

**UNIVERSIDADE ESTADUAL PAULISTA – UNESP  
CAMPUS DE JABOTICABAL**

**APLICAÇÃO DE ENERGIA TÉRMICA NO CONDICIONADOR  
NA EXTRUSÃO DE ALIMENTOS PARA CÃES**

**Peterson Dante Gavasso Pacheco**

Zootecnista

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**Peterson Dante Gavasso Pacheco**

**Orientador: Prof. Dr. Aulus Cavalieri Carciofi**

**Coorientadora: Dra. Thaila Cristina Putarov**

Dissertação apresentada à Faculdade de Ciências Agrárias e Veterinárias – Unesp, Campus de Jaboticabal, como parte das exigências para a obtenção do título de Mestre em Zootecnia

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TÍTULO: APLICAÇÃO DE ENERGIA TÉRMICA NO CONDICIONADOR NA EXTRUSÃO  
DE ALIMENTOS PARA CÃES

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## **DADOS CURRICULARES DO AUTOR**

Peterson Dante Gavasso Pacheco, nascido em 24 de janeiro de 1990, na cidade de Alta Floresta - MT, ingressou no curso de Zootecnia da Faculdade de Medicina Veterinária e Zootecnia da Universidade Estadual Paulista “Júlio de Mesquita Filho” (UNESP), campus de Botucatu - SP, em março de 2009, graduando-se em dezembro de 2013. Em março de 2014, iniciou o curso de Mestrado em Zootecnia na Faculdade de Ciências Agrárias e Veterinárias da UNESP, campus de Jaboticabal - SP. Durante esse período, desenvolveu parte do seu projeto de pesquisa na Kansas State University, Manhattan - KS, EUA.

“ Descobri como é bom chegar quando se tem paciência. E para se chegar, onde quer que seja, aprendi que não é preciso dominar a força, mas a razão. É preciso, antes de mais nada, querer. ”

Amyr Klink

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## SÚMARIO

|  | Página |
|--|--------|
| CEUA .....   | iii    |
| RESUMO.....  | iv     |
| ABSTRACT .....   | vi     |
| CAPÍTULO 1 – Considerações gerais.....   | 1      |
| 1. Introdução .....  | 1      |
| 2. Revisão de Literatura .....   | 3      |
| 2.1. Efeito da extrusão no amido .....   | 3      |
| 2.2. Efeito da extrusão nos lipídeos.....  | 3      |
| 2.3. Efeito da extrusão nas proteínas .....  | 4      |
| 2.4. Efeito da extrusão na palatabilidade .....  | 5      |
| 2.5. Implicações da aplicação de energia mecânica e térmica .....                              | 6      |
| 2.6. Justificativa .....   | 8      |
| 3. REFERÊNCIAS.....  | 9      |
| CAPÍTULO 2 – Thermal energy application to the conditioner on the extrusion of dog foods ..... | 13     |
| Abstract.....  | 14     |
| 1. Introduction .....  | 15     |
| 2. Material and Methods.....   | 17     |
| 2.1. Experimental design and food formulation.....   | 17     |
| 2.2. Extrusion processing and experimental treatments .....                                    | 19     |
| 2.3. Specific mechanical energy and specific thermal energy calculations .....                 | 21     |
| 2.4. Kibble macrostructure, starch cooking and in vitro digestibility .....                    | 24     |
| 2.5. Food palatability test.....   | 24     |
| 2.6. Digestibility protocol.....   | 25     |
| 2.7. Faecal pH and fermentation products.....  | 26     |
| 2.8. Statistical analysis.....   | 27     |
| 3. Results .....   | 28     |
| 3.1. Extrusion traits .....  | 28     |
| 3.2. Kibble macrostructure, starch gelatinization and in vitro digestibility .....             | 33     |
| 3.3. Nutrient intake, apparent total tract digestibility and fecal characteristics ....        | 36     |
| 3.4. Faecal characteristics and fermentation products.....                                     | 38     |
| 3.5. Food palatability test.....   | 40     |

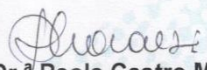
4. Discussion.....43  
5. Conclusion .....47  
References.....47

## CEUA – COMISSÃO DE ÉTICA NO USO DE ANIMAIS

### CERTIFICADO

Certificamos que o Protocolo nº 018909/14 do trabalho de pesquisa intitulado **"Adição de diferentes quantidades de energia térmica no condicionador sobre o cozimento do amido, digestibilidade e palatabilidade de rações extrusadas para cães"**, sob a responsabilidade do Prof. Dr. Aulus Cavalieri Carciofi, está de acordo com os Princípios Éticos na Experimentação Animal adotado pelo Conselho Nacional de Controle de Experimentação Animal (CONCEA) e foi aprovado pela COMISSÃO DE ÉTICA NO USO DE ANIMAIS (CEUA), em reunião ordinária de 09 de outubro de 2014.

Jaboticabal, 09 de outubro de 2014.

  
**Prof.ª Dr.ª Paola Castro Moraes**  
Coordenadora – CEUA

## APLICAÇÃO DE ENERGIA TÉRMICA NO CONDICIONADOR NA EXTRUSÃO DE ALIMENTOS PARA CÃES

**RESUMO** – O uso generalizado da tecnologia de extrusão em alimentos para cães deve-se ao fato desta promover mudanças físicas e químicas no ingrediente tornando possível seu uso na alimentação animal. Estas mudanças ocorrem principalmente devido a aplicação de energia térmica específica (ETE) e energia mecânica específica (EME) aplicada ao processo de extrusão. Diversos estudos evidenciam o uso e a quantidade de EME para o adequado processamento de alimentos, contudo, ainda se desconhecem as diferenças nutricionais e a influência na macroestrutura dos kibbles resultantes do cozimento por ETE em relação a aqueles obtidos pela aplicação de EME. Desta forma, os objetivos deste estudo foram avaliar a aplicação de seis quantidades de energia térmica específica (ETE) no pré-condicionador, em dois sistemas de extrusão, sobre os parâmetros de processo, a gelatinização do amido, a macroestrutura dos kibbles, a digestibilidade *in vitro* e *in vivo* de alimento para cães e produtos da fermentação microbiana no cólon. O estudo foi dividido em dois experimentos. O primeiro experimento avaliou o efeito da aplicação de ETE em dois sistemas de extrusão sobre os parâmetros de processo e a partir destes resultados, as dietas que apresentaram maior variação em relação ao cozimento do amido foram utilizadas para o segundo experimento que avaliou a digestibilidade, produtos da fermentação e palatabilidade de rações para cães. Uma dieta padrão para cães adultos em manutenção foi formulada de acordo com as recomendações nutricionais do Fédération Européenne de l'industrie des Aliments pour Animaux Familiars (FEDIAF, 2014). As rações foram produzidas em extrusora Wenger X-20 (extrusora A) e extrusora Manzoni Mex-250 (extrusora B) por meio da modulação de adição de água e vapor para obtenção das temperaturas desejadas que corresponderiam a aplicação de seis ETE (Temperaturas: 45°C; 55°C; 65°C; 75°C; 85°C; 95°C). Para o primeiro experimento foi utilizado um delineamento inteiramente casualizado, em esquema fatorial 6 (ETE) x 2 (extrusora) com 12 tratamentos e 4 repetições (amostra de ração coletada a cada 10 minutos durante o tempo de processamento) por tratamento. Para o segundo experimento, 36 cães adultos foram divididos em seis tratamentos, o experimento seguiu um delineamento em blocos casualizado, com 3 blocos de 12 animais cada e duas repetições por bloco. As variáveis de processo estudadas foram ETE, EME, energia específica total (EET), relação ETE/EME e pressão da saída da extrusora. As variáveis para avaliação da macroestrutura do kibble foram gelatinização do amido (GA) da massa do condicionador e do produto seco, digestibilidade *in vitro* da matéria orgânica, comprimento específico, taxa de expansão radial, densidade aparente e densidade específica. A digestibilidade dos nutrientes e a palatabilidade do alimento foram avaliados nos alimentos produzidos na extrusora B devido a maior amplitude dos resultados de gelatinização do amido. Os dados de processo e macroestrutura foram submetidos a análise de variância, considerando-se os efeitos de extrusora, ETE e suas interações, sendo as médias comparadas por contrastes polinomiais ( $P < 0,05$ ). Os dados do segundo experimento foram submetidos a análise de variância considerando-se os efeitos de ETE e bloco e as médias comparadas por contrastes polinomiais ( $P < 0,05$ ). Houve interação entre extrusora e ETE para umidade na massa no pré-condicionador e umidade no canhão da extrusora. Não houve efeito de

interação para a produtividade do pré-condicionador e extrusora. Houve efeito quadrático em ambas as extrusoras para temperatura da massa antes da matriz e relação ETE/EME, sendo que a aplicação de ETE elevou os valores. A amperagem e pressão na saída da extrusora reduziram quadraticamente, enquanto que não houve efeito de interação para SME, ETE, EET. Houve efeito de extrusora para EME e EET, a extrusora A apresentou os maiores valores. Houve efeito de ETE para EME e EET, a EME reduziu linearmente com a aplicação de ETE e as demais energias aumentaram linearmente. Houve interação entre extrusora e ETE para todas as características de macroestrutura. A densidade específica e a taxa de expansão radial reduziram quadraticamente nos dois sistemas de extrusão com a aplicação de ETE. O comprimento específico apresentou comportamento quadrático para a extrusora B, sendo que o menor comprimento específico foi verificado a temperatura de 74,4 °C, não ocorrendo o mesmo para a extrusora A. A densidade aparente reduziu linearmente nas duas extrusoras com o aumento de ETE, e a extrusora A apresentou os menores valores em todas as temperaturas estudadas. A GA da massa do pré-condicionador aumentou quadraticamente para as duas extrusoras. A GA do produto seco aumentou quadraticamente para a extrusora A e linearmente para a extrusora B com o aumento da ETE. Ocorreu aumento linear da digestibilidade *in vitro* em ambas as extrusoras, sendo que os valores da extrusora B foram maiores. Não foi verificado alteração para a palatabilidade, qualidade fecal, digestibilidade *in vivo* dos nutrientes e produtos da fermentação com a aplicação de ETE. A aplicação de ETE no pré-condicionador parecer ter mais efeito sobre a macroestrutura dos kibbles e parâmetros de processo de alimentos para cães do que sobre a digestibilidade de nutrientes e qualidade fecal. Porém, mais estudos são necessários para avaliar a ETE em outras condições de extrusão.

**Palavras-chave:** energia térmica específica, extrusão, gelatinização do amido

## THERMAL ENERGY APPLICATION TO THE CONDITIONER ON THE EXTRUSION OF DOG FOODS

**ABSTRACT** – The generalized use of extrusion in pet food is due to the modification of the physical and chemical characteristics of the ingredient, which make possible its use in animal food. These changes occur mainly due to the application of specific thermal energy (STE) and specific mechanical energy (SME) to the extrusion process. Several studies demonstrate the suitable use and quantity of SME for proper pet food processing, however, there are no information about the STE application to the conditioner on the nutritional differences and its influences on the macrostructure of kibbles of dog food. Thus, the objectives of this study were to evaluate the application of six amounts of specific thermal energy (STE) in the preconditioner, in two extrusion systems, on the process parameters, starch gelatinization, macrostructure of kibbles, *in vitro* and *in vivo* digestibility and fermentation products of dog food. The study was divided in two experiments. The first experiment evaluated the effect of STE application in two extrusion systems on process parameters and from these results, the diets with higher amplitude of starch cooking were used for the second experiment, which evaluated the digestibility, fermentation products and palatability of dog food. A standard diet for adult dogs in maintenance was formulated according to the nutritional recommendations of the Fédération Européenne de l'industrie des Aliments pour Animaux familiers (FEDIAF, 2014). The diets were produced in the extruder Wenger X-20 (extruder A) and extruder Manzoni Mex-250 (extruder B) by the modulation of water and steam addition to reach the desired temperatures which correspond to application of six STE (temperatures: 45°C, 55°C, 65°C, 75°C, 85°C, 95°C). For the first experiment, a completely randomized design in a factorial arrangement of treatments 6 (STE) x 2 (extruder) was used, totaling 12 treatments and 4 replications (feed sample collected every 10 minutes during the processing time) for treatment. For the second experiment, 36 adult dogs were divided into six treatments, the experiment followed a randomized block design with three blocks of 12 animals each and two repetitions per block. The studied processing variables were STE, SME, total specific energy (TSE), STE/SME ratio, motor load and the extruder output pressure. The variables for the evaluation of the macrostructure of the kibble were starch gelatinization (SG) of the dough from the conditioner and the dried product, *in vitro* digestibility, specific length, radial expansion ratio, bulk density and piece specific density. The digestibility of nutrients, fermentation products and food palatability were evaluated in the foods produced in the extruder B due to the higher range of the results of the SG. For the first experiment the effects of extruder, STE and their interactions were considered, and the means were compared by polynomial contrasts ( $P < 0.05$ ). The second experiment data were submitted to analysis of variance considering the effects of STE and block and means compared by polynomial contrasts ( $P < 0.05$ ). There was interaction between extruder and STE for preconditioner discharge mass moisture and in-barrel moisture. No significant effects of interaction were verified for productivity from the preconditioner and extruder. There was a quadratic effect on both extruders for mass temperature before the die and STE/SME ratio; the application of STE increased the values. The motor load and pressure showed a quadratic reduction in both extruders, while there were no interaction effect for SME, STE and TSE. There was extruder effect for SME and TSE, the extruder A showed the highest values. There was STE effect for SME, STE and

