federation)
g	Grama
GA	Gelatinização do amido
IBGE	Instituto brasileiro de geografia e estatística
IVND	*In vivo* nutriente digestibilty
Kgf	Kilo grama força
Kw-h	Kilo watts hora
MGD	Mean geometric diameter

mm	Milímetro
mm^2	Milímetro quadrado
OM	Organic matter
RAR	Restrição após rosca da extrusora
RASc	Restriction after extruder screw
SAS	Statistical analysis system
SEM	Standard error of the mean
SME	Specific mechanical energy
SG	Starch gelatinization
STE	Specific thermal energy
ton	Tonelada

CAPÍTULO 1 – Considerações Gerais

1. Introdução

Dados da Associação brasileira da indústria de produtos para animais de estimação revelam que o segmento de “pet food” possui uma considerável importância econômica e social, pois representa valor considerável no PIB brasileiro e promove benefícios para a saúde e bem estar na interação entre homens e animais (ABINPET, 2016).

Atualmente 95% dos alimentos secos para cães e gatos são produzidos com tecnologia de extrusão termoplástica (SPEARS; FAHEY, 2004). O processo de extrusão é amplamente utilizado pela indústria, pois permite o processamento de vários ingredientes e coprodutos de origem vegetal e animal. Este consiste na cocção de mistura homogênea de ingredientes, promovendo sanitização, texturização e formatação, que ocorrem pela presença de umidade, pressão, temperatura e fricção mecânica em curto espaço de tempo (RIAZ, 2007; TRAN et al., 2008). O processo inclui as etapas de condicionamento, extrusão propriamente dita, corte e secagem, com funções específicas no cozimento e na qualidade do produto final.

O uso generalizado da extrusão termoplástica na indústria “pet food” deve-se ao fato dela promover mudanças físicas e químicas nos ingredientes, alterando sua qualidade e propriedades físicas, aumentando seu valor nutricional com eficiência e baixo custo relativo (GRIFFIN, 2003; TRAN et al., 2008). A elevada aplicação de energia termomecânica no processo induz alterações vantajosas e desejáveis em alimentos para gatos, como: aumento da digestibilidade dos cereais, melhora da palatabilidade do alimento, modificações de atributos texturais favorecendo a apreensão e mastigação, inativação de fatores antinutricionais, destruição de microrganismos, aumento da vida de prateleira, ampliação das possibilidades de uso de matérias primas, desnaturação de proteínas com melhora de sua digestibilidade (CHEFEL, 1986; LANKHORST et al., 2007). O ganho em digestibilidade e palatabilidade dos cerais talvez seja o efeito mais notório, promovido pela gelatinização e plasticização do amido, que se torna mais digerível pelas enzimas digestivas dos carnívoros (MURRAY et. al., 2001).

No entanto, a extrusão também pode promover efeitos indesejáveis, incluindo destruição de vitaminas, oxidação de lipídeos, destruição e redução na disponibilidade de aminoácidos, principalmente da lisina envolvida na reação de “Maillard” (LANKHORST et al., 2007). Devido a isto, e também de modo a se evitar gastos desnecessários, o balanço entre os efeitos desejáveis e indesejáveis deve sempre ser buscado. Para que isso ocorra todas as etapas do processo necessitam ser controladas e seus efeitos conhecidos.

O mercado “pet food” está cada vez mais competitivo e exigente, impulsionado principalmente por proprietários que consideram seus animais como membros da família e se interessam em fornecer alimentos de alta qualidade e sabor. Além disso, deve-se considerar o fato do crescente conhecimento entre as pessoas da estreita relação da alimentação com a promoção de saúde, bem estar e expectativa de vida. Segundo Carciofi e Jeremias (2010) é crescente o número de pesquisas direcionadas ao conhecimento de diversas áreas que envolvem a nutrição de cães e gatos. Contudo, provavelmente um dos campos onde exista maior escassez de informações científicas é relativa ao processamento dos alimentos extrusados e suas implicações nutricionais e nas respostas metabólicas promovidas nos animais.

Dentro deste contexto, a presente Tese de Doutorado teve como objetivo geral estudar as implicações nutricionais, à macroestrutura de extrusados e aos parâmetros de processo de alimentos para gatos produzidos a partir de diferentes graus de moagem da matéria prima e configurações de extrusora.

2. Revisão de Literatura

2.1 Mercado de alimentos industrializados para animais de estimação

Segundo pesquisa do IBGE (2013), o Brasil possui 52,2 milhões de cães e 22,1 milhões de gatos, o que confere ao país a segunda maior população desses animais no mundo, atrás dos Estados Unidos que apresentam respectivamente 74,1 e 73,6 milhões. O número de gatos no Brasil vem aumentando ao longo do tempo e acompanha uma tendência mundial, sendo que em muitos países o número de felinos já superou o de caninos. O setor “pet” é o segmento do agronegócio relacionado com o desenvolvimento das atividades de criação, produção e comercialização de animais de estimação e representa 0,38% do PIB brasileiro. Dentro desse setor, o de maior destaque é o mercado de alimentos industrializados que representou em 2015 a área de maior faturamento (67,3%) e gerou um montante de 18 bilhões de reais, crescimento de 7,6% em relação a 2014 (ABINPET, 2016). Esses dados demonstram a importância crescente do segmento para a economia brasileira.

A produção brasileira de alimentos destinados a cães e gatos foi 2,53 milhões de toneladas em 2015 (ABINPET, 2016). Acredita-se que mais de 95% do volume desses alimentos comercializados no Brasil são do tipo seco, o qual apresenta máximo de 12% de umidade. O alimento seco assim como o semi úmido são produzidos a partir da tecnologia de extrusão.

2.2 Princípios gerais do processo de extrusão

A aplicação de extrusão em alimentos para o homem inclui principalmente o uso de farinhas e féculas que são destinadas para a produção de alimentos práticos, como os cereais matinais, "snacks", alimentos infantis, sopas instantâneas, farinhas para empanamento, gomas de mascar, proteína vegetal texturizada, bebidas, amido modificado, doces, macarrões e macarrões instantâneos (ASCHERI et al., 2000; CAPRILES; ARÊAS, 2005; CHANG and NG, 2009). De acordo com Allam e colaboradores (2016) a extrusão está se tornando popular em relação a outros métodos de processo, devido ao seu controle automatizado, capacidade de operação contínua, alta produtividade, adaptação e versatilidade e eficiência energética. Além disso, também permite o desenvolvimento de produtos novos e de alta qualidade com aparência e forma únicas e diferenciadas, aliado a um baixo custo e sem geração de efluente (Faraj et al., 2004).

Na alimentação animal a extrusão é largamente empregada para a produção de alimentos destinados à organismos aquáticos (peixes, camarões etc), cães e gatos. Estimativas apontam que 95% dos produtos para essas duas últimas espécies utilizam esse tipo de tecnologia (SPEARS; FAHEY, 2004). Para gatos, inclusive acredita-se que no Brasil em termos percentuais a participação dos alimentos extrusados seja maior do que para cães, com a oferta de alimento do tipo caseiro sendo mais restrita para esta espécie.

De modo geral, o processo de extrusão consiste na cocção em um fluxo constante de mistura homogênea de ingredientes, promovendo sanitização, texturização e formatação, que ocorrem pela presença de umidade, pressão, temperatura e fricção mecânica em curto espaço de tempo (RIAZ, 2007; TRAN et al., 2008). A mistura de ingredientes segue uma sequência lógica, acompanha diferentes equipamentos e inclui as etapas de mistura, moagem, condicionamento, extrusão propriamente dita, corte e secagem. Esse processo permite a modificação estrutural, funcional e nutricional dos ingredientes utilizados.

O processo de fabricação de alimentos extrusados para cães e gatos segue uma sequência lógica e acompanha diferentes equipamentos. Primeiramente os ingredientes de maior inclusão são dosados em sistemas automatizados ou de forma manual e os de menor inclusão geralmente são pesados de forma manual, sendo que a inclusão de cada um deles é baseada em uma formulação pré-estabelecida. Posteriormente esse conjunto de matérias primas é encaminhado para um misturador horizontal de pás ou de fitas e em seguida moído em moinhos de martelos. Estas são etapas iniciais importantes do processamento. Não se conhece ainda o melhor tamanho geométrico de matéria prima que alie eficiência de mistura, custo competitivo de moagem e adequado processamento de extrusão (BAZOLLI et al., 2015). Através do alimentador, a mistura previamente moída e homogeneizada chega ao condicionador, onde se adiciona umidade e energia térmica por meio da injeção de vapor direto e água. No condicionador essa mistura é homogeneizada e transformada em massa uniforme, mediante a ação de sistema de barras cilíndricas com pás dispostas radialmente girando a velocidade variável (BAZOLLI, 2007). A adição de água e vapor tem como objetivo aumentar a umidade (que pode variar entre 15 a 35%) e temperatura da massa (que pode variar entre 64 a 99°C), iniciando o cozimento do amido. A permanência no condicionador favorece a hidratação interna das partículas de matéria prima, plasticização e sanitização, com aumento da estabilidade e produtividade da extrusora e da qualidade do produto final. Além disso, favorece vantagens econômicas, pois diminui a fricção da massa e aumenta a produtividade, reduzindo o consumo de energia elétrica e o desgaste mecânico das peças do equipamento (RIAZ, 2000).

Em seguida ao condicionador, a massa em processamento é conduzida para o canhão da extrusora, um tubo com sistema de rosca sem fim girando a velocidade variável em seu interior. Este sistema irá comprimir a massa gerando a produção de energia mecânica, pela rotação do parafuso da extrusora, que promove cisalhamento da mistura contra seu revestimento, ou camisa e a comprime contra a matriz, na extremidade do cilindro, criando pressão, fricção e temperatura. O atrito provocado pela ação da rosca sobre as partículas criará a energia mecânica, responsável pelo aumento da temperatura e cozimento do amido. As pressões e temperaturas no final do canhão podem atingir, respectivamente, mais de 60 bars e 160°C, mas usualmente trabalha-se com pressões de 20 a 40 bars e temperaturas de 120 a 140°C. Toda esta energia e compressão em um fluxo laminar modificam profundamente os amidos e proteínas. Ela permite o cozimento completo do amido em poucos segundos e a baixa umidade, entre 20% e 35% de água, o que é bastante vantajoso em relação ao cozimento em pressão atmosférica, quando são necessárias mais de 10 minutos e duas partes de água para uma de amido para que este se gelatinize completamente (GIBSON; ALAVI, 2013). Na saída da extrusora existe, por fim, o sistema de corte. Este é composto por facas em disposição perpendicular e rotação variável, que cortam a massa no tamanho desejado.

O processo de extrusão na indústria de “pet food” está bastante difundido e promove alterações vantajosas e econômicas que incluem desde a ampliação do uso de matérias primas em especial os cereais, a sua aceitação e o seu bom aproveitamento nutricional pelos cães e gatos (CHEFEL, 1986;

GRIFFIN, 2003; LANKHORST et al., 2007; TRAN, 2008). No entanto, a extrusão também pode promover efeitos indesejáveis, incluindo destruição de vitaminas, oxidação de lipídeos, destruição e redução na disponibilidade de aminoácidos, principalmente da lisina envolvida na reação de “Maillard” (LANKHORST et al., 2007). Devido a isto, e também de modo a se evitar gastos desnecessários, o balanço entre os efeitos desejáveis e indesejáveis deve sempre ser buscado e para tanto o conhecimento de todas as etapas do processo produtivo e suas implicações devem ser minuciosamente estudadas e compreendidas.

2.3 Efeito da extrusão sobre os nutrientes

2.3.1 Amido

Os cereais (arroz, milho e trigo) são as fontes de amido mais utilizadas em rações extrusadas para gatos. Nos grãos as moléculas de amilose e amilopectina, compostas por cadeias de glicose, organizam-se em estruturas cristalinas altamente ordenadas denominadas grânulos. Esta organização confere a característica de birrefringência, típica de substâncias cristalinas organizadas. Durante a extrusão, na presença de água, calor, cisalhamento e pressão, os grânulos de amido sofrem o fenômeno de gelatinização, quando incham, derretem e perdem a sua estrutura cristalina (ZENG et al., 1997). O amido gelatinizado é solúvel em água e mais susceptível à degradação enzimática do que o amido não cozido (DONA et al., 2010). Dados de nosso grupo de pesquisa demonstraram que se extrusados adequadamente, o amido dos cereais apresenta digestibilidade aparente superior a 95% para gatos (DE-OLIVEIRA et al., 2008) e 98% para cães (CARCIOFI et al., 2008).

A extensão da gelatinização do amido promovida pelo processo de extrusão pode ser expressa em porcentagem, sendo análise importante para aferição e monitoramento da eficiência do processo de cozimento do alimento. Esta pode ser feita por dois métodos principais, por Calorimetria Diferencial de Varredura (CDV) e pelo método enzimático com emprego de amiloglicosidase (SÁ et al., 2013). A CDV é uma técnica de análise térmica que registra o fluxo de energia calorífica associado a transições nos materiais em função da temperatura. É um método de variação entálpica (ΔH), a diferença no fornecimento de energia calorífica entre uma substância e um material de referência é medida em função da temperatura, enquanto ambas são submetidas a um mesmo programa de aquecimento ou arrefecimento, rigorosamente controlado (DE PILLI et al., 2008). Estas medidas fornecem dados qualitativos e quantitativos em processos endotérmicos (absorção de energia calorífica) e exotérmicos (liberação de energia calorífica), o que permite obter informações referentes a alterações das propriedades físicas e/ou químicas, como: temperaturas características (fusão, cristalização, transição vítrea), grau de cristalinidade de um polímero, diagrama de fases, entalpias de transição de fase e de reação, estabilidade térmica e oxidativa, grau de pureza e cinética de reações. Esse método parece ser mais interessante para materiais homogêneos, como quando se processam cereais simples. Para matrizes complexas de composição química, como alimentos completos e balanceadas para cães e gatos com múltiplos ingredientes o método parece não ser tão eficiente.

O método da amiloglicosidase baseia-se no princípio de que esta enzima atua somente nas cadeias de amilose e amilopectina gelatinizadas, não degradando amido na forma de grânulos cristalinos crus (CHIANG; JOHNSON, 1977; SÁ et al., 2013). Assim, numa amostra composta de ingredientes, como os alimentos para cães e gatos, determina-se o teor total de amido e sua fração cozida e crua. Por se tratar de método enzimático, admite-se que apresente associação com a digestão enzimática nos animais, sendo representativo do ponto biológico e interessante como medida da intensidade e qualidade do processamento (GIBSON; ALAVI, 2013). No entanto, a gelatinização do amido não é apresentada na maioria dos estudos científicos conduzidos nesses alimentos e tampouco é uma análise de rotina aplicada pela indústria de “pet food”, sendo aspecto que ainda requer mais estudos.

Não se conhece quanto é necessário gelatinizar o amido para que cães e gatos apresentem adequada digestibilidade dos alimentos extrusados, nem mesmo se a extensão de gelatinização necessária varia entre os diferentes cereais (BAZOLLI et al., 2015; SÁ, 2015). Além da digestibilidade, a extensão do processamento do amido guarda relação com a resposta pós-prandial induzida de glicose e insulina (JENKINS et al., 1981).

