**UNIVERSIDADE ESTADUAL PAULISTA - UNESP**
**CÂMPUS DE JABOTICABAL**

# Selênio e mucosa intestinal e cozimento do amido, digestibilidade dos nutrientes e produtos de fermentação nas fezes de cães

**Michele Cristina de Camargo Oliveira**
Médica Veterinária

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

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TÍTULO: SELÊNIO E MUCOSA INTESTINAL E COZIMENTO DO AMIDO, DIGESTIBILIDADE DOS NUTRIENTES E PRODUTOS DE FERMENTAÇÃO NAS FEZES DE CÃES

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*Dedico*

Ao meu marido, *Cícero Marcelo dos Santos*, por seu amor, companheirismo e confiança e por sempre acreditar em mim, me auxiliando e me apoiando em minhas decisões, por estar ao meu lado mesmo nas horas mais difíceis.

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Jaboticabal, 04 de Fevereiro de 2013.

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**Selênio e mucosa intestinal e cozimento do amido, digestibilidade dos nutrientes e produtos de fermentação nas fezes de cães**

## RESUMO

Este trabalho incluiu um estudo sobre os efeitos da adição de selênio em dietas secas extrusadas na mucosa intestinal de cães. Utilizou-se uma única formulação para produzir cinco dietas sendo uma dieta controle com adição de 0,13ppm de selenito de sódio, uma segunda dieta com 0,33ppm de selênio levedura, outra com 0,66ppm de selênio levedura, a quarta contendo 0,99ppm de selênio levedura e a quinta com 0,66ppm de selenito de sódio. Foram utilizados 30 cães em três blocos de 10 cães e cada período teve duração de 210 dias incluindo adaptação e fase experimental. Ao final de cada período foram coletadas amostras de mucosa do trato gastrintestinal por endoscopia e colonoscopia para avaliação. Um segundo estudo avaliou o efeito da gelatinização do amido na digestibilidade aparente dos nutrientes, produção de fezes e produtos de fermentação de cães alimentados com dietas extrusadas incluindo dois estudos. No estudo 1 uma mesma formulação foi processada de quatro maneiras diferentes resultando em três dietas extrusadas com moagens de 0.5, 1.8 e 2.0 mm e uma dieta peletizada com moagem de 1.8mm com diferentes graus de gelatinização. Foram utilizados 24 cães, seis por tratamento e avaliou-se a digestibilidade e os produtos de fermentação. No estudo 2 foi utilizada uma base de dados publicados e a digestibilidade de dietas preparadas para construir regressões polinomiais entre o grau de gelatinização do amido e a digestibilidade aparente dos nutrientes. No primeiro estudo a análise histológica demonstrou diferença significativa com a dieta suplementada com selênio orgânico 0,66ppm comparado com as dietas suplementadas com selênio orgânico 0,33ppm e 0,99ppm. Não houve diferença entre as dietas suplementadas e a dieta controle. É possível que a suplementação de selênio possa trazer benefícios a saúde intestinal. No estudo 1 do segundo estudo houve aumento linear da proteína bruta ($p<0.002$), e aumento quadrático da digestibilidade do amido ($p<0.001$) seguindo o aumento da gelatinização do amido das dietas. As concentrações fecais de acetato, propionato, butirato, ácido valérico e isobutírico reduziram linearmente

enquanto o lactato reduziu quadraticamente com aumento da gelatinização do amido ($p<0.001$). No estudo 2 a digestibilidade da matéria orgânica ($R^2$ de 0,63 a 0,26), proteína bruta ($R^2$ de 0,91 a 0,26), amido ou extrativos não nitrogenados ($R^2$ de 0,95 a 0,23), e gordura ($R^2$ d 0,84 a 0,46) aumentou com a maior gelatinização do amido ($p<0.05$). A digestibilidade da proteína parece ser mais influenciada pela gelatinização do amido. A gelatinização do amido entre 83% a 87% parece ser capaz de maximizar a digestibilidade aparente dos nutrientes.

**Palavras-chave**: suplementação, inflamação, cozimento do amido, digestibilidade

# Selenium and intestinal mucosa and starch cooking, nutrient digestibility and fecal fermentation products of dogs


## ABSTRACT

This work included a study on the effects of the addition of selenium in extruded dry diets in the gastrointestinal tract of dogs. It was used a single formulation to produce five diets and a control diet with the addition of 0,13ppm sodium selenite, a second diet with selenium yeast of 0,33ppm, another diet with 0,66ppm selenium yeast, the fourth diet containing 0, 99ppm of selenium yeast and the fifth with 0,66ppm sodium selenite. It was used a total of 30 dogs in three blocks of 10 dogs and each period lasted 210 days including adaptation and experimental phase. At the end of each period mucosa samples were collected from the gastrointestinal tract by endoscopy and colonoscopy for evaluation. A second study evaluated the effect of starch gelatinization in the apparent digestibility of nutrients, production of feces and dog fermentation products fed extruded diets including two studies. In Study 1 the same formulation was processed in four different ways, resulting in three extruded diets of grindings 0.5, 1.8 and 2.0 mm and a pelleted diet with grinding of 1.8mm with different degrees of gelatinization. Twenty-four dogs were used, six dogs per treatment and digestibility and fermentation products were evaluated. In the second study it was used a published database and diet digestibility prepared to construct polynomial regressions between the degree of starch gelatinization and apparent nutrient digestibility. In the first study histological analysis showed a significant difference with the diet supplemented with organic selenium 0,66ppm compared with the diets supplemented with organic selenium 0,33ppm and 0,99ppm. There was no difference between the supplemented diets and the control diet. It is possible that selenium supplementation may benefit intestinal health. In Study 1 of the second study crude protein increased linearly ($p < 0.002$), and starch digestibility increase quadratically ($p < 0.001$) following the increased starch gelatinization. Fecal concentrations of acetate, propionate, butyrate, isobutyric acid and valeric reduced linearly while the lactate reduced quadratically with an increase in starch gelatinization ($p < 0.001$). In study 2 the organic matter digestibility ($R^2$

0.63 to 0.26), crude protein (R2 0.91 to 0.26), starch or nitrogen free extract ($R^2$ .95 to .23) and fat ($R^2$ 0.84 to 0.46) increased with higher starch gelatinization ($p < 0.05$). The digestibility of the protein seems to be more influenced by gelatinization. The gelatinization from 83% to 87% seems to be able to maximize the apparent digestibility of nutrients.

**Keywords**: supplementation, inflammation, starch gelatinization, digestibility

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## LISTA DE ABREVIATURAS

| | |
|---|---|
| **AAFCO** | Association of American Feed Control Officials |
| **AGCC** | Ácidos graxos de cadeia curta |
| **AGCR** | Ácidos graxos de cadeia ramificada |
| **AGV** | Ácidos graxos voláteis |
| **AOAC** | Association of the Official Analitical Chemists |
| **AR** | Amido resistente |
| **CEUA** | Comissão de Ética no Uso de Animais |
| **COBEA** | Colégio Brasileiro de Experimentação Animal |
| **CP** | Crude protein |
| **DGM** | Diâmetro geométrico médio |
| **DM** | Dry matter |
| **EEHA** | Extrato extract in acid hydrolisis |
| **EME** | Energia mecânica específica |
| **EPM** | Erro padrão da média |
| **ETE** | Energia térmica específica |
| **FDA** | Food and Drug Administration |
| **FEDIAF** | Federação Europeia das Indústrias de Pet Food (The European Pet Food Industry Federation) |
| **g** | Gramas |
| **GSH-Px** | Glutationa peroxidase |
| **GE** | Gross energy |
| **h** | Hora |
| **kcal** | Kilocalorias |
| **kg** | Kilogramas |

| | |
|---|---|
| **ME** | Metabolizable energy |
| **MGD** | Mean geometric diameter |
| **mL** | Mililitros |
| **MM** | Mineral matter |
| **NRC** | Nacional Research Council |
| **RCFA** | Reduced chain fatty acids |
| **SAS** | Statistical Analysis System |
| **SCFA** | Short chain fatty acids |
| **SEM** | Standard error of the mean |
| **SOD** | Superóxido desmutase |
| **T3** | Triiodotironina |
| **T4** | Tiroxina |
| **UFC** | Unidades formadoras de colônia |
| **VA** | Volatile acids |

# CAPÍTULO 1 – Considerações Gerais

## 1. Introdução

O convívio com animais de estimação tem se estreitado cada vez mais ao longo dos anos. Hoje, muitos cães e gatos são considerados “membros da família” e por esse motivo a preocupação com a saúde e bem-estar desses animais vem aumentando consideravelmente. A alimentação é ponto chave quando se considera a qualidade de vida e prevenção de doenças. Cães e gatos apresentam necessidades nutricionais que devem ser atendidas através de sua alimentação, levando-se em consideração diversos aspectos da dieta como a palatabilidade, composição nutricional, densidade energética, digestibilidade e uso ingredientes de boa qualidade. A intervenção nutricional em cães é importante para promover bem estar e saúde e, em muitas situações para retardar ou prevenir doenças, maximizando sua expectativa de vida. Desse modo, diversos estudos procuram entender como a dieta pode influenciar os mecanismos fisiológicos, morfológicos e de defesa das diversas partes do organismo e, como estas ações são exercidas pelos nutrientes presentes nos alimentos.

O selênio é um dos nutrientes que vem sendo estudado na alimentação de animais de companhia. É essencial para o funcionamento adequado do sistema reprodutivo e na produção e regulação da atividade dos hormônios da tireóide, além de apresentar importante função imunológica influenciando tanto a resposta imunológica natural/inata quanto adaptativa (TURNER e FINCH, 1991; SURAI, 2003). Reconhecido por suas características antioxidantes, na medicina humana, o selênio tem recebido muita atenção por também possuir propriedades anti-inflamatórias e anticancerígenas, podendo atuar na prevenção de enfermidades como doença inflamatória intestinal (AVINASH et al., 2015) e câncer colonrectal (ATHAR et al., 2007; REID et al., 2006; KUNE et al., 2006).

Considerando-se o uso de nutrientes como o selênio na prevenção de doenças, um aspecto de grande relevância é a saúde intestinal. O trato gastrintestinal é uma estrutura complexa que fornece nutrientes ao organismo e

que contém diversos tipos celulares especializados que cumprem papel importante na proteção contra o ambiente externo (CUNNINGHAM-RUNDLES & LIN, 1998). Os ingredientes da dieta e a atividade da microbiota intestinal contribuem para o fornecimento dos compostos que são absorvidos pelo lúmen e que constituem a principal fonte de nutrientes para a mucosa intestinal. Assim a dieta deve fornecer nutrientes em níveis adequados para que haja bom desenvolvimento e funcionamento desse órgão. De um modo geral nos alimentos comerciais, as doses de selênio adicionadas são recomendadas para a manutenção do animal e não para uma ativação ou melhora do sistema imune e ou antioxidante. Sabendo-se que a fisiologia e a morfologia da mucosa intestinal podem mudar de acordo com fatores como estresse, doença e o tipo de dieta e que estas mudanças podem afetar a função do intestino, influenciando a absorção e metabolização de nutrientes, bem como sua capacidade de proteger o organismo (CARCIOFI; GOMES, 2010) é importante estudar como a suplementação de selênio através do alimento pode alterar características morfométricas de células intestinais dos cães promovendo benefícios à saúde intestinal e possivelmente prevenindo doenças tais como o câncer, assim como já relatado em humanos.

Outro fator importante a ser considerado, pensando em saúde e bem estar dos animais de companhia é a qualidade dos alimentos oferecidos diariamente. A indústria de rações para cães vem crescendo substancialmente a cada ano e cada vez mais busca aprimorar esses alimentos para atender as exigências de mercado. Atualmente 95% dos alimentos secos para cães e gatos são processados com tecnologia de extrusão termoplástica (SPEARS; FAHEY, 2004). Todo processamento de alimento visa promover modificações físicas, químicas, térmicas ou bacterianas para modificar sua qualidade (CRANE et al., 2000). Dessa forma, diferentes quantidades de energia térmica e mecânica são aplicadas durante o processamento induzindo mudanças na composição química e na estrutura física dos ingredientes (MOSCICKI; WÓJTOWICZ, 2011), influenciando o grau de cozimento da ração e consequentemente a gelatinização do amido. Esse processamento promove alterações na digestibilidade, valor energético e palatabilidade (GRIFFIN, 2003) da ração, determinando assim também sua qualidade.

O grau de moagem dos ingredientes também está relacionado com a eficiência de produção dos alimentos. A moagem objetiva reduzir o tamanho das partículas dos ingredientes, melhorando sua mistura e o processamento. Os diferentes graus de moagem, de acordo com os ingredientes utilizados modificam a qualidade do extrusado, alterando o grau de gelatinização do amido e quantidade de amido resistente (BAZOLLI, 2007) podendo promover maior ou menor digestibilidade.