TSE, the SME linearly reduced with the application of STE and STE and TSE increased linearly. There was interaction between extruder and STE for all the macrostructure traits. The piece specific density and radial expansion rate had a quadratic reduction in both extrusion systems with the application of STE. The specific length had a quadratic behavior in extruder B, and the lowest specific length was verified at temperature of 74.4°C, which did not occur with extruder A. The bulk density had a linear decrease with increasing STE, independent on the extruders; the extruder A showed the lowest values at the studied temperatures. The SG of the dough from conditioner had a quadratic augment in both extruders. The SG of the dried product showed a quadratic and linear increase in extruder A and B, respectively, with increasing the STE. There was a linear increase for *in vitro* digestibility in both extruders, with higher values for extruder B. There were no significant effects on palatability, fecal quality, nutrients digestibility and fermentation products, except for starch digestibility that reduced linearly with the application of STE. The application of STE into preconditioner seems to have more effect on the macrostructure of kibbles and pet food process parameters than on nutrient digestibility and fecal quality. However, more studies are needed to better understand the STE in other extrusion conditions.

**Keywords:** specific thermal energy, extrusion, starch gelatinization

## **CAPÍTULO 1 – Considerações gerais**

### **1. Introdução**

As rações comerciais para animais de estimação podem ser classificadas, quanto ao processamento, em três tipos básicos: seca, semi-úmida e úmida. Os alimentos secos compõem o maior segmento da indústria *pet* e 95% desses são obtidos pela tecnologia de extrusão (SPEARS; FAHEY, 2004). O Brasil é o segundo maior produtor de alimentos extrusados para cães e gatos. Segundo a Associação Brasileira da Indústria de Produtos para Animais de Estimação (Abinpet), em 2015, foram produzidas 2,53 milhões de toneladas de pet food, com faturamento de 18 bilhões de reais (ABINPET, 2016).

Extrusão é um processo no qual a mistura de ingredientes é cozida, sanitizada, texturizada e formatada na presença de umidade, pressão, temperatura e fricção mecânica, em curto espaço de tempo (RIAZ, 2007; TRAN; HENDRIKS; VAN DER POEL, 2008). O processo inclui as etapas de pré-condicionamento, extrusão propriamente dita, corte e secagem. Cada uma desempenha função específica tanto no cozimento quanto na formação do produto final.

No pré-condicionador, adiciona-se energia térmica à mistura de ingredientes moídos, pela injeção de vapor direto e água, obtendo-se uma massa uniforme, mediante a ação de um sistema de barras cilíndricas com pás dispostas radialmente, girando a velocidade variável (BAZOLLI, 2007). A adição de energia térmica que tem, como objetivo, aumentar a umidade e temperatura da massa, promove o início do cozimento do amido e assim favorece a hidratação interna dos grânulos, a plasticização, a sanitização, a estabilidade da extrusora e a qualidade do produto final. Além disso, a adição de energia térmica na forma de vapor é vantajosa em termos econômicos, por ser mais barata e simples e resultar em menos desgaste do equipamento e menor consumo de energia elétrica mediante a aplicação de energia mecânica (RIAZ, 2000).

Em seguida, a massa em processamento é conduzida para o canhão da extrusora, um tubo com sistema de rosca sem fim, que gira a velocidade ajustável em

seu interior. No canhão da extrusora a massa recebe energia mecânica, adicionada pela rotação do parafuso da extrusora, que promove cisalhamento da massa contra seu revestimento e a comprime contra a matriz, na extremidade do cilindro, criando pressão, fricção e temperatura. As pressões e temperaturas no final do canhão podem atingir, respectivamente, mais de 60 bars e 160°C, embora seja usual trabalhar com pressões de 20 a 40 bars e temperaturas de 120 a 140°C. Toda essa energia e compressão em um fluxo laminar modificam profundamente os amidos e as proteínas, como será discutido mais adiante. A energia aplicada permite o cozimento completo do amido em poucos segundos e a baixa umidade, entre 20% e 35%, o que é bastante vantajoso em relação ao cozimento em pressão atmosférica, que necessita mais de 10 minutos e duas partes de água para uma de amido, para que este se gelatinize completamente (GIBSON; ALAVI, 2013).

O uso generalizado da tecnologia de extrusão na indústria *pet food* deve-se ao fato de 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, 2008). A elevada aplicação de energia termomecânica no processo induz alterações vantajosas e desejáveis em alimentos para cães e gatos, como: aumento da digestibilidade dos cereais, melhora da palatabilidade do alimento, modificações de atributos texturais que favorecem a apreensão e a mastigação, inativação de fatores antinutricionais, destruição de microrganismos, aumento da vida de prateleira, ampliação das possibilidades de uso de matérias primas e desnaturação de proteínas com melhora de sua digestibilidade (CHEFTEL, 1986; LANKHORST et al., 2007). O ganho em digestibilidade e palatabilidade dos cereais talvez seja o efeito mais notório do processo de gelatinização e plasticização do amido, que se torna mais digerível pelas enzimas digestivas dos carnívoros (MURRAY et. al., 2001).

A extrusão também pode promover efeitos indesejáveis, como destruição de vitaminas, oxidação de lipídeos, redução na disponibilidade de aminoácidos, principalmente da lisina envolvida na reação de Maillard (LANKHORST et al., 2007). Devido a isso, e também de modo a evitar gastos desnecessários, o balanço entre os efeitos desejáveis e indesejáveis deve sempre ser buscado com a aplicação necessária de energia térmica e mecânica, mas não excessiva.

Pretendeu-se, com este projeto, levantar informações sobre a aplicação de energia térmica no processo de extrusão de alimentos para cães, dados que permitirão definir melhores parâmetros de processamento, que aliem eficiência energética e de custos com adequada transformação das matérias primas, garantindo elevada digestibilidade e palatabilidade ao alimento. Desta forma, os objetivos dessa dissertação foram avaliar os efeitos da adição de seis quantidades de energia térmica no pré-condicionador, em dois sistemas de extrusão, sobre a gelatinização do amido, digestibilidade aparente dos nutrientes e da energia, produtos da fermentação microbiana no cólon, palatabilidade e macroestrutura de alimentos extrusados para cães.

## **2. Revisão de Literatura**

### **2.1. Efeito da extrusão no amido**

Os grãos de cereais (arroz, milho, trigo e sorgo) são as fontes de amido mais utilizadas em rações para animais de companhia. Estruturalmente, o amido dos cereais se encontra como partículas semi-esféricas altamente ordenadas, denominadas grânulos, formados por cadeias de amilose e amilopectina (RATNAYAKE; JACKSON, 2003). O amido é o principal substrato para que o processo de extrusão ocorra de forma apropriada (CRANE; GRIFFIN; MESSENT, 2000). Durante esse processo, grânulos de amido são umedecidos e recebem calor, atrito mecânico, corte e pressão, sofrendo o fenômeno de gelatinização: incham, derretem e perdem sua estrutura cristalina (ZENG et al., 1997; RATNAYAKE; JACKSON, 2009). O amido gelatinizado, perdendo sua ordenação e estrutura, torna-se solúvel em água e mais suscetível à degradação enzimática do que o amido cru (DONA et al., 2010). Estudos demonstraram que o amido dos cereais, se extrusado adequadamente, apresenta digestibilidade aparente superior a 95% para gatos (DE-OLIVEIRA et al., 2008) e 98% para cães (CARCIOFI et al., 2008).

### **2.2. Efeito da extrusão nos lipídeos**

O processo de gelatinização do amido também influencia os lipídeos do alimento. No canhão da extrusora o amido gelatinizado, especificamente as cadeias de amilose, formam complexos com a gordura naturalmente presente nos ingredientes (GIBSON; ALAVI, 2013). Os complexos constituídos de amilose e lipídeos são formados pela encapsulação do triglicerídeo na molécula de amilose e suas implicações no aproveitamento do alimento para cães e gatos ainda não foram adequadamente estudadas. Stroucken et al. (1996) não consideram que o processo influencie a digestibilidade da gordura, sugerindo que tais complexos seriam facilmente digeríveis, o que estaria de acordo com a elevada digestibilidade de lipídeos comumente verificada em dietas para cães e gatos (HULLÁR; FEKETE; SZÖCS, 1998). Ademais, os complexos, formados por amilose e lipídeos, alteram a textura e expansão dos kibbles. Quanto maior a quantidade de gordura interna na ração, menor será a eficiência de transferência de energia mecânica e da extrusão em si, reduzindo o cozimento e promovendo a formação de kibbles pouco expandidos e duros (CHEFTEL, 1986). Por fim, a formação destes complexos impede a remoção dos lipídeos pelo éter na análise de extrato etéreo, tornando necessária uma pré-hidrólise ácida da amostra para sua posterior extração pelo éter.

Quando lipídeos ou alimentos contendo lipídeos são aquecidos na presença de oxigênio sofrem oxidação, devido à degradação dos ácidos graxos. A oxidação lipídica é um 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; HSIEH; HUFF, 1998; DEFFENBAUGH, 2007). Os radicais livres que resultam dessa oxidação podem reagir com proteínas, vitaminas ou outros constituintes e reduzir a qualidade nutricional do alimento. Além disso, ocorrem perda pronunciada de sabor e alteração da cor (LILLARD, 1983).

### **2.3. Efeito da extrusão nas proteínas**

Os efeitos da extrusão sobre os componentes proteicos podem ser benéficos ou prejudiciais para as características físicas e nutricionais da mistura alimentar, a depender de sua intensidade. Ela pode inativar fatores antinutricionais à base de

proteínas, destruindo a integridade de sua estrutura e, conseqüentemente, evitando sua ação contrária à nutrição (VAN DER POEL et al., 1990; ALONSO; AGUIRRE; MARZO, 2000). A desnaturaçãõ 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, de modo que sua inativaçãõ pela extrusãõ contribui para estabilizar o armazenamento e aumentar a vida de prateleira de alimentos secos para animais (CHEFTEL, 1986).

Os efeitos indesejáveis do tratamento termomecânico incluem a destruiçãõ e a perda de aminoácidos, as ligações inter peptídicas e extra peptídicas e uma sêrie de reações químicas, tais como a de Maillard e a de formaçãõ de complexos proteína-lipídeo e proteína-carboidrato (BJÖRCK; ASP, 1983). Essas reações podem danificar a estrutura da proteína, com perda de seu valor nutricional.

Umidade, temperatura e tempo de extrusãõ parecem ser os parâmetros mais importantes para a reaçãõ de Maillard, reaçãõ que aumenta ao se reduzir a umidade, elevar a temperatura e ampliar o tempo de processo. Segundo Cheftel, (1986), a temperatura do produto deve ser mantida abaixo de 180°C, para minimizar as perdas nos alimentos.