De acordo com a resistência à hidrólise *in vitro*, o amido é dividido em três classificações: amidos de digestão rápida e completa, amidos de digestão lenta e completa e amidos resistentes (ENGLYST; KINGMAN; CUMMINGS; 1992). Este último se refere à soma do amido e de seus produtos de degradação que não são digeridos e absorvidos no intestino delgado de indivíduos sadios (LOBO; LEMOS SILVA, 2003), sendo fermentados por bactérias anaeróbicas no intestino grosso (SALGADO et al., 2005). Dessa maneira, essa fração do amido apresenta comportamento similar ao da fibra alimentar, e tem sido relacionada a efeitos benéficos locais e sistêmicos (LOBO & LEMOS SILVA, 2003).

Existem três tipos de amido resistente: amidos fisicamente inacessíveis (AR1), grânulos de amido nativo cru (AR2) e amido retrogradado (AR3). Mamíferos são capazes de digerir, ou pelo menos fermentar, os tipos AR2 e AR3 (ENGLYST; KINGMAN; CUMMINGS; 1992).

A dissertação de Roberti-Filho (2013) avaliou em dietas para cães o efeito de quatro diâmetros geométricos (169; 248; 252 e 290µm) da matéria prima e duas configurações de extrusora que geraram áreas abertas de 24 e 64mm^2. Foi observado que a intensidade da gelatinização do amido e a quantidade de amido resistente gerado foram influenciados de forma significativa tanto pelo tamanho da partícula quanto pela área aberta.

A gelatinização do amido não é fenômeno isolado durante o processamento. No interior do canhão da extrusora, o amido já gelatinizado, principalmente as cadeias de amilose, podem se associar com a gordura presente naturalmente nos ingredientes e originar complexos, denominados amido-lipídio. Estes são formados pelo encapsulamento de moléculas de triglicerídeos no interior de cadeias de amilose (GIBSON; ALAVI, 2013). Suas consequências para o animal e aproveitamento do alimento não estão ainda adequadamente estudadas para cães e gatos. Stroucken et al. (1996) não encontraram efeito do processo sobre a digestibilidade da gordura, sugerindo que estes complexos seriam facilmente digeridos, o que estaria de acordo com a elevada digestibilidade de lipídeos comumente verificada em dietas para cães e gatos (HULLÁR et al., 1998).

2.3.2 Proteínas

Gatos são considerados carnívoros estritos e sua necessidade de proteínas é superior em relação aos cães tanto na fase de crescimento como na de manutenção (NRC, 2006). Por isso a composição das rações destinadas à essa espécie apresenta um conteúdo maior deste nutriente. Para atender essa exigência a fórmula desses produtos apresenta quantidade significativa de ingredientes proteicos, sendo as farinhas de origem animal (farinha de vísceras de frango, farinha de carne e ossos de bovinos e farinhas a base de suínos) e os farelos de origem vegetal (glúten de milho, farelo de soja, concentrado proteico de soja, glúten de trigo) os mais comumente utilizados.

Ao longo do processo de extrusão, a força de cisalhamento, pressão e temperatura elevadas modificam profundamente as proteínas (CAMIRE, 1991). Os efeitos da extrusão sobre os componentes proteicos podem ser benéficos ou prejudiciais para as características físicas e nutricionais da mistura alimentar, à depender de sua intensidade. A extrusão pode inativar fatores antinutricionais à base de proteínas, destruindo a integridade de sua estrutura e, consequentemente, evitando sua ação antinutricional (VAN DER POEL et al., 1990; ALONSO et al., 2000; SOUZA, 2013). A desnaturação das proteínas pode torná-las mais suscetíveis a enzimas digestivas e, portanto, melhorar a sua digestibilidade (HENDRIKS; SRITHARAN, 2002). As enzimas presentes nos ingredientes podem causar efeitos deteriorantes durante a sua armazenagem. Assim sua inativação pela extrusão contribui para estabilizar o armazenamento e aumentar a vida de prateleira de alimentos secos para animais (CHEFTEL, 1986; GUY, 2001).

No entanto, a extrusão também pode promover efeitos indesejáveis, incluindo destruição e redução na disponibilidade de aminoácidos, principalmente da lisina envolvida na reação de “Maillard” (LANKHORST et al., 2007).

2.3.3 Lipídios

Quando lipídios ou alimentos contendo lipídios são aquecidos na presença de oxigênio estes sofrem oxidação, devido à degradação dos ácidos graxos. A oxidação lipídica é desafio para a conservação de alimentos para animais de estimação, uma vez que a taxa de oxidação é afetada por muitos fatores, como: tipo de gordura, teor de gordura, teor de umidade e grau de expansão dos “kibbles” (LIN et al, 1998). Os radicais livres que resultam dessa oxidação podem reagir com proteínas, vitaminas ou outros constituintes e reduzir a qualidade nutricional do alimento. Além disso, ocorre perda pronunciada de sabor e alteração da cor do alimento (LILLARD, 1983). Por fim, a extrusão também pode induzir hidrogenação, isomerização e polimerização das gorduras (ROKEY; PLATTNER, 1995).

A complexação de lipídios com carboidratos também altera a textura do extrusado e limita em muito sua expansão, promovendo formação de extrusados duros e densos (LLO et al., 2000; LALUSH et al., 2005). Além disso, quanto maior a quantidade de gordura presentes nos ingredientes da dieta, menor será a eficiência de transferência de energia mecânica e da extrusão em si, reduzindo o cozimento e promovendo formação de “kibbles” pouco expandidos e duros (CHEFTEL, 1986). A extrusão interfere, ainda, na recuperação de gordura durante a análise química do produto final, tornando necessário um passo prévio de hidrólise ácida da amostra com ácido clorídrico antes da extração por éter. Esta hidrólise rompe os complexos amido-lipídio possibilitando a quantificação completa da gordura (AOAC, 2002). Estas interações entre lipídeo e amidos também interferem no comportamento e nas propriedades de gelatinização e retrogradação dos amidos, sendo a formação destes complexos influenciada pelo tipo de lipídeo e amido presentes no alimento, assim como pelas condições em que os mesmos são processados (SOUZA, 2013).

2.4 Redução do tamanho de partículas da matéria prima

A eficiência do processo de extrusão dos alimentos está diretamente relacionada à eficiência na moagem dos ingredientes. Dados antigos indicam que a indústria de alimentos para cães empregava, em geral, moagem dos ingredientes em moinhos de martelos com telas de 1,6mm (ROKEY; HUBER, 1994). Acredita-se que, no Brasil, as empresas de “pet food” utilizem peneiras com 1,0 a 1,2mm de abertura dos furos, porém nada foi publicado a esse respeito. Infelizmente ainda, a redução da granulometria é utilizada principalmente para melhorar a apresentação visual e acabamento do produto final, sem se ater ao custo financeiro e aspectos nutricionais, como por exemplo, influência na digestibilidade.

A redução do tamanho das partículas tem como principais funções no processo proporcionar mistura homogênea dos ingredientes e facilitar sua extrusão (FRAILHA, 2005). O principal método de redução do tamanho das partículas é a moagem com moinhos de martelo (COWELL et al., 2000). Segundo Bellaver e Nones (2000), a moagem e a mistura dos ingredientes são importantes processos em uma fábrica de ração e a consistência dessas etapas promove forte impacto na qualidade final dos produtos.

O moinho de martelos consiste basicamente em um conjunto de facas rombas, denominados de martelos. Esses martelos possuem alguns milímetros de espessura. São perfilados paralelamente uns aos outros e fixos a um eixo com capacidade de girar a alta rotação. Ao redor desse sistema é fixada uma peneira. O diâmetro dos furos das peneiras varia, dependendo da característica final desejada para o produto. A peneira faz a seleção do tamanho de partículas, permitindo passar as que já estão pequenas o suficiente (menores que seu diâmetro) e retendo as partículas grandes na câmara de moagem até que seu tamanho seja reduzido o suficiente para que passem adiante (ALLES, 2003).

Os ingredientes que serão moídos entram na câmara de moagem por ação da gravidade e fluxo positivo de ar aspirado, encontrando os martelos que estão em alta rotação. Esse contato irá reduzir o material, dependendo da característica do ingrediente utilizado. Isso ocorre devido ao grande diferencial de velocidade entre os martelos e o material que entra. Além disso, o material será propelido contra a placa de impacto, promovendo seu rompimento. Após essa primeira ação, as partículas que restam igualam sua velocidade à dos martelos. O atrito gerado entre os ingredientes e os martelos e placa de impacto irá proporcionar a redução do tamanho das partículas. Uma vez reduzidas, as partículas irão passar pelos forames da peneira, impulsionadas pelo ar em movimento que é sugado e retirado de dentro da câmara de moagem (OWENS; HEIMANN, 1994).

Diferentes estudos foram conduzidos sobre a influência do tamanho das partículas da matéria-prima na qualidade de cereais matinais e "snacks" extrusados para o homem, e todos concordaram que redução do tamanho de partículas corresponde a aumento da gelatinização do amido, expansão do extrusado e aumento de sua capacidade de absorção de água. Por exemplo, Chauhan e Bains (1985), verificaram que a diminuição da granulometria da matéria prima de 0,542mm para 0,175mm melhorou a gelatinização do amido, expansão do extrusado, índice de absorção de água e índice de solubilidade em água de "snacks" à base de arroz. Desrumaux et al. (1998) avaliaram grãos de milho de diferentes tamanhos de partículas (de 0,1 a 0,6mm), demonstrando que quando há aumento do tamanho de partícula da matéria-prima o extrusado adquire textura mais dura e reduzido coeficiente de expansão longitudinal. Estudando a granulometria da fibra em extrusados compostos por mistura de farinha de milho e fibra de beterraba com diferentes tamanhos (de 2 a 0,074mm), Lue et al. (1991) concluíram que com a diminuição do tamanho do material fibroso há maior expansão radial e menor expansão longitudinal do extrusado, sem diferenças em relação às frações de fibra solúvel, insolúvel e total. No estudo de Fouhse (2011) foram comparados diferentes tamanhos de partículas da ervilha (de 0,407 a 0,288mm), os extrusados com partículas menores tiveram menor quantidade de amido resistente e digestão *in vitro* mais rápida do amido. Em um estudo com "pet food", diferentes tamanhos de partículas de milho, variando entre 1,5 e 0,75mm foram avaliadas por Mathew et al. (1999), que verificaram que quanto menores são as partículas, maior será a expansão e o índice de absorção de água dos “kibbles” extrusados.

A redução de partículas, no entanto, consome grande quantidade de energia. Pozza et al. (2005) mensuraram a energia gasta em 10 granjas de suínos, produtoras de ração e observaram que os gastos variaram entre 6 e 20 kWh/t de ração, ilustrando a necessidade de se aperfeiçoar a moagem de forma a maximizar o aproveitamento do alimento e racionalizar o uso da energia. O tamanho final desejado para as partículas tem enorme impacto no custo, Healy et al. (1994) demonstraram que a energia elétrica gasta para a moagem do grão de milho a 900µm foi de 5,3 kWh/t, com produção de 1,76 t/h. Com a redução do tamanho de partículas para 300µm ocorreu aumento no consumo de energia elétrica para 24,5kWh/t e redução na produção de ração para 0,65t/h. O ingrediente utilizado tem também influência direta na produtividade e consumo de energia dos moinhos de martelo. Healy et al. (1994) observaram que a energia elétrica consumida para a moagem do grão de milho a 500µm foi superior à energia gasta para a moagem do sorgo na mesma granulometria. Enquanto para moer uma tonelada de milho foram gastos 15,7kWh, para moer o sorgo foram gastos apenas 3,8kWh. A mesma relação foi encontrada para a produtividade dos moinhos, em uma hora, foram moídas 0,63 toneladas de milho, mas 2,37 toneladas de sorgo.

Em um trabalho recente do nosso grupo de pesquisa (MONTI, 2015) foi avaliado em beagles adultos o efeito de dois tamanhos geométricos da fibra de cana (394 e 196µm) e farelo de trigo (345 e 143µm) sobre a digestibilidade dos nutrientes, produtos da fermentação, qualidade fecal, tempo de retenção gastrointestinal (TRGI) e palatabilidade. Foi observado em ambas fibras que o menor tamanho de partículas não resultou em melhoras quanto à digestibilidade, e tampouco alterou o TRGI; entretanto, interferiu na formação de ácidos graxos de cadeia curta. Com relação a preferência alimentar os cães optaram pela fibra de cana longa em relação à curta (P<0,01), mas não demonstraram preferência quanto ao tamanho do farelo de trigo.

Existem poucos trabalhos disponíveis a respeito do consumo de energia elétrica na moagem em função da formulação, inclusão de proteína de origem animal ou vegetal, teor de gordura, fibra, tipo de cereal e tamanho geométrico final desejado. Assim como a influência do tamanho de partículas na eficiência de extrusão, macroestrutura dos extrusados e nas respostas metabólicas. E para a espécie felina esses dados não estão disponíveis no momento.

2.5. Efeitos do processamento sobre a digestibilidade dos nutrientes

Em um dos poucos estudos sobre o tamanho de partículas de matérias-primas para rações extrusadas para cães, Bazolli e colaboradores (2015) avaliaram arroz, milho e sorgo moídos entre 0,277 e 0,619mm de diâmetro geométrico médio, que foram posteriormente incorporados em dietas completas que foram extrusadas. O aumento da granulometria da matéria-prima resultou em reduções na gelatinização do amido de todas as dietas. A relação entre tamanho de partícula e cozimento pôde ser explicada por:

Gelatinização do amido = 106.32 – [0.0699 * tamanho geométrico médio do cereal (R2 =0,85; P < 0,001). A gelatinização foi dependente apenas do tamanho das partículas de cereal, não diferindo entre os três grãos estudados. A digestibilidade dos nutrientes, entretanto, mudou de acordo com o ingrediente. Dietas a base de quirera de arroz não se alterou a digestibilidade dos nutrientes em função do grau de gelatinização ou do tamanho geométrico do cereal. Entretanto, dietas a base de milho apresentaram aumento quadrático na digestibilidade, já as a base de sorgo foi observada aumento linear na digestibilidade dos nutrientes com a redução da granulometria e aumento do cozimento dos cereais. Alimentos para cães adultos à base de milho foram estudadas por Hilcko et al. (2009), estes moeram a mistura completa de ingredientes em peneiras de 0,8, 1,0, 1,2, e 1,5mm e verificaram redução quadrática da digestibilidade de gordura e do extrativo não nitrogenado, com o aumento no tamanho das partículas.