O consumo e o aproveitamento dos nutrientes são influenciados pelo grau de cozimento do alimento, promovido pela extrusão do mesmo e pela qualidade de moagem dos ingredientes. Quando se obtém boas condições de processamento pode-se otimizar a palatabilidade e a digestibilidade do alimento e dessa forma modificar a qualidade das fezes e a produção dos produtos de fermentação, como os ácidos graxos de cadeia curta resultando em benefícios à saúde intestinal e melhora na qualidade de vida dos cães. A alimentação de cães deve servir para promover saúde e qualidade de vida sendo fundamental conhecer quais as melhores condições de processamento e quais graus de moagem permitem melhor aproveitamento dos nutrientes para se otimizar a saúde do intestino, o que poderia ter importantes implicações práticas para a indústria de alimentos.

## 2. Revisão de Literatura

### 2.1- O Selênio

O selênio é um elemento químico não metálico descoberto por J. J. Berzelius em 1817 que apresenta propriedades químicas semelhantes as do enxofre e na natureza pode ser encontrado tanto em sua forma orgânica quanto inorgânica dependendo do agente ligante que pode ser um metal ou um não metal.

É encontrado nos tecidos vegetais em concentrações bastante variáveis. Isso ocorre porque seu teor e sua forma nos solos dependem do pH, do potencial redox e da composição mineral do meio, além da fertilização artificial e das chuvas (LYONS et al., 2007). Assim uma mesma espécie vegetal cultivada em solos diferentes apresenta distintos teores de selênio e espécies

diferentes cultivadas em um mesmo solo apresentam concentrações distintas deste mineral, já que cada planta absorve e armazena o selênio de forma variada. No solo o selênio encontra-se principalmente em sua forma inorgânica; nas plantas o que predomina é a forma orgânica como selenometionina ou selenocisteína, sendo esta a principal maneira que os animais o ingerem na natureza (COMBS e COMBS, 1986).

Além de participar na conversão do T4 (tiroxina) em T3 (triiodotironina) e aumentar a resistência do sistema imunológico, o selênio também tem capacidade antioxidante. Dentre os diferentes grupos de selenoproteínas, o das glutationas peroxidases (GSH-Px) é o mais abundante e encontrado em todos os tecidos de mamíferos em que ocorrem processos oxidativos. O selênio compõe as GSH-Px e age juntamente com o tocoferol na regulação da peroxidação lipídica. Hoje são conhecidos quatro membros da família da GPx. A glutationa peroxidase clássica (GSH-Px 1) presente no citosol das células. A glutationa peroxidase gastrintestinal (GSH-Px 2), considerada a selenoproteína antioxidante mais importante no cólon e protege o organismo de mamíferos da toxicidade causada por hidroperóxidos lipídicos. A glutationa peroxidase extracelular ou plasmática (GSH-Px 3) apresenta-se em grandes concentrações nos rins e a glutationa peroxidase fosfolipídeo hidroperóxido (GSH-Px 4), responsável pela destruição redutiva de hidroperóxidos lipídicos (BROWN & ARTHUR, 2001; TAPIERO et al., 2003; GONZAGA et al., 2005).

## 2.2- Níveis adequados e metabolismo do selênio

A falta de selênio resulta em diversas doenças, que podem variar de acordo com a espécie e o status de vitamina E. No entanto, em cães e gatos, a deficiência deste nutriente não parece ser um grande problema, uma vez que a maioria desses animais é alimentada com rações comerciais que, provavelmente, contem níveis adequados de selênio (SIMCOCK et al., 2005). A toxicidade por selênio nessas espécies também é improvável de ocorrer naturalmente (CASE et al., 1998). O maior risco de intoxicação por selênio em animais de companhia é o uso de tabletes suplementares de selênio destinados a humanos. A intoxicação por selênio é caracterizada pelo odor de

alho na respiração, queda de pelos, excesso de salivação, cegueira, paralisia e morte (HILL e ALDRICH, 2003).

O uso do selênio na alimentação animal é regulado nos Estados Unidos pelo Food and Drug Administration (FDA, 2015), permitindo o limite máximo de 0,3 ppm como adição suplementar para animais de produção (MATEOS et al., 2004). Enquanto não há regulamentações especificas para *Pet Food* admite-se nos Estados Unidos este mesmo valor na nutrição de cães e gatos (HILL e ALDRICH, 2003). No Brasil não existe legislação a respeito, o que deveria ser modificado pelos órgãos governamentais competentes.

Os animais obtêm o selênio dos cereais e grãos ou de tecidos de outros animais, dependendo do seu hábito alimentar (TODD e HENDRIKS, 2005). As formas de selênio encontradas em plantas e animais incluem uma variedade de compostos orgânicos e inorgânicos (WHANGER, 2002). As formas inorgânicas são os sais de selênio: selenido ($Se^{-2}$), selenito ($Se^{-4}$) e selenato ($Se^{-6}$). As plantas absorvem o selênio em sua forma inorgânica a partir do solo e o converte para a forma orgânica, gerando compostos metilados de baixo peso molecular, além de selenometionina e selenocisteína. A selenometionina é a principal fonte de compostos de selênio encontrada em plantas e o principal precursor para a síntese de selenocisteína (COMINETTI & COZZOLINO, 2009).

Uma vez absorvido, o selênio da dieta de origem orgânica ou inorgânica possui diferentes destinos, podendo ser estocado, utilizado na síntese de selenoproteínas ou excretado. Todas as formas de selênio são convertidas em única forma de selênio, o hidreto de selênio ($H_2Se$), e depois encaminhadas para possíveis destinos (FRANKENBERGER e BENSON, 1994; SIMCOCK et al., 2002). As formas inorgânicas quando não usadas na síntese de selenoproteínas sofrerão o processo de metilação e serão excretadas pelo pulmão ou pelos rins. Selenometionina e selenocisteína podem ser incorporadas às proteínas como, por exemplo, em músculos, no lugar da metionina e da cisteína (TODD e HENDRIKS, 2005), mas também sofrem o processo de metilação (NAKAMURO et al., 2000).

O selênio é carreado no plasma, associado à albumina e selenoproteína P até os tecidos alvos, está associado às proteínas no organismo sendo primeiramente encontrado nos músculos e tecidos ricos em proteínas e pouco

encontrado no tecido adiposo. Os níveis sanguíneos respondem as mudanças nas concentrações das dietas (HILL e ALDRICH, 2003). Nos tecidos é armazenado principalmente como selenocisteína e selenometionina e sua concentração nos diversos órgãos varia com o consumo. A principal via de excreção do selênio é pela urina, mas também podem ocorrer perdas pelo pulmão e fezes. As perdas fecais são constituídas do selênio não absorvido.

## 2.3- Selênio na nutrição de cães

O selênio é necessário e essencial para funções fisiológicas básicas. O organismo animal sofre constantes ataques de moléculas denominadas radicais livres, formadas a partir da atividade metabólica normal como estratégia do sistema imune para se proteger contra microorganismos invasores. Os radicais livres são moléculas instáveis e por isso altamente reativos. Elas apresentam um elétron com carga negativa que tende a se associar muito rapidamente a outras moléculas de carga positiva com as quais podem reagir ou oxidar e estão envolvidas na iniciação, propagação e manutenção dos processos inflamatórios agudos e crônicos (HALLIWELL et al., 1982; MUGESH and SINGH, 2000). Quando em excesso provocam danos celulares e podem levar a doenças inflamatórias e alguns tipos de câncer (SHARADAMMA et al., 2011).

Nas células de mamíferos, a primeira linha de defesa antioxidante é o sistema de enzimas superóxido desmutase (SOD), glutationa peroxidase (GSH-Px) e a catalase. O selênio é componente essencial da enzima GSH-Px (ROTRUCK et al., 1973) e o nível de atividade desta enzima no fígado ou sangue total são indicativos do suprimento de selênio do organismo. A GSH-Px protege as membranas celulares dos radicais livres e outros danos oxidativos, desativando a formação de peróxidos durante a oxidação dos lipídeos da membrana celular. O sitio ativo da GSH-Px contém selenocisteína, um aminoácido raro no qual o átomo de enxofre da cisteína foi substituído pelo átomo de selênio (MCDOWELL, 1992).

O selênio, juntamente com a vitamina E, é necessário para o funcionamento adequado do sistema imune. Seu efeito imunomodulatório ocorre pelos mecanismos de efeitos antiinflamatórios, sistema antioxidante e

por propriedades citostáticas e anticancerígenas. Mckenzie et al., (2002) verificaram que doses suplementares de selênio melhoraram a resposta imunológica e protegeram contra certas infecções virais conferindo benefícios adicionais à saúde. Surai (2003) verificou que sua deficiência pode afetar tanto a imunidade natural quanto a imunidade adquirida.

Estudos em humanos verificaram que baixas concentrações de selênio e reduzida expressão de selenoproteínas estão associadas com alto risco de câncer (ALMONDES et al., 2010) e que há uma forte correlação entre o nível de selênio no soro e o risco de câncer colonretal (LENER et al., 2013). A suplementação de selênio promove efeito anticarcinogênico. Em pequenas doses, funciona como um componente essencial da selenocisteína em várias selenoproteínas específicas promovendo proliferação celular, importante para a resposta imune. Em doses altas, porém não tóxicas, pode reduzir o risco de câncer, impedindo o ciclo celular tumoral, estimulando a apoptose e inibindo a migração e invasão celular tumoral (ZENG & COMBS, 2008). Hiller et al (2015) sugeriram que a forma do selênio e a duração da suplementação terapêutica pode ser importante para conferir seus efeitos benéficos. Independente da forma de selênio utilizada, Avinash et al (2015) relatam que a inflamação é um fator chave durante a deficiência de selênio que leva a patofisiologia no intestino e a qual pode ser suavizada pela expressão aumentada de selenoproteínas.

### 2.4- Intestino e nutrição

O trato gastrointestinal é um órgão que além de fornecer nutrientes ao organismo e apresentar um sistema imune muito ativo, tem uma estrutura bastante complexa com muitos tipos celulares especializados para absorção, digestão e proteção contra o ambiente externo (CUNNINGHAM-RUNDLES & LIN, 1998).

Histologicamente o trato digestório é constituído de quatro camadas distintas: mucosa, submucosa, muscular e serosa. A mucosa é subdividida em três camadas: o revestimento epitelial, a lâmina própria (formada por tecido conjuntivo) e a camada muscular. A submucosa é constituída por tecido conjuntivo e pelo plexo nervoso subcutâneo. O revestimento epitelial forma

uma barreira seletivamente permeável entre o conteúdo luminal e os tecidos do organismo, além de facilitar o transporte e a digestão do alimento, promover a absorção dos produtos dessa digestão, produzir hormônios e muco (JUNQUEIRA & CARNEIRO, 2009).

Na porção do intestino delgado, a mucosa forma as vilosidades intestinais ou vilos que são projeções alongadas abrangendo o epitélio e a lâmina própria e entre os vilos formam-se as criptas (glândulas de Lieberkuhn). No epitélio dos vilos e criptas são encontrados diversos tipos celulares: células absortivas (enterócitos), células caliciformes, células enteroendócrinas, células de Paneth e células tronco.

Diversos fatores contribuem para o funcionamento adequado do intestino. A microbiota intestinal apresenta um papel importante já que participa da regulação de processos como maturação e proliferação de células intestinais, digestão de alimentos, proteção contra bactérias patogênicas e modulação da resposta imune local (POSSEMIERS et al., 2009; HATTORI and TAYLOR, 2009). Muitos estudos em humanos mostram evidências de que desequilíbrios nos microorganismos intestinais estão correlacionados com doenças como câncer e doença inflamatória intestinal (TAKAISHI et al., 2008; TANNOCK, 2008). Intervenções nutricionais como variações na composição da dieta, além de modificar a morfologia intestinal, podem modular a microbiota intestinal influenciando a saúde como um todo (FLINT et al., 2007).

Kasaikina et al (2011) demonstraram em humanos que a suplementação de selênio pode ser benéfico para a prevenção de câncer de cólon já que o selênio na dieta afetou a composição da microbiota e a colonização do trato gastrointestinal. Avinash et al (2015) verificaram que na doença inflamatória intestinal, a deficiência de selênio e selenoproteína, afetou os processos inflamatórios, o estresse oxidativo e alterou a microbiota piorando o quadro de colite experimental. Gong et al (2012) associa os efeitos da suplementação de selenoproteínas nos mecanismos inflamatórios nas células epiteliais com os efeitos do consumo de selênio na susceptibilidade de desenvolvimento de câncer colonretal, inflamação e carcinogenese.

## 2.5- A gelatinização do amido, o amido resistente e produtos de fermentação

Na indústria de alimentos para animais de companhia, os grãos, compostos por 50 a 90% de amido, são os ingredientes quantitativamente mais utilizados. O milho e o arroz são os mais empregados no Brasil. Estruturalmente, o amido se encontra como partículas semi-esféricas altamente ordenadas denominadas grânulos, formadas por cadeias de amilose e amilopectina. Sua estrutura altamente ordenada lhes confere a característica de birrefringência, típica de substâncias cristalinas. De forma geral, os grânulos de amido são compostos majoritariamente por amilopectina, sendo esta fração mais susceptível à ação enzimática que a amilose. Grânulos de amido são insolúveis em água à temperatura ambiente, possivelmente devido às várias pontes de hidrogênio presentes em sua estrutura. Isto limita o uso de grânulos crus na alimentação, tanto humana como animal (ROBERTI-FILHO, 2013).