#### **2.4. Efeito da extrusãõ na palatabilidade**

Para os animais, a palatabilidade, que engloba fatores como sabor, aroma, apreensãõ e sensaçãõ de mastigaçãõ (textura, forma e tamanho dos extrusados) é normalmente referida como valor de preferências alimentares e comportamento de ingestãõ. É fator chave na seleçãõ da dieta pelos cães e gatos, estando estreitamente relacionada com seu sucesso comercial. Dos fatores que determinam a palatabilidade dos alimentos, os mais estudados sãõ a composiçãõ dos nutrientes (teor de gordura, proteína e carboidratos) e os tipos de ingredientes (proteínas e gorduras de origem animal, ingredientes de origem vegetal, ingredientes fibrosos) (HULLÁR; FEKETE; SZÖCS, 1998; CASE et al., 2000). No entanto, o processamento por extrusãõ é igualmente importante, pois determina vários aspectos estreitamente ligados à palatabilidade e preferênciam alimentar, como crocância, dureza, forma, tamanho, odor

e sabor da ração. Todas essas características são fortemente influenciadas pelas condições de processamento da extrusão (CARCIOFI et al., 2012), embora dados de sua influência sejam praticamente inexistentes.

As características macroestruturais dos extrusados são o resultado da sua formulação (teores de amido, proteína, gordura e fibra), do tipo de ingredientes usados (fontes de proteína de origem vegetal ou animal) e das condições de processamento, incluindo: umidade no canhão da extrusora, 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 trafila 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 determinam a macroestrutura do kibbles: expansão radial e longitudinal, densidade aparente e específica, comprimento, estrutura celular, dureza e crocância (TRIVEDI; BENNING, 2003). Infelizmente, são poucas as publicações que identifiquem as características macroestruturais de melhor aceitação pelos animais, tampouco informações sobre as melhores disposições e configurações de processamento para que essas características sejam obtidas. Esses dados seriam importantes para fabricantes, pois possibilitariam ajustar formulações, desenhos e configuração de equipamentos, bem como condições de operação que resultassem em alimentos com maior aceitação e palatabilidade.

## **2.5. Implicações da aplicação de energia mecânica e térmica**

As mudanças físico-químicas promovidas pelo processo de extrusão nos ingredientes estão diretamente ligadas à quantidade de energia específica total (EET) transferida para a massa que, por sua vez, é composta pela soma das implementações de energia mecânica específica (EME) e energia térmica específica (ETE). Quando se iniciou o emprego da extrusão termoplástica de médio cisalhamento, 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* correspondem à EME, sendo que o percentual restante diz respeito ao uso de

energia térmica. No entanto, no Brasil, isso ainda não está claro. Não se mede de rotina a transferência de energia e a capacidade de funcionamento dos condicionadores. Alguns condicionadores são pequenos, não há controle de variação da velocidade de rotação das pás e o tempo de residência do produto em seu interior não é medido ou controlado. Alguns sistemas, inclusive, não trabalham com vapor, sendo o processo 100% baseado em energia mecânica, o que torna o processo ineficiente e caro.

A aferição ou cálculo da EME e ETE aplicadas torna possível compreender, parametrizar, controlar e replicar o processo de fabricação dos produtos (RIAZ, 2007). Do ponto de vista científico, no entanto, não se estudou, ainda, a relação mais adequada entre ETE e EME para a extrusão de alimentos para cães e gatos. Não existem informações disponíveis a este respeito, nos estudos publicados não há descrição das condições de extrusão ou menção da EME, ETE e transferência 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 SÁ (2015) foi pioneira em avaliar os efeitos de diferentes aplicações de EME e ETE na produção de alimentos para cães e gatos. Com aplicação de mais ETE e redução proporcional de EME, várias vantagens foram observadas: ganho em palatabilidade para cães e gatos; menor custo de processamento, somando-se o menor custo decorrente do uso de ETE (vapor) e o menor custo com desgaste de equipamentos; menor perda de nutrientes, pois aminoácidos e selênio sofreram maiores perdas e a formação de lisina reativa (que indica dano à proteína) foi maior no tratamento com mais EME. Como a menor aplicação de EME foi compensada com o emprego de mais ETE, o cozimento do amido e a digestibilidade dos nutrientes apresentaram-se de igual forma nas diferentes rações estudadas. O ganho em palatabilidade diferiu do relatado por Trivedi e Benning (2003), ao observarem que gatos manifestaram preferência por alimentos processados com mais EME. No entanto, estes autores não apresentaram os dados resultantes da aplicação de ETE e EET, de modo que é possível supor um subprocessamento, interferindo nos resultados.

## 2.6. Justificativa

Todas essas informações são novas, verificando-se, portanto, que ainda se desconhecem as implicações nutricionais e a macroestrutura dos kibbles resultantes do cozimento por energia térmica em relação a aqueles obtidos pela aplicação de energia mecânica. A presente Dissertação de Mestrado, a partir dos dados iniciais de Sá (2015), tem como hipótese que a transferência inadequada de ETE no pré-condicionador limite a eficiência da extrusão, afetando o cozimento, a digestibilidade e a palatabilidade de alimentos para cães. Por outro lado, acredita-se que a correta e suficiente aplicação de ETE no condicionador resultará em melhor cozimento do amido, promovendo-se, assim, adequada digestibilidade da dieta. Esta possível vantagem nutricional deverá ser acompanhada de ganhos em palatabilidade, provavelmente decorrentes da formação de compostos de odor (reação de Maillard) e indução de aspectos desejáveis na macroestrutura dos extrusados.

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## **CAPÍTULO 2 – Thermal energy application to the conditioner on the extrusion of dog foods<sup>1</sup>**

<sup>1</sup>Artigo redigido conforme as normas de publicação do *Animal Feed Science and Technology*, exceto o posicionamento das tabelas.

## THERMAL ENERGY APPLICATION TO THE CONDITIONER ON THE EXTRUSION OF DOG FOODS

### **Abstract**

The aims of this study were to verify the effects of six amounts of specific thermal energy (STE) application to the preconditioner in two extrusion systems on the processing parameters, starch gelatinization, kibble macrostructure, nutrients and energy apparent digestibility, fermentation products and palatability of extruded dog foods. The diets were produced by the modulation of water and steam addition to reach the desired temperatures which corresponded to the application of six STE (temperatures: 45°C, 55°C, 65°C, 75°C, 85°C, 95°C). Data were submitted to analysis of variance and means compared by polynomial contrasts ( $P < 0.05$ ). There was interaction between extruder and STE for preconditioner discharge mass and in-barrel moisture. There was a quadratic effect on both extruders for mass temperature before the die and STE/SME ratio; the application of STE increased the values. The motor load and pressure showed a quadratic reduction in both extruders, while there were no interaction effect for SME, STE and TSE. There was extruder effect for SME and TSE, the extruder A showed the highest values. There was STE effect for SME, and TSE, the SME reduced linearly with the application of STE and STE and TSE increased linearly. The piece specific density and radial expansion rate had a quadratic reduction in both extrusion systems with the application of STE. The specific length had a quadratic behavior in extruder B, and the lowest specific length was verified at temperature of 74.4°C, which did not occur with extruder A. The bulk density had a linear decrease with increasing STE, independent of the extruders. The starch

gelatinization (SG) of the dough from preconditioner had a quadratic augment in both extruders. The SG of the dried product showed a quadratic and linear increase in extruder A and B, respectively, with increasing the STE. There was a linear increase for *in vitro* digestibility in both extruders, with higher values for extruder B. There were no significant effects on palatability, fecal quality and nutrient digestibility with the application of STE. The application of STE into preconditioner seems to have more effect on the macrostructure of kibbles and pet food process parameters than on nutrient digestibility and fecal quality. However, more studies are needed to better understand the STE in other extrusion conditions.

*Keywords:* extrusion, macrostructure, pet food, starch gelatinization

*Abbreviations:* STE, specific thermal energy; SME, specific mechanical energy; TSE, total specific thermal energy; SG, starch gelatinization

## **1. Introduction**

The extrusion process has been investigated to improve the nutritional quality of a variety of raw materials into a high quality cooked food by gelatinization of starch, denaturation of protein, and inactivation of thermal labile anti-nutritional factors (Alonso et al., 2000).

Food extruders provide specific mechanical energy (SME) via main drive motor and specific thermal energy (STE) from steam and water injection during the processing, which together promote changes on the physicochemical properties of the nutrients and final product (Griffin, 2003). These changes may affect the pet food digestibility and palatability, and can have an impact in the animal's health. However,

few studies evaluated the impact of the extrusion processing for dog foods (Carciofi et al., 2012).

Extrusion cooking includes the steps of preconditioning, extrusion, cutting and drying. Each step performs a specific function in cooking and formation of the final product (Riaz, 2000). Preconditioning is an important part of the extrusion system, it starts the process of starch gelatinization, favor the internal hydration of food granules, the plasticization and sanitization of the dough, increase extruder stability and improve the quality of the final product (Gibson and Alavi, 2013; Guy, 2001).

The raw material transformation during the extrusion are directly linked and dependent on the total specific energy (TSE) applied to the dough, which is composed by the SME and STE input during the processing. It is believed that between 60% and 75% of the TSE applied to the pet food process corresponds to the STE, and the remainder is SME. Preconditioning of raw material increases the life of the equipment, due to a reduction in the wearing of barrel and screw components, and reduces the SME application, electric power consumption and processing cost (Riaz, 2000; Huber and Rokey, 1990). The throughput efficiency of an extruder can also be significantly increased if the starting raw material is properly preconditioned with steam and water for a proper period of time (Guy, 2001). However, the authors were unable to find published studies that evaluated the better TSE and STE:SME ratio to produce pet foods.

Therefore, the objectives of the present study were to compare the effects of six amounts of thermal energy application to the preconditioner in two extrusion systems on the processing parameters, starch gelatinization, kibble macrostructure, nutrients and energy apparent digestibility, and palatability of extruded dog foods.

## 2. Material and Methods

The study was conducted in two locations, at the Extrusion Laboratory of the Kansas State University, Manhattan, Kansas, USA (extrusion system A) and at the Faculdade de Ciências Agrárias e Veterinárias, UNESP – Univ Estadual Paulista, Jaboticabal, Brazil (extrusion system B). In both locations, a single formula of dog food was extruded with six applications of thermal energy on preconditioner, to obtain six treatments. The implications of the different STE application on extrusion parameters, kibble macrostructure, starch gelatinization, and *in vitro* digestibility were studied.

Additionally, the six foods produced at UNESP were submitted to *in vivo* digestibility trials with dogs and three of them to palatability evaluations in a qualified dog panel. They were selected due to the target linear differences on starch gelatinization after processing. 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 018909/14).

### 2.1. Experimental design and food formulation

The study of extrusion was organized in a 6 x 2 factorial arrangement of treatments, composed by six amounts of thermal energy application to the preconditioner and two extrusion systems (two locations), totaling 12 treatments. The experiment followed a completely randomized design. The experimental unit (repetition) was considered the food sampling taken every 10 minutes along the extrusion process, with four samplings per food. The digestibility study was organized in a completely randomized block design, with six diets and three blocks of 12 dogs;

there were two dogs per diet in each block, totalizing six dogs per diet. The experimental unit was considered one dog.

For each location a dog food was formulated for maintenance (Table 1), following the nutritional recommendations of FEDIAF (2014). The ingredients were purchased, mixed and ground in a hammer mill fitted with a 0.8 mm screen sieve size, compounding a single production lot. Thereafter, the mixed raw material were split into six parts to be extruder with six STE application.

Table 1

Ingredient and chemical composition of the experimental diets for dogs produced at the Kansas State University (KSU) and Univ Estadual Paulista (UNESP)

| Item  | Experimental diets |                  |
|---|--------------------|------------------|
|   | KSU                | UNESP            |
| <i>Ingredient Composition (g/kg, as-fed basis)</i>  |                    |                  |
| Corn, grain   | 547.6              | 557.1            |
| Chicken by-product meal   | 300.0              | 300.0            |
| Beet pulp   | 40.0               | -                |
| Sugarcane fiber   | -                  | 30.0             |
| Common salt   | 5.0                | 5.0              |
| Potassium chlorite  | 4.5                | 4.5              |
| Choline chloride  | 2.0                | 2.0              |
| Vitamin-mineral premix <sup>a</sup>   | -                  | 5.0              |
| Trace mineral premix <sup>b</sup>   | 2.2                | -                |
| Vitamin premix <sup>c</sup>   | 1.5                | -                |
| Potassium sorbate   | 1.0                | -                |
| Mold inhibitor  | -                  | 1.0              |
| Antioxidant   | 0.5 <sup>d</sup>   | 0.4 <sup>e</sup> |
| L-lysine  | 0.5                | -                |
| DL-methionine   | 0.2                | -                |
| Added by coating  |                    |                  |
| Chicken fat   | 85.0               | 85.0             |
| Palatant enhancer   | 10.0               | 10.0             |
| <i>Chemical composition of the kibbles after extrusion, before coating (g/kg, DM-basis)</i> |                    |                  |
| Moisture  | 61.2               | 70.3             |
| Ash   | 91.8               | 70.0             |
| Crude Protein   | 297.8              | 309.2            |
| Acid-hydrolyzed fat   | 94.8               | 80.1             |
| Starch  | 478.2              | 473.1            |

<sup>a</sup> Rovimix®, added per kg of food. DSM Produtos Nutricionais Brasil S/A.