O estudo de Roberti-Filho (2013) avaliou dietas para cães à base de milho e farinha de vísceras de frango, utilizando para isto arranjo fatorial de tratamentos com 4 diferentes moagens (peneiras de 0,5mm; 0,8mm, 1,4 mm e 2,0mm em um moinho de martelos) e duas configurações de extrusão (área aberta da matriz de 63,9mm^2 e 23,7mm^2). Foi verificado que a configuração da extrusora afetou a gelatinização do amido, mas não influenciou a digestibilidade dos nutrientes. Por outro lado, a digestibilidade dos nutrientes reduziu linearmente quando o tamanho das partículas da dieta aumentou. A redução da digestibilidade verificada foi atribuída à redução na gelatinização do amido (redução de 86% para 69% de gelatinização) e à indisponibilidade dos grânulos inteiros, grosseiramente moídos com o aumento do tamanho de partículas. Nestas condições existe limitação do contato entre as enzimas e os substratos no trato digestivo, com menor aproveitamento da dieta (AMERAH et al., 2008).

Outro aspecto relativo ao tamanho de partículas da matéria-prima é o consumo de energia mecânica específica (EME) para a extrusão do alimento. No experimento de Roberti-Filho (2013), a implementação de EME diminuiu linearmente com o aumento no tamanho de partículas de milho. Isto pode ser explicado pela redução da viscosidade consequente à menor gelatinização do milho grosseiramente moído, reduzindo a resistência da massa ao fluxo no interior da extrusora. Esta limitação de aplicação de energia pode parecer vantajosa, por reduzir os custos de produção, mas na realidade representa a ocorrência de processamento incompleto, as partículas grosseiramente moídas não hidratam e seu amido permanece inacessível ao ataque enzimático e com digestibilidade limitada.

Para os animais a palatabilidade engloba fatores como sabor, aroma, apreensão e sensação de mastigação (textura, forma e tamanho dos extrusados). Esta é normalmente referida como aceitabilidade, valor de preferência alimentar e comportamento de ingestão. É fator chave na seleção da dieta pelos cães e gatos, estando estreitamente relacionada com seu sucesso comercial. Os fatores mais estudados que determinam a palatabilidade dos alimentos são composição de nutrientes (teores de gordura, proteína e carboidratos) e tipo de ingredientes (proteínas e gorduras de origem animal, ingredientes de origem vegetal, ingredientes fibrosos) (HULLÁR et al., 1998). No entanto, o processamento por extrusão é igualmente importante, pois determina vários aspectos estreitamente ligados à palatabilidade e preferência alimentar, como crocância, dureza, forma, tamanho, odor e sabor da ração. Todas estas características são fortemente influenciadas pelas condições de processamento, embora dados de sua influência sejam praticamente inexistentes para cães e gatos.

As características macroestruturais dos “kibbles” extrusados são o resultado da formulação (teor de amido, proteína, gordura e fibra), tipo de ingredientes (proteína vegetal ou de origem animal) e condições de processamento, incluindo a umidade no canhão extrusor, tempo de residência no condicionador e no canhão da extrusora, transferência de energias mecânica e térmica, velocidade de rotação e configuração da rosca, relação entre área aberta da matriz e produção horária de alimentos, temperatura do cilindro ou camisa, temperatura e pressão da massa em processamento e tipo e velocidade de corte das facas (RIAZ, 2007). Juntos, todos esses parâmetros determinarão a expansão radial e longitudinal do “kibbles”, sua densidade aparente e específica, comprimento específico, estrutura celular, dureza e crocância (TRIVEDI e BENNING, 2003). Para gatos não existem quase publicações que identificaram as características macroestruturais que resultam em melhor aceitação pelos animais, nem tampouco informações sobre as melhores disposições e configurações de processamento para que estas características sejam alcançadas. Estes dados seriam importantes para os fabricantes, possibilitando ajustes nas formulações, desenhos e configurações de equipamentos e condições de operação que resultassem em melhor aceitação e palatabilidade do alimento.

2.7. Implicações das aplicações de energia mecânica e térmica

As mudanças físico químicas promovidas pela extrusão nos ingredientes estão diretamente ligadas à quantidade de energia específica total (EET) transferida para a massa. Esta, por sua vez é composta pela soma das implementações de Energia Mecânica Específica (EME) e Energia Térmica Específica (ETE). No início da extrusão termoplástica de médio cisalhamento, utilizada para produzir “pet food”, muita ênfase se dava à transferência de EME. Esta continua sendo fundamental, mas hoje é quantitativamente menos importante que a ETE. Estima-se que entre 20% e 35% da energia total aplicada no processo “pet food” corresponda à EME, sendo a restante energia térmica. Contudo, talvez essa realidade não seja condizente a todas as empresas. Alguns dos sistemas de extrusão podem não ter tecnologia para transferir quantidades expressivas de energia térmica, pois os condicionadores são pequenos, as pás não são eficientes em homogeneizar a massa, não há controle e variação da velocidade de rotação das pás e mesmo o tempo de residência do produto em seu interior nem sempre é medido ou controlado. Alguns sistemas, inclusive, não trabalham com vapor sendo o processo 100% baseado em energia mecânica, o que os torna ineficientes e caros quanto ao custo de processamento. Adicionalmente, não se computam as aplicações de energia, controlando a extrusão de modo insipiente pela aferição da temperatura do condicionador e medição do tamanho, densidade e cor dos “kibbles” extrusados. Mesmo o grau de cozimento, ou de gelatinização do amido não é atualmente parâmetro de controle para a maioria dos estabelecimentos.

A aferição ou cálculo da EME e ETE aplicadas torna possível compreender, parametrizar, controlar e replicar o processo de fabricação dos produtos (RIAZ, 2007). Na grande maioria dos estudos publicados não há descrição das condições dos parâmetros de extrusão ou quantificação da EME, ETE e transferência de EET. Provavelmente também não exista uma proporção única, pois as condições adequadas de processo variam em função da composição de nutrientes e dos tipos de matérias primas empregadas (TRAN, 2008).

Tese de Doutorado de nosso grupo de pesquisa foi pioneira em avaliar os efeitos de diferentes aplicações de EME e ETE na produção de alimentos para cães e gatos (SÁ et al., 2013b). Várias vantagens foram verificadas quando mais ETE foi implementada, com redução proporcional da EME, como: ganho em palatabilidade, tanto para cães como para gatos; menor custo de processamento, somando o menor custo relativo do vapor em relação à energia elétrica e o menor custo com desgaste de equipamentos, que é maior à medida que mais EME é implementada; menor perda de nutrientes, pois aminoácidos e selênio tiveram maiores perdas e a formação de lisina ligada (que indica dano à proteína) foi maior no tratamento com mais EME. Como a menor aplicação de EME foi compensada com maior ETE, o cozimento do amido foi igual entre rações, bem como a digestibilidade dos nutrientes. O ganho em palatabilidade difere do relatado por Trivedi e Benning (2003), que observaram para gatos preferência por alimentos processados com mais EME. No entanto, estes últimos autores não apresentaram a aplicação de ETE e ETE, de modo que é possível que sub processamento tenha ocorrido, interferindo nos resultados.

3. Objetivos

Considerando-se o exposto, o presente estudo teve como objetivo avaliar o efeito do tamanho de partículas da matéria prima e a configuração da extrusora sobre os parâmetros de processo, características dos extrusados e digestibilidade de rações para felinos. Para a conclusão desses objetivos dois experimentos foram conduzidos e apresentados na forma de dois capítulos. No primeiro experimento uma mesma formulação foi moída em duas aberturas de peneira (0,5 e 1,2 mm) e extrusadas com seis áreas abertas. Estudou-se o impacto conjunto destes fatores nas variáveis de extrusão, macroestrutura dos extrusados, cozimento do amido, digestibilidade *in vitro* da matéria orgânica e *in vivo* e quantificação da lisina ligada em rações para gatos. No segundo experimento a mesma formulação foi moída em peneira de aberturas 0,9 mm e foi avaliado a influência conjunta do efeito de duas restrições ao final da rosca extrusora e quatro áreas abertas nas variáveis de extrusão, macroestrutura dos extrusados, cozimento do amido e digestibilidade *in vitro* da matéria orgânica e *in vivo*.

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CAPÍTULO 2

Raw material particle size and extruder open area on processing parameters, kibble traits, and digestibility of extruded cat foods[1]

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Abstract

The aims of the study were to evaluate the effect of raw material particle size and extruder open area (Eoa) on processing parameters, starch gelatinization (SG), *in vitro* digestibility of organic matter, kibble macrostructure, *in vivo* nutrient digestibility (IVND) and reactive lysine of extruded cat foods. The experiment followed a 6 x 2 factorial arrangements, with 6 Eoa and 2 raw material particle sizes, a total of 12 diets. A single food was formulated for cat maintenance, and ground in hammer mill to 195 μm or 254 μm of mean geometric diameter (MGD). The material was manufactured in a single screw extruder with target open areas of 59, 118, 177, 236, 294, 353 mm^2/ton/h. All other extruder software and hardware parameters were unchanged. IVND was measured by the total collection of feces only in 4 diets, dependent of extreme MGD and Eoa. Lysine were measured in 5 treatments (raw material and in 4 diets chosen to measure IVND). Data were analyzed via ANOVA, considering MGD and Eoa effects, their interactions and compared by polynomial contrasts ($P<0.05$). In the IVND an interaction was considered significant when $P<0.15$. Results of lysine were compared using test of Dunnet ($P<0.05$) and raw material was considered the reference treatment. All data were analyzed using procedure of SAS. An interaction between MGD and Eoa was observed for mash pressure before die and temperature of last extruder jacket, with smaller values for larger MGD. Water flash off was similar between MGD and decreases linearly with increase Eoa. SME (specific mechanical energy) and STE (specific thermal energy) suffered interaction between MGD and Eoa. In both MGD there were a quadratic reduction on SME and increase on STE application with the increase on Eoa. An interaction ($P<0.05$) between MGD and Eoa was observed for all kibble traits. The larger MGD presented higher bulk and piece density, specific length and lower expansion. SG and *in vitro* digestibility of organic matter were not influenced by Eoa, only by MGD. For IVND an interaction (P<0.15) between MGD and Eoa were verified for dry and organic matter and crude protein, with lower results for minor MGD and restrictive Eoa. Total and reactive lysine increase in all extruder diets, but not lysine bound. To summarize, MGD of raw material and Eoa have strong influence on extrusion parameters, SME transference and kibble traits. SG greater than 88.5% or MGD below 254 µm not increase IVND.

Keywords: grind, extrusion, starch gelatinization, reactive lysine.

Abbreviations: Eoa, extruder open area; IVND, *In vivo* nutrient digestibility; MGD, mean geometric diameter; SG, starch gelatinization; SME, specific mechanical energy; STE, specific thermal energy.

1. Introduction

During the manufacturing of extruded commercial pet food, several factors interfere on the quality and appearance of the kibbles and the bioavailability of nutrients for dogs and cats. Although the quality of raw materials and food formulation are usually the main points considered, processing also have an important role (Tran et al., 2008), especially the grinding and extrusion conditions (Bazolli et al., 2015).

The grinding of raw material promotes the reduction of the ingredients particle size, which aims to provide more homogeneous mixing and facilitate the extrusion (Fraiha et al., 2005). The main equipment used to grind is the hammer mill, of which efficiency varies depending on the ingredient and chemical composition of the food, the screen open area, the configuration, number, and peripheral speed of the hammers, the presence and configuration of the impact zones, and the air flux inside the milling chamber (Cowell, 2000). Particle reduction is relevant to production cost (Amerah et al., 2008), and due this is important to optimize the process, considering the food utilization for animals and the efficiency of the extrusion processing. The extruder production efficiency, starch gelatinization, kibble expansion rate and appearance are influenced by the raw material particle size (Desrumaux et al. 1998; Mathew et al., 1999). Another effect of grinding is the ability to induce changes in the digestibility of nutrients, as observed in several species (Amerah et al. 2008; Wondra et al., 1995) including dogs (Hilcko et al., 2009; Bazolli et al., 2015). However, this information is not available for cats fed extruded kibble diets. In general, it is assumed that small raw material particle sizes increase the gelatinization of the starch during extrusion and the nutrient digestibility. This concept has been used by the pet food industry, but scientific data are necessary to allow more driven decisions balancing the possible nutritional benefits with reduced production cost.

Another critical point in the production of pet food is the extrusion. In the extruder, specific mechanical energy (SME) via main drive motor and specific thermal energy (STE) from steam and water injection are applied to the mash, inducing important physiochemical modifications on the raw material (Guy, 2001; Gibson and Alavi, 2013;). Variable operation conditions as well as hardware configurations are usually adjusted to maximize productivity, reduce production costs and improve kibble formation and appearance. The hardware configuration, specially the parameters related with higher or lower resistance to mash flow directly determines the residence time, shear force, temperature and pressure of the mash inside the extruder barrel, parameters that in turn will determine starch gelatinization, and kibble expansion and physical attributes (Riaz, 2007; Carciofi et al., 2012). The resistance against mash flow in extruder is important to allow adequate SME transference (Deffenbaugh, 2007), and it is usually controlled by screw and die configuration. The die configuration will determine the extruder open area, and ultimate the restriction of the mash to flow out extruder. It is defined according to production rate, food composition, and the desired characteristics of the final kibble (Riaz, 2007). Unfortunately, the authors were unable to find scientific publications on the implications of the extruder open area on kibble formation and the nutritional aspects of extruded foods for cats.

Taken these in consideration, the present study aimed to evaluate the implications of two raw material particle sizes and six extruder open areas on the extrusion traits, kibble, starch gelatinization degree, reactive lysine content, and digestibility of a cat food formulation.

2. Material and Methods

2.1. Diets and experimental design

The study was organized in a 6 x 2 factorial arrangement of treatments, composed by six extruder open areas (Eoa) and two raw material particle sizes, totaling 12 treatments. A cat food was formulated (Table 1) for maintenance according to the nutritional recommendations of the European Pet Food Industry Federation (FEDIAF, 2014). A single lot of ingredients was mixed and ground in two different particle sizes, using a hammer mill (Sistema Tigre de Mistura e Moagem, Tigre, Sao Paulo, Brazil) fitted with two different sieve screens sizes: 1.2 or 0.5 mm. The mean geometric diameters (MGD) of the raw materials were analyzed according to Zanotto and Bellaver (1996), modifying the screen sizes on the test: 1 mm; 0.840 mm; 0.595 mm; 0.500 mm; 0.420 mm; 0.297 mm; 0.210 mm; 0.125 mm; and plate. The program Gransuave (Embrapa, Brasilia, Brazil) was used to calculated the MGD values (Table 2). These two raw material mixtures were extruded using six different die configurations, targeting the following six extruder open areas: 59; 118; 177; 236; 294; 353 mm^2/ton/h.