Durante a extrusão o amido é particularmente alterado. Na presença de água, calor, cisalhamento e pressão, ocorre a ruptura das pontes de hidrogênio que estabilizam sua estrutura cristalina interna. Se o aquecimento prossegue com quantidade suficiente de água, rompe-se a região cristalina e a água entra, fazendo o grânulo romper-se e perder a birrefringência, processo este conhecido como gelatinização (ZENG et al., 1997). O amido gelatinizado é solúvel em água e mais susceptíveis à degradação enzimática do que o amido não cozido (DONA et al., 2010) o que melhora a digestibilidade do alimento. Jouglin et al. (1992), em estudo sobre o processamento dos carboidratos, compararam a digestibilidade de quatro carboidratos nas suas formas crua e gelatinizada. Utilizaram oito dietas contendo diferentes tipos de amido. O processo de gelatinização resultou em melhora significativa da digestibilidade de todas as dietas. Mais recentemente estudos com rações extrusadas demonstraram que o amido dos cereais, adequadamente processado apresenta digestibilidade superior a 98% para cães (CARCIOFI et al. 2008).

A retrogradação ocorre quando o amido é cozido em água, acima de sua temperatura de gelatinização e em seguida resfriado, ocorre reestruturação do amido que pode resistir à atividade hidrolítica da amilase, passando inalterada

pelo intestino delgado (BROWN, 2004), sendo denominado amido resistente tipo Ⅳ.

De acordo com Englyst et al. (1992), o amido divide-se em três classificações de acordo com a velocidade a qual o alimento é digerido in vitro: a primeira rapidamente digerível, quando, ao ser submetido à incubação com as enzimas amilase pancreática e amiloglicosidase à temperatura de 37ºC, converte-se em glicose em 20 minutos; a segunda lentamente digerível, se, nas condições anteriores, é convertido em glicose em 120 minutos; e a terceira: amido resistente (AR), que resiste à ação das enzimas digestivas

O AR tem sido definido, em termos fisiológicos, como a soma do amido e dos produtos da sua degradação que não são digeridos e absorvidos no intestino delgado de indivíduos sadios. Deste modo, esta fração do amido apresenta comportamento similar ao da fibra alimentar, e tem sido relacionada a efeitos benéficos locais principalmente no intestino grosso e sistêmicos, através de uma série de mecanismos (LOBO e LEMOS SILVA, 2003). Dessa forma o AR é fermentado no intestino grosso e produz, principalmente, gases, como o dióxido de carbono, metano e hidrogênio, ácidos orgânicos, incluindo o ácido lático e os AGCC (NUGENT, 2005; WALTER, 2005). São classificados em quatro tipos. O tipo I representa o amido fisicamente inacessível à digestão, devido à presença de paredes celulares intactas em grãos, sementes ou tubérculos. O tipo Ⅱ são grânulos de amido não cozidos e nativos, tais como batata crua ou a banana, cuja cristalinidade torna-os pouco susceptível a hidrólise. O Ⅲ se refere ao amido retrogradado, resultante de aplicações de processamento de alimentos. O qual é de grande interesse, devido à sua estabilidade térmica. Os processamentos dos alimentos que envolvem calor e umidade, na maioria dos casos destrói o tipo I e o Ⅱ, mas pode formar o Ⅲ. O tipo Ⅳ representa amidos que são quimicamente modificados para se obter resistência à digestão enzimática (FUENTES-ZARAGOZA, 2010).

De acordo com Conway (1994), existem três componentes importantes que determinam a saúde intestinal: a dieta, a mucosa intestinal e sua microbiota. A porção intestinal que apresenta a maior colonização é o cólon, mantendo uma ampla população de bactérias estimadas entre 1010 e 1012 unidades formadoras de colônias por mililitro (CFU/mL) (RASTALL, 2004). Bactérias colônicas tem importante papel na prevenção de colonização de

bactérias patogênicas e na fermentação das fibras dietéticas, resultando na produção dos ácidos graxos de cadeia curta (AGCC), predominantemente acetato, propionato e butirato, ácidos graxos de cadeia ramificada (AGCR), amônia, lactato e outros compostos. Este processo de fermentação e formação de AGCC é essencial para manter o estado de eubiose e saúde do intestino, com influência direta sobre a digestão dos nutrientes, a síntese de vitaminas, a estimulação do sistema imunitário, a proteção da mucosa como barreira contra invasão de microrganismos e absorção de toxinas, e efeitos antagonistas contra microrganismos patogênico (WENK, 2006).

Estudos *in vitro* e *in vivo* demonstram que a produção desses produtos finais de fermentação dependem amplamente da composição química do alimento que chega ao intestino grosso (McBURNEY, et al., 1991; HIELI et al., 1991). A quantidade produzida desses AGCC varia de indivíduo para indivíduo de acordo com o substrato e com a população microbiana e, apresenta atividades metabólicas específicas para o organismo animal. O butirato, por exemplo, é usado primariamente pelos colonócitos como fonte de energia além de auxiliar no desenvolvimento e crescimento do intestino delgado e grosso pela estimulação da proliferação das células epiteliais (MONTAGNE, et al.,2003). Já os ácidos acéticos e propiônico são absorvidos e metabolizados, proporcionando substratos para a lipogênese e cetogênese (SALGADO et al., 2005). O acetato promove aumento do fluxo sanguíneo no cólon e a motilidade do íleo (SCHEPPACH, 1994). Acetato, propionato e butirato são importantes ânions no lúmen do intestino grosso: afetam a forma e função do colonócito; estimulam a absorção de sódio e fluidos e exercem efeitos proliferativos nos colonócitos (SCHEPPACH, 1994; NUGENT, 2005). As células epiteliais da mucosa do cólon chegam a obter de 60% a 70% de sua energia a partir de produtos de fermentação bacteriana gerados no lúmen do órgão (CUMMINGS e MACFARLANE, 1991), pois o epitélio que reveste o trato gastrointestinal conta mais com o fornecimento de energia luminal do que vascular (SCHEPPACH, 1994).

## 2.6- Processamento de rações para cães

As tecnologias no processamento e fabricação de rações têm avançado no sentido de maximizar a eficiência produtiva, minimizar a perda de nutrientes e a formulação de produtos mais elaborados, com maior valor agregado e que confiram melhor qualidade aos animais de estimação.

A moagem e a mistura dos ingredientes são etapas de extrema importância do processamento do alimento, objetivando-se adequada homogeneidade e uniformidade de partículas e balanceamento da mistura. Com relação ao tamanho das partículas após a moagem, acredita-se, como regra geral, que quanto mais finamente moído, melhor a digestibilidade do ingrediente, no entanto isto não é sempre verdadeiro (CARCIOFI, 2008). Em um estudo feito por Bazolli (2007) avaliou-se o efeito da moagem de três cereais (arroz, milho e sorgo) em alimentos extrusados para cães. Verificou-se que o aumento da granulometria das dietas, de 277 µm para 521 µm para o arroz, de 360 a 619 µm para o milho e de 314 a 594 µm para o sorgo resultou em diminuição do índice de gelatinização do amido e redução do coeficiente de digestibilidade aparente de alguns nutrientes. Foi observado que este efeito variou segundo o ingrediente testado, sendo o tamanho de partículas determinante para o sorgo, mas menos importante para o arroz. Particularmente o milho e o sorgo moídos mais grosseiramente induziram expressivo aumento do butirato nas fezes dos cães avaliados. Em outro trabalho Roberti-Filho (2013) verificou que em ração para cães contendo milho como cereal, o aumento do tamanho de partículas da matéria prima e a redução da transferência de energia mecânica específica resultaram em grânulos de amido mais intactos, nos quais a hidratação e transferência de calor são mais difíceis. Esta situação resultou em redução na porcentagem de gelatinização do amido e aumento de mais de 4 vezes na quantidade de AR das rações. Como resultado houve diminuição da digestibilidade dos nutrientes, aumento de butirato e AGCC totais nas fezes.

Os alimentos para cães são hoje processados por extrusão. O processo de extrusão é composto por um silo de alimentação, pré-condicionador, extrusor e conjunto de matriz e corte A mistura primeiramente é transportada para o pré-condicionador da extrusora, onde recebe líquidos e vapor, responsáveis por aumentar a umidade (que pode variar de 15 a 35%) e a temperatura (que pode variar de 64 a 99 °C) dos ingredientes. No pré-

condicionador inicia-se o processo de gelatinização do amido e é preparada a massa para ser extrusada. Após, o material é conduzido para o canhão da extrusora, onde a massa pode alcançar temperaturas e pressões acima de 130ºC e 40bars, respectivamente. Esta parte do equipamento é composta por um cilindro com rosca interna, que pode ser simples ou dupla. A rosca é firmemente montada sobre um eixo que promove sua circunvolução, internamente e em contato com camisas cuja configuração geométrica pode aumentar ou diminuir o atrito da massa com esses componentes internos. O movimento da rosca comprime a massa contra uma matriz, fazendo com que esta seja pressurizada, aquecida e derretida, adquirindo consistência semilíquida. No final da extrusora o material derretido passa através dos orifícios da matriz. Ao ganhar a atmosfera a água se vaporiza e expande a estrutura plástica criada pelo amido e proteínas funcionais fundidos que retém parcialmente o vapor de água promovendo a expansão e formação da estrutura celular dos extrusados

A extrusão modifica a estrutura das moléculas, principalmente a do amido, resultando em diferenças na digestibilidade dos nutrientes e da energia (ANDRIGUETTO et al., 1985). Estas modificações estão diretamente ligadas e dependem da energia total transferida para a massa. Segundo Guy (2001), a energia total do sistema de extrusão é composta pela Energia Mecânica Específica (EME) e pela Energia Térmica Específica (ETE). A ETE é implementada por injeção direta de vapor. Isto ocorre principalmente no pré-condicionador, mas também o vapor pode ser injetado na massa, no interior do canhão extrusor . A EME é implementada pela revolução da rosca da extrusora, que transporta e comprime a massa. Esse transporte constante causa o cisalhamento da massa contra a camisa interna e comprime a massa contra a matriz na extremidade do canhão. Este processo cria pressão, fricção e aumento da temperatura. Variações na aplicação de EME e ETE durante a extrusão influenciam o grau de cozimento da ração (gelatinização do amido),a digestibilidade do amido e da energia, a palatabilidade e a formação de fezes. No entanto, apesar deste conhecimento geral, pouco se conhece ainda como e em que extensão os parâmetros de processamento, moagem das matérias primas e grau de cozimento do amido interferem na digestibilidade dos alimentos para cães.

Outro processo de produção de alimentos para animais é a peletização. Esta consiste em compactar mecanicamente a dieta, por meio do aquecimento pelo atrito mecânico durante a prensagem dos alimentos pelos rolos compressores contra a matriz (MILLAN, 1987). A massa misturada é colocada em contato com umidade e temperatura dentro de um condicionador dotado de único eixo, ou condicionador duplo, ou condicionador de diâmetro e velocidade variáveis. Em razão da exposição ao calor e à umidade, ocorre alteração nas cadeias de amido. Essa pré-gelatinização dos amidos é moderada, maior ou menor, em função do tempo de exposição a essas condições. Após o condicionamento, o produto é levado para uma câmara de compressão e comprimido, forçando a sua passagem por matrizes que formam os peletes, que são resfriados. O processo de peletização promove baixos percentuais de gelatinização do amido (HONORATO et al., 2010) e possivelmente índices mais baixos de digestibilidade e pior qualidade de fezes comparado com o processo de extrusão.

O processamento do amido, incluindo sua moagem e cozimento é fundamental para determinar fatores inerentes a qualidade do produto final: a digestibilidade aparente dos nutrientes, a palatabilidade do alimento, a produção de produtos de fermentação intestinal e a qualidade das fezes dos cães. Até o presente momento existem poucos estudos a respeito da influência do grau de cozimento sobre o aproveitamento dos nutrientes para animais de companhia.

## 3. **Objetivos**

Considerando-se o exposto, este estudo incluiu dois experimentos distintos, o primeiro sobre selênio e morfologia intestinal onde foi investigado o efeito do consumo de rações com teores crescentes de selênio sobre a morfologia da mucosa intestinal. O segundo experimento estudou o efeito da gelatinização do amido sobre a digestibilidade aparente dos nutrientes, produção e características das fezes e formação de produtos de fermentação bacteriana.

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## CAPÍTULO 2 - Concentration and sources of selenium on intestinal mucosa characteristics of dogs[1]

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**Abstract**

This study evaluated the effects of selenium amount and source on the intestinal mucosa of dogs fed extruded diets. The same formulation was used to produce five diets: control diet (CO) – sodium selenite added to diet presented 0.13ppm of Se; selenium yeast (SY) added to diet presented 0.33ppm Se; SY added to diet presented 0.66ppm Se; SY added to diet present 0.99ppm Se; sodium selenite added to diet presented 0.66ppm Se. Thirty adult Beagle dogs were used and the experiment was conducted as a randomized complete block design, with three blocks of 10 animals, and five diets. Periods lasted 210 days which included an adaptation phase of 90 days, and an experimental phase that lasted 120 days. At the end of this period, samples of the intestinal tract mucosa was collected by endoscopy and colonoscopy to morphometric assessments from all the dogs.Histological results of intestinal biopsies were evaluated using Kruskal-Wallis test and Mann-Whitney t test, considering the effects of each treatment (food) and non-parametric statistics ($P<0.05$). Food intake was adequate. Histological analysis showed a significant difference concerning the diets supplemented with 0.66ppm organic selenium compared to the diets supplemented with organic selenium 0.33ppm and 0.99ppm. In the comparison between the control diet and the supplemented with SY ($P>0.05$) no differences were found and the comparison between SY and $Na_2SeO_3$ also did not find significant differences between dogs ($P>0.05$). It is possible that selenium supplementation in healthy dogs can prevent inflammatory diseases but more studies are needed.