<sup>b</sup> KS Dog & Cat Trace Mineral, added per kg of food. Lortscher Animal Nutrition Inc., Bern, Kansas, USA.

<sup>c</sup> KS Dog & Cat Vitamin Premix, added per kg of food. Lortscher Animal Nutrition Inc., Bern, Kansas, USA.

<sup>d</sup> Mold Zap® Citrus: Ammonium dipropionate, acetic acid, sorbic acid and benzoic acid. Alltech do Brasil Agroindustrial Ltda.

<sup>e</sup> Naturox®: Amorphous silicon dioxide, citric acid, natural mixed tocopherols, vegetable oil and rosemary extract. Kemin Nutrisurance, Inc.

<sup>f</sup> Banox®: Butylated hydroxyanisole, butylated hydroxytoluene, propyl gallate and calcium carbonate. Alltech do Brasil Agroindustrial Ltda.

## *2.2. Extrusion processing and experimental treatments*

At the Laboratory of Extrusion of the Kansas State University, a Wenger X-20 pilot-scale single-screw extruder (Wenger Manufacturing, Sabetha, Kansas, USA) and a circular die with an opening of 7.8 mm diameter was used to process all diets. The operating conditions were set at a feed rate of 160 kg/h of raw material, 400 rpm preconditioner cylinder speed, 1525 rpm knife speed and 637 rpm extruder shaft speed. The mean extruder open area was 271.5 mm<sup>2</sup>/ton/h. The extruded screw profile have five sections: initial - single flight screw and small steam lock; second – single flight screw and small steam lock; third – single flight screw and medium steam lock; fourth - double flight uncut screw and large steam lock; fifth - double flight uncut cone screw. These parameters were kept constant for all treatments.

At the Faculdade de Ciências Agrárias e Veterinárias, UNESP – Univ. Estadual Paulista, a Mex-250 single-screw extruder (Manzoni Industrial Ltda, Campinas, Sao Paulo, Brazil) and a circular die with an opening of 8.0 mm diameter was used to process all diets. The operations conditions were set at feed rate of 158 kg/h of raw material, 45.5 rpm preconditioner cylinder speed, 1026 rpm knife speed and 643 rpm extruder shaft speed. The mean extruder open area was 261.4 mm<sup>2</sup>/ton/h. The extruded screw profile have five sections: initial - single flight screw and no steam lock;

second – single flight screw and small steam lock; third – double flight uncut screw and small steam lock; fourth - double flight uncut screw and medium steam lock; fifth - double flight cut cone screw. These parameters were also kept constant for all treatments.

The thermal energy was added to the preconditioner through direct steam injection. Six different amounts of steam were injected, which implicated in six different temperatures of the mass out the preconditioner and constituted the experimental treatments as following: mass temperature of 45°C (T45); mass temperature of 55°C (T55); mass temperature of 65°C (T65); mass temperature of 75°C (T75); mass temperature of 85°C (T85); mass temperature of 95°C (T95).

In order to compensate the moisture of the mass during processing, the treatments with lower steam application had greater water addition on preconditioner to achieve similar in-barrel moisture content. Water addition in extruder barrel was kept constant for all treatments, around 7.5% of the total feed rate in both locations.

After the stabilization of the processing (30 to 40 min) the following parameters were recorded every 10 minutes: feed screw speed (rpm); preconditioner cylinder speed (rpm); preconditioner steam flow (kg/h); preconditioner water flow (kg/h); preconditioner water temperature (°C); preconditioner discharge mass temperature (°C); extrusion shaft speed (rpm); motor load (A); extruder water flow (kg/h); extruder water temperature (°C); knife speed (rpm); mass temperature before the die (°C); mass pressure before the die (Bars); steam pressure (psig); dryer temperature (°C) and retention time (min); ambient temperature (°C); air moisture (%). Kibble bulk density after extruder and after drier (g/L) were also recorded (measured as the weight of food corresponded to 1 L volume). At each observation time, food samples were collected

from the preconditioner, the extruder and dryer and stored at -20°C for further analysis. After extrusion, the kibbles were dried in a forced air dryer at 105°C for approximately 20 minutes.

### 2.3. Specific mechanical energy and specific thermal energy calculations

The SME (kW-h/ton) was calculated for each treatments using the equation A for Wenger X-20 extruder, and equation B for Manzoni Mex-250 extruder:

(A)

$$SME = \frac{\left(\frac{\tau - \tau_0}{100}\right) \times P_{rated} \times \left(\frac{N}{N_{rated}}\right)}{M}$$

Where:

$\tau$  = % torque

$\tau_0$  = % torque at no-load (34%)

$P_{rated}$  = rated motor power (37.3 kW)

$N$  = screw speed (rpm)

$N_{rated}$  = rated screw speed (508 RPM)

$M$  = mass flow rate from extruder (kg/s)

(B)

$$SME = \frac{\left(\sqrt{3} \times \text{Voltage} \times (A_t - A_v) \times (\cos\phi_i \div 1000)\right) \times 1000}{M}$$

Where:

Voltage = 220 V

$A_t$  = torque load working amperage (A)

$A_v$  = no torque load working amperage (A)

$\cos\phi_i$  = power factor (0.76)

$M$  = mass flow rate from extruder (kg/h)

The STE (kW-h/ton) was calculated by mass and energy balance equations in the conditioner and extruder according to Riaz (2000). The feed, water and steam total input and output mass amounts were determined. These mass values and the corresponded specific heats from each component of the system were used to calculate the amount of heat produced, as described below.

#### A. Mass Balance:

##### 1) For Preconditioner

$$M_r + M_{sp} + M_{wp} = M_p + M_{slp}$$

Where:

$M_r$  = raw material feed rate (kg/hr);

$M_{sp}$  = steam injection into preconditioner (kg/hr);

$M_{wp}$  = water injection into preconditioner (kg/hr);

$M_p$  = preconditioner product flow rate (kg/hr);

$M_{slp}$  = steam loss from preconditioner (kg/hr).

##### 2) For Extruder

$$M_p + M_{we} = M_{sle} + M_e$$

Where:

$M_p$  = preconditioner product flow rate (kg/hr);

$M_{we}$  = water injection into extruder (kg/hr);

$M_{sle}$  = steam loss from extruder (kg/hr);

$M_e$  = product flow rate (kg/hr).

## B. Energy Balance:

### 1) For Preconditioner

$$Q_r + Q_{sp} + Q_{wp} = Q_p + Q_{slp} + Q_{\Sigma\Delta h} + Q_{LP}$$

Where:

$Q_r$  = energy flow with raw material (kJ/hr);

$Q_{sp}$  = energy flow with steam injection (kJ/hr);

$Q_{wp}$  = energy flow with water injection (kJ/hr);

$Q_p$  = energy flow with flow rate (kJ/hr);

$Q_{slp}$  = energy flow with steam loss (kJ/hr);

$Q_{\Sigma\Delta h}$  = energy flow to cook starch and protein (kJ/hr);

$Q_{LP}$  = preconditioner energy loss (kJ/hr).

### 2) For Extruder

$$Q_p + Q_{we} + Q_{SME} = Q_{sle} + Q_{\Sigma\Delta h} + Q_{LE} + Q_e$$

Where:

$Q_p$  = energy flow with raw flow rate (kJ/hr);

$Q_{we}$  = energy flow with water injection (kJ/hr);

$Q_{SME}$  = energy flow with specific mechanical energy (kJ/hr);

$Q_{sle}$  = energy flow with product flow rate (kJ/hr);

$Q_{\Sigma\Delta h}$  = energy flow to cook starch and protein (kJ/hr);

$Q_{LE}$  = extruder energy loss (kJ/hr);

$Q_e$  = energy flow with flow rate (kJ/hr).

The TSE (kW-h/ton) was obtained by the summation of SME and STE.

#### 2.4. Kibble macrostructure, starch cooking and *in vitro* digestibility

For each treatment, the length ( $l_e$ ), diameter ( $d_e$ ) and mass ( $m_e$ ) of 20 extrudate kibbles were measured by using a vernier caliper. Data were used to obtain the radial expansion ratio (ER), specific length ( $l_{sp}$ ) and piece density ( $\rho$ ), as described below (Karkle et al., 2012). The die diameter ( $d_d$ ) used was 7.8 mm and 8.0 mm, respectively, for extruder A and B.

$$ER = \frac{d_e^2}{d_d^2} \quad l_{sp} (m/kg) = \frac{l_e}{m_e} \quad \rho (kg/m^3) = \frac{4 m_e}{\pi \cdot d_e^2 \cdot l_e}$$

Samples from preconditioner and after dryer were collected from each treatment for the measurement of the starch gelatinization, which was determined by the amyloglucosidase method (Sá et al., 2013). The *in vitro* digestibility 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.

#### 2.5. Food palatability test

The palatability testes were performed at Panellis Latin America (Descalvado, SP, Brazil) using a qualified panel of dogs. Three preference tests were performed, with foods produced at UNESP: T45 *versus* T75, T45 *versus* T95, and T75 *versus* T95. The first preference (first product consumed) and palatability (product consumed in greater amount) were compared using the two-pan method (Griffin, 2003). For the study, 36 dogs of different breeds and body weight, were housed individually, and tested on two consecutive meals. In the morning, after 12 hours fasting, the animals received the first meals in two pans, each one containing one of the experimental

foods, and were allowed to eat during 30 minutes. The position of the food bowls was changed at the evening meal. The amount of food offered in each bowl surpassed the consumption capacity of the animal to ensure there would be leftovers to measure. After 30 minutes, the bowls were removed, the remains weighted, and the consumption was calculated by taking the difference. Due to the differences in body weight, the results were calculated as relative consumption of each diet, and the mean intake of the two meals was compared.

### *2.6. Digestibility protocol*

The six diets produced at UNESP were used for the digestibility test. Thirty-six Beagle dogs with  $3.6 \pm 2.2$  years old and  $11.3 \pm 1.5$  kg of body weight were used. The health of the animals were confirmed prior to beginning of the study. The digestibility tests were carried out through the quantitative collection of feces without urine collection method according to FEDIAF (2014) recommendations. Dogs were allowed a 10-d diet adaptation phase, after a 5-d total fecal collection was conducted for determination of nutrient and energy digestibilities. During the adaptation, dogs were housed in 1.5 x 4.0 m kennels with a solarium. During the fecal collection period, dogs were individually housed in 1 x 1 x 1 m stainless steel metabolic cages. Animals were fed twice daily (10h00 am and 16h00 pm) in amounts sufficient to satisfy their metabolizable energy requirements ( $ME, Kcal/d = 130 \times BW^{0.75}$ ), as recommended by the NRC (2006). Feces were quantitatively collected and weighed at each feeding time, and scored using the system described by Carciofi (2008).

Feces were immediately frozen at  $-20^{\circ}C$  and a pool was composed by dog at the end of each collection period. Before analysis, all feces samples were dried using

a forced-air oven (Fanem, São Paulo, Brazil) at 55°C for 72 h. After that, feces and diets were ground to pass a 1-mm screen sieve in a cutting mill (MA-350, Marconi, Piracicaba, Brazil), and were analyzed according to AOAC (2010) for dry matter (DM) by drying the samples overnight in a forced-air oven at 105°C, crude protein (CP) using a Leco nitrogen/protein determination, with a Soxhlet apparatus extraction for acid hydrolyzed ether extract, and by muffle furnace incineration for ash content. Organic matter (OM) of the samples was calculated as DM minus ash. Gross energy (GE) of diets and feces were determined by an adiabatic bomb calorimeter (model 1281, Parr Instrument, USA). Starch content was determined according to the method of Hendrix (1993). All samples were analyzed in duplicate and the analyses were repeated when the variation among duplicates was greater than 5%.