The extruder open area (mm^2/ton/h) was calculated as:

$$\text{Extruder open area} = \frac{\text{Die open area (mm2)}\times 1000}{\text{Extruder output mass (kg/h)}}$$

Table 1

Ingredient and chemical composition of the experimental cat foods

Item		***g/kg, as-fed basis***
Maize		408.0
Poultry by-product meal		338.0
Corn gluten meal		104.5
Beet pulp		40.0
Sodium Chloride		5.0
Potassium Chloride		4.0
Vitamin and mineral premix[a]		5.0
Choline Chloride		3.5
L-lysine		2.5
DL-Methionine		2.0
Taurine		1.6
Antioxidant [b]		0.5
Mold Inhibitor [c]		0.4
Poultry fat		75.0
Palatant enhancer[d]		10.0
Chemical composition (g/kg, DM-basis) of the ground raw material with hammer mill screen sieve of 0.5 or 1.2 mm, excluding the poultry fat and palatant enhancer		
	0.5	***1.2***
Moisture	53.7	81.5
Ash	80.4	76.1
Crude protein	367.1	356.1
Starch	32.9	34.2

[a] Added per kg of diet: vitamin A 10.000 IU; vitamin D_3 1.350 IU; vitamin E 60 mg; vitamin K_3 0.5 mg; vitamin B1 15 mg; vitamin B2 8 mg; vitamin B6 10 mg; vitamin B12 75 mcg; pantothenic acid 20 mg; folic acid 1.2 mg; biotin 0.12 mg, niacin 80 mg; copper 5 mg; iron 60 mg; manganese 14 mg; iodine 1.2 mg; zinc 125 mg; selenium 0.2 mg.

[b] Petox: butyl hydroxyanisole; butylated hydroxytoluene; citric acid and phosphoric acid. Kemin do Brasil Ltda, Indaiatuba, Brazil.

[c] Shield: calcium propiante. Kemin do Brasil Ltda, Indaiatuba, Brazil.

[d] Liquid palatant: AFB do Brasil, Jaguariúna, Brazil.

Table 2

Particle size distribution of the raw material mixtures and ground with screen sieve sizes of 0.5 and 1.2 mm

Item	**Hammer mill screen sieve size (mm)**	
	0.5	1.2
Sieve screen size *(µm)*	*Retained particles (%)*	
1000	0.0	0.0
840	0.0	0.0
595	0.0	3.0
500	0.0	5.0
420	0.0	9.9
297	2.0	13.9
210	43.9	35.6
125	52.0	29.7
plate	2.0	3.0
Mean geometric diameter (µm)	*195*	*254*
Geometric standard deviation (µm)	*1.33*	*1.58*

The diets were extruded in a single screw extruder (MEX 250, Manzoni, Campinas, Brazil), with a processing capacity of 250 kg/h, at the Feed Facility of the College of Agrarian and Veterinarian Science, UNESP – Univ Estadual Paulista, Jaboticabal, Brazil. The extruded screw had a diameter of 80 mm and five sections: initial - single flight and no steam lock; second – single flight and small steam lock; third – double flight uncut and small steam lock; fourth - double flight uncut and medium steam lock; fifth - double flight cut cone. Water was injected into the preconditioner at a rate of 12 L/h and the shaft speed was set at 21 rpm for all treatments. Thermal energy was implemented on the mash at preconditioner by direct steam infusion, targeting a mean preconditioning temperature of 80 ºC for all treatments. Raw material was fed into the preconditioner using a volumetric delivery system at a target rate of 120 kg/hr. For all treatments, the extruder screw speed was set to 535 rpm. No water or steam was injected into the extruder barrel. To obtain the desired extruder open area, the following dies open diameter were used: 7.1; 14.1; 21.3; 28.3; 35.3 and 42.4 mm^2.

For the extrusion of each treatment, the equipment was initially stabilized and the basal processing conditions established (minimum of 45 min). After this step, production parameters were recorded every 15 minutes, with four measurements per treatment, totaling four experimental units. The parameters registered were preconditioner exit temperature and moisture, motor load amperage, dough temperature, pressure before die, dough temperature at extruder exit, extruder output mass and bulk density after extruder and after dryer. Other parameters recorded included ambient temperature, working water temperature, mash feed temperature, and steam pressure. At each observation time, food samples were collected from the preconditioner, the extruder and the dryer and stored at -20 ºC for further analysis. Following the extrusion, the kibbles were dried in a forced air dryer system at 105 °C for 20 minutes.

With recorded data, the SME (kW-h/ton) was calculated for each treatment in accordance with Riaz (2007), with the following formula:

$$\text{SME}=\frac{(\sqrt{3}\times \text{Voltage}\times(\text{WA-EA})\times(\text{cosFi}\div 1000))\times 1000}{\text{Throughput}}$$

where;

Voltage = (220V)

WA = working amperage or the motor load during processing (A)

EA = motor on load amperage (A)

cosFi = power factor (0.76)

Throughput = raw material feed rate into the preconditioner (ton/h)

The STE (kW-h/ton) was calculated as the net thermal energy contributed via steam absorption by mash inside the preconditioner divided by the raw material throughput (ton/h). The net steam absorption (kg/h) was calculated from mass balance according to Riaz (2007), and corresponding thermal energy was calculated by multiplying by the steam enthalpy (kJ/kg) from steam tables and adjusting for average mash temperature inside the preconditioner. Finally, the Total Specific Energy (TSE; kW-h/ton) was obtained by the sum of the SME and STE. The water flash off after extruder was calculated by the difference between in barrel moisture and the moisture of kibbles collected immediately from extruder exit. It was assumed that the moisture measured in conditioner corresponded in barrel moisture, since no injection of water or steam into the extruder barrel.

2.2. Kibble macrostructure, starch gelatinization, in vitro digestibility, and reactive lysine.

For each treatment, the length (le), diameter (de) and mass (me) of 20 kibbles were measured with a vernier caliper and used to obtain the radial expansion ratio (RE), specific length (l_{sp}) and piece density (ρ), as described below:

$$RE=\frac{d_e^{\,2}}{d_d^{\,2}}$$

$$l_{sp} = \frac{le}{me} \quad (\mathrm{mm/g})$$

$$\rho = \frac{4\ me}{\pi \times de^2 \times le} \quad (\mathrm{kg/m^3})$$

where: d_d= die open area.

The hardness of the kibbles were evaluated by a cutting test. It was performed with a texturometer (TA-XT2 SMS, Stable Micro Systems, Godalming, UK) set to operation mode strength/compression, return to start option enabled, pre-test speed of 2 mm/s, speed during the test of 0.5 mm/s and speed before test of 10 mm/s. Twenty kibbles of each sample were used, with a probe with a blade set with a Warner Bratzler knife (heavy duty platform/blade set) with a cutting distance of 10 mm. The data were analyzed with the software Texture Expert (Stable Micro Systems, Godalming, UK). The cutting force was measured in 20 kibbles per treatment.

The *in vitro* digestibility of the organic matter (OM) was determined as describe by Hervera et al. (2007) in samples collected after the dryer of each treatment. Incubations conditions simulate the digestion process in two steps, stomach and small intestine, using an enzymatic system with the pepsin and pancreatin enzymes, respectively. Samples after dryer were also collected from each treatment for the measurement of the starch gelatinization, which was determined by the amyloglucosidase method (Sá et al., 2013).

Lysine reactive analysis was conducted to evaluate the influence of processing conditions on the dietary protein. For each of the two raw material particle sizes, the extremes extruder open areas (respectively 59 and 353 $\mathrm{mm^2}$/ton/h) were selected. In addition, a sample compounded by the two ground raw material mixtures, before extrusion was also analyzed, totaling five samples. The reactive lysine analysis was performed in the laboratory Agricultural Experiment Station Chemical Laboratories, which belongs to University of Missouri – Columbia, Missouri, USA and was conducted according to the method of O-methylisourea (OMLU) as described by Moughan and Rutherfurd (1996). In this method, lysine ε-free amino group is converted into homoarginine by using OMLU and reactive lysine is calculated from the molar amount of formed homoarginine. This analysis involved incubating the sample (5 mg) with 0.5 to 1 mL of OMLU in a water bath with shaking at 21 °C for three days.

2.3. In vivo digestibility protocol

Four diets were selected to be tested on cats, represented by the extremes extruder open areas (respectively 59 and 353 mm^2/ton/) of the two raw material particle sizes. They presented similar chemical composition, after coated with poultry fat and palatant enhancer. The diets had the following mean composition (as-fed basis): 50 g/kg of moisture; 335 g/kg of crude protein; 165 g/kg of fat; 271 g/kg of starch; 21 g/kg of crude fiber; 75 g/kg of ash.

Twenty-four cats, with 3.7 ± 2.9 years old and 4.0 ± 0.7 kg of body weight were used. The health of the animals were confirmed prior to the beginning of the test.

The study followed a randomized block design, with two blocks of 12 cats and 3 cats per food in each block, totaling 6 animals per treatment. The blocking factor was the period. All the procedures with animals were previously approved by the Ethics and Animal Welfare Committee of the College of

Agrarian and Veterinarian Science, UNESP – Univ Estadual Paulista, Jaboticabal, Brazil (protocol number 2070/16).

The total tract apparent digestibility of nutrients and energy were determined by the method of total feces collection without urine collection, according to recommendations of the FEDIAF (2014). Cats were fed their respective diets for 18 days; diet adaptation was done from days 1 to 10, and total fecal collection for digestibility from days 11 to 18.

During the adaptation period, the cats remained from 18:00 h to 08:00 h individually housed in metabolic cages of stainless steel with dimensions of 0.9 m x 0.8 m x 1.0 m, equipped with apparatus to separate feces and urine for collection, and from 08:00 h to 18:00 h in a collective cattery to socialize and exercise. During the collection period, cats remained restricted to their metabolic cages.

The amount of food supplied was individually calculated according to the energy value of the food and the energy requirement of the animal (100 kcal x $kg^{0,67}$; NRC, 2006). Food was provided once a day, being provided at 18:00 h when the cats were restricted to the cages. Leftovers were collected on the next morning (08:00 h), weighted and the consumption registered. Water was available *ad libitum*.

Feces were totally collected at least twice a day, weighed and stored at -15 ^{o}C for further analysis.

At the end of the collection period, feces were thawed, mixed and dried using a forced-air oven (Fanem, São Paulo, Brazil) at 55 °C for 72 h. Food and feces were ground in a cutting mill (Mod MA-350, Marconi, Piracicaba, Brazil) fitted with a 1 mm screen and analysed by oven-drying for dry matter (DM) (method 934.01), by muffle furnace incineration for ash content (method 942.05), crude protein (CP) using a Leco nitrogen/protein determination, and with a Soxhlet apparatus extraction for acid hydrolysed ether extract (method 954.02), following the methods described by the Association of Official Analytical Chemists (AOAC, 1995). OM of the samples was calculated as DM minus ash. The starch content was determined according to Hendrix (1993). Gross energy (GE) of diets and feces were determined by an adiabatic bomb calorimeter (model 1281, Parr Instrument, USA). All samples were analyzed in duplicate and the analyses were repeated when the variation among duplicates was greater than 5%.

The quality of the feces were indirectly evaluated during the collection period by the following score 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; 5 = hard, dry pellets – small, hard mass.

2.4. Statistical analysis

Data of the extrusion test were analyzed as a 6 x 2 factorial arrangement of treatments, in a completely randomized design. The experimental unit was considered the sample collected at each 15 min interval, with 4 repetitions per treatment. For kibble macrostructure and cutting force the experimental unit was one kibble, with 20 repetitions per treatment. Results were submitted to analysis of variance, model sums of squares were separated into the effects of the raw material particle size, Eoa and their interactions. When differences were found on F test, means were compared by polynomial contrasts. The results of digestibility in cats were analyzed as a 2 x 2 factorial arrangement of treatments, in a completely randomized block design. The experimental unit was considered one cat, with six repetitions per treatment. Results were submitted to analysis of variance, model sums of squares were separated into the effects of the raw material particle size, Eoa and their interactions. The results of reactive lysine was analyzed in a completely randomized design, with five treatments. The ground raw material was considered the reference treatment, according to Dunnet test. All data were found to comply with the assumptions of the ANOVA model, and were analyzed using GLM procedure of SAS software (SAS Inst. Inc., Cary, NC). Values of $P < 0.05$ was considered significant. For digestibility data, an interaction was considered significant when $P < 0.15$.

3. Results

3.1. Extrusion traits

The raw material ground with a screen sieve size of 1.2 mm presented 31.8% of particles greater than 297 µm, while the raw material ground with the 0.5mm sieve size less than 2%. On the other side, particles lower than 210 µm amounted 54% of the raw material grond with 0.5mm, and 32.7% of the ground with the screen size of 1.2 mm. These particle distributions resulted in MGD of 195 µm and 254 µm, respectively (Table 2).

During the extrusion runs the mean production for all treatments were 117.8 ± 1.14 kg/h (as-fed basis), very close to the established target rate of 120 kg/h. Due this, the obtained Eoa were close to the target established in the experiment (Table 3). During the production of the coarse ground diets (MGD of 254 µm) a slightly higher production was obtained, resulting in smaller extruder open area for these treatments ($P<0.001$). In both MGD, a linear increase on Eoa was verified ($P<0.001$).

Preconditioner temperature did not change according to MGD or Eoa. The extruder in barrel moisture was a little higher for the coarse ground diets ($P<0.001$), and increased linearly with the extruder open area ($P<0.004$). This increase was not expected, but was small. An interaction between particle size and Eoa was observed for the motor load, mash pressure before die, and the temperature of the last extruder jacket ($P<0.001$). The pressure before die was smaller for the large MGD diets ($P<0.001$), with a mean value of 20 bars lower. The pressure of the mass reduced quadratically ($P<0.001$) with the increase on Eoa. The relation between mass pressure and Eoa was described for the 195 µm MGD food as: Mass pressure = $97.29 – 0.28(\mathrm{Eoa}) + 0.0005(\mathrm{Eoa}^2)$; $R^2 = 0.89$; $P<0.000$. For the 254 µm MGD food, it was: Mass pressure = $60.01 - 0.16(\mathrm{Eoa}) - 0.0003(\mathrm{Eoa}^2)$; $R^2 = 0.58$; $P<0.001$. The derivation of this equations resulted on minimum theoretical pressure with an extruder open area of 350mm^2/ton/h for the 195 µm MGD food, and 267 mm^2/ton/h for the 254 µm MGD.