**Keywords**: inflammation, intestinal morphology, supplementation

## INTRODUCTION

Dietary strategies have been emphasized to promote health benefits and quality of life to dogs. The diet composition can alter the physiology and morphology of the intestinal mucosa. These changes affect the absorption and metabolism of nutrients, as well as the gut ability to protect the organism (CARCIOFI; GOMES, 2010). Changes on microelements intake, through diet supplementation may also interfere on mucosa nutrition, development and integrity, potentially promoting health benefits to animals

Selenium is an essential trace element in dogs (NRC, 2006). It is related to important aspects of canine physiology including thyroid hormone metabolism (DUNTAS, 2010), immune function (MCKENZIE et al., 2002), antioxidant protection (TINGGI, 2008) and inhibition of cancer development in a variety of experimental animal models (COMBS et al., 1998; IP et al., 1991; IP et al., 2000). Combs and Gray (1998) suggest that humans with nutritionally adequate selenium intake may benefit from its supplementation due to its role in cancer prevention as an essential component of antioxidant enzymes and as anticarcinogenic metabolites. Studies in humans demonstrate that low levels of selenium are associated with increased risks of colorectal cancer (RUSSIAN et al.1997; JACOBS et al 2004; RAYMAN 2005; PETERS & TAKATA 2008) and selenium serum concentration in people is strongly correlated with the chance of cancer development on colon (LENER et al., 2013).

PILARCZYK et al (2013) verified that healthy dogs have significant higher mean serum selenium concentration compared to dogs diagnosed with malignant neoplasm and that low levels of selenium contributes to the incidence of neoplasms. Other study observed that increasing concentrations of selenium could vary the metabolic response of canine mammary tumor cell lines and sensitivity to selenium increased with increasing concentrations of this trace element in all of the neoplastic lines studied (FICO et al., 1986).

KIM et al (2014) observed that treatment with selenium was beneficial to the small intestinal mucosa since that it may enhance glutathione peroxidase activity and attenuate lipid peroxidation. Selenium also helps to neutralize the

excessive superoxide radicals and lipid peroxidation in addition to present modulating effect in affected inflammatory and immune states (VOLP et al., 2010). Moreover, AVINASH et al (2015) found that an increased expression of selenoproteins on macrophages can favor a reduction on gut inflammation, and improve the integrity of the intestinal barrier impaired by a pro-inflammatory state in the intestinal environment. In dogs little is known about the effect of selenium supplementation on gut mucosa characteristics and function. Thus, the present study evaluated the effect of selenium amount and source on dry extruded food on the morphological characteristics of the intestinal mucosa of dogs.

## MATERIAL AND METHODS

The experimental protocol is in accordance with the ethical principles adopted by the National Council for Animal Experiments Control (CONCEA) and was approved by the Ethics Committee on Animal Use (CEUA), College of Agricultural and Veterinary Sciences / UNESP - Jaboticabal (Protocol No. 01632/13).

### Animals and Experimental design

The experiment was conducted at the Laboratory of Research on Nutrition and Nutritional Diseases of Dogs and Cats "Prof. Dr. Flavio Prada", Department of Veterinary Clinic and Surgery, College of Agricultural and Veterinary Sciences, of UNESP – Univ Estadual Paulista, Jaboticabal, Brazil.

Thirty adult Beagle dogs were used, with a mean age of 7.3 ± 2.2 years old and average body weight of 12.6 ± 1.6 kg. The health of the animals were verified prior to the study, by physical examination, blood and serum biochemical analysis, that resulted normal for all dogs. The experiment was conducted as a randomized complete block design, with three blocks of 10 animals, and five diets. The blocking factor was considered the period. In each block two dogs were fed each one of the five diets, totaling six dogs per diet.

Dogs were fed individually calculated amounts of food, according to the energy value of the diet, estimated based on their chemical composition and the

individual energy requirement of each animal, according to the recommendations of the National Research Council (2006). Food was offered twice daily at 8:00 a.m and 4:00 p.m, and fresh water was available all the time. Each period lasted 210 days which included an adaptation phase which lasted 90 days, when all dogs were fed with the control diet, and an experimental phase that lasted 120 days when animals were fed experimental diets. At the end of this period samples of the intestinal tract mucosa was collected by endoscopy and colonoscopy from all the dogs.

Experimental diets and selenium determination

A same diet formulation with low selenium content was used to produce all experimental diets (Table 1). For this, ingredients were analyzed for selenium content and after selected for diet formulation. The basal diet was formulated following the nutritional recommendations for dog maintenance of AAFCO (2011). A specific vitamin and mineral premix without selenium and suppling only the minimum requirement of vitamin E (30 IU/kg of diet) was designed for the experiment, and produced by Mcassab (Sao Paulo, Brazil). Animals were submitted to five diets: Control (CO) – the basal formulation with added sodium selenite to achieve 0.13 ppm Se in the diet; SY33 - basal diet with added selenium yeast (SY) totaling 0.33 ppm Se; SY66 - basal diet with added SY totaling 0.66 ppm Se; SY99 - basal diet with added SY totaling 0.99 ppm Se; $Na_2SeO_3$66 - basal diet with added sodium selenite ($Na_2SeO_3$) totaling 0.66 ppm Se. Sodium selenite and selenium yeast were also analyzed for their selenium levels before being added to the diet.

To produce the foods, all the ingredients excluding the sources of selenium were mixed and ground in a hammer mill fitted with a screen sieve size of 1.0mm (Tigre, Moinhos Tigre, São Paulo, Brazil). The selenium content was determined on this premixed raw material before adding the selenium sources in study. The inclusion amount required of SY and $Na_2SeO_3$ was then calculated considering that the raw material mixture presented 0.07ppm of Se. Diets were processed at the animal feed plant of UNESP – Univ Estadual Paulista, Jaboticabal, Brazil in a single screw extruder (Mab 400S, Extrucenter, Monte Alto, Brazil), with an average extrusion capacity of 150 kg/h. After 30 min

of a stable production, the kibble density out of extruder was adjusted for all diets to be between 320 and 340 g/L (on as is basis) every 15 min, to ensure similar and proper processing quality. The extruder feed rate was approximately 100 kg/h, the extruder screw speed was 450 rotations per minute, and the die open area was 63.9 mm2. The extruder preconditioning temperature was maintained above 90°C through direct steam injection. The mass temperature at the end of the extruder varied between 130°C and 135°C. After extrusion, the kibbles were dried in a forced-air dryer at 105 °C for 20 minutes, and further coated with poultry fat and liquid flavor enhancer.

**Table 1**. Ingredient and chemical composition of the experimental diets for dogs with different amounts and sources of selenium.

| Item | Diets [1] | | | | |
|---|---|---|---|---|---|
| | **CO** | **SY33** | **SY66** | **SY99** | **Na2SeO366** |
| Ingredients (%) | | | | | |
| Maize, grain | 46.03 | 46.03 | 46.03 | 46.03 | 46.03 |
| Maize gluten meal | 19.20 | 19.20 | 19.20 | 19.20 | 19.20 |
| Soybean meal | 17.00 | 17.00 | 17.00 | 17.00 | 17.00 |
| Poultry fat | 8.54 | 8.54 | 8.54 | 8.54 | 8.54 |
| Sugarcane fiber | 2.50 | 2.50 | 2.50 | 2.50 | 2.50 |
| Palatant enhancer [2] | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 |
| Dicalcium phosphate | 1.70 | 1.70 | 1.70 | 1.70 | 1.70 |
| Vitamin-mineral premix [3] | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| Common salt | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 |
| Calcium carbonate | 0.38 | 0.38 | 0.38 | 0.38 | 0.38 |
| Fish oil | 0.35 | 0.35 | 0.35 | 0.35 | 0.35 |
| Choline chloride | 0.15 | 0.15 | 0.15 | 0.15 | 0.15 |
| Potassium chloride | 0.12 | 0.12 | 0.12 | 0.12 | 0.12 |
| Mold inhibitor [4] | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 |
| L-tryptophan | 0.09 | 0.09 | 0.09 | 0.09 | 0.09 |
| Antioxidant [5] | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 |
| L-Lysine | 0.29 | 0.29 | 0.29 | 0.29 | 0.29 |
| Sodium selenite [6] | 0.0002 | - | - | - | 0.001 |
| Selenium yeast [7] | - | 0.09 | 0.21 | 0.33 | - |
| Analyzed chemical composition (DM-basis) | | | | | |
| Dry Matter (%) | 92.80 | 92.40 | 92.20 | 92.20 | 92.50 |
| Crude Protein (%) | 25.30 | 24.70 | 24.80 | 25.10 | 24.80 |
| Fat (%) | 14.90 | 15.10 | 14.10 | 15.20 | 14.30 |
| Ash (%) | 5.90 | 5.80 | 5.70 | 5.50 | 5.40 |
| Crude Fiber (%) | 5.40 | 4.90 | 4.80 | 5.34 | 5.20 |
| Vitamin E (UI/kg) [8] | 30.00 | 30.00 | 30.00 | 30.00 | 30.00 |
| Selenium (mg/kg) [9] | 0.13 | 0.34 | 0.67 | 0.97 | 0.67 |

[1] - CO - basal formulation with added sodium selenite to achieve 0.13 ppm Se; SY33 - basal diet with added selenium yeast (SY) totaling 0.33 ppm Se; SY66 - basal diet with added SY totaling 0.66 ppm Se; SY99 - basal diet with added SY totaling 0.99 ppm Se; Na2SeO366 - basal diet with added sodium selenite (Na2SeO3) totaling 0.66 ppm.
[2] - Liquid Palatant, SPF do Brasil, Descalvado, Brazil
[3] - Addition per kilogram of diet: Iron 150 mg; Copper 15 mg; Manganese 7 mg; Zinc 150 mg; Iodine 2 mg; Vitamin A 15,000 IU; Vitamin D 1,000 IU; Thiamine 4 mg; Riboflavin 5 mg; Pantothenic acid 20 mg; Niacin 30 mg; Pyridoxine 4 mg; Folic acid 0.3 mg; cobalamin 0.04 mg.
[4] - Mold Zap: Ammonium dipropionate, acetic acid, sorbic acid and benzoic acid. Alltech do Brazil Agroindustrial Ltda.
[5] - Banox: butylated hydroxyanisole, butylated hydroxytoluene, propyl gallate and calcium carbonate. Alltech do Brasil Agroindustrial Ltda
[6] - 448.7mg of selenium per gram of product. VETEC Química Fina Ltda., Duque de Caxias, Brazil.
[7] - 998.7 mg of selenium per kilogram of product. Selemax (Biorigin, Lençóis Paulista, Brazil).
[8] – Calculated addition via premix.
[9] - Analyzed in quadruplicate, under a coefficient of variation bellow 5%.

Selenium determinations were conducted at the Laboratory of Applied Atomic Spectrometry, Department of Chemistry and Biochemistry, UNESP – Univ Estadual Paulista, Botucatu, Brazil. The technique of atomic spectrometry and the method by graphite furnace atomization were used (Silva et al, 2007; Moraes et al., 2009). In addition, diets were analyzed for dry matter, crude protein, acid-hydrolyzed fat, crude fiber, and ash content following the methodologies recommended by AOAC (1995).

Histological analysis of the stomach, small and large intestine

At the end of each period, after 12 hours fasting, the dogs were anesthetized for endoscopic and colonoscopy procedure. Dogs received intramuscularly chlorpromazine (Sanofi-Aventis Pharmaceutical Ltd., Suzano, Brazil) at a dose of 1.0 mg/kg, as a pre-anesthesia. After sedation, anesthesia was induced with propofol (Cristália ltd pharmaceutical chemicals, São Paulo, Brazil), in the dose 5.0 mg/kg, slowly administered intravenously. After relaxation of the jaw and the loss of laryngotracheal reflection, endotracheal intubation was performed with a tracheal probe of appropriate size for each animal. Anesthesia was maintained with isoflurane (Virbac Animal Health of

Brazil, Jurubatuba, Brazil), diluted in oxygen in sufficient concentration to keep the animal in anesthesia.

For the biopsy procedure by endoscopy and colonoscopy, animals remained in left lateral decubitus position. A flexible endoscope (Karl Stoz, 60914NKS, Karl Stoz GmbH & Co. KG, Tuttlingen, Germany) and flexible alligator grasper of 2.2 mm (Karl Stoz, 60252LX, Karl Stoz GmbH & Co. KG, Tuttlingen, Germany) were used. From the mucosa of stomach, duodenum and jejunum up to 15 samples were removed. After performing an enema, until 6 ileal mucosa fragments and 14 fragments of each segment of the colon (ascending, transverse and descending) of each animal were collected. After the procedure, the animals were given 4 mg/kg of tramadol (Pfizer, Grünenthal GmbH, Stolberg, Germany) and 25 mg/kg of butylscopolamine (Boehringer Ingelheim, Sant Cugat Del Vallés, Spain) as pain relief medication after the procedure. Dogs were assisted until fully recovered from the anesthesia.