### *2.7. Faecal pH and fermentation products*

Fresh faecal samples (collected and processed within 15 min of elimination) were collected on three consecutive days to measure pH, short-chain fatty acids (SCFAs), lactic acid and ammonia. Faecal pH was measured immediately after collection by mixing 2 g of fresh faeces with 6 mL of ultrapure water with 3 g of fresh faeces (1:3 w/v) in a pH metre (model DM20, Digicrom Analítica Ltda, São Paulo, Brazil). For SCFA and branched-chain fatty acids (BCFAs) analyses, 10 g of fresh faeces were mixed in 30 mL of formic acid solution at 4.2 N (1:3 w/v), precipitated at 4 °C for 72 h, and supernatant was centrifuged three times at 5000 G at 15 C for 15 minutes. The concentration of SCFAs and BCFAs were performed by gas chromatography (model GC-2014, Shimadzu, Kyoto, Japan) according to Erwin et al. (1961), using a flame ionization detector (FID) and fused silica capillary column (HP

INNOwas – 19091N), which was 30 m in length and 0.32 mm diameter and had a film thickness of 0.50  $\mu\text{m}$ . Nitrogen was used as a carrier gas at a flow of 3.18 mL/min. A 1- $\mu\text{L}$  aliquot of the sample was injected into a “split” at a flow of 30 and a temperature of 250°C. The temperature of oven was programmed to remain at 80°C for 3 min and then increase to 240°C at 20°C/min for 15 min, while detector was at 250°C. Lactic acid was measured by mixing 3 g of faeces with 9 mL of distilled water (1:3 w/v), using a colorimetric method (Spectrophotometer Quick – Lab, Drake, São José do Rio Preto, Brazil) according to Pryce (1969). The concentration of ammonia ( $\text{NH}_3$ ) was assessed in the same extracts prepared for SCFAs and BCFAs analysis, according to Vieira (1980). The extracts were thawed at room temperature, diluted into distilled water (2:13 v/v) and then ammonia was distilled in a nitrogen system (TE Tecnal – 036/1, Piracicaba, Brazil).

## *2.8. Statistical analysis*

Results of the extrusion parameters were analyzed as a 6 x 2 factorial arrangement of treatments, in a complete randomized design. Data were submitted to analysis of variance, model sums of squares were separated into the effects of the extruder, preconditioner temperature and their interactions. When differences were found on F test, means were compared by polynomial contrasts. For the palatability study, the first preference was evaluated through the  $\text{Chi}^2$  test, and for the food intake rate the Student test was applied. Data of digestibility experiment and fermentation products were submitted to analysis of variance, in a completely randomized block design. Models sums of square were separated on diet and block effects. When differences were found on F test, means were also compared by polynomial contrasts.

All data were found to comply with the assumptions of the analysis of variance, and were analyzed using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC).

### **3. Results**

#### *3.1. Extrusion traits*

The chemical composition of the diets produced at KSU and UNESP was similar, with very close protein and starch contents, only ash and fat content were a slight higher for the diet made at KSU. The only difference on ingredient composition was the fiber sources, as beet pulp was used at KSU and sugarcane fiber at UNESP.

During extrusion, the target temperatures of the experimental treatments was obtained with very small differences (Table 2). The values were also very close in both locations of extrusion. Despite the procedures adopted to control the preconditioner mass moisture, it reduced two points of percentage for extruder A and increased two points of percentage of extruder B ( $P<0.001$ ). The product flow rate, on the other hand, did not change for the different STE application, but was different for both extruders ( $P<0.001$ ).

Table 2

Processing traits and starch gelatinization after preconditioner of dog foods extruded in two locations with different specific thermal energy applications

| Item                             | Extruder <sup>a</sup> | Preconditioner Temperature (°C) <sup>b</sup> |       |       |       |       |       | Mean  | SEM <sup>c</sup> | P value          |                   |            | Contrasts <sup>f</sup> |       |
|----------------------------------|-----------------------|--|-------|-------|-------|-------|-------|-------|------------------|------------------|-------------------|------------|------------------------|-------|
|                                  |                       | 45   | 55    | 65    | 75    | 85    | 95    |       |                  | Ext <sup>d</sup> | Temp <sup>e</sup> | Ext x Temp | Linear                 | Quad  |
| <i>Preconditioner</i>            |                       |  |       |       |       |       |       |       |                  |                  |                   |            |                        |       |
| Discharge mass temperature (°C)  |                       |  |       |       |       |       |       |       |                  |                  |                   |            |                        |       |
|                                  | A                     | 44.3   | 55.2  | 64.9  | 74.0  | 85.6  | 95.3  | 69.8  | 0.13             | 0.030            | <.001             | <.001      | <.001                  | 0.409 |
|                                  | B                     | 43.5   | 54.3  | 64.5  | 75.7  | 86.0  | 93.0  | 69.4  | 0.13             |                  |                   |            | <.001                  | <.001 |
|                                  | Mean                  | 43.9   | 54.7  | 64.7  | 74.8  | 85.8  | 94.0  |       | 0.23             |                  |                   |            |                        |       |
| Discharge mass moisture (%)      |                       |  |       |       |       |       |       |       |                  |                  |                   |            |                        |       |
|                                  | A                     | 25.5   | 23.6  | 22.8  | 24.7  | 23.5  | 22.2  | 23.7  | 0.18             | <.001            | 0.055             | 0.002      | 0.003                  | –     |
|                                  | B                     | 20.5   | 20.7  | 20.7  | 21.4  | 21.6  | 22.5  | 21.2  | 0.19             |                  |                   |            | 0.001                  | –     |
|                                  | Mean                  | 23.0   | 22.1  | 21.7  | 23.1  | 22.5  | 22.3  |       | 0.33             |                  |                   |            |                        |       |
| Product flow rate (kg/h)         |                       |  |       |       |       |       |       |       |                  |                  |                   |            |                        |       |
|                                  | A                     | 160.8  | 172.5 | 173.4 | 168.4 | 173.0 | 171.2 | 169.9 | 1.58             | <.001            | 0.586             | 0.215      |                        |       |
|                                  | B                     | 192.1  | 185.9 | 189.3 | 193.6 | 191.1 | 195.5 | 191.2 | 1.62             |                  |                   |            |                        |       |
|                                  | Mean                  | 176.4  | 179.2 | 181.3 | 181.0 | 182.1 | 183.3 |       | 2.77             |                  |                   |            |                        |       |
| Starch gelatinization (%)        |                       |  |       |       |       |       |       |       |                  |                  |                   |            |                        |       |
|                                  | A                     | 18.4   | 19.3  | 19.1  | 20.8  | 21.7  | 25.1  | 20.7  | 0.11             | <.001            | <.001             | <.001      | <.001                  | <.001 |
|                                  | B                     | 26.1   | 28.0  | 29.0  | 31.1  | 33.6  | 30.8  | 29.8  | 0.11             |                  |                   |            | <.001                  | <.001 |
|                                  | Mean                  | 22.3   | 23.7  | 24.0  | 25.9  | 27.7  | 28.0  |       | 0.19             |                  |                   |            |                        |       |
| <i>Extruder</i>                  |                       |  |       |       |       |       |       |       |                  |                  |                   |            |                        |       |
| Motor amperage (A)               |                       |  |       |       |       |       |       |       |                  |                  |                   |            |                        |       |
|                                  | A                     | 33.3   | 34.0  | 33.2  | 33.0  | 32.5  | 31.7  | 32.9  | 0.09             | <.001            | <.001             | <.001      | <.001                  | 0.025 |
|                                  | B                     | 42.5   | 41.0  | 39.2  | 38.3  | 36.4  | 36.8  | 39.0  | 0.09             |                  |                   |            | <.001                  | 0.002 |
|                                  | Mean                  | 37.9   | 37.6  | 36.2  | 35.7  | 34.5  | 34.3  |       | 0.17             |                  |                   |            |                        |       |
| Pressure (Bars)                  |                       |  |       |       |       |       |       |       |                  |                  |                   |            |                        |       |
|                                  | A                     | 33.7   | 39.6  | 39.3  | 33.4  | 34.4  | 26.8  | 34.5  | 0.55             | <.001            | <.001             | 0.004      | 0.001                  | 0.001 |
|                                  | B                     | 50.2   | 50.7  | 50.0  | 43.2  | 38.5  | 37.5  | 45.0  | 0.55             |                  |                   |            | <.001                  | 0.002 |
|                                  | Mean                  | 42.0   | 45.1  | 44.6  | 38.3  | 36.4  | 32.1  |       | 0.95             |                  |                   |            |                        |       |
| Mass temperature before die (°C) |                       |  |       |       |       |       |       |       |                  |                  |                   |            |                        |       |
|                                  | A                     | 109.7  | 113.2 | 113.8 | 115.4 | 116.3 | 115.6 | 114.0 | 0.14             | <.001            | <.001             | <.001      | <.001                  | <.001 |
|                                  | B                     | 112.5  | 115.5 | 119.0 | 122.2 | 123.2 | 125.5 | 119.6 | 0.14             |                  |                   |            | <.001                  | 0.002 |

|                                  |       |       |       |       |       |       |       |      |       |       |       |        |       |  |
|----------------------------------|-------|-------|-------|-------|-------|-------|-------|------|-------|-------|-------|--------|-------|--|
| Mean                             | 111.1 | 114.3 | 116.4 | 118.8 | 119.8 | 120.5 |       | 0.25 |       |       |       |        |       |  |
| Product flow rate (kg/h)         |       |       |       |       |       |       |       |      |       |       |       |        |       |  |
| A                                | 167.2 | 178.6 | 179.1 | 175.5 | 181.3 | 176.8 | 176.4 | 1.24 | <.001 | 0.471 | 0.068 |        |       |  |
| B                                | 195.5 | 190.2 | 189.5 | 192.7 | 194.5 | 192.0 | 192.4 | 1.24 |       |       |       |        |       |  |
| Mean                             | 181.3 | 184.4 | 184.3 | 184.1 | 187.9 | 184.4 |       | 2.15 |       |       |       |        |       |  |
| In-barrel moisture (%)           |       |       |       |       |       |       |       |      |       |       |       |        |       |  |
| A                                | 26.7  | 27.0  | 26.7  | 26.8  | 26.8  | 26.7  | 26.8  | 0.10 | <.001 | 0.009 | 0.005 | –      | 0.002 |  |
| B                                | 23.5  | 23.8  | 23.8  | 24.4  | 24.6  | 25.4  | 24.2  | 0.10 |       |       |       | 0.0009 | –     |  |
| Mean                             | 25.1  | 25.4  | 25.2  | 25.6  | 25.7  | 26.0  |       | 0.17 |       |       |       |        |       |  |
| <i>Energy Balance (kW-h/ton)</i> |       |       |       |       |       |       |       |      |       |       |       |        |       |  |
| Specific mechanical energy       |       |       |       |       |       |       |       |      |       |       |       |        |       |  |
| A                                | 44.0  | 43.5  | 40.6  | 40.9  | 37.9  | 36.3  | 40.5  | 0.42 | <.001 | <.001 | 0.652 |        |       |  |
| B                                | 18.5  | 16.8  | 14.1  | 12.5  | 9.6   | 10.3  | 13.6  | 0.42 |       |       |       |        |       |  |
| Mean                             | 31.2  | 20.3  | 27.4  | 26.7  | 23.7  | 23.3  |       | 0.73 |       |       |       | <.001  | –     |  |
| Specific thermal energy          |       |       |       |       |       |       |       |      |       |       |       |        |       |  |
| A                                | 10.6  | 24.5  | 29.7  | 36.0  | 45.8  | 59.9  | 34.4  | 1.75 | 0.065 | <.001 | 0.849 |        |       |  |
| B                                | 19.6  | 23.7  | 35.1  | 44.0  | 47.2  | 64.6  | 39.0  | 1.67 |       |       |       |        |       |  |
| Mean                             | 15.1  | 24.1  | 32.4  | 40.0  | 46.5  | 62.3  |       | 2.97 |       |       |       | <.001  | –     |  |
| Total specific energy            |       |       |       |       |       |       |       |      |       |       |       |        |       |  |
| A                                | 53.7  | 66.4  | 70.3  | 77.3  | 83.6  | 89.8  | 73.5  | 1.64 | <.001 | <.001 | 0.538 |        |       |  |
| B                                | 38.1  | 40.5  | 49.3  | 56.5  | 56.8  | 75.5  | 52.8  | 1.60 |       |       |       |        |       |  |
| Mean                             | 45.9  | 53.5  | 59.8  | 66.9  | 70.2  | 82.6  |       | 2.81 |       |       |       | <.001  | –     |  |
| STE/SME ratio                    |       |       |       |       |       |       |       |      |       |       |       |        |       |  |
| A                                | 0.2   | 0.5   | 0.7   | 0.8   | 1.2   | 1.6   | 0.8   | 0.12 | <.001 | <.001 | <.001 | <.001  | –     |  |
| B                                | 1.0   | 1.4   | 2.4   | 3.5   | 4.8   | 6.0   | 3.2   | 0.12 |       |       |       | <.001  | –     |  |
| Mean                             | 0.6   | 0.9   | 1.6   | 2.2   | 3.0   | 3.8   |       | 0.21 |       |       |       |        |       |  |

<sup>a</sup> Extruder: A = Wenger X-20; B = Manzoni Mex-250.