Table 3

Processing parameters of a cat food ground to two mean geometric diameters (MGD) of raw materials and extruded with six extruder open areas (Eoa)

Item	MGD[a] (µm)	Target extruder open area (mm^2/ton/h)						Mean	SEM[b]	P value			Contrast[e]	
		59	118	177	236	294	353			MGD	Eoa[c]	MGD x Eoa[d]	Lin	Quad
Extruder open area (mm^2/ton/h)														
	195	64.4	126.0	190.0	261.5	322.3	372.6	222.8	22.5	<0.001	<0.001	<0.001	<0.001	-
	254	56.6	112.4	172.4	227.6	275.1	350.6	199.1	20.5				<0.001	-
	Mean	60.5	119.2	181.2	244.5[f]	298.7[f]	361.6							
Preconditioner														
Temperature (ºC)														
	195	79.7	77.8	78.8	78.7	79.5	80.2	79.1	0.3	0.102	0.066	0.172		
	254	76.9	78.8	78.4	78.1	78.4	80.3	78.5	0.4					
	Mean	78.3	78.3	78.6	78.4	79.0	80.3							
Extruder														
In barrel moisture (%)														
	195	24.7	25.9	25.2	27.1	25.4	25.2	25.6	0.2	0.001	0.003	0.001	0.004	-
	254	26.4	25.5	26.6	26.9	27.6	26.8	26.6	0.2				<.001	-
	Mean	25.6[f]	25.7	25.9[f]	27.0[f]	26.5[f]	26.0[f]							
Motor load (A)														
	195	42.5	40.4	39.3	37.5	37.5	37.6	39.1	0.4	<0.001	<0.001	0.005	<0.001	<0.001
	254	44.0	40.4	39.3	39.0	40.0	38.4	40.2	0.4				<0.001	<0.001
	Mean	43.2[f]	40.4	39.3	38.2[f]	38.6[f]	38.0							
Pressure before die (bar)														
	195	83.0	70.4	63.3	57.0	57.0	57.0	64.6	2.1	<0.001	<0.001	<0.001	<0.001	<0.001
	254	51.2	46.1	44.0	43.3	35.1	47.0	44.4	1.1				<0.001	<0.001
	Mean	67.0[f]	58.2[f]	53.3[f]	50.1[f]	46.0[f]	51.6[f]							
Last jacket temperature (°C)														
	195	141	130	132	127	130	130	132	1.0	<0.001	<0.001	<0.001	<0.001	<0.001
	254	139	132	128	128	123	124	128	1.2				<0.001	<0.001

	Mean	140	131	130[f]	128	127[f]	127[f]							
Mass temperature before die (ºC)														
	195	116	116	119	124	124	122	122	1.4	0.656	0.409	0.066	-	0.024
	254	121	125	122	124	116	123	121	1.2				-	-
	Mean	118.5	120,5	120	124	120	122.5							
Water flash off (%)														
	195	4.4	3.3	3.5	3.4	1.7	2.3	3.0	0.3	0.871	<0.001	0.947		
	254	4.3	3.3	3.0	3.7	2.2	1.9	3.0	0.2					
	Mean	4.4	3.3	3.3	3.6	2.0	2.1						<0.001	-
Energy balance (kW-h/ton)														
Specific mechanical energy														
	195	28.9	22.9	20.1	15.9	15.7	15.5	19.8	1.1	0.694	<0.001	0.007	<0.001	<0.001
	254	28.9	20.5	18.2	17.4	18.5	16.4	20.0	0.9				<0.001	<0.001
	Mean	28.9	21.7[f]	19.1	16.7	17.1[f]	15.9							
Specific thermal energy														
	195	98.3	108.4	102.7	114.8	101.3	102.6	104.7	1.7	0.710	0.018	0.002	-	0.035
	254	103.6	94.7	103.2	106.6	113.2	102.9	104.0	1.4				0.011	-
	Mean	100.9	101.6[f]	102.9	110.7	107.3[f]	102.8							
Total specific energy														
	195	127.3	131.4	122.6	130.7	117.0	118.0	124.5	8.7	<0.001	<0.001	<0.001	<0.001	<0.001
	254	132.6	115.1	121.5	124.0	131.8	119.3	123.6	1.6				-	-
	Mean	129.9	123.3[f]	122.1[f]	126.1[f]	124.4[f]	118.7[f]							
STE/SME ratio														
	195	3.4	4.7	5.1	7.2	6.5	6.8	5.6	0.3	0.189	<0.001	0.077		
	254	3.6	4.6	5.7	6.1	6.1	6.3	5.4	0.2					
	Mean	3.5	4.7	5.4	6.7[f]	6.3	6.5						<0.001	<0.001

[a] MGD = mean geometric diameter of the raw material.

[b] SEM= standard error of the mean (n= 4 repetitions per treatment).

[c] Eoa = extruder open area.

[d] MGD x Eoa = interaction between raw material particle size and extruder open area.

[e] Linear or quadratic effects of the extruder open area.

[f] MGD in the same column considering each item were different (P<0.05).

Greater temperatures of the last jacket were verified for the foods with small MGD ($P<0.001$). The relation between jacket temperature and Eoa was describe for the 195 µm MGD as: Jacket temperature = 147.47 – 0.15(Eoa) + 0.0003(Eoa^2); $R^2 = 0.70$; $P<0.001$. For the 254 µm MGD food was: Jacket temperature = 147.21 - 0.17(Eoa) + 0.0003(Eoa^2); $R^2 = 0.69$; $P<.001$. The derivation of these equations resulted on minimum theoretical jacket temperature for an Eoa of 250 mm^2/ton/h for 195 µm MGD food, and 284 mm^2/ton/h for the 254 µm MGD food.

The temperature of the mass before the die was similar among treatments. Water flash off corresponds to the difference between in barrel moisture and the kibble moisture immediately after extrusion, it was similar between raw material particle sizes, and decreased linearly with the increase on Eoa: Water flash off = 4.47 - 0.005(Eoa); $R^2 = 0.44$; $P<0.001$.

On energy balance, SME, STE and TSE application presented an interaction between raw material particle size and Eoa ($P<0.001$). For SME and STE, no effect on raw material MGD were verified, only of Eoa ($P<0.02$). For both MGD, there was a quadratic reduction on SME application with the increase on Eoa, with a mean reduction of approximately 45%. The relation between SME and Eoa for the 195 µm MGD food was described by the following equation: SME = 35.78 - 0.13(Eoa) + 0.0002(Eoa^2); $R^2 = 0.92$; $P<0.001$. For the 254 µm MGD food was: SME = 34.55 - 0.13(Eoa) - 0.0002(Eoa^2); $R^2 = 0.83$; $P<0.001$. The derivate of these equations were similar, with a minimum SME application with an Eoa of 325 mm^2/ton/h. For STE, although an increase was verified with the increase on Eoa ($P=0.018$), the variation was numerically low and possibly not relevant. Due to the reduction on the SME application, the TSE transference also reduced with the increase on Eoa ($P<0.001$), this reduction, however, was numerically small. An interaction between raw material MGD and Eoa was observed, with a quadratic reduction on TSE application for the 195 µm MGD food ($P<0.001$), but with similar TSE for the 254 µm MGD food, with values did not differ according to Eoa. The STE/SME ratio increased quadratically with the increase on the Eoa ($P<0.001$).

3.2. Kibble macrostructure, starch gelatinization and In vitro digestibility

A significant interaction ($P<0.01$) between raw material particle size and Eoa was observed for all analyzed kibble macrostructure characteristics (Table 4). Kibbles produced with the coarse ground raw material presented lower expansion, verified by higher bulk density, piece density, and specific length ($P<0.001$), and lower radial expansion ($P<0.001$). For the 195 µm MGD food, the kibbles bulk density increased from 237g/L to 441g/dL with the increase on Eoa (Bulk density = $180.7 + 0.98(\text{Eoa}) - 0.0008(\text{Eoa}^2)$; $R^2 = 0.95$; $P<0.001$). For the 254 µm MGD food, it increased from 272 g/L to 451 g/L (Bulk density = $219.7 + 1.31(\text{Eoa}) - 0.002(\text{Eoa}^2)$; $R^2 = 0.88$; $P<0.001$). The derivation of these equations resulted on maximum theoretical bulk density with an Eoa of 613 mm^2/ton/h and 328 mm^2/ton/h for the 195 and 254 µm MGD foods, respectively. Piece density also reduced quadratically, been described for the 195 µm MGD food as: Piece density = $0.27 + 0.004(\text{Eoa}) - 0.000001(\text{Eoa}^2)$; $R^2 = 0.64$; $P=0.016$. For the 254 µm MGD food, was: Piece density = $0.35 + 0.002(\text{Eoa}) - 0.000004(\text{Eoa}^2)$; $R^2 = 0.42$; $P<0.001$).

Table 4
Kibble macrostructure and characteristics of a cat food ground to two mean geometric diameters (MGD) of raw materials and extruded with six extruder open areas (Eoa)

Item	**MGD [a] (µm)**	**Target extruder open area (mm^2/ton/h)**						**Mean**	**SEM[b]**	**P value**			**Contrast [e]**	
		59	**118**	**177**	**236**	**294**	**353**			**MGD**	**Eoa [c]**	**MGD x Eoa [d]**	**Lin**	**Quad**
Bulk density after drier (g/L)														
	195	237	277	358	354	406	441	345	14.8	<0.001	<0.001	<0.001	<0.001	<0.001
	254	272	373	400	406	407	451	385	11.6				<0.001	<.0001
	Mean	255[f]	325[f]	379[f]	380[f]	407	446							
Piece density (g/ cm3)														
	195	0.35	0.41	0.52	0.50	0.54	0.60	0.49	0.01	<0.001	<0.001	<0.001	<0.001	0.016
	254	0.41	0.59	0.57	0.56	0.57	0.59	0.55	0.01				<0.001	<0.001
	Mean	0.38[f]	0.50[f]	0.54[f]	0.53[f]	0.55	0.60							
Specific length (cm/ g)														
	195	121.1	108.1	107.7	119.1	118.4	120.2	115.8	0.8	0.011	<0.001	0.006	0.007	<0.001
	254	118.4	108.0	116.7	120.0	122.0	123.0	118.0	0.7				<0.001	0.016
	Mean	119.7	108	112.2[f]	119.5	120.2	121.6							
Radial expansion rate														
	195	13.9	12.9	10.3	9.5	8.9	8.0	10.6	0.2	<0.001	<0.001	<0.001	<0.001	<0.001
	254	11.9	8.9	8.5	8.5	8.2	7.8	9.0	0.1				<0.001	<0.001
	Mean	12.9[f]	10.9[f]	9.4[f]	9.0[f]	8.5[f]	7.9							
Cutting force (kg.f)														
	195	2.5	2.6	2.6	2.8	4.0	3.5	3.0	0.1	<0.001	<0.001	<0.001	<0.001	-

254	2.7	2.6	2.4	2.2	2.3	2.5	2.5					0.013	0.015
Mean	2.8	2.6	2.5	2.5[f]	3.1[f]	3.0[f]							
Starch gelatinization (%)													
195	95.3	96.0	97.3	95.9	96.7	96.2	96.4	0.5	<0.001	0.866	0.341		
254	89.4	86.3	86.5	87.8	87.3	88.5	87.6	0.4				-	-
Mean	92.4	91.2	91.9	91.9	92.0	92,4							
In vitro digestibility of organic matter													
195	0.862	0.860	0.858	0.853	0.871	0.875	0.863	0.003	<0.001	0.611	0.210		
254	0.853	0.849	0.843	0.854	0.845	0.848	0.849	0.003					
Mean	0.856	0.855	0.851	0.854	0.858	0.862	0.856					-	-

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[a] MGD = mean geometric diameter of the raw material.
[b] SEM= standard error of the mean (n= 4 repetitions per treatment).
[c] Eoa = extruder open area.
[d] MGD x Eoa = interaction between raw material particle size and extruder open area.
[e] Linear or quadratic effects of the extruder open area.
[f] MGD in the same column considering each item were different (P<0.05).

The kibbles specific length increased quadratically with the increase on Eoa for both raw material particle size (For the 195 µm MGD: Specific length= 124.2 - 0.14(Eoa) + 0.0004(Eoa^2); $R^2 = 0.17$; $P<0.001$. For the 254 µm MGD: Specific length= 116.3 - 0.03(Eoa) - 0.0002(Eoa^2); $R^2 = 0.22$; $P<0.016$). The radial expansion rate reduced quadratically after the increase on Eoa, with a mean reduction of 39.7% (For the 195 µm MGD food: Radial expansion rate = 16.25 - 0.04(Eoa) - 0.0001(Eoa^2); $R^2 = 0.79$; $P<0.001$. For the 254 µm MGD food: Radial expansion rate = 13.5 - 0.04(Eoa) - 0.00007(Eao^2); $R^2 = 0.65$; $P<0.001$). The derivation of these equations resulted on maximum theoretical radial expansion rate with an open area of 200 mm^2/ton/h and 286 mm^2/ton/h, for the 195 and 254 µm MGD foods, respectively. The cutting force exhibited a decrease followed by an increase for the 254 µm MGD food ($P<0.015$). However, for the 195 µm MGD food the kibbles cutting force increased linear with the increase on Eoa (Cutting force = 2.37 + 0.0006 Eoa; $R^2 = 0.31$; $P<0.001$).

The starch gelatinization and *in vitro* digestibility of OM were not influenced by Eoa, only by raw material particle size. Foods with 195 µm of raw material MGD presented starch gelatinization degree almost 9 points of percentage higher than the foods with 254 µm MGD ($P<0.001$). A higher *in vitro* digestibility of OM was also verified for the 195 µm MGD foods ($P<0.001$).

3.3. Reactive lysine content

When the extremes Eoa for each raw material MGD were compared with the raw material mixture, a significant increase on total lysine content was verified for all extruded diets ($P<0.05$; Table 5). The reactive lysine content also increased for all extruded diets (P<0.05), but not the linked lysine amount and the linked lysine: total lysine ratio.

Table 5

Total lysine, reactive lysine, and linked lysine content of the raw material mixture and cat foods ground to two mean geometric diameters (MGD) and extruded with different extruder open areas (mm^2/ton/h). Values in g/kg of dry matter

Item	**Raw material**	**MGD of 195 µm**		**MGD of 254 µm**	
		59 mm^2/ton/h	**353 mm^2/ton/h**	**59 mm^2/ton/h**	**353 mm^2/ton/h**
Total lysine	1.51±0.01^a	1.93±0.02^b	1.82±0.02^b	1.80±0.04^b	1.94±0.01^b
Reactive lysine	1.42±0.00^a	1.81±0.03^b	1.70±0.00^b	1.60±0.04^b	1.81±0.01^b
Linked lysine	0.10±0.01	0.13±0.01	0.12±0.01	0.12±0.00	0.13±0.00
Linked lysine: Total lysine ratio	0.94±0.01	0.93±0.00	0.94±0.00	0.93±0.00	0.93±0.00

$^{a,\,b}$ Means in the row no sharing a common superscript differ ($P<0.05$).

3.4. Nutrient intake, apparent total tract digestibility and fecal characteristics

All of the diets were readily consumed by the cats, without episodes of refusal, diarrhea or vomiting. During the experiment the body weight of the cats did not change ($P>0.05$; data not shown). Cats presented similar food intake, without differences among diets (Table 6). For the cats digestibility study, interaction between raw material particle size and Eoa were verified for DM, OM, and crude protein digestibility ($P<0.15$). For the extruded foods with more restrictive output area (Eoa of 59 mm^2/ton/h), the raw material ground to 195 µm of MGD resulted in lower DM, OM, and crude protein digestibility than the more coarser ground food (254 µm of MGD).

Feces production, on DM and as-is basis, and moisture content were similar between cats fed the four diets. On fecal score, a raw material particle size effect was verified, with firmer feces for cats fed the coarse ground food ($P=0.016$).