The mucosa fragments were fixed in 10% buffered formalin and processed according to standard technique with paraffin embedding. Subsequently, 5 µm serial sections were performed and fragments stained with hematoxylin and eosin (HE). Microscopic examination was conducted by a qualified observer, without access to any information on the identity and characteristics of each animal used in the experiment. The samples were examined by light microscopy using an optical microscope (Nikon, Nill model, Japan).

Samples were first analyzed for the adequacy of the biopsy, using a score from zero to two, on which zero is an inappropriate fragment and two a suitable fragment. Then, mucosal fragments were analyzed qualitatively and semi-quantitatively for the occurrence and severity of morphological, inflammatory and circulatory changes according to parameters described by Day et al. (2008). The stomach was analyzed for epithelial changes, number of intraepithelial lymphocytes, type and intensity of inflammatory cells in the lamina propria and circulatory disorders. The segments of the small intestine were evaluated for epithelium changes, villus: crypt occurrence and crypt hyperplasia intensity, crypt dilatation, number of intraepithelial lymphocytes, type and intensity of inflammatory cells in the lamina propria and circulatory changes. The segments of the large intestine were analyzed for epithelial changes,

occurrence and crypt hyperplasia intensity, crypt dilation, presence of crypt abscesses, presence or absence of lymphocytes on crypt, type and intensity of inflammatory cells in the lamina propria and circulatory alterations.

## STATISTICAL ANALYSIS

Results of the histological traits of the mucosa fragments were analyzed following a randomized blind block design by the Kruskal Wallis test followed by the Mann-Whitney t-test for group comparisons. Additionally, orthogonal contrast analysis were used to compare the CO diet with the selenium yeast supplemented diets (CO *versus* SY33 + SY66 + SY99), and the selenium yeast supplemented diets with the sodium selenite diet (SY33 + SY66 + SY99 *versus* $Na_2SeO_3$66). All analysis was conducted on the Graphpad Prism (version 4.5, Graphpad Software Inc, California, USA). Values of $P<0.05$ were considered significant.

## RESULTS

The consumption of the experimental diets was adequate, with no episodes of diarrhea, vomiting or refusal to eat throughout the experiment. The body weight of the dogs did not differ between treatments, and remained constant throughout the experiment ($P>0.05$; data not shown). A total of 56 fragments were collected from the stomach, 470 from the small intestine, and 330 from the large intestine. The fragments were considered 100% suitable for analysis.

The statistical evaluation of the semi-quantitative analysis of the inflammatory process showed lower lymphocytic infiltration on the duodenal region of dogs fed SY66 diet ($P<0.05$), as presented on Table 2. No significant differences were verified for the other parameters assessed ($P>0.05$). No differences were verified in the comparison between the control diet and the diets supplemented with SY ($P>0.05$). The comparison between SY and $Na_2SeO_3$ also did not find significant differences between dogs ($P>0.05$).

**Table 2.** Histological evaluation of the gastrointestinal mucosa of dogs fed diets with different amounts and sources of selenium.

| Item | Diets [1] | | | | | |
|---|---|---|---|---|---|---|
| | CO | SY33 | SY66 | SY99 | Na2SeO366 | P value |
| *Lymphocytic infiltration* | | | | | | |
| Stomach | 0 (0-2) [2] | 0 (0-1) | 0.5 (0-1) | 0.5 (0-1) | 0.5 (0-2) | 0.602 |
| Duodenum | 1 (0-1)[ab] | 1 (0-1) [a] | 0 (0-1) [b] | 1 (0-2) [a] | 1 (0-1) [ab] | <0.050 |
| Jejunum and Ileum | 1 (0-1) | 1 (0-2) | 1 (0-1) | 0.5 (0-1) | 1 (0-2) | 0.329 |
| Colon | 0 (0-1) | 0 (0-0) | 0 (0-0) | 0 (0-1) | 0 (0-1) | 0.708 |
| *Intraepithelial lymphocytes* | | | | | | |
| Stomach | 0 (0-0) | 0 (0-0) | 0 (0-0) | 0 (0-0) | 0 (0-0) | 1.000 |
| Duodenum | 0.5 (0-1) | 0 (0-1) | 0 (0-1) | 0 (0-1) | 0 (0-1) | 0.708 |
| Jejunum and Ileum | 0 (0-1) | 0.5 (0-1) | 0 (0-1) | 0 (0-1) | 0.5 (0-1) | 0.746 |
| *Lymphocyte in crypt* | | | | | | |
| Colon | 0 (0-0) | 0 (0-1) | 0 (0-0) | 0 (0-0) | 0 (0-1) | 0.540 |
| *Hyperplasia of crypt* | | | | | | |
| Colon | 1 (0-1) | 1 (0-1) | 1 (0-1) | 1 (0-1) | 1 (0-2) | 0.945 |
| *Crypt dilatation* | | | | | | |
| Colon | 0 (0-0) | 0 (0-1) | 0 (0-1) | 0 (0-0) | 0 (0-1) | 0.419 |

[1] - CO - basal formulation with added sodium selenite to achieve 0.13 ppm Se; SY33 - basal diet with added selenium yeast (SY) totaling 0.33 ppm Se; SY66 - basal diet with added SY totaling 0.66 ppm Se; SY99 - basal diet with added SY totaling 0.99 ppm Se; Na2SeO366 - basal diet with added sodium selenite (Na2SeO3) totaling 0.66 ppm.
[2] Results are median (min-max) of the following score: 0, absence; 1, discrete infiltration; 2, moderate infiltration; 3, severe infiltration (Day et al., 2008),
[ab] Means in the same row not sharing a common superscript differ (P<0.05)

## DISCUSSION

In this study there was no experimental induction of inflammation, and the animals that had mild or moderate inflammation possibly had chronic inflammation of unknown cause. The gastrointestinal tract is constantly exposed to numerous antigens arising from nutrition, microbial components and pathogenic organisms. Experimental studies suggest that changes in the mucosa which forms the intestinal barrier against the external environment, the local immune system and the gut microbiota may result in chronic inflammation (ELSON 1999; WATANABE et al., 1998). The intestinal immune system

performs modulation according to the nature of the antigenic stimulus blocking pathogens while preserving tolerance for harmless substances. If this balance is broken a chronic inflammatory state may be settled (GERMAN et al., 2003). Thus during the animal´s life it is likely to develop gastric and/or bowel chronic inflammatory conditions of varying intensities without clinical manifestations as observed in this study.

According to the results found there is a difference in the inflammatory bowel aspect of the mucosa in the duodenum region of the dogs fed diets with addition of 0.66 ppm of organic selenium (SY66). Selenium acts as an agent with both antioxidant and anti-inflammatory activity. This is because in its role of antioxidant, mainly as glutathione peroxidase, it can reduce hydrogen peroxide, hydroperoxides of lipids and phospholipids, thus reducing the spread of free radicals and reduce hydroperoxide intermediates in the cyclooxygenase and lipoxygenase by decreasing production of inflammatory prostaglandins and leukotrienes (SPALLHOLZ et al., 1990). For this reason it is believed that selenium causes an impact on the course and development of a number of inflammatory diseases (DUNTAS, 2009). The results of this experiment may be in accordance with data found by PERETZ et al (1992) who found that selenium supplementation in the form of selenium yeast in a small group of human patients with rheumatoid arthritis caused a significant reduction of pain and joint involvement.

Considering the difference of species selenium has been shown to reduce inflammatory effect. In other study selenium supplementation resulted in increased glutathione peroxidase activity, reduction of oxidative damage and improves the clinical condition in humans (ANGSTWURM et al., 1999). Unlike the studies mentioned, in this work the experimental dogs were healthy animals, although showing slight gastrointestinal inflammation, they did not have selenium depletion, nor had severe chronic disease, which may explain the absence of other evidence of inflammatory improvement in the stomach or in the bowel.

In the present study, no differences were observed regarding the inorganic and organic selenium source in the inflammatory parameters analysed contrary to what was observed by PUTAROV (2014). In her work with

dogs she noted that the intake of organic selenium source had higher antioxidant properties compared to high intake of sodium selenite.

In terms of health benefits, low selenium levels in the body are associated with increased malignancy risk (RAYMAN, 2005; RAYMAN, 2012), chronic diseases such as inflammatory bowel disease and some kinds of cancer are correlated with selenium deficiency in both human and animals. Thus the findings of this study may be an indication that supplementation bringing intestinal health benefits and improving inflammatory status can be used as a possible preventive in chronic diseases.

## CONCLUSION

Considering the effects observed, possibly selenium supplementation in healthy dogs can prevent inflammatory diseases of the gastrointestinal tract, although more studies are needed to meet the long-term effects of its supplementation and appropriate doses of this trace element.

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## CAPÍTULO 3 – Starch gelatinization, nutrient digestibility and fermentation products on the feces of dogs[1]

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**Abstract**

A combination of raw material particle size and extrusion parameters, including thermal and mechanical energy application promotes starch gelatinization (SG), and protein texturization. The extension of SG required for a proper digestibility of dog diets is not completely studied. The effect of SG on apparent nutrients and energy digestibility, fecal traits and fermentation products on the feces of dogs fed extruded diets were studied. On study 1 a formula with maize as the cereal was grind and extruded to obtain three SG degrees: SG99 (SG=99.9%; mean geometric diameter [MGD]=224mm; SME=22kW-h/t); SG76 (SG=76%; MGD=283mm; SME=12kW-h/t); SG63 (SG=63%; MGD=312mm; SME=11kW-h/t). A fourth treatment was constituted by a pelleted diet, not extruded (SG26; with 26% of SG, and raw material MDG of 312mm). Twenty-four dogs were used, six per treatment. Digestibility was measured by total fecal collection, and fermentation products on fresh fecal samples. Results were evaluated by polynomial contrast ($P<0.05$). On study 2 a database form published data and digestibility of prepared diets (n=60) was used to build polynomial regressions among SG degree and apparent digestibility of nutrients. On study 1 a linear increase on crude protein ($P<0.002$), and a quadratic increase on starch digestibility $P<0.001$) followed the increase on SG of the diets, without differences for the other nutrients. Fecal production reduced quadractically with the increase on SG ($P=0.045$), and the fecal concentration of acetate, propionate, butyrate, valerate, and isobutirate reduced linearly, and lactate quadractically with the increase on SG ($P<0.001$). On Study 2 apparent digestibility of organic matter ($R^2$ from 0.63 to 0.26), crude protein ($R^2$ from 0.91 to 0.26), starch or nitrogen-free extract ($R^2$ from 0.95 to 0.23), and fat ($R^2$ from 0.84 to 0.46) increased with higher SG ($P<0.05$). The apparent digestibility of crude protein seems to be the most influenced by SG. Starch gelatinization from 83% to 87% seems be able to maximize the apparent digestibility of nutrients, although for some diets a linear response was described. Reduced SG increase fermentation on colon, and lactate and most short-chain fatty acids, including butyrate, fecal concentration, aspect that can be explored as a nutritional intervention to promote gut health.

**Keywords**: butyrate, cooking, extrusion, particle size, pellet

## INTRODUCTION

The majority of commercial dry foods for dogs are currently produced by the thermoplastic extrusion process (SPEARS and FAHEY, 2004; MOSCICKI, 2011). During extrusion, thermal and mechanical energy are implemented which, together with compression in a laminar flow promote extensive changes on starch and proteins, allowing cooking in a few seconds and with low moisture (CARCIOFI et al., 2012; GIBSON and ALAVI, 2013). The process is energetically efficient, reducing microbial counts, inactivating thermolabile, antinutritional factors, shaping and texturizing the food in a short time (CHEFTEL, 1986; RIAZ, 2000).

The endosperm of cereals is mainly starch, organized in semicrystalline granules (SVIHUS, 2005). Starch facilitates the extrusion process, in the presence of water, heat, shear and pressure they swell, melt and lose their crystaline structure (ZENG et al., 1997). The process increase the viscosity and mass resistance to flow inside the extruder barrel, allowing for mechanical energy transference from the screw and promoting proper cooking, adequate kibble expansion, cellular structure formation, and kibble crispness (CRANE et al., 2000; RIAZ, 2007).

The gelatinized starch is soluble in water and more susceptible to enzymatic degradation than the uncooked starch (DONA et al., 2010). In properly extruded diets, dogs can digest more that 98% of the starch from cereals (CARCIOFI et al. 2008). However, very little is known about the extension of starch gelatinization required for adequate nutrient digestibility and fecal formation in dogs. The assumption is that extensive starch gelatinization during extrusion increases nutrient digestibility and promotes better feces formation. A recent study on dogs, however, verified that different cereals require different grind (raw material particle size) and starch gelatinization to be properly digested (BAZOLLI et al., 2015). In their study, that authors found that 75% of starch gelatinization in diets based on maize or broken rice was enough for proper apparent nutrient digestibility and feces formation, but for sorghum, higher starch gelatinization and small raw material particle size is necessary.