<sup>b</sup> Target of the temperature.

<sup>c</sup> Standard error of the mean (n = 4 repetitions per treatment).

<sup>d</sup> Extruder effect.

<sup>e</sup> Preconditioner temperature effect.

<sup>f</sup> Linear or quadratic effects of the preconditioner temperature.

The ground raw material presented different starch gelatinization degrees; the raw mixture of extruder A and B presented 13.4% and 22.8% of starch gelatinization, respectively. This was probably due to the type and configuration of the hammer mill used, that resulted in higher or lower exposure of the starch granules content. Due this, although apparently, the cooking differed in both preconditioners, the results were strongly influenced by the initial gelatinization of the raw material, and in both extruders a quadratic increase on starch gelatinization followed the increase on STE application (preconditioner temperature). For extruder A, starch gelatinization as a function of preconditioner temperature was described as: Starch gelatinization in the preconditioner =  $21.24 - 0.34(\text{Temp}) + 0.0034(\text{Temp}^2)$ ;  $r^2 = 0.98$ ;  $P < 0.001$ . For extruder B was described as: Starch gelation on preconditioner =  $23.8 - 0.38(\text{Temp}) + 0.0005(\text{Temp}^2)$ ;  $r^2 = 0.97$ ;  $P < 0.001$ .

The extruder motor amperage and mass pressure before the die reduced in both extruders with the increase on preconditioner temperature ( $P < 0.001$ ). The reductions of motor amperage followed a quadratic regression, as the reduction was greater until 65°C but proportionally smaller after this temperature. The mass pressure as a function of preconditioner temperature was described for extruder A as: Mass pressure =  $-5.01 + 1.39(\text{Temp}) - 0.011(\text{Temp}^2)$ ;  $r^2 = 0.54$ ;  $P < 0.001$ . For extruder B was: Mass pressure =  $47.15 + 0.28(\text{Temp}) - 0.004(\text{Temp}^2)$ ;  $r^2 = 0.87$ ;  $P < 0.001$ . The derivation of this equations resulted on maximum theoretical pressure with a preconditioner temperature of 63.1°C for extruder A, and 33.5°C for extruder B, and after these temperatures, the mass pressure exhibits a decrease.

The temperature of the mass before the die, on the other hand increased in both places with the increase on STE application ( $P < 0.001$ ). An interaction was verified with

higher temperature of extrusion for extruder B ( $P < 0.001$ ). The mass temperature as a function of preconditioner temperature was describe for extruder A as: Mass temperature =  $89.68 + 0.04(\text{Temp}) - 0.0035(\text{Temp}^2)$ ;  $r^2 = 0.91$ ;  $P < 0.001$ . For extruder B was: Mass temperature =  $90.05 + 0.61(\text{Temp}) - 0.0025(\text{Temp}^2)$ ;  $r^2 = 0.97$ ;  $P < 0.001$ . The derivation of this equations resulted on maximum extrusion temperature with a preconditioner temperature of  $87.1^\circ\text{C}$  and  $121.0^\circ\text{C}$  for extruder A and B, respectively. As  $121.0^\circ\text{C}$  is not conceivable, is possible to conclude that the maximum temperature of extrusion was not achieved for this particular condition of operation or equipment.

The product flow rate was similar between preconditioner temperatures, but varied for different extruders ( $P < 0.001$ ). The in-barrel moisture also varied between extruders, been higher for extruder A ( $P < 0.001$ ). Although it varied between preconditioner temperatures ( $P < 0.01$ ), the variation was numerically small.

On energy balance, SME application reduced linearly with the increase on preconditioner temperature ( $P < 0.001$ ). No interaction was verified, but an extruder effect was observed with higher SME application for extruder A ( $P < 0.001$ ). To be more reliable, separate equations of the effect of preconditioner temperature on SME were tested. For extruder A:  $10.98 + 0.360(\text{Temp})$ ;  $r^2 = 0.85$ ;  $P < 0.001$ . For extruder B:  $4.03 + 0.09(\text{Temp})$ ;  $r^2 = 0.78$ ;  $P < 0.001$ . The STE application did not differ between locations, but increased linearly according preconditioner temperature: STE application =  $-2.96 + 0.23(\text{Temp})$ ;  $r^2 = 0.79$ ;  $P < 0.001$ . The STE application increase from  $15 \text{ kW-h/ton}$  on treatment T45 to  $62 \text{ kW-h/ton}$  on treatment T95. Due this, a linear increase on TSE was observed ( $P < 0.001$ ), without a treatment x extruder interaction. The TSE application differ among extruder ( $P < 0.001$ ), been higher for extruder A due to the

higher SME application. The difference on SME application also induced differences between extruders on the STE/SME ratio ( $P < 0.001$ ).

### 3.2. Kibble macrostructure, starch gelatinization and *in vitro* digestibility

A significant interaction ( $P < 0.001$ ) between extruder and preconditioner temperature was observed for all analyzed kibble characteristics (Table 3). In addition, an extruder effect was also verified ( $P < 0.001$ ). For extruder A the piece density reduced, specific length did no change, and expansion rate increased quadratically with the increase on preconditioner temperature ( $P < 0.001$ ). For extruder B the kibble structure changed in the same way, but more clearly with a quadratic reduction on piece density, and quadratic increase on expansion rate ( $P < 0.001$ ). Bulk density of the dry kibbles reduced linearly in both extruders ( $P < 0.001$ ), reducing from 346 g/L to 310 g/L for extruder A (Bulk density =  $327.1 + 1.15(\text{Temp})$ ;  $r^2 = 0.53$ ;  $P < 0.001$ ) and from 444 g/L to 355 g/L for extruder B (Bulk density =  $527.6 - 1.92(\text{Temp})$ ;  $r^2 = 0.97$ ;  $P < 0.001$ ). The bulk density was also related with TSE application (Extruder A:  $r^2 = 0.56$ ;  $P < 0.001$ . Extruder B:  $r^2 = 0.62$ ;  $P < 0.001$ ).

Starch gelatinization increased in both extruders with the increase on preconditioner temperature ( $P < 0.001$ ). It increased quadratically for extruder A (Starch gelatinization =  $39.74 + 1.17(\text{Temp}) - 0.007(\text{Temp}^2)$ ;  $r^2 = 0.88$ ;  $P < 0.001$ ), with an estimated derivation of 82.4 °C, and in a linear manner for extruder B (Starch gelatinization =  $52.19 + 0.35(\text{Temp})$ ;  $r^2 = 0.96$ ;  $P < 0.001$ ). Starch gelatinization was also related with TSE application (Extruder A: starch gelatinization =  $26.13 - 1.39(\text{TSE}) - 0.008(\text{TSE}^2)$ ;  $r^2 = 0.78$ ;  $P < 0.001$ ; derivate = 87.8. Extruder B: starch gelatinization =  $63.16 - 0.205(\text{TSE})$ ,  $r^2 = 0.59$ ,  $P = 0.0001$ ).

The *in vitro* digestibility of the OM of the diets increased linearly ( $P < 0.001$ ) in both extruders with the increase on preconditioner temperature (Extruder A: *in vitro* digestibility =  $78.98 + 0.038(\text{Temp})$ ;  $r^2 = 0.52$ ;  $P < 0.001$ . Extruder B: *in vitro* digestibility =  $81.13 + 0.106(\text{Temp})$ ;  $r^2 = 0.76$ ;  $P < 0.001$ ). The OM *in vitro* digestibility also increased with the TSE application (Extruder A: *in vitro* digestibility =  $77.54 + 0.07(\text{TSE})$ ,  $r^2 = 0.33$ ,  $P = 0.023$ . Extruder B: *in vitro* digestibility =  $83.59 + 0.059(\text{TSE})$ ;  $r^2 = 0.41$ ;  $P = 0.005$ ).

Table 3

Kibble macrostructure, starch gelatinization after drier, and *in vitro* digestibility of the OM of dog foods extruded in two locations with different specific thermal energy applications

| Item  | Extruder <sup>a</sup> | Preconditioner Temperature (°C) <sup>b</sup> |      |      |      |      |      | Mean | SEM <sup>c</sup> | P value          |                   |            | Contrasts <sup>f</sup> |       |
|---|-----------------------|--|------|------|------|------|------|------|------------------|------------------|-------------------|------------|------------------------|-------|
|   |                       | 45   | 55   | 65   | 75   | 85   | 95   |      |                  | Ext <sup>d</sup> | Temp <sup>e</sup> | Ext x Temp | Linear                 | Quad  |
| Piece density (g/cm <sup>3</sup> )          |                       |  |      |      |      |      |      |      |                  |                  |                   |            |                        |       |
|   | A                     | 0.44   | 0.44 | 0.42 | 0.41 | 0.40 | 0.43 | 0.42 | 0.001            | <.001            | <.001             | <.001      | <.001                  | <.001 |
|   | B                     | 0.55   | 0.51 | 0.49 | 0.47 | 0.46 | 0.43 | 0.49 | 0.001            |                  |                   |            | <.001                  | 0.027 |
|   | Mean                  | 0.50   | 0.48 | 0.46 | 0.44 | 0.43 | 0.43 |      | 0.003            |                  |                   |            |                        |       |
| Specific length (cm/g)                      |                       |  |      |      |      |      |      |      |                  |                  |                   |            |                        |       |
|   | A                     | 2.27   | 2.03 | 2.19 | 2.25 | 2.30 | 2.19 | 2.20 | 0.010            | <.001            | <.001             | <.001      | –                      | –     |
|   | B                     | 1.85   | 1.81 | 1.81 | 1.80 | 1.81 | 1.87 | 1.82 | 0.010            |                  |                   |            | –                      | 0.002 |
|   | Mean                  | 2.06   | 1.91 | 2.00 | 2.02 | 2.05 | 2.03 |      | 0.018            |                  |                   |            |                        |       |
| Expansion rate                              |                       |  |      |      |      |      |      |      |                  |                  |                   |            |                        |       |
|   | A                     | 2.1  | 2.3  | 2.3  | 2.3  | 2.3  | 2.2  | 2.2  | 0.01             | <.001            | <.001             | <.001      | <.001                  | <.001 |
|   | B                     | 2.1  | 2.2  | 2.4  | 2.5  | 2.5  | 2.6  | 2.4  | 0.01             |                  |                   |            | <.001                  | <.001 |
|   | Mean                  | 2.1  | 2.2  | 2.3  | 2.4  | 2.4  | 2.4  |      | 0.01             |                  |                   |            |                        |       |
| Bulk density (g/L)                          |                       |  |      |      |      |      |      |      |                  |                  |                   |            |                        |       |
|   | A                     | 346  | 361  | 342  | 321  | 340  | 310  | 336  | 1.42             | <.001            | <.001             | <.001      | <.001                  | –     |
|   | B                     | 444  | 426  | 405  | 391  | 376  | 355  | 400  | 1.46             |                  |                   |            | <.001                  | –     |
|   | Mean                  | 395  | 393  | 373  | 356  | 358  | 332  |      | 2.50             |                  |                   |            |                        |       |
| Starch gelatinization after drier (%)       |                       |  |      |      |      |      |      |      |                  |                  |                   |            |                        |       |
|   | A                     | 78.0   | 82.8 | 85.9 | 86.1 | 89.7 | 86.1 | 84.8 | 0.25             | <.001            | <.001             | <.001      | <.001                  | <.001 |
|   | B                     | 69.0   | 72.2 | 77.8 | 80.1 | 85.3 | 89.4 | 79.0 | 0.26             |                  |                   |            | <.001                  | –     |
|   | Mean                  | 73.5   | 77.5 | 81.8 | 83.1 | 87.5 | 87.8 |      | 0.44             |                  |                   |            |                        |       |
| <i>In vitro</i> digestibility of the OM (%) |                       |  |      |      |      |      |      |      |                  |                  |                   |            |                        |       |
|   | A                     | 80.9   | 80.3 | 81.3 | 81.7 | 82.2 | 81.9 | 81.4 | 0.08             | <.001            | <.001             | 0.009      | <.001                  | –     |
|   | B                     | 84.8   | 85.6 | 86.1 | 86.2 | 86.3 | 86.9 | 86.0 | 0.08             |                  |                   |            | <.001                  | –     |
|   | Mean                  | 82.8   | 82.9 | 83.7 | 83.9 | 84.2 | 84.4 |      | 0.14             |                  |                   |            |                        |       |

<sup>a</sup> Extruder: A = Wenger X-20; B = Manzoni Mex-250.