Table 6

Intake and apparent total tract digestibility, and fecal characteristics of cat foods ground to two mean geometric diameters (MGD) of raw materials and extruded with two extruder open areas (Eoa)

Item	**MGD (µm)**[a]	**Target extruder open area (mm^2/ton/h)**		**Mean**	**SEM**[b]	**P value**		
		59	**353**			**MGD**	**Eoa** [c]	**MGD x Eoa** [d]
Food intake (g of DM/kg/d)								
	195	11.5	12.0	11.8	0.8	0.566	0.709	0.998
	254	10.8	11.3	11.1	0.9			
	Mean	11.2	11.7					
Apparent total tract digestibility								
Dry matter	195	0.768^{Ef}	0.793^{Ee}	0.781	1.3	0.045	0.151	0.115
	254	0.804^{Ee}	0.803^{Ee}	0.804	0.1			
	Mean	0.786	0.798					
Organic matter								
	195	0.812^{Ef}	0.832^{Ee}	0.822	1.0	0.032	0.337	0.097
	254	0.844^{Ee}	0.841^{Ee}	0.843	0.2			
	Mean	0.828	0.837					
Crude fat								
	195	0.888	0.893	0.891	0.3	0.222	0.268	0.785
	254	0.898	0.915	0.907	0.8			
	Mean	0.893	0.904					
Crude protein								
	195	0.801^{Ef}	0.831^{Ee}	0.816	1.5	0.025	0.434	0.037
	254	0.851^{Ee}	0.836^{Ee}	0.844	0.7			
	Mean	0.826	0.834					
Starch								
	195	0.999	0.999	0.999	0.01	0.648	0.891	0.808

	254	0.999	0.999	0.999	0.00			
	Mean	0.999	0.999	0.999				
Gross energy								
	195	0.834	0.854	0.844	1.0	0.052	0.266	0.111
	254	0.870	0.860	0.865	0.5			
	Mean	0.852	0.857					
Fecal characteristics								
Excretion (g/kg/d, DM basis)								
	195	2.7	2.5	2.6	0.22	0.194	0.933	0.595
	254	2.1	2.6	2.2	0.18			
	Mean	2.4	2.6					
Excretion (g/kg/d, As-is basis)								
	195	8.3	8.2	8.3	0.8	0.194	0.664	0.594
	254	6.2	7.3	6.8	0.7			
	Mean	7.3	7.8					
Moisture (g/kg)								
	195	0.649	0.652	0.651	0.014	0.512	0.338	0.403
	254	0.616	0.656	0.636	0.016			
	Mean	0.633	0.654					
Score								
	195	3.5	3.4	3.5	0.1	0.016	0.415	0.674
	254	4.1	3.9	4.0	0.2			
	Mean	3.8	3.7					

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[a] MGD = mean geometric diameter of the raw material.

[b] SEM= standard error of the mean (n= 6 cats per food).

[c] Eoa = extruder open area.

[d] MGD x Eoa = interaction between raw material particle size and extruder open area.

[E] Means in the row not sharing a common uppercase letter differ (P<0.05).

[e,f] Means in the column not sharing a common uppercase letter differ. Comparison valid inside a variable (P<0.05).

4. Discussion

The differences in raw material particle distribution resulted in alterations of practically all extrusion parameters, with the exception of the extrusion temperature and water flash off. Among the parameters that varied, extruder in barrel moisture is dependent of water and steam infusion, and not related to MGD. This variation, however, was a little higher for only two or three treatments, with the remaining showing close values. Water is a fluidizing agent, and variations on in barrel moisture significantly alter extrusion parameters (Pansawat et al., 2008). Due this, attempting was done to avoid variations on in barrel moisture and the observed changes were unexpected.

The higher pressure before die and last jacket temperature for the 195 µm MGD food may be explained by a higher viscosity generation, increasing resistance to flow and shear transference to the dough (Riaz, 2001). Viscosity is a characteristic of swollen starch, which molten with the application of temperature, pressure, and shear on the extrusion system (Crane et al., 2000; Tran et al., 2008). This was greater for the lower particle size raw material probably due to the increase efficiency of starch granules hydration when fine ground, with lower MGD (Bazolli, et al., 2015). Due to the limited residency time of food particles on the extrusion system, the hydration of the starch granules on the center of the ingredients is important, and dependent of the geometric diameter of the materials (Móscicki and Wójtowicz, 2011).

Observing the energy balance, although the mean value of SME application was similar between raw material particle sizes, on the food with 254 µm MGD the reduction of SME application with the increase on Eoa was smaller than the verified for the 195 µm MGD. This suggests that the mass flow on extruder varied according to the particle size of raw materials, when the die restriction is low (elevated Eoa) the resistance to flow seems to be higher for coarser particles. On the other hand, when the restriction is high, with small Eoa, the differences between MGD on mass flow and SME application were not relevant.

On the same way as verified for the extrusion traits, the MGD of raw materials influenced all parameters of kibble macrostructure. Important differences on kibble expansion were verified, with higher values for the 195 µm MGD foods. This higher kibble expansion is probably explained by several outcomes, including the greater starch gelatinization, jacket temperature, and mass pressure of the 195 µm MGD foods. Together with the smaller geometric size of raw materials, these results favored the formation of a mass of molten starch, which is easily deformed by the water vaporization out the extruder, creating the inner cell structure of the kibbles (Guy, 2001). This is a relevant aspect for pet food companies, which target the production of well-formed kibbles, with specific macro and microstructure characteristics. The kibbles with greater expansion and higher gelatinization of the 195 µm MGD foods, however, were harder, as they presented higher cutting force than the 254 µm MGD foods. This might be consequent of the kibbles cell structure characteristic, with more or less resistant cell walls to deformation (Karkle, 2011). Unfortunately, the cellular structure was not evaluated on the present study.

It is interesting that Eoa did not influence starch gelatinization and in vitro digestibility of OM, only the raw material particle size. These highlights the importance of ingredients MGD for the extrusion of cat foods, as already shown for dogs (Bazolli, et al., 2015). Comparing the Eoa of 59 and 353 mm^2/ton/h, even with the reduction of 13.4kW-h/t for the 195 µm MGD foods, and 12.5kW-h/t for the 254 µm MGD foods on SME application, enough energy was transferred to the mass to ensure cooking and *in vitro* digestibility. This can be explained by STE being the main energy applied to mass, being 5.5 times greater than the SME application on the present study. Moreover, authors were unable to find studies that quantified the amount of SME or STE required to promote the gelatinization of the starch of cat food formulations with maize as the cereal source. These results suggests that 15 kW-h/t of SME with approximately 118 kW-h/t of TSE were enough to process this particular cat food recipe, but the results of starch gelatinization and *in vitro* digestibility will be dependent of the MGD of the raw materials.

The restriction of mass flow by the die configuration influenced practically all extrusion parameters. The reduction on motor load, mass pressure, jacket temperature, and SME application with the increase on Eoa is explained by the lower limitation, favoring the flow of the mass out extruder (Camire et al., 1991). On the extrusion system, the mechanical energy is provided by the extruder screw rotation, its application to the mass on processing, however, depends on several aspects including food chemical composition, production rate, and the configuration of screw elements, jacket and the die (Riaz, 2007). Considering that the main objective is the SME transference, as the resulted shear force will explain the changes on temperature and pressure, data of the present study suggest that an Eoa of 325 mm^2/ton/h will minimize the SME application, and higher restrictions are necessary to improve energy transference and kibble formation.

Bulk density, for example, is usually a critical parameter adopted by pet food companies for product development and processing monitoring. In the present study, the coefficient of determination of the polynomial regressions between Eoa and bulk density were high, respectively 0.95 for the 195 µm MGD foods, and 0.88 for the 254 µm MGD foods, reinforcing the strengthen relationship between these variables and the importance of die conformation for processing configuration. The STE application varied according only to Eoa. There was not different inclusion of steam or water among the diets, so the differences on STE were related with mechanical energy dissipation resulted by shear force due die open area and was consequent to fluctuations on the processing conditions. The mean value, however, was numerically close and probably did not interfere on the obtained results.

The increase in lysine content after the extrusion of the diets was previously reported by Sá (2015) in extruded diets for dogs and cats and in poultries by Son and Ravindran (2012) and Ahmed et al. (2014). This phenomenon can be attributed to the breakdown of high-energy covalent bonds such as disulfide together with breaking weak covalent bonds such as hydrophobic bonds, promoted during the extrusion process (Bhattacharya and Hanna, 1988; Hayakawa et al, 1996, Camiré, 2000). Lysine is routinely used as an indicator for the evaluation of protein quality deterioration by the Maillard reaction (Hendriks et al., 1999; Tran et al., 2011), which corresponds a non-enzymic browning and flavouring reaction that can occur during the processing and storage of foods. In general, the rate of Maillard reaction increases with temperature and time, resulting in a decrease in reactive lysine content (Mauron, 1981). The data of Tran et al. (2008), however, did not show the effect of lower or higher extrusion temperature on total and reactive lysine content in foods or its ingredients. Lankhorst et al. (2007) reported that extrusion of an experimental dog food had no effect on total lysine content, but reactive lysine content was increased by 20.3 % after extrusion at 110°C and 35.6 % after extrusion at 150°C. In our study the complexation of the carbonyl group of lysine (bound lysine) did not increase after the processing, not varying according particle size or Eoa, suggesting that the process did not induce further changes on amino acid or the method is not sensible enough to evaluate the effects of extrusion on them. According to Rooijen et al. (2013) additional studies are, required to assess the importance of extrusion in pet foods.

Studies in dogs about raw material particle size found increase on nutrient digestibility with the reduction of MGD (Hilcko, et al., 2009; Bazolli, et al., 2015). The present study did not confirm it for cats. However, only two particle sizes of the raw material were evaluated, and the treatment with greater raw material particle size presented a MGD of 254 µm, smaller than the lower treatment tested by Bazolli et al. (2015) that was 360 µm for the maize based diet and Hilcko et al. (2009), that was 468 µm. This way, it is possible that even the coarse ground raw material used in the present study was reduced enough to be properly digested by the cats, as only 8% of the raw material particles were greater than 500 µm on the 254 µm MGD foods. The more intensive processing, represented by the combination of smaller MGD and more restrictive Eoa had, on the opposite, lowered the protein digestibility, which probably explain the lower DM and OM digestibilities also verified for this food. Apart from the destruction of some vitamins, the reactions of food proteins are the main chemical reactions that occur during food processing (Moughan, 2003). Exposure to denaturation temperatures may increase digestibility of native proteins by unfolding the polypeptide chain and rendering the protein more susceptible to digestive enzymes. On the other hand, when proteins are exposed to higher temperatures, digestibility could be reduced as observed by Opstvedt (1989) in fish feeds. Undesirable effects of heat treatment involve destruction of amino acids, racemization of amino acids, inter- and extra-peptide linkages and a number of chemical reactions such as Maillard reactions and cross linking reactions of protein-protein, protein lipid and protein-carbohydrate complexes (Björck and Asp, 1983). It also enables formation of bonds that resist in-vivo hydrolysis. These reactions between amino acids and other compounds and intra-molecular reactions between amino acids within the protein molecule that cannot be split by digestive enzymes (Mauron, 1990).

Starch gelatinization is an analysis that can be used to monitor the extrusion processing. Considering the four foods fed to cats, 88.5% of starch gelatinization seems to be adequate to nutrient digestibility and the combination of very high starch gelatinization (>95%) and very low MGD may be undesirable. It is interesting that a very high gelatinization but of a raw material mixture that is not too fine grind (254 µm MGD) did not reduce the protein digestibility, probably due to not favor the development of damage to proteins. All these observations, however, are relatively new. No other studies could be localized that compared raw material particle size, extrusion conditions, or starch gelatinization for kibble diets for cats. Another observation was that the kibble macrostructure characteristics did not have relation with nutrient digestibility. For example, the bulk of density of the diets fed to the cats varied from 237 to 451 g/L, without differences.

Some limitations of the present study must also be considered, only one formulation based on maize was tested, and for other cereals the particle reduction and extrusion conditions might be different, as verified for dogs by Bazolli et al. (2015) comparing maize, sorghum and rice. The digestibility was studied in only 4 diets, and not in all the 12 experimental treatments. The method of *in vitro* digestibility of OM utilized on the experiment was validated for dogs (Hervera, et al., 2007), but not for cats. The method resulted in higher digestibility for the lower MGD foods, but this was not verified on cats. As in the present study only the total tract apparent nutrient digestibility was evaluated, the interference of OM fermentation on the colon was not verified and is possible that the ileal digestibility of the diets were different.

5. Conclusions

The raw material particle size and extruder open area have important influence on extrusion parameters, SME transference and kibble formation. Smaller MGD and more restrictive Eoa induce higher kibble expansion, and starch gelatinization. However, nutrient digestibility by cats do not improve when the starch gelatinization increase from 88.5% or the MGD reduce below 254 µm.

6. References

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CAPÍTULO 3

Extruder open area and screw configuration on processing parameters, kibble traits, and digestibility of extruded cat foods [1]

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Abstract

The present study evaluated the effect of different extruder configuration on the processing parameters, starch gelatinization, *in vitro* digestibility of organic matter, kibble macrostructure, and *in vivo* nutrient digestibility (IVND) of extruded cat foods. The experiment followed a 4 x 2 factorial arrangements of treatments, with 4 extruder open areas (Eoa) and 2 restrictions after extruder screw (RASc), a total of 8 treatments. A single food was formulated for cat maintenance, and processed in a single screen extruder with a combination of 60% or 90% RASc, and 4 Eoa: 59, 118, 236, 353 mm^2/ton/h. All other extruder software and hardware parameters was unchanged during the study. IVND was measured by the total collection of feces method on the diets processed with 90% RASc and 353 mm^2/ton/h, and 60% RASc and 59 mm^2/ton/h, using 6 cats per diet. Data were submitted to analysis of variance considering RASc and Eoa effects, as well their interactions. Means were compared by polynomial contrasts ($P<0.05$). For motor load was verified an interaction between RASc and Eoa, with greater means for the 90% RASc diets and a reduction with increase Eoa. In barrel moisture was similar among treatments. Die pressure did not change according to RASc, only Eoa, it decreased. Flash off was different only in diets with 90% of RASc and decreased with the increase Eoa. There was a reduction in mean temperatures of forth extruder jacket with increase Eoa. Specific mechanical energy (SME) application did not suffer influence of the interaction between RASc and Eoa, but was higher for 90% RASc diets. Specific thermal energy was similar among treatments. Total specific energy application was similar for all Eoa on 60% RASc, but decreased with the increase on Eoa for 90% RASc. A significant effect of Eoa was observed for all analyzed kibble traits. There was an interaction for bulk and piece density and both item showed an increase with increase on Eoa. Higher expansion were verified for 90% RASc diets. Starch gelatinization and *in vitro* digestibility were not influenced by the interaction of RASc and Eoa. *In vitro* digestibility changed according to Eoa, but IVND did not alter with Eoa or RASc. To summarize, RASc an Eoa had significant impact on extrusion parameters, SME transference and kibble formation. IVND was not influenced by kibble expansion.

Keywords: cats, digestibility, extrusion, extruder configuration, open area.

Abbreviation: Eoa, extruder open area; IVND, *in vivo* nutrient digestibility; RASc, restriction after extruder screw, SME: specific mechanical energy.

1. Introduction

Approximately 95% of the dry foods for dogs and cats are produced with thermoplastic extrusion technology (Spears and Fahey, 2004). The extrusion process is widely adopted by the pet food industry due to its flexibility, allowing the use of various ingredients and co-products of plant and animal origin, elevated thermodynamic efficiency, low operational cost and low space required per metric unit of production (Lankhorst et al., 2007). Moreover, extrusion promotes beneficial characteristics on the foods, including the formation of desired physical form and features, the inactivation of thermolabile anti-nutritional factors, increased product shelf life, improved nutrient digestibility and enhanced palatability (Lankhorst et al., 2007; Gibson and Alavi, 2013).