In addition, protein quality can be affected by some chemical reactions induced during the food processing, such as protein cross-linking formation (STANLEY, 1989; AREAS, 1992) or Maillard reactions between the amino group of lysine and the carbonyl group of other compounds (BJORCK and ASP, 1983; VAN BARNEVELD, 1993). Some studies have shown indicatives that the over processing can affect lysine reactivity and nitrogen digestibility in commercial and experimental dog foods (STROUCKEN et al., 1996; WILLIAMS et al., 2006; LANKHORST et al., 2006). On the other hand, the protein digestibility can be improved by the reorganization of the molecule structure and the pre-hydrolysis by linkages rupture (DOZIER, 2001), being controversy the effect of the extrusion process on protein quality.

Starch gelatinization depends on cereal particle size (BAZOLLI et al., 2015) and extrusion parameters such as residence time, moisture, mechanical and thermal energy application (RIAZ, 2007). All this industrial transforming process require energy and increase production cost, making important to understand the influence of starch gelatinization on apparent nutrient digestibility to pet food companies do informed decisions about processing requirements and conditions. Taking these in consideration, the present study evaluated the effect of starch gelatinization on apparent nutrients and energy digestibility, fecal traits and fermentation products on the feces of dogs.

## MATERIAL AND METHODS

Two studies were conducted to evaluate the effect of starch gelatinization. The study 1 evaluated nutrient digestibility, fecal characteristics and fermentation products of dogs fed the same formulation processed under different conditions to obtain four starch gelatinization degrees. The study 2 evaluated the relationship between starch gelatinization and nutrient digestibility using data from literature and two databases from commercial kibble diets for dogs, with a total of 60 diets.

**Study 1 - Effect of starch gelatinization for a dog food based on maze on nutrient digestibility, and fecal production and fermentation products.**

The experimental protocol performed is in accordance with the ethical principles adopted by the National Council for Animal Experiments Control (CONCEA) and was approved by the Ethics Committee on Animal Use (CEUA), Faculty of Agricultural and Veterinarian Sciences, UNESP – Univ Estadual Paulista, Jaboticabal, Brazil (Protocol 09954/13).

Twenty-four Beagle dogs were used, males or females with a mean of 5.5 ± 0.8 years old, and 11.9 ± 0.3 kg of body weight. Dogs were considered healthy after physical examination, blood, and serum biochemistry analysis (urea, creatinine, alanine aminotransferase, alkaline phosphatase, and albumin). The experiment followed a randomized block design, consisted of two blocks of 12 animals, and four diets. Each block included three dogs per food, totaling six dogs per diet. The blocking factor was the period. Each block lasted 20 days, organized as follows: the first 10 days was used for diet adaptation; total feces collection for digestibility was conducted from the $11^{th}$ to the $16^{th}$ day; feces was collected immediately after produced (maximum 10 min) from days $17^{th}$ to $20^{th}$ to evaluate fermentation products.

During the adaptation period, dogs remained in kennels of 1.5m x 4.5m with a solarium, being released daily to voluntary exercise in a grass area. During fecal collection periods, dogs were housed in individual cages of stainless steel, with dimensions of 90 x 90 x 90 cm, equipped with apparatus for separate feces and urine for collection. The amount of food fed to animals was individually calculated according to the energy value of the food, estimated based on their chemical composition, and the energy needs of kennels dogs, (130 kcal/$kg^{0.75}$/day), according to the recommendations of the Nutrient Requirements of Dogs and Cats (NRC, 2006). The dogs were then weighed weekly and the offered amount of food adjusted to maintain constant body weight. The total daily amount of food was divided into two equal meals, provided at 9h and 16h. Water was offered ad libitum.

## Experimental diets

The study included four experimental diets, three extruded foods and one pelleted food. A diet for dog maintenance was formulated following the nutritional recommendations of FEDIAF (2014), as described on Table 1. From this unique formulation, the four experimental diets were produced by varying the degree of raw material particle size reduction, their extrusion parameters, and one diet was pelletized. The experimental diets were: SG99 – raw materials ground with a 0.5mm screen sieve size and extruded with high thermal and mechanical energy application; SG76 – raw material ground with a 1.8mm screen sieve size and extruded with moderate thermal energy and low mechanical energy application; SG62– raw material ground with a 2.0mm screen sieve size and extruded with low thermal and mechanical energy application; SG26 - raw material ground with a 1.8mm screen sieve size and pelleted.

**Table 1.** Ingredient composition of the dog foods used in study 1, to produce diets with different starch gelatinization degrees.

| **Ingredient** | **%** |
|---|---|
| Maize grain | 54.35 |
| Poultry by-product meal | 25.00 |
| Poultry fat | 9.00 |
| Maize gluten meal 60% protein | 5.00 |
| Sugarcane fiber | 2.00 |
| Liquid palatant[a] | 3.00 |
| Vitamin and mineral premix [b] | 0.30 |
| Common Salt | 0.50 |
| Potassium chloride | 0.48 |
| Choline chloride | 0.20 |
| Mold inhibitor [c] | 0.10 |
| Antioxidant [d] | 0.04 |
| L-lysine | 0.03 |

[a] – SPF do Brasil, Descalvado, Brasil.
[b] – Addition per Kg of product: Iron, 100 mg; Copper, 9.25 mg; Manganese, 6.25 mg; Zinc, 150 mg; Iodine, 1.87 mg; Selenium, 0.13 mg; Vitamin A, 18.750 UI; Vitamin D, 1.500 UI; Vitamin E, 225 mg; Vitamin K, 0.15 mg; Thiamin, 5 mg; Riboflavin, 16 mg; Pantothenic acid, 35.75 mg; Niacin, 62.5 mg; Pyridoxine, 7.5 mg; Cobalamin, 45 mcg; Folic acid, 0.75 mg.
[c] – Mould Zap: Ammonium dipropionate, acetic acid, sorbic acid and benzoic acid. Alltech do Brasil Agroindustrial Ltda.
[d] – Banox: butylated hydroxyanisole, butylated hydroxytoluene, propyl gallate and calcium carbonate. Alltech do Brasil Agroindustrial Ltda

All the ingredients were purchased and mixed, forming a single lot of product. The mixture was then divided into three parts, ground in a hammer mill (Tigre, Sao Paulo, SP) fitted with screen sieve sizes of 0.5 mm, 1.8 mm or 2.0 mm, generating mixtures with three different raw material particle sizes distributions (Table 2). The raw material mean geometric diameter (MGD) was calculated by the sieve method according to Zanotto and Bellaver (1996).

**Table 2**. Particle size distribution of a same formulation for dogs used on study 1 to ground in a hammer mill fitted with different screen sieve sizes.

| Item | Scree sieve size | | |
|---|---|---|---|
| | 0.5 mm | 1.8 mm | 2.0 mm |
| Scree sieve size (µm) | Percentage of particles retained | | |
| 2000 | na [1] | na | 0.1 |
| 1400 | na | 0.0 | 01 |
| 840 | na | 0.1 | 1.5 |
| 500 | 4.3 | 12.7 | 24.7 |
| 425 | 0.2 | na | na |
| 300 | 4.5 | 16.3 | 33.5 |
| 210 | 63.9 | 29.7 | 20.4 |
| 125 | 25.9 | 20.7 | 18.8 |
| 53 | 1.1 | 0.9 | 0.9 |
| Plate | 0 | 0 | 0 |
| Mean geometric diameter (µm) | 224 | 283 | 312 |
| Geometric standard deviation (µm) | 1.5 | 1.6 | 1.7 |

[1] na = not analyzed

Each raw material mixture was extruded in a single screw extruder with a processing capacity of 250 kg per hour (MEX 250, Manzoni, Campinas, Brazil). During extrusion, water addition, feed rate, and extruder screw speed variable parameters were unchanged for the three extruded diets. Preconditioner temperature was maintained by direct steam injection, to modulate thermal energy application, the mean temperature of the mass out of the preconditioner was 84 °C for the SG99 diet, 68 °C for the SG76 diet, and 58 °C for the SG62 diet. After 45 minutes of stable processing, the following parameters were measured at each 10 min: preconditioner temperature, moisture of the preconditioner discharge mass, extruder motor amperage, pressure and temperature of extrusion, and extrudate bulk density. The in-barrel moisture was the same for the three diets, around 25.3%. The implementation of specific mechanical energy (SME) was determined according to RIAZ (2007). It was modulated by adjusting extruder die open area, which was 232 $mm^2$/ton/h for the SG99 diet, and 340 $mm^2$/ton/h for the SG76 and SG62 diets. Due to the

interaction between raw material particle size and die open area, SME application was 22.0±0.5 kW-h/ton for the SG99 diet, 13±0.6 kW-h/ton for the SG76 diet, and 12±0.4 kW-h/ton for the SG62 diet. The temperature of the mass before the die was 113 ºC, 107 ºC, and 108 ºC, and the pressure of the mass was 42 bars, 34 bars, and 38 bars, respectively, for the SG99, SG76, and SG62 diets. Additionally, one portion of the raw material mixture ground with the 1.8 mm screen sieve size was pelletized in an equipment with a processing capacity of 300 kg per hour (California Pellet Mill, Brazil). The pelletizer preconditioner temperature was kept around 79 °C by direct steam injection, and the pellet temperature out the pelletizer was 78 °C. After processing the extrudates and pellets were dried for 20 minutes in a continuous forced air dryer system, heated to 105 ° C, and subsequently coated with fats and palatants. The chemical composition of the experimental diets are presented on Table 3.

**Table 3**. Starch gelatinization degree and chemical composition of a same formulation for dogs processed under different conditions. Values on dry matter basis.

| Item | Diets [1] | | | |
|---|---|---|---|---|
| | **SG26** | **SG62** | **SG76** | **SG99** |
| Starch gelatinization, % | 26.3 | 62.6 | 76.0 | 99.9 |
| Chemical composition, % | | | | |
| Dry Matter | 92.3 | 95.2 | 95.3 | 95.2 |
| Ash | 8.0 | 8.0 | 8.6 | 8.1 |
| Crude Protein | 27.1 | 27.0 | 27.1 | 27.04 |
| Fat | 14.5 | 14.3 | 14.2 | 14.1 |
| Starch | 41.5 | 39.4 | 39.5 | 39.5 |

[1] - Pel1.8 - raw material ground with a 1.8mm screen sieve size and pelleted; Ext2.0 – raw material ground with a 2.0mm screen sieve size and extruded with low thermal and mechanical energy application; Ext1.8 – raw material ground with a 1.8mm screen sieve size and extruded with moderate thermal energy and low mechanical energy application; Ext0.5 – raw material ground with a 0.5mm screen sieve size and extruded with high thermal and mechanical energy application.

Determination of the coefficient of total tract apparent digestibility of nutrients

The coefficient of total tract apparent digestibility (CTTAD) of nutrients and energy of the diets were determined by the method of total collection of feces without urine collection, following recommendations of FEDIAF (2014). Food intake was recorded daily, weighing the food quantities offered and refused in every meal. For five consecutive days, feces were quantitatively collected at 08:00h and at 17:00h, weighed and stored in a freezer (-20°C) for later analysis.

At the end of the collection period, feces were thawed and homogenized, compounding one sample per dog and period. Feces were then dried in a forced air oven (Model 320-SE, FANEM, São Paulo, Brazil) at 55 °C for 72 hours. The dried feces and diets were ground in a knife type mill (Model MOD 340, ART LAB, São Paulo, Brazil), fitted with a 1mm screen sieve size for laboratory analysis. Feces and food were analyzed according to the

methodology described by AOAC (1995) for dry matter (DM), crude protein (CP), acid-hydrolyzed fat and ash. The organic matter was calculated as DM – ash. The total starch was evaluated by a technique adapted from KARKALAS (1985) and HOLM et al, (1986). The gross energy (GE) content were determined by an adiabatic calorimetry pump (1281, PARR Instruments, Moline, USA). Furthermore, the starch gelatinization degree was analyzed on foods by the method of amiloglucosidase (SA, et al., 2013). All laboratory analysis were conducted in duplicate and repeated when the variation between duplicates was greater than 5%.

Based on laboratory results the metabolizable energy content and CTTAD of dry matter, crude protein, organic matter, fat, starch and gross energy were calculated for each diet, following the calculation procedures described in FEDIAF (2014).

Fecal traits and fermentation products

Feces quality was indirectly assed by the following score (CARCIOFI, et al., 2008): 0 = liquid feces; 1 = soft feces, formless; 2 = soft feces , malformed, which assume the shape of the container; 3 = soft, formed feces and wet, which retains shape; 4 = well formed and consistent feces, which do not adhere to the floor; 5 = dry and hard feces, with reduced volume.

Fresh samples of feces, collected and processed in a maximum of 10 minutes after deletion, were used to measure pH and determine some fermentation products. The pH was determined using two grams of feces diluted (1:3 w/w) in six ml of MilliQ water, and the pH measured with a pH meter (Digicrom Analytical Inc., model DM20, São Paulo, Brazil). The procedure was performed in each of the three collection days, and the mean value used on the study.