<sup>b</sup> Target of the temperature.

<sup>c</sup> Standard error of the mean (n = 4 repetitions per treatment).

<sup>d</sup> Extruder effect.

<sup>e</sup> Preconditioner temperature effect <sup>f</sup> Linear or quadratic effects of the preconditioner temperature.

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

To study digestibility, the foods produced at UNESP were coated with poultry fat and liquid palatant, as described on Table 1. The analyzed chemical composition of the diets after coating are on Table 4. Their chemical composition were similar, with the exception of a slightly reduced fat content for diet T75. Nutrient intake did not differ among diets (Table 5). The apparent total tract digestibility was similar for all the diets, with the exception of a linear reduction on starch digestibility with the increase on preconditioner temperature ( $P < 0.001$ ). This reduction, however, was small and only 0.1%. Feces production and characteristics were also similar among the dogs fed the experimental diets.

Table 4

Analyzed chemical composition of the experimental diets for dogs extruded with different specific thermal energy. Diets produced at UNESP. Values of the foods after coating with poultry fat and palatant enhancer

| Item   | Preconditioner Temperature, °C |       |       |       |       |       |
|--|--------------------------------|-------|-------|-------|-------|-------|
|  | 45                             | 55    | 65    | 75    | 85    | 95    |
| <i>Chemical Composition (g/kg, DM-basis)</i> |                                |       |       |       |       |       |
| Moisture                                     | 79.8                           | 77.5  | 71.8  | 79.6  | 73.2  | 74.6  |
| Ash  | 68.2                           | 69.0  | 69.1  | 70.7  | 70.8  | 71.1  |
| Crude Protein                                | 274.9                          | 273.6 | 276.3 | 285.8 | 278.6 | 281.7 |
| Acid-hydrolyzed fat                          | 161.1                          | 153.4 | 159.1 | 146.1 | 163.9 | 161.3 |
| Starch                                       | 398.6                          | 419.0 | 399.2 | 412.5 | 394.8 | 395.3 |

Table 5

Nutrient intake and apparent total tract digestibility coefficients of dogs fed experimental diets extruded with different specific thermal energy. Diets produced at UNESP

| Item                                      | Preconditioner Temperature, °C |       |       |       |       |       | SEM <sup>a</sup> | P value | Contrast <sup>b</sup> |      |
|---|--------------------------------|-------|-------|-------|-------|-------|------------------|---------|-----------------------|------|
|   | 45                             | 55    | 65    | 75    | 85    | 95    |                  |         | Linear                | Quad |
| <i>Nutrient intake (g/dog/d)</i>          |                                |       |       |       |       |       |                  |         |                       |      |
| Dry Matter                                | 161.8                          | 168.4 | 160.4 | 175.4 | 162.0 | 161.5 | 6.96             | 0.617   | –                     | –    |
| Organic Matter                            | 163.9                          | 170.1 | 161.0 | 177.7 | 162.9 | 162.7 | 7.02             | 0.546   | –                     | –    |
| Acid-hydrolyzed Fat                       | 28.3                           | 29.4  | 27.8  | 30.7  | 28.1  | 28.1  | 1.21             | 0.546   | –                     | –    |
| Protein                                   | 48.3                           | 50.1  | 47.5  | 52.4  | 48.0  | 47.9  | 2.07             | 0.546   | –                     | –    |
| Starch                                    | 70.1                           | 72.7  | 68.8  | 76.0  | 69.6  | 69.5  | 3.00             | 0.546   | –                     | –    |
| <i>Apparent total tract digestibility</i> |                                |       |       |       |       |       |                  |         |                       |      |
| Dry Matter                                | 0.830                          | 0.833 | 0.830 | 0.836 | 0.833 | 0.828 | 0.58             | 0.932   | –                     | –    |
| Organic Matter                            | 0.864                          | 0.868 | 0.866 | 0.869 | 0.868 | 0.863 | 0.47             | 0.919   | –                     | –    |
| Acid-hydrolyzed Fat                       | 0.924                          | 0.927 | 0.918 | 0.924 | 0.922 | 0.929 | 0.89             | 0.966   | –                     | –    |
| Crude Protein                             | 0.885                          | 0.889 | 0.890 | 0.896 | 0.895 | 0.893 | 0.45             | 0.544   | –                     | –    |
| Starch                                    | 0.998                          | 0.998 | 0.997 | 0.997 | 0.997 | 0.997 | 0.01             | <.001   | <.001                 | –    |
| Gross Energy                              | 0.873                          | 0.878 | 0.877 | 0.880 | 0.879 | 0.875 | 0.45             | 0.901   | –                     | –    |

<sup>a</sup> Standard error of the mean (n = 6 dogs per food).

<sup>b</sup> Linear or quadratic effects of the preconditioner temperature.

#### *3.4. Faecal characteristics and fermentation products*

The faecal characteristics and fermentation products were similar among the dogs fed the experimental diets (Table 6).

Table 6  
Faecal characteristics and fermentation products of dogs fed experimental diets extruded with different specific thermal energy. Diets produced at UNESP

| Item   | Preconditioner Temperature, °C |       |       |       |       |       | SEM <sup>a</sup> | P value | Contrast <sup>b</sup> |      |
|--|--------------------------------|-------|-------|-------|-------|-------|------------------|---------|-----------------------|------|
|  | 45                             | 55    | 65    | 75    | 85    | 95    |                  |         | Linear                | Quad |
| <i>Faecal characteristics (g/dog/d)</i>            |                                |       |       |       |       |       |                  |         |                       |      |
| g/dog/d (as-is basis)                              | 69.7                           | 70.8  | 66.1  | 72.2  | 61.6  | 63.4  | 5.08             | 0.625   | –                     | –    |
| g/dog/d (DM-basis)                                 | 27.6                           | 27.9  | 27.2  | 28.7  | 26.9  | 27.7  | 1.63             | 0.975   | –                     | –    |
| Dry matter (g/kg)                                  | 397.4                          | 399.3 | 412.3 | 406.5 | 438.6 | 445.5 | 1.69             | 0.228   | –                     | –    |
| Score <sup>c</sup>                                 | 3.9                            | 3.9   | 4.0   | 3.9   | 4.0   | 4.0   | 0.02             | 0.387   | –                     | –    |
| pH   | 6.5                            | 6.3   | 6.3   | 6.4   | 6.4   | 6.6   | 0.07             | 0.119   | –                     | –    |
| <i>Fermentation products (mMol/g of faecal DM)</i> |                                |       |       |       |       |       |                  |         |                       |      |
| Acetic acid  | 229.8                          | 265.1 | 286.7 | 245.9 | 226.7 | 224.0 | 17.06            | 0.086   | –                     | –    |
| Propionic acid                                     | 122.1                          | 138.4 | 147.0 | 115.9 | 117.0 | 110.2 | 9.49             | 0.069   | –                     | –    |
| Butyric acid                                       | 49.5                           | 53.3  | 58.2  | 64.8  | 48.6  | 50.3  | 5.41             | 0.271   | –                     | –    |
| Total SCFA <sup>d</sup>                            | 401.4                          | 456.9 | 491.9 | 426.6 | 392.3 | 384.5 | 27.95            | 0.076   | –                     | –    |
| Isobutyric acid                                    | 7.6                            | 7.7   | 8.2   | 8.0   | 7.1   | 8.6   | 0.60             | 0.596   | –                     | –    |
| Isovaleric acid                                    | 11.1                           | 11.4  | 11.6  | 11.9  | 10.5  | 12.7  | 0.85             | 0.568   | –                     | –    |
| Valeric acid                                       | 0.02                           | 0.03  | 0.02  | 0.02  | 0.02  | 0.02  | 0.01             | 0.485   | –                     | –    |
| Total BCFA <sup>e</sup>                            | 18.7                           | 19.2  | 19.8  | 20.0  | 17.6  | 21.3  | 1.44             | 0.579   | –                     | –    |
| Total VFA <sup>f</sup>                             | 420.1                          | 476.0 | 511.8 | 446.6 | 409.9 | 405.8 | 28.86            | 0.091   | –                     | –    |
| Ammonia (mMol/Kg of faecal DM)                     | 116.6                          | 146.2 | 140.9 | 131.3 | 117.8 | 132.9 | 12.99            | 0.528   | –                     | –    |
| Lactate (mMol/Kg of faecal DM)                     | 8.5                            | 8.2   | 10.1  | 8.2   | 8.1   | 8.1   | 0.46             | 0.029   | –                     | –    |

<sup>a</sup> Standard error of the mean (n = 6 dogs per food).

<sup>b</sup> Linear or quadratic effects of the preconditioner temperature.

<sup>c</sup> According to the following system: 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.

<sup>d</sup> SCFA – short-chain fatty acids.

<sup>e</sup> BCFA – branched-chain fatty acids.

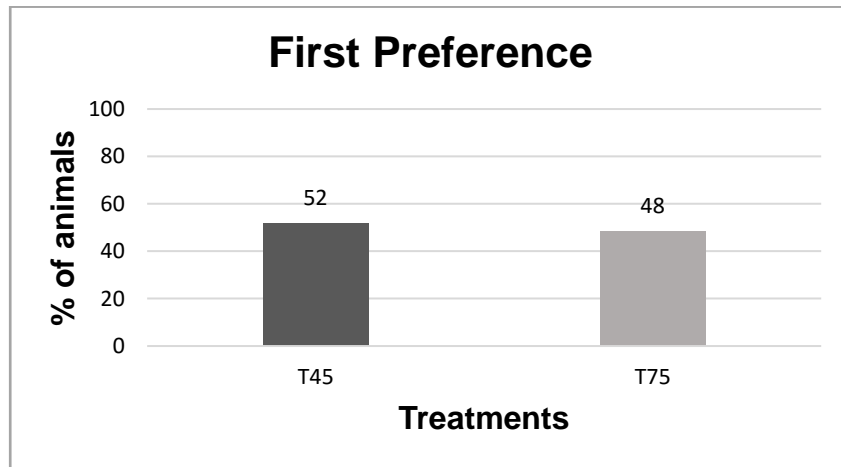
<sup>f</sup> VFA – volatile fatty acids.

### 3.5. Food palatability test

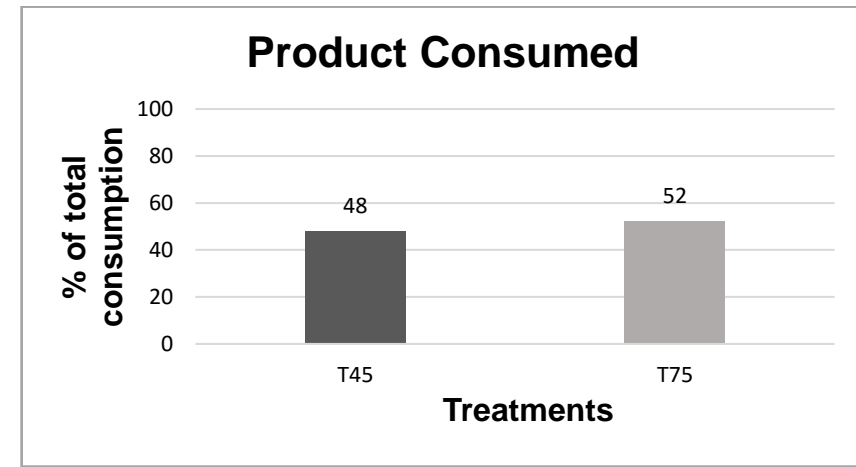
Before the palatability test, the foods were coated with 8.5% of poultry fat and 1% of a standard liquid palatant for dogs (2% D'Tech 2L 0512901, SPF Palatability, Descalvado, Brazil). The palatability study did not find important differences on dog acceptance of the tested foods. When comparing T45 with T95, although dogs revealed higher first consumption (first preference) for the T45 food ( $P < 0.01$ ), no significant difference was observed on the total intake among animals (Figure 1). No differences were observed in the other comparisons.