Two energy sources are applied to transform the raw materials, food extruders provide specific mechanical energy (SME) via extruder screw rotation by the main drive motor and specific thermal energy (STE) from steam and water injection during the processing. Together, these energy sources promote changes on the physico-chemical properties of the nutrients and final product (Guy, 2001; Riaz, 2007). The hardware configuration and the operation conditions are adjusted to maximize productivity, reduce production costs and improve kibble formation and appearance. Important aspect of hardware configuration is the restrictions to mass flow inside extruder. The resistance against mass flow can be controlled by the conformation of the screw elements and barrel, and the die configuration (Moscicki, 2011). Combining these elements higher or lower resistance to mass flow can be created, been defined according to production rate, food composition, and the desired characteristics of the final kibble (Riaz, 2007).

Higher or lower resistance to flow directly determines the residence time of the dough inside the equipment, the transference of shear force, and the temperature and pressure of the mass inside the extruder barrel (Deffenbaugh, 2007). These parameters will ultimate determine the gelatinization amount of the starch, and the physical attributes of the kibbles (Harper, 1981; Riaz, 2007; Carciofi et al., 2012; Alam et al., 2016).

Although enough SME transference is important to achieve adequate processing, an elevation of this energy may increase the production cost due to high requirement of electricity, equipment wear and limitation of productivity (Rokey et al., 2010) Due this, a balance between SME application and cost reduction is important. Taken this in consideration, the present study evaluated the implications of two restrictions after screw and four extruder open areas on the extrusion traits, kibble formation, starch gelatinization, and digestibility of a cat food formulation.

2. Material and Methods

2.1. Diets and experimental design

A cat food was formulated (Table 7) for maintenance according to the nutritional recommendations of the European Pet Food Industry Federation (FEDIAF, 2014). Ingredients were mixed and ground, using a hammer mill (Sistema Tigre de Mistura e Moagem, Tigre, Sao Paulo, Brazil) fitted with a sieve screens sizes of 0.9 mm. The mean geometric diameters of the raw materials were analyzed according to Zanotto and Bellaver (1996), resulting in 209 ± 1.3 µm.

A 4 x 2 factorial arrangement of treatments was used to the study of extrusion, composed by four extruder open areas (Eoa) and two restrictions after screw, totaling 8 treatments. The restriction after screw was obtained with the use of a two rings with different open diameters. The extruded screw have a diameter of 80 mm and five sections: initial - single flight and no steam lock; second – single flight and small steam lock; third – double flight uncut and small steam lock; fourth - double flight uncut and medium steam lock; fifth - double flight cut cone, starting with 80 mm and ending with 61 mm of diameter. The restrictions after screw (RASc) was calculated considering the open area at the end of the jacket (63 mm of diameter, or 3.019 mm^2) and the open area of the rings: ring 1 with 39 mm of open diameter, resulting in 1.195 mm^2 and a restriction of 60% of the jacket open area; ring 2 with 20 mm of open diameter, resulting in 314 mm^2 and a restriction of 90% of the jacket open area. The diets produced with these two RASc were extruded using four different die configurations, targeting the following four Eoa: 59; 118; 236; and 353 mm^2/ton/h. The Eoa (mm^2/ton/h) was calculated as:

$$\text{Extruder open area} = \frac{\text{Die open area (mm2)}\times 1000}{\text{Extruder output mass (kg/h)}}$$

Table 7

Ingredient and chemical composition of the experimental diet for cats

Item	***g/kg, as-fed basis***
Maize	408.0
Poultry by-product meal	338.0
Corn gluten meal	104.5
Beet pulp	40.0
Sodium Chloride	5.0
Potassium Chloride	4.0
Vitamin and mineral premix[a]	5.0
Choline Chloride	3.5
L-lysine	2.5
DL-Methionine	2.0
Taurine	1.6
Antioxidant [b]	0.5
Mold Inhibitor [c]	0.4
Poultry fat	75.0
Palatant enhancer[d]	10.0
Chemical composition of the ground raw material, excluding the poultry fat and palatant enhancer	
Moisture	62.8
Ash	355.2
Crude protein	78.0
Starch	302.3

[a] Added per kg of diet: vitamin A 10.000 IU; vitamin D_3 1.350 IU; vitamin E 60 mg; vitamin K_3 0.5 mg; vitamin B1 15 mg; vitamin B2 8 mg; vitamin B6 10 mg; vitamin B12 75 mcg; pantothenic acid 20 mg; folic acid 1.2 mg; biotin 0.12 mg, niacin 80 mg; copper 5 mg; iron 60 mg; manganese 14 mg; iodine 1.2 mg; zinc 125 mg; selenium 0.2 mg.

[b] Petox: butyl hydroxyanisole; butylated hydroxytoluene; citric acid and phosphoric acid. Kemin do Brasil Ltda, Indaiatuba, Brazil.

[c] Shield: calcium propiante. Kemin do Brasil Ltda, Indaiatuba, Brazil.

[d] Liquid palatant: AFB do Brasil, Jaguariúna, Brazil.

The diets were extruded in a single screw extruder (MEX 250, Manzoni, Campinas, Brazil), with a processing capacity of 250 kg/h, at the Feed Facilty of the Faculdade de Ciências Agrárias e Veterinárias, UNESP – Univ Estadual Paulista, Jaboticabal, Brazil. For all treatments water was injected into the preconditioner at a rate of 12 L/h and the shaft speed was set at 21 rpm, thermal energy was implemented on the preconditioner by direct steam infusion, targeting a mean preconditioning temperature of 80 ºC. Raw material was fed into the preconditioner using a volumetric delivery system at a target rate of 120 kg/hr. For all treatments, the extruder screw speed was set to 540 rpm. No water or steam was injected into the extruder barrel. To obtain the desired extruder open area, the following dies open diameters were used: 7.1 mm^2; 14.1 mm^2; 28.3 mm^2; and 42.4 mm^2.

For the extrusion of each treatment, the equipment was initially stabilized and the basal processing conditions established (minimum of 45 min). After this step, production parameters were recorded every 15 minutes, with four measurements per treatment, totaling four experimental units. The parameters registered were preconditioner exit temperature and moisture, motor load amperage, dough temperature, pressure before die, dough temperature at extruder exit, extruder output mass and bulk density after extruder and after dryer. Other parameters recorded included ambient temperature, working water temperature, mash feed temperature, and steam pressure. At each observation time, food samples were collected from the preconditioner, the extruder and the dryer and stored at -20 ºC for further analysis. Following the extrusion, the kibbles were dried in a forced air dryer system at 105 °C for 20 minutes.

With recorded data, the SME (kW-h/ton) was calculated for each treatment in accordance with Riaz (2007), with the following formula:

$$\text{SME}=\frac{(\sqrt{3}\times\text{Voltage}\times(\text{WA-EA})\times(\text{cosFi}\div1000))\times1000}{\text{Throughput}}$$

where;

Voltage = (220V)

WA = working amperage or the motor load during processing (A)

EA = motor on load amperage (A)

cosFi = power factor (0.76)

Throughput = raw material feed rate into the preconditioner (ton/hr)

The STE (kW-h/ton) was calculated as the net thermal energy contributed via steam absorption by mash inside the preconditioner divided by the raw material throughput (ton/hr). The net steam absorption (kg/hr) was calculated from mass balance according to Riaz (2007), and corresponding thermal energy was calculated by multiplying by the steam enthalpy (kJ/ kg) from steam tables and adjusting for average mash temperature inside the preconditioner. Finally, the Total Specific Energy (TSE; kW-h/ton) was obtained by the sum of the SME and STE. The water flash off after extruder was calculated by the difference between the calculated in barrel moisture and the moisture of kibbles collected immediately at extruder exit. It was assumed that the moisture measured in conditioner corresponded in barrel moisture, since no injection of water or steam into the extruder barrel.

2.2. Kibble macrostructure, starch gelatinization degree, and in vitro digestibility

For each treatment, the length (le), diameter (de) and mass (me) of 20 kibbles were measured with a vernier caliper and used to obtain the radial expansion ratio (RE), specific length (l_{sp}) and piece density (ρ), as described below:

$$RE=\frac{d_e^2}{d_d^2}$$

$$l_{sp}=\frac{le}{me} \quad (mm/g)$$

$$\rho=\frac{4\ me}{\pi\times de^2\times le}\ (kg/m^3)$$

where: d_d= die open area.

The hardness of the kibbles were evaluated by a cutting test, performed with a texturometer (TA-XT2 SMS, Stable Micro Systems, Godalming, UK). The equipment was set to operation mode strength/compression, return to start option enabled, pre-test speed of 2 mm/s, speed during the test of 0.5 mm/s and speed before test of 10 mm/s. Twenty kibbles of each sample were used, with a probe with a blade set with a Warner Bratzler knife (heavy duty platform/blade set) with a cutting distance of 10 mm. The data were analyzed with the software Texture Expert (Stable Micro Systems, Godalming, UK). The cutting force was measured in 20 kibbles per treatment.

The *in vitro* digestibility of the organic matter (OM) was determined as describe by Hervera et al. (2007) in samples collected after the dryer of each treatment. Incubations conditions simulate the digestion process in two steps, stomach and small intestine, using an enzymatic system with the pepsin and pancreatin enzymes, respectively. Samples after dryer were also collected from each treatment for the measurement of the starch gelatinization, which was determined by the amyloglucosidase method (Sá et al., 2013).

2.3. In vivo digestibility protocol

Eight diets were produced, but only two diets were selected to be tested on cats: 60% of RASc and 353 mm^2/ton/h of Eoa and 90% of RASc and 59 mm^2/ton/h of Eoa. Twelve cats, with 5.8 ± 4.1 years old and 4.2 ± 0.8 kg of body weight were used in a completely randomized design, with six cats per food. The health of the animals were confirmed prior to the beginning of the test. All the procedures with animals were previously approved by the Ethics and Animal Welfare Committee of the Faculdade de Ciências Agrárias e Veterinárias, UNESP – Univ. Estadual Paulista (protocol number 2070/16).

The total tract apparent digestibility of nutrients and energy were determined by the method of total feces collection without urine collection, according to recommendations of the FEDIAF (2014). Cats were fed with their respective diets for 18 days: from days 1 to 10 diet adaptation was performed; total feces collection was done form days 11 to 18. During the adaptation period, the cats remained from 18:00h to 08:00h individually housed in metabolic cages of stainless steel with dimensions of 0.9 m x 0.8 m x 1.0 m, equipped with apparatus to separate feces and urine for collection, and from 08:00h to 18:00hs in a collective cattery to socialize and exercise. During the collection period, cats remained restricted to their metabolic cages.

The amount of food supplied was individually calculated according to the energy value of the food and the energy requirement of the animal (100kcal x $kg^{0,67}$; NRC, 2006). Food was provided once a day, at 18:00h when the cats were restricted to the cages. Leftovers were collected on the next morning (08:00h), weighted and the consumption registered. Water was available *ad libitum*. Feces were totally collected at least twice a day, weighed and stored at -15ºC for further analysis.

At the end of the collection period, feces were thawed, mixed and dried at 55 °C for 72 h using a forced-air oven (Fanem, São Paulo, Brazil). Food and dry feces were ground in a cutting mill (Mod MA-350, Marconi, Piracicaba, Brazil) fitted with a 1 mm screen. Samples were analysed by oven-drying for dry matter (DM) (method 934.01), by muffle furnace incineration for ash content (method 942.05), crude protein (CP) using a Leco nitrogen/protein determination, and with a Soxhlet apparatus for acid hydrolysed ether extract (method 954.02), according to the Association of Official Analytical Chemists (AOAC, 1995). Organic matter (OM) of the samples was calculated as DM minus ash. The starch content was determined according to Hendrix (1993). Gross energy (GE) of diets and feces were determined by an adiabatic bomb calorimeter (model 1281, Parr Instrument, USA). Samples were analyzed in duplicate and the analyses repeated when the variation of duplicates was greater than 5%.

The quality of the feces were indirectly evaluated during the collection period by the following score 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; 5 = hard, dry pellets – small, hard mass.

2.4. Statistical analysis

Data of the extrusion test were analyzed as a 4 x 2 factorial arrangement of treatments, in a completely randomized design. The experimental unit was considered the sampling time, collected at each 15 min interval, with four repetitions per treatment. The experimental unit for kibble macrostructure and cutting force was one kibble, with 20 repetitions per treatment. Results were submitted to analysis of variance, model sums of squares were separated into the effects of the restriction after screw, extruder open area and their interactions. When differences were found on F test, means were compared by polynomial contrasts. The results of digestibility in cats was analyzed as in a completely randomized design. The experimental unit was considered one cat, with six repetitions per treatment. Results were submitted to analysis of variance. All data were found to comply with the assumptions of the ANOVA model, and were analyzed using the GLM procedure of SAS software (SAS Inst. Inc., Cary, NC). Values of $P < 0.05$ was considered significant.

3. Results

3.1. Extrusion traits

The mean extruder production did not differ between treatments, with a mean value of 117.8 ± 1.14 kg/h (as-fed basis), close to the 120 kg/h established target rate. Due this, the obtained Eoa were very close to the proposed in the experiment (Table 8), and increased linearly ($P<0.001$). Some fluctuations on preconditioner temperature were verified, with an increase on 60% RASc diets according to the Eoa ($P<0.001$) and an increase followed by a decrease for the 90% RASc ($P<0.001$). This was not expected, and probably was consequent to changes on steam infusion on preconditioner. In-barrel moisture did not change for any treatments (P>0.05).

For main motor load an significant interaction RASc x Eoa was verified (P=0.029), with a greater mean motor load for the 90% RASc foods (P<0.001). With the increase on Eoa the motor load decreased linearly for the 60% RASc. The pressure before the die did not change according to the RASc, after the increase on Eoa, it decreased quadratically: Pressure before die = 53.07 – 0.004(Oa) – 0.00008(Oa2); $R^2 = 0.48$; P<.001. The derivate of this equation estimate a lower pressure before die with an Eoa of 250 mm^2/ton/h. The water flash off out extruder did not change for the 60% RASc treatments, but decreased quadratically for the 90% RASc diets with the increase on Eoa (P=0.04).

The mean temperature of the fourth extruder jacket was higher for the 90% RASc treatments (P=0.032), and decreased quadratically with the increase on Eoa for both RASc (P<0.001). For the fourth extruder jacket temperature, no interaction RASc x Eoa or RASc effects were verified, only a quadratic decrease with the increase on the Eoa (P=0.001). The mass temperature before the die had no RASc x Eoa interaction, it mean value was almost 10 °C higher for the 60% RASc than the 90% RASc treatments (P<0.001). For both RASc a linear decrease on the mass temperature was verified with the increase on Eoa.