For the analysis of short-chain fatty acids (SCFA) and branched-chain fatty acids (BCFA), 10 grams of fresh feces were homogenized and mixed with 30 ml of 4.2 N formic acid (1:3 w/w). The procedure was repeated for each of the three days of collection. The mixture was centrifuged three times at 4,500 G for 15 minutes at 15 °C, taking the supernatant and pellet was discarded. The supernatant was then centrifuged at 20,000 G for 1 hour at 5 °C. After

extraction, the samples were identified and stored in a freezer (-20 °C). The concentration of SCFA and BCFA were determined by gas chromatography according to Erwin et al. (1961), using a glass column of 30 meters in length, 0.32 mm internal diameter, packed with HP-Innowax column (Movie: 0,5µm). The chromatograph (Shimatzu, CG-2014, Shimatzu Scientific Instruments, Tokyo, Japan) was calibrated by the injection of 1 uL of a standard mixed solution and the curve pre-established in software (version GC Solution 2:41:00 SU1, Shimadzu, Tokio, Japan). Nitrogen was the carrier gas with a flow rate of 50 mL/min. Working temperatures were 200°C at injection, 240°C in the column, and 250 °C in the flame ionization detector.

For lactic acid analysis was used approximately three grams of fresh feces, rapidly homogenized and mixed with nine milliliters of distilled water (1:3 w/v). The mixture was kept at 4 °C for one day, and then centrifuged three times at 4,500 G and 15 °C, during 15 minutes. Supernatant was tapped and the sediment discarded. Lactic acid was analyzed according PRYCE (1969) by the spectrophotometric method with reading at 565 nm (500 to 570nm). Blank was used to calibrate the spectrophotometer (QUICK-Lab, DRAKE, São José do Rio Preto, Brazil). The samples were quantified by comparing the readings with a standard lactic acid solution of 0.08%. To measure fecal ammonia concentration was adapted a methodology from VIEIRA (1980). The extracts prepared for determination of SCFA were used. The extracts were thawed at room temperature, diluted into distilled water (2:13 v/v), and ammonia distilled in a nitrogen system (TE Tecnal - 036/1, Piracicaba , Brazil).

**Study 2 – Starch gelatinization degree and nutrient digestibility of extruded diets for dogs**

To study the relationship between starch gelatinization degree and nutrient digestibility, data from literature and two databases of the digestibility of prepared kibble diets for dogs were used. From the literature, the study of Bazolli et al. (2015) included three formulations with similar compositions, based on maize, broken rice, or sorghum. Each formulation was ground to produce raw material mixtures with three different MGD. Each mixture was later extruded under similar conditions originating nine experimental diets. The diets

had starch gelatinization degrees ranging from 62% to 90%, by the amiloglucosidase method. Each diet was fed to six dogs, and the CTTAD of nutrients calculated.

A database composed of 18 commercial extruded diets for dogs, with different chemical composition, and ingredient sources were used (Table 4). All diets came from Brazil, and the digestibility trials were carried out from 2011 to 2014 at UNESP – Universidade Estadual Paulista, Jaboticabal, Brazil. A database composed of 33 commercial extruded diets for dogs, with different chemical composition, and ingredient sources that were evaluated at the University of Zaragoza, Zaragoza, Spain, were also used (Table 5). The diets were evaluated in dogs on the period of 2013 to 2014. No information about the processing conditions of the foods was available for both database.

For all the diets, results of chemical composition and CTTAD of nutrients were provided. All digestibility trials were carried out in a minimum of six dogs, including a minimum of five days of adaptation and five days of total fecal collection, following the recommendations of FEDIAF (2011). In addition, for all the diets the starch gelatinization degree was determined by the amyloglucosidase method (Sá et al., 2013).

**Table 4**. Chemical composition, coefficients of total tract apparent digestibility of nutrients, and starch gelatinization degree of prepared diets for dogs evaluate at UNESP – Universidade Estadual Paulista (n = 18).

| Item | Mean | Min – max | SEM[1] |
|---|---|---|---|
| Chemical Composition (%, As-fed basis) | | | |
| Dry matter | 93.7 | 90.9 – 98.1 | 0.35 |
| Crude protein | 28.7 | 20.3 – 42.9 | 1.42 |
| Fat | 14.0 | 9.6 – 21.7 | 0.74 |
| Ash | 8.9 | 6.7 – 12.6 | 0.48 |
| Crude fiber | 3.2 | 1.8 – 5.5 | 0.27 |
| Starch | 30.5 | 16.2 – 37.9 | 1.37 |
| Starch gelatinization degree (%) | 89.5 | 81.7 – 99.8 | 1.69 |
| Coefficients of total tract apparent digestibility (%) | | | |
| Dry matter | 77.5 | 70.6 – 86.4 | 0.62 |
| Organic matter | 83.1 | 75.9 – 91.1 | 0.83 |
| Crude protein | 82.6 | 74.1 – 90.2 | 0.82 |
| Fat | 89.4 | 78.4 – 93.2 | 0.74 |
| Nitrogen-free extract[2] | 85.4 | 77.0 – 95.8 | 0.99 |

[1] – SEM – standard error mean (n= 18 foods)

[2] – Nitrogen-free extract was calculated as: DM – (CP + Fat + Ash + crude fiber). It was used for digestibility calculation due the lack of information on starch digestibility of the foods from the database.

**Table 5.** Chemical composition, coefficients of total tract apparent digestibility of nutrients, and starch gelatinization degree of prepared diets for dogs evaluate at University of Zaragoza (n = 33).

| Item | Mean | Min – max | SEM [1] |
|---|---|---|---|
| Chemical Composition (%, As-fed basis) | | | |
| Dry matter | 92.1 | 89.7 – 94.8 | 0.20 |
| Crude protein | 29.6 | 25.5 – 40.6 | 0.49 |
| Fat | 18.4 | 10.6 – 28.1 | 0.58 |
| Ash | 7.8 | 9.8 – 6.5 | 0.15 |
| Crude fiber | 2.3 | 1.0 – 5.9 | 0.19 |
| Starch | 32.2 | 20.8 – 42.2 | 0.82 |
| Starch gelatinization degree (%) | 85.6 | 72.4 - 95.9 | 1.19 |
| Coefficients of total tract apparent digestibility (%) | | | |
| Dry matter | 82.3 | 72.3 – 87.9 | 0.56 |
| Organic matter | 86.7 | 77.6 – 91.5 | 0.54 |
| Protein | 84.1 | 75.8 – 89.6 | 0.57 |
| Fat | 92.9 | 86.5 - 96.4 | 0.36 |
| Nitrogen-free extract [2] | 89.8 | 80.9 – 94.4 | 0.52 |

[1] – SEM – standard error mean (n= 33 foods)

[2] – Nitrogen-free extract was calculated as: DM – (CP + Fat + Ash + crude fiber). It was used for digestibility calculation due the lack of information on starch digestibility of the foods from the database.

**Statistical analysis**

The assumptions of homogeneity of variance and normality of errors were verified for all variables. The study 1 was analyzed as a randomized block design, with four diets and six dogs per diet. The experimental unit was considered one dog. Data were subjected to analysis of variance and the model sums of squares were separated into treatment (diet) and block effects. When treatment effects were verified at the F test, means were compared by polynomial contrasts according to the starch gelatinization degree of the diets. The study 2 was analyzed by polynomial contrasts considering the starch gelatinization degree of the diets as the independent variable and the nutrient digestibility as the dependent variable. The experimental unit was considered

one prepared food. For both studies values of $P<0.05$ was considered significant. The statistics procedures were performed using the Statistical Analysis System software (version 9.0, SAS Institute Inc., Cary, NC, USA).

## RESULTS

### Study 1 - Effect of starch gelatinization for a dog food based on maze on nutrient digestibility, and fecal production and fermentation products.

The consumption of the experimental diets was adequate, with no episodes of diarrhea, vomiting or refusal to eat throughout the experiment. Even the pelleted diet was eaten, without differences on nutrient intake between diets (Table 6). Crude protein and starch digestibilities changed between diets ($P<0.01$), the CP digestibility increased linearly ($R^2=0.40$) and starch digestibility quadratically ($R^2=0.58$) with the increase on starch gelatinization degree ($P<0.002$). The digestibility of dry matter, organic matter, fat and crude energy did not differ according to starch gelatinization.

Feces production (As-is basis) reduced quadratically (Table 7) as the starch gelatinization degree increased ($R^2= 0.23$; $P=0.045$). This increase, however, was small and lower than 1g of feces/kg/day. Fecal score ($R^2=0.29$; $P=0.012$), and feces pH ($R^2= 0.86$; $P=0.003$) increased quadratically with higher starch gelatinization. Other parameters as feces production on DM-basis and fecal moisture content did not differ for the diets.

**Table 6.** Body weight, food intake, coefficient of total tract apparent digestibility of nutrients, and metabolizable energy content of a same formulation for dogs processed under different conditions to obtain four starch gelatinization degrees.

| Item | Diets[1] | | | | SEM [2] | P-value | Contrasts [3] | |
|---|---|---|---|---|---|---|---|---|
| | SG26 | SG62 | SG76 | SG99 | | | Linear | Quadratic |
| Body weight (kg) | 12.4 | 12.3 | 10.5 | 12.4 | 0.27 | 0.012 | - | - |
| Food intake (g/kg/day) | | | | | | | | |
| Dry Matter | 15.6 | 15.6 | 15.9 | 15.1 | 0.22 | 0.593 | - | - |
| Crude Protein | 4.2 | 4.2 | 4.3 | 4.2 | 0.06 | 0.572 | - | - |
| Fat | 2.3 | 2.2 | 2.1 | 2.1 | 0.04 | 0.600 | - | - |
| Crude Fiber | 0.4 | 0.4 | 0.4 | 0.3 | 0.01 | 0.276 | - | - |
| Starch | 6.5 | 6.2 | 6.3 | 5.9 | 0.10 | 0.215 | - | - |
| Coefficient of total tract apparent digestibility (%) | | | | | | | | |
| Dry Matter | 79.6 | 80.4 | 79.5 | 79.6 | 0.25 | 0.536 | - | - |
| Organic Matter | 84.8 | 85.4 | 84.3 | 84.9 | 0.20 | 0.375 | - | - |
| Crude Protein | 83.7 | 85.8 | 86.1 | 86.7 | 0.35 | 0.010 | 0.002 | 0.213 |
| Fat | 92.3 | 92.1 | 92.2 | 92.5 | 0.17 | 0.849 | - | - |
| Starch | 99.7 | 99.8 | 99.8 | 99.8 | 0.01 | 0.004 | 0.002 | <0.001 |
| Crude Energy | 85.7 | 86.2 | 85.7 | 85.7 | 0.15 | 0.448 | - | - |
| Metabolizable energy (kcal/g of DM) | 4.1 | 4.2 | 4.1 | 4.1 | 0.09 | 0.056 | - | - |

[1] – SG26 - raw material ground with a 1.8mm screen sieve size and pelleted, 26.3% of starch gelatinization; SG62 – raw material ground with a 2.0mm screen sieve size and extruded with low mechanical energy application, 62.6% of starch gelatinization; SG76 – raw material ground with a 1.8mm screen sieve size and extruded with low mechanical energy application, 76.0% of starch gelatinization; SG99 – raw material ground with a 0.5mm screen sieve size and extruded with high mechanical energy application, 99.9% of starch gelatinization.[2] – SEM – standard error mean (n= 6 dogs per diet)
[3] – Linear or quadratic effects of starch gelatinization degree

**Table 7.** Characteristics and fermentation products concentration on feces of dogs fed a same formulation processed under different conditions to obtain four starch gelatinization degrees.

| Item | Diets[1] | | | | SEM [2] | P-value | Contrasts [3] | |
|---|---|---|---|---|---|---|---|---|
| | SG26 | SG62 | SG76 | SG99 | | | Linear | Quadratic |
| Feces | | | | | | | | |
| g/kg/day (As-is basis) | 8.0 | 7.0 | 7.1 | 7.1 | 0.13 | 0.029 | 0.026 | 0.045 |
| g/kg/day (DM basis) | 3.7 | 3.2 | 3.1 | 3.3 | 0.08 | 0.933 | - | - |
| Moisture (%) | 59.0 | 58.3 | 52.1 | 56.7 | 0.37 | 0.268 | - | - |
| Score | 3.8 | 3.9 | 3.9 | 3.9 | 0.02 | 0.028 | 0.012 | 0.089 |
| pH | 6.44 | 7.00 | 7.11 | 7.32 | 0.07 | <0.001 | <0.001 | 0.003 |
| Fermentation products (mMol/kg of feces DM) | | | | | | | | |
| Acetic acid | 309.2 | 304.2 | 258.2 | 252.7 | 10.32 | 0.013 | 0.002 | 0.988 |
| Propionic acid | 167.7 | 143.4 | 122.9 | 111.5 | 4.56 | <0.001 | <0.001 | 0.308 |
| Butiric acid | 72.5 | 68.7 | 60.6 | 44.3 | 2.91 | <0.001 | <0.001 | 0.165 |
| Total SCFA | 549.9 | 527.2 | 441.7 | 410.1 | 15.01 | <0.001 | <0.001 | 0.840 |
| Isobutiric acid | 7.6 | 7.5 | 7.6 | 7.9 | 0.37 | 0.959 | - | - |
| Isovaleric acid | 11.7 | 11.6 | 11.3 | 11.9 | 0.52 | 0.897 | - | - |
| Valeric acid | 2.6 | 1.9 | 1.4 | 0.9 | 0.20 | 0.122 | 0.001 | 0.783 |
| Total BCFA | 21.8 | 20.9 | 20.3 | 20.8 | 0.93 | 0.766 | - | - |
| Total volatile fatty acids | 571.8 | 548.5 | 462.0 | 430.4 | 15.43 | <0.001 | <0.001 | 0.850 |
| Ammonia | 148.3 | 145.1 | 141.8 | 142.1 | 2.37 | 0.771 | - | - |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Lactate | 36.9 | 6.0 | 8.5 | 5.4 | 3.29 | <0.001 | <0.001 | 0.002 |

[1] – SG26 - raw material ground with a 1.8mm screen sieve size and pelleted, 26.3% of starch gelatinization; SG62 – raw material ground with a 2.0mm screen sieve size and extruded with low mechanical energy application, 62.6% of starch gelatinization; SG76 – raw material ground with a 1.8mm screen sieve size and extruded with low mechanical energy application, 76.0% of starch gelatinization; SG99 – raw material ground with a 0.5mm screen sieve size and extruded with high mechanical energy application, 99.9% of starch gelatinization.
[2] – SEM – standard error mean (n= 6 dogs per diet)
[3] – Linear or quadratic effects of starch gelatinization degree

The acetate (acetate = 208.1 +0.691SG; $R^2$ = 0.30), propionate (propionate = 145.9 _ 0.281SG; $R^2$ = 0.68), butyrate (butyrate = 59.63 – 0.053SG; $R^2$ = 0.46), total amount of SCFA (total SCFA = 418.9 + 0.30SG; $R^2$ = 0.49), and valerate (valerate = 2.77 – 0.017SG; $R^2$ = 0.27) content of the feces reduced linearly as the starch gelatinization degree of the diets increased (P<0.01). Especially different was the lactic acid content of the feces of dogs fed the Pel1.8 diet, that was 6.8 times higher than the verified for dogs fed the Ext0.5 food (lactate = 71.6 – 1.58SG + 0.01$SG^2$; $R^2$ = 0.67). No alterations of fecal ammonia concentration were verified.

**Study 2 – Starch gelatinization degree and nutrient digestibility of extruded diets for dogs**

When polynomial contrasts between nutrient digestibility and starch gelatinization degree were established for the diets of the study of Bazolli et al. (2015), significant variation of the digestibility of OM (CTTAD OM = 5.71 +1.91SG -0.01$SG^2$; $R^2$ = 0.63; P=0.048), CP (CTTAD CP = -15.28 + 2.25SG – 0.01S $G^2$; $R^2$ = 0.91; P<0.001), Fat (CTTAD Fat = 44.31 + 1.18SG – 0.006 $SG^2$; $R^2$ = 0.84; P=0.003), Starch (CTTAD Starch = 66.14 + 0.80SG – 0.004 $SG^2$; $R^2$ = 0.95; P<0.001), and GE (CTTAD GE =20.27 +1.51SG -0.008 $SG^2$; $R^2$ = 0.65; P=0.04) could be explained by starch gelatinization (Figure 1). When the optimal amount of SG was deducted from the polynomial equations, a SG of 95.5% results in maximum CTTAD of OM, 86.5% of SG for the CTTAD of CP, 85.5% of SG for the CTTAD of Fat, 82.8% of SG for the CTTAD of Starch and 86.4% of SG for the CTTAD of GE.

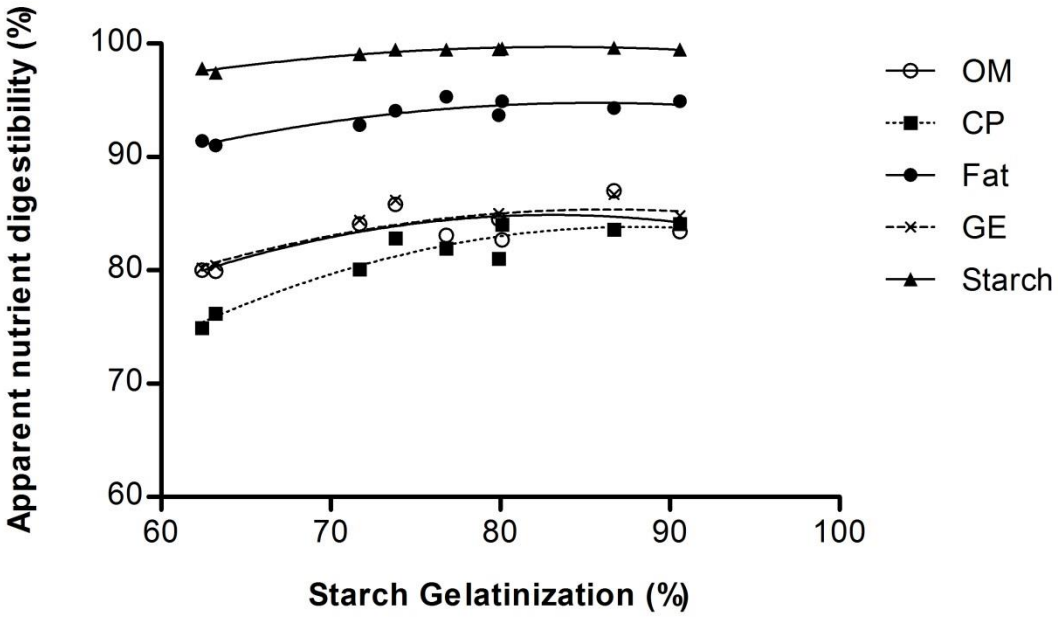


**Figure 1**. Relationship between starch gelatinization (SG) degree (%) and the coefficient of total tract apparent digestibility (CTTAD) of nutrients (%) verified on the study of Bazolli et al. (2015). Organic matter (CTTAD OM = 5.71 +1.91SG -0.01$SG^2$; $R^2$ = 0.63; P=0.048), crude protein (CTTAD CP = -15.28 + 2.25SG – 0.01S $G^2$; $R^2$ = 0.91; P<0.001), fat (CTTAD Fat = 44.31 + 1.18SG – 0.006 $SG^2$; $R^2$ = 0.84; P=0.003), starch (CTTAD Starch = 66.14 + 0.80SG – 0.004 $SG^2$; $R^2$ = 0.95; P<0.001), and gross energy (CTTAD GE =20.27 +1.51SG -0.008 $SG^2$; $R^2$ = 0.65; P=0.04).

For the first database (Figure 2) of prepared diets (UNESP), starch gelatinization also explained some of the variation on the CTTAD of OM (CTTAD OM = 48.3 + 0.38 SG; $R^2$ = 0.26; P=0.032), CP (CTTAD CP = 50.2 + 0.36 SG; $R^2$ = 0.26; P=0.031 ), Fat (CTTAD Fat = -343.9 + 9.19SG – 0.048 $SG^2$; $R^2$ = 0.46; P=0.009), and nitrogen-free extract (CTTAD NFE = 46.3 + 0.42 SG; $R^2$ = 0.23; P=0.042). It is possible to see that although significant, the coefficients of determination were smaller, explaining less the differences on digestibilities between products. For the CTTAD of OM, CP, and NFE a linear relationship with SG was found, suggesting the higher the SG is, more complete would be the nutrient digestibilities. Fat digestibility adjusted to a quadratic equation, and a SG of 95.7% would be enough to maximize it digestibility.

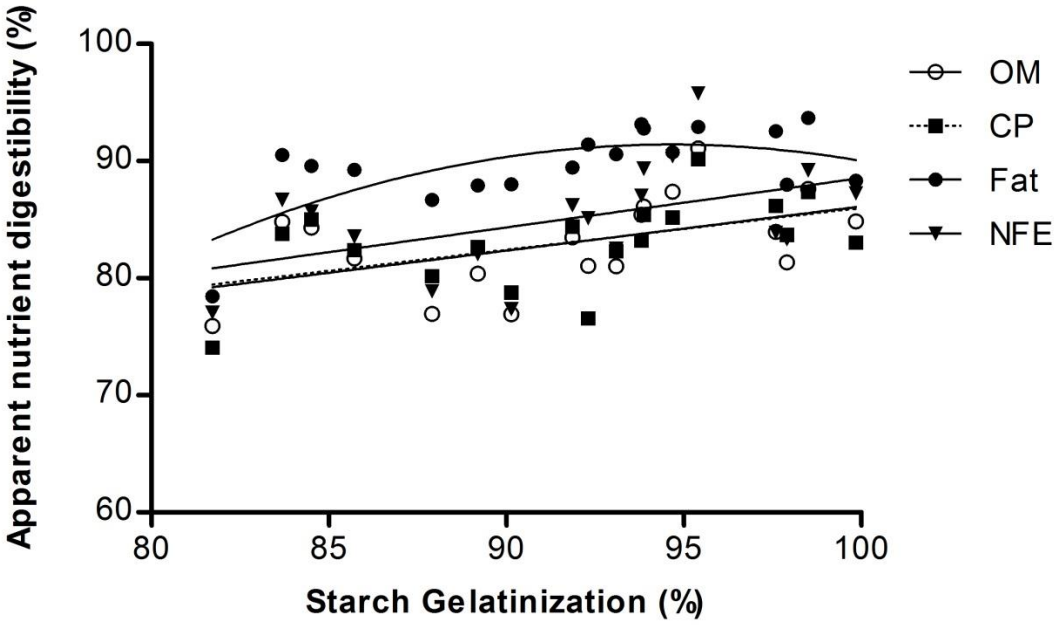


**Figure 2.** Relationship between starch gelatinization (SG) degree (%) and the coefficient of total tract apparent digestibility (CTTAD) of nutrients (%) of prepared diets on database of UNESP. Organic matter (CTTAD OM = 48.3 + 0.38 SG; $R^2$ = 0.26; P=0.032), crude protein (CTTAD CP = 50.2 + 0.36 SG; $R^2$ = 0.26; P=0.031 ), fat (CTTAD Fat = -343.9 + 9.19SG – 0.048 $SG^2$; $R^2$ = 0.46; P=0.009), and nitrogen-free extract (CTTAD NFE = 46.3 + 0.42 SG; $R^2$ = 0.23; P=0.042).

For the second database (University of Zaragoza), the variation of the digestibility of OM (CTTAD OM = -208.0 + 6.99SG – 0.04 $SG^2$; $R^2$ = 0.34; P = 0.001), CP (CTTAD CP = -229.9 + 7.27SG – 0.041$SG^2$; $R^2$ = 0.55; P<0.001), and NFE (CTTAD NFE = -165.3 + 6,08SG - 0.036$SG^2$; $R^2$ = 0.26; P=0.009) could also be explained by SG (Figure 3). When the optimal amount of SG was deducted from the polynomial equations, a SG of 84.9% results in maximum CTTAD of OM, 86.7% of SG for the CTTAD of CP, and 84.4% of SG for the CTTAD of NFE.

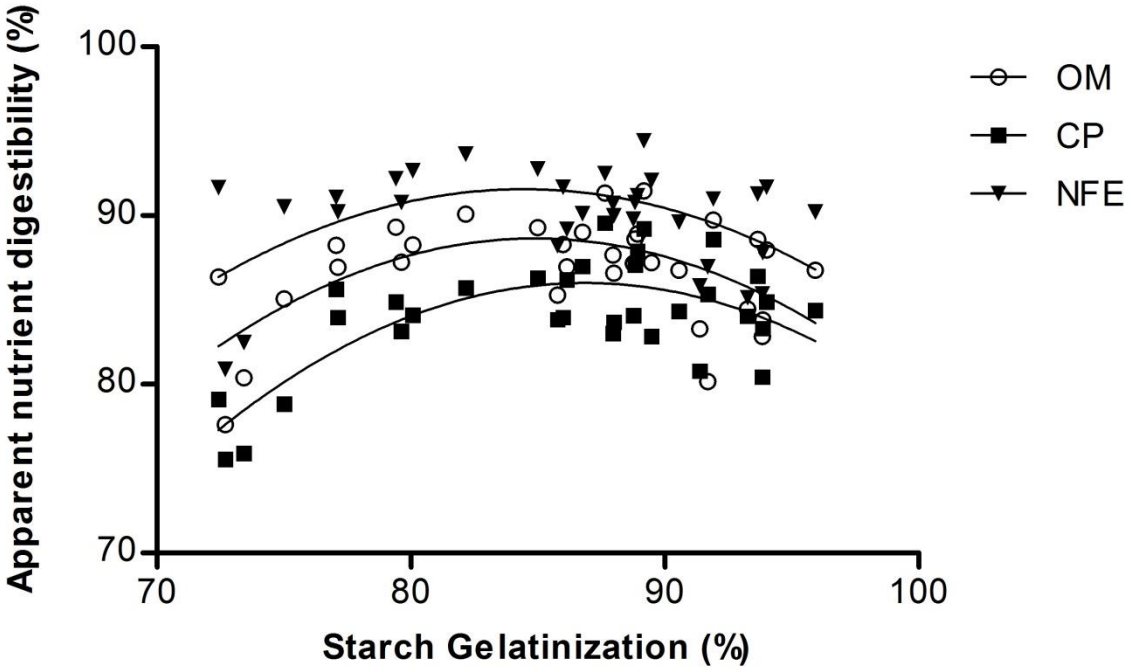


**Figure 3.** Relationship