There was also no difference in palatability between T45 *versus* T75 foods, nor between T75 *versus* T95.

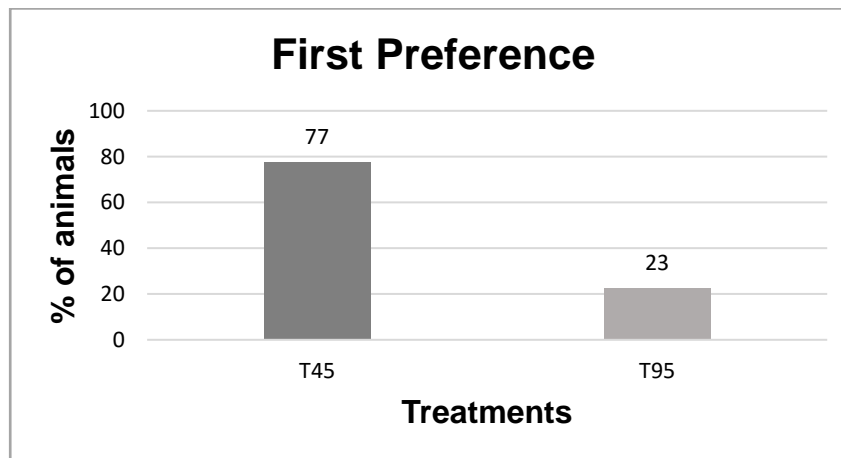
Figure 1  
Food palatability test of the experimental diets with different specific thermal energy application produced at UNESP



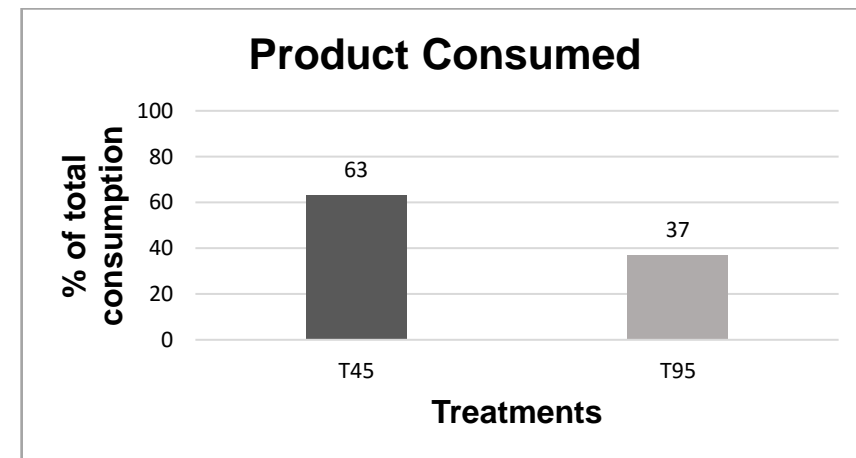
A



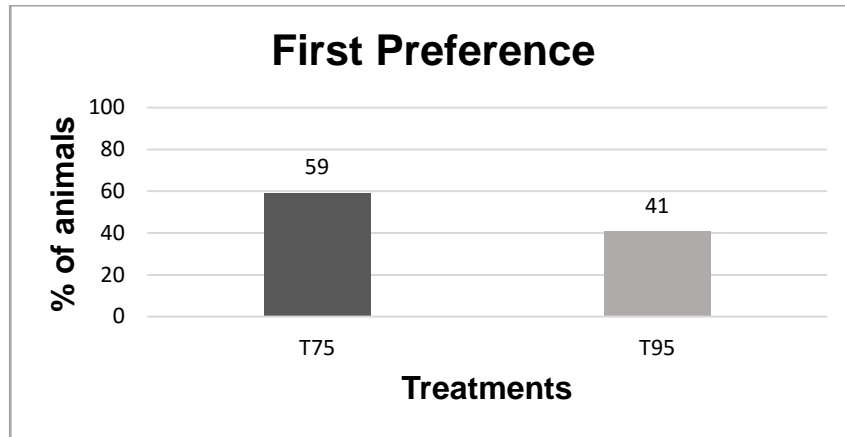
B



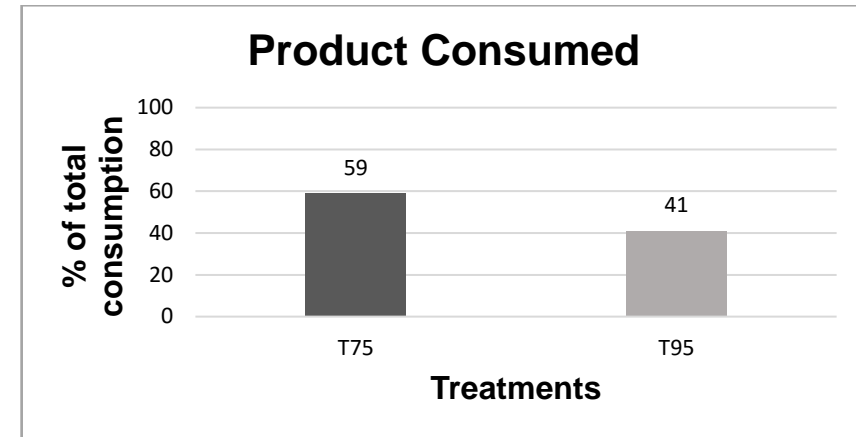
C



D



E



F

Experimental diets: T45 – preconditioner mass temperature target 45 °C; T75 – preconditioner mass temperature target 75 °C; T95 – preconditioner mass temperature target 95 °C

#### 4. Discussion

Important alterations on extrusion parameters were verified after the increase of STE implementation (or preconditioner temperature). In both extruders, the effects were similar with reductions on SME, but an increase in the TSE. In the extruder A the SME reduced approximately 18%, and in the extruder B 44%. This effect was already described (Riaz, 2000), and could potentially reduce the processing cost due to a reduction on electric energy consumption and equipment wear (Streit, 2015). The reduction on SME application can be explained by an increase on mass fluidity. The supplementary STE and TSE when the preconditioner temperature were higher, probably promoted better internal hydration of the food particles and induced higher starch gelatinization on preconditioner, reducing the mechanical energy required to deform and push forward the mass on extruder barrel, and the shear stress. This is reinforced by the reduction of the pressure of the mass before the die with the increase of STE application, showing that the mass resistance to flow was lower.

Possible confounding factors for the reduction on SME application and mass pressure were the die open area and in-barrel moisture, which are critical software parameters to understand and compare the extrusion conditions (Riaz, 2000). The product flow rate, and consequently die open area did not change according to preconditioner temperature. For extruder A, the in-barrel moisture was similar among preconditioner temperatures, and not related with SME application ( $r^2=0.03$ ;  $P=0.746$ ). However, in extruder B the in-barrel moisture increased with the increase on preconditioner temperature, and its relation with the SME application was significant ( $r^2=0.35$ ;  $P=0.014$ ). Moisture increased in extruder B and since water is a fluidizing agent, it contributed together the higher TSE input to the reduction on SME (Suknark

et al., 1998; Pansawat et al., 2008). This increase on moisture content of extruder B was not expected, and occurred even after the procedures adopted to avoid moisture variation during the extruder operation. Anyway, the fact that for extruder A the moisture did not vary evidences that a better preconditioning of raw materials induce lower SME during extrusion.

In the present study was possible to verify that kibble formation can be greatly improved by preconditioning the raw ingredients (Riaz, 2001). Most published studies evaluated the impact the SME on kibble formation and quality, specifically altering moisture content, extruder screw speed, and die open area (Iwe et al., 2001; Pansawat et al., 2008). All of the studies, however, were conducted for extruded human snacks or cereals, and none was found for dog foods. For cereals and starch based formulations, mechanical energy is more relevant than for pet food recipes, to which thermal energy assumes greater relevance due the high protein, fat, and also fiber contents (Kazemzadeh, 2001; Monti et al., 2016). On the present study, the kibble density reduced, and the kibble expansion increased on both studied extruders. These alterations can be explained by the greater STE and TSE applications with the increase on preconditioner temperature. It is interesting to observe that the increase in expansion arose even with the reduction on SME application and the mass pressure, highlighting that the STE and TSE implementation are important parameters to consider when studying and comparing different extrusion conditions. Probably, the increase on kibble expansion can be attributed to the higher starch gelatinization verified after greater STE application, the mass of molten gelatinized starch is more easily deformed by the water vaporization out the extruder, creating the inner cell structure of the kibbles (Guy, 2001).

Important parameters of processing quality such starch gelatinization after drier and *in vitro* digestibility of the OM increased for both extruders with the higher preconditioner temperature and TSE application. It was verified that the temperature of the mass before the die also increased in both extruders with the increase on preconditioner temperature, and can be related with the greater gelatinization and *in vitro* digestibility. The temperature and pressure of extrusion usually are directly linked with the SME application and mass resistance to flow (Riaz, 2000; Pansawat et al, 2008). However, in the present study the SME and the pressure of the mass before the die reduced. Due this, the increase on mass temperature can be probably attributed to the supplementary STE application and the consequent higher mass temperature at the entrance of the extruder. Therefore, the higher starch gelatinization after drier is probably the result of the increased starch cooking on the preconditioner, the higher temperature of extrusion, and the greater TSE application on the product. The better preconditioning of the raw material create conditions to favor the starch gelatinization, which occurred with less SME application. Considering that the same production lot was used for all diets, the increase on OM digestibility can be mainly attributed to an increased cooking efficiency of the food, verified by the higher starch gelatinization.

Even differing on *in vitro* OM digestibility, the diets presented similar digestibilities for dogs. Considering that the starch gelatinization increased from 69% (diet T45) to 89% (diet T95), this was not expected. Evaluating the available literature, the effect of starch gelatinization on nutrient digestibility of extruded diets for dogs is little studied. Most publications did not report the starch gelatinization, and information about how much it is necessary to gelatinize the starch during extrusion to obtain proper digestibilities is not available for dogs (Bazolli et al., 2015). Studying rice, corn,

and sorghum to dogs, Bazolli et al. (2015) verified that for a corn based formulation the apparent total tract nutrient digestibility did not change for diets with 73.8% or 79.9% of starch gelatinization. Due this, it is possible that a modest cooking is enough to achieve adequate digestibility of a dog food when corn is the cereal source. One methodological limitation is the measurement of only the total tract apparent digestibility of the nutrients in the present study. Nutrients not digested and absorbed in small intestine can be fermented in colon, and they will not be recovered in feces (Silvio et al., 2000). However, in the present study, the fermentation products, feces production and traits also did not change. The method used to study the *in vitro* OM digestibility has been validated to dogs (Hervera et al., 2007), an enzymatic method. The obtained results increased with the increase on starch gelatinization. The lack of relation between the *in vitro* OM digestibility with the *in vivo* digestibility in dogs can be explained by: the numerically small differences on *in vitro* digestibilities among diets; the addition by coating of 8.5% of poultry fat to diets before the *in vivo* digestibility trials, which changed their composition; and the *in vitro* method used do not consider the OM fermentation in the colon.

There are few studies evaluating the effects of mechanical and thermal energy input during the extrusion on the palatability of dog foods. Dunsford et al. (2002) and Sá (2015) evaluated different SME and STE application, and verified that dogs preferred a more thermically cooked product. However, Trivedi and Benning (2003) founded opposite response, with dogs preferring a diet with higher SME. The results of the present study were also different, as the dogs did not shown preferences for foods with a high range of STE/SME ratio, from 1.0 to 6.0 (foods of extruder B). Palatability is a complex characteristic in which several aspects, dependent on the

ingredients used and processing parameters, are involved, including food texture, shape, size, taste and flavor (Trivedi et al., 2000).

## 5. Conclusion

The increase of the STE application by direct steam injection on preconditioner decreased the SME required to cook, to form and to structure the kibbles of a dog food. Linear or quadratic responses were verified to TSE application, depending on the extrusion system studied, and values similar or higher than 65 kW-h/ton can be used to produce dog foods. This might reduce the processing cost, and increase the efficiency of the processing with higher expansion and starch gelatinization.

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