Table 8

Processing parameters of a cat food extruded with two restrictions after screw (RASc) and four extruder open areas (Eoa)

Item	**RASc** [a]	**Target open area (mm^2/ton/h)**				**Mean**	**SEM** [b]	**P Value**			**Contrast** [e]	
		59	**118**	**236**	**353**			**RASc**	**Eoa** [c]	**RAScxEoa**[d]	**Lin**	**Quad**
Extruder open Area (mm^2/ton/h)												
	60%	61.6	116.7	233.7	360.3	193.1	29.6	0.470	<.001	0.222		
	90%	60.0	117.4	242.9	358.1	194.6	29.8					
	Mean	60.8	117	238.9	359.2						<.001	-
Preconditioner												
Temperature (ºC)												
	60%	82.0	86.4	84.3	87.4	85.0	0.6	<.001	<.001	<.001	0.001	-
	90%	81.4	82.3	85.9	78.2	81.9	0.8				-	0.001
	Mean	81.7	84.4[f]	85.1	82.8[f]							
Extruder												
In barrel moisture (%)												
	60%	24.8	25.1	25.0	26.3	25.3	0.2	0.126	0.888	0.136		
	90%	26.1	26.5	27.1	24.9	26.1	0.2					
	Mean	25.4	25.8	26.0	25.7						-	-
Motor load (A)												
	60%	42.6	40.4	38.9	38.0	40.0	0.5	<.001	<.001	0.029	<.001	-
	90%	44.7	40.5	39.5	39.9	41.2	0.6				<.001	<.001
	Mean	43.7[f]	40.5	39.2	38.9[f]							
Pressure before die (bar)												
	60%	51.8	56.7	48.3	41.2	49.5	1.9	0.244	<.001	0.129		
	90%	50.7	51.6	44.5	44.9	47.9	0.9					
	Mean	51.3	54.2	46.4	43.0[f]						<.001	0.030
Water flash off (%)												
	60%	3.3	2.1	1.7	3.0	2.5	0.4	0.148	0.043	0.013	-	-
	90%	4.8	4.6	3.9	0.9	3.4	0.6				0.001	0.040
	Mean	4.1	3.4[f]	2.8	2.0[f]							
Fourth jacket temperature (°C)												

60%	133.5	112.5	97.0	95.3	109.6	4.0	0.032	0.001	0.004	<.001	<.001
90%	137.5	113.0	104.8	92.0	111.8	1.9				<.001	<.001
Mean	135.5[f]	112.8	100.9[f]	93.7							
Last jacket temperature (°C)											
60%	147.5	139	133.8	129.8	137.5	4.3	0.445	<.001	0.055		
90%	151.3	137	132.3	126.8	136.8	9.5					
Mean	141.5[f]	112.8	126.0	123.5						<.001	0.001
Mass temperature before die (°C)											
60%	149.0	133.0	133.0	128.0	135.3	2.4	<.001	0.001	0.629		
90%	134.0	127.0	119.0	119.0	124.7	2.8					
Mean	141.5[f]	130.0	126.0[f]	123.5						<.001	-
Energy Balance (kW-h/ton)											
Specific mechanical energy											
60%	28.1	21.2	17.8	16.0	20.8	1.3	<.001	<.001	0.144		
90%	32.6	21.7	20	20.5	23.7	1.3					
Mean	30.3[f]	21.5	18.9	18.2[f]						<.001	<.001
Specific thermal energy											
60%	103.1	102.8	110.2	111.8	104.4	2.9	0.665	0.778	0.029	-	-
90%	107.2	111.9	113.3	92.3	106.2	3.0				-	-
Mean	105.2	107.3	106.7	102.1							
Total specific energy											
60%	131.3	123.9	118.0	127.8	125.3	2.7	0.340	0.017	0.001	-	-
90%	139.8	133.6	133.2	108.3	128.8	3.7				0.001	-
Mean	135.5	128.8	125.6[f]	118.1[f]							
STE/SME ratio											
60%	3.7	4.9	5.6	7.1	5.3	0.4	0.292	<.001	0.306		
90%	3.3	5.1	5.7	5.8	5.0	0.3					
Mean	3.5	5.0	5.7	6.4[f]						<.001	-

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[a] RASc = restriction after extruder screw.

[b] SEM= standard error of the mean (n= 4 repetitions per treatment).

[c] Eoa = extruder open area.

[d] RASc x Eoa = interaction between restriction after screw and extruder open area.

[e] Linear or quadratic effects of the extruder open area.

[f] MGD means in the same column considering each item were different ($P<0.05$).

On energy balance, the SME application did not present interaction RASc x Eoa. The mean SME application was higher for the 90% RASc treatments ($P<0.001$), and decreased quadratically with the increase on Eoa (SME application = 36.78 - 0.15(Doa) - 0.0003(Doa2); $R^2 = 0.74$; $P<.001$). The derivate of this equation estimate a lower SME application with an Eoa of 250 mm^2/ton/h. The STE application did not vary according to RASc or Eoa. Due to the variations on SME and STE applications, and interaction RASc x Eoa was verified for the TSE implementation ($P=0.001$). The TSE application was similar for all Eoa on the 60% RASc treatments, but it decreased linearly with the increase on Eoa for the 90% RASc diets ($P<0.001$). Finally, the STE/ SME ratio was not influenced by RASc, and increased linearly with the increase on the Eoa.

3.2. Kibble macrostructure, starch gelatinization and in vitro digestibility

A significant effect of the Eoa was observed for all analyzed kibble macrostructure characteristics ($P<0.01$; Table 9). For bulk density and piece density an interaction between RASc x Eoa were also verified ($P<0.01$). Higher expansion were verified for the kibbles processed with 90% RASc, with lower bulk density and piece density and higher specific length than kibbles of the 60% RASc treatment ($P<0.01$). The kibble bulk density reduced approximately 56 g/L comparing the 90% and 60% RASc.

With the increase on Eoa, a quadratic increases on bulk density were verified for the 60% RASc, that elevated from 281 to 384 g/L (Bulk density = 201.30 + 0.55(Oa) - 0.001(Oa2); $R^2 = 0.99$; $P<.000$) and 90% RASc diets, that increased from 214 to 307 g/L (Bulk density = 222.74 - 0.21(Oa) - 0.001(Oa2);

$R^2 = 0.90$; $P<.000$. The derivate of this equation would result in lower bulk densities with an Eoa of 105 for the 60% RASc.

The mean kibbles piece density increased approximately 70% with the increase on Eoa, this increase can be described for the 60% RASc as: Piece density = $0.32 + 0.0001(Oa) - 0.000001\ (Oa^2)$; $R^2 = 0.67$; $P<.0007$. For the 90% RASc was: Piece density = $0.32 + 0.00008(Oa) - 0.0000008(Oa^2)$; $R^2 = 0.62$; $P<.0002$. Radial expansion of the kibbles showed a mean reduction of approximately 60% (Radial expansion = $19.49 - 0.012\ (Oa) - 0.00003(Oa^2)$; $R^2 = 0.77$; $P<.001$). The derivate of this equation resulted on the lower kibble radial expansion with an Eoa of 200 mm^2/ton/h. The kibbles cutting force was influenced only by the Eoa, a quadratic reduction on cutting force was verified with it increase ($P=0.01$). No interaction was verified between RASc x Eoa for starch gelatinization degree and *in vitro* digestibility of OM. Starch gelatinization was similar among Eoa, with only a tendency ($P= 0.06$) to be higher for foods extruded with 90% RASc in comparison with 60% RASc ($P=0.053$). For the *in vitro* digestibility of OM no effect of RASc was verified, only a quadratic reduction with the increase on Eoa ($P=0.006$).

Table 9
Kibble macrostructure and characteristics of a cat food extruded with two restrictions after screw (RASc) and four extruder open areas (Eoa)

Item	**RASc** [a]	**Target open area (mm^2/ton/h)**				**Mean**	**SEM** [b]	**P Value**			**Contrast** [e]	
		59	**118**	**236**	**353**			**RASc**	**Eoa**[c]	**RAScxEoa**[d]	**Lin**	**Quad**
Bulk density after drier (d/L)												
	60%	231	270	322	384	302	59.6	<.001	<.001	<.001	<.001	0.006
	90%	214	219	242	307	246	10.1				<.001	0.001
	Mean	223[f]	245[f]	282[f]	346[f]							
Piece density (g/cm^3)												
	60%	0.33	0.37	0.40	0.49	0.39	0.01	0.004	<.001	0.008	<.001	0.002
	90%	0.32	0.35	0.37	0.45	0.38	0.01				<.001	0.027
	Mean	0.33	0.36	0.39[f]	0.47[f]							
Specific length (cm/g)												
	60%	94.5	90.8	99.4	104.7	96.7	0.9	0.046	<.001	0.090		
	90%	94.2	90.1	101	107.3	98.5	0.9					
	Mean	94.3	90.5	100.2[f]	106.6[f]						<.001	<.001
Radial expansion rate												
	60%	18.5	17.7	13.8	11.2	15.5	0.4	0.276	<.001	0.192		
	90%	19.1	17.1	15.4	11.6	15.8	0.3					
	Mean	18.8	17.4	14.6	11.4						<.001	<.001
Cutting force (kg.f)												
	60%	3.9	3.5	3.0	3.3	3.4	0.1	0.130	<.000	0.495		
	90%	3.8	3.2	3.0	2.9	3.2	0.7					
	Mean	3.9	3.4	3.0	3.1						<.001	0.010

Starch gelatinization (%)												
	60%	97.2	96.6	96.1	93.0	95.7	0.2	0.053	0.117	0.388		
	90%	97.8	98.2	96.6	96.9	97.4	0.1					
	Mean	97.5	97.4	96.4	95.0							
In vitro digestibility of the OM												
	60%	0.882	0.872	0.867	0.873	0.873	0.1	0.065	0.002	0.837		
	90%	0.865	0.865	0.865	0.868	0.868	0.1					
	Mean	0.874	0.869	0.866	0.871						0.003	0.006

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[a] RASc = restriction after extruder screw.

[b] SEM= standard error of the mean (n= 4 repetitions per treatment).

[c] Eoa = extruder open area.

[d] RASc x Eoa = interaction between restriction after screw and extruder open area.

[e] Linear or quadratic effects of the extruder open area

[f] MGD in the same column considering each item were different (P<0.05).

3.3. Nutrient intake, apparent total tract digestibility and fecal characteristics

The two diets selected to study the effects of processing conditions on digestibility presented similar chemical compositions. After coated with poultry fat and palatant enhancer, the diets presented the following mean composition (as-fed basis): 45 g/kg of moisture; 346 g/kg of crude protein; 162 g/kg of fat; 288 g/kg of starch; 20 g/kg of crude fiber, 79 g/kg of ash. Nutrient intake during the digestibility tests were similar between diets (Table 10), without episodes of refusal, diarrhea or vomiting.

The body weight of the cats did not change during the study ($P>0.05$; data not shown). No effect of RASc or Eoa was verified for nutrient digestibility, and fecal production and traits, with similar values between the two foods tested.

Table 10

Intake and apparent total tract digestibility, and fecal characteristics of cats fed a diet extruded with two restrictions after screw (RASc) and four extruder open areas (Eoa)

Item	***Diets***		***SEM*** [a]	***P Value***
	60% RASc and 353 mm^2/ton/h	***90% RASc and 59 mm^2/ton/h***		
Food intake				
g of DM/kg/d	11.7	11.6	0.51	0.684
Apparent total tract digestibility				
Dry matter	0.796	0.804	0.89	0.644
Organic matter	0.838	0.846	0.81	0.638
Crude fat	0.908	0.904	0.10	0.824
Crude protein	0.829	0.840	0.10	0.619
Starch	0.998	0.999	0.03	0.131
Gross energy	0.851	0.859	0.81	0.664
Fecal characteristics				
g/kg/d (DM basis)	2.4	2.4	0.13	0.974
g/kg/d (As-fed basis)	8.0	6.9	0.61	0.358
Moisture content (%)	66.4	62.2	1.56	0.196
Score	3.3	3.8	0.20	0.163

[a] SEM= standard error of the mean (n= 6 cats per food).

4. Discussion

The importance of the restrictions to mass flow were confirmed by the results of the present study, RASc and Eoa influenced practically in all extrusion parameters, with the exception of the STE. The STE is transferred to mass by steam and water injections (Riaz, 2007), so changes in this energy source were not expected. When the output area of the extruder screw was restricted in 90%, in comparison with 60% an increase on motor load was verified resulting in higher SME application. This elevated mechanical energy application induce higher shear force, explaining the higher fourth jacket temperature. However, no effect of RASc were verified on last jacket temperature, and mass pressure. The mass temperature, on the contrary, was greater for the 60% RASc treatments. One explanation to these results is the screw filling. With a 90% restriction of screw output area, more material accumulated on the fourth and last sections of the screw elements, creating more shear, temperature and pressure on this sections. When only 60% of the area was restricted, the mass flow was easier and it accumulated on the final parts of the extruder, at the die. With this, more shear was developed at the end of the equipment, by the larger amount of mass accumulated explaining the higher temperature before the die.

The increase on Eoa from 59 to 353 mm^2/ton/h reduced in 40% de SME application, explaining the proportional reduction on mass pressure, jacket temperature, and mass temperature before die. The impact of the Eoa on the extrusion parameters was even greater than verified by the RASc. The die open area is the ultimate determinant of the restriction to mass flow, and need to be calculated considering the chemical composition of the mass, equipment production rate, and desired macrostructure of the kibbles (Moscicki, 2011). In the conditions of the present study, and considering the food formulation used, an Eoa of 250 mm^2/ton/h should minimize the mechanical energy application, and more restrictive Eoa need to be considered to improve SME application to the mass. This effect, however, is dependent of the screw configuration as the SME was greater for the 90% RASc for all tested Eoa.

The screw and die configurations clearly influenced the kibble macrostructure. Only the cutting force and radial expansion were not influenced by RASc, and all evaluated parameters were affected by Eoa. When greater restrictions to flow is placed, more SME is dissipated by the extruder screw rotation and more motor load is required to surpass the restriction. The resulted increased temperature and pressure favor the water vapor flash off, and the formation of the cellular structure of the kibbles. The importance of this can be seen for bulk density, one important parameter for the development and production monitoring of kibble diets in the industry. In the present study, varying the RASc a 20% change in bulk density was verified, and altering the Eoa, variations between 30 to 25% can be obtained. The coefficient of determination of the polynomial regressions between bulk density and Eoa were high, respectively 0.99 for the 60% RASc and 0.90 for the 90% RASc diets.

Although determinant for kibble macrostructure, the applied restriction did not alter starch gelatinization or clearly influenced the *in vitro* digestibility of the OM. The digestibility in cats were also similar between the two foods tested. Nutrient intake and feces formation and characteristics were also similar, in a way that the different processing conditions tested resulted in food with similar nutritional value. This allows to speculate that more energy is required to kibbles structure formation than for starch cooking or nutrient digestibility. No publications were find to compare these results, highlighting the importance of studies about cat food extrusion.

5. Conclusions

The restriction after screw and extruder open area combined have a great influence on extrusion parameters, SME transference and kibble formation. It is possible to modulate the kibble expansion through a greater or lower restriction to mass flow. However, nutrient digestibility by cats do not necessarily change according the higher or lower kibble expansion, considering that enough energy is applied and the starch cooking is adequate.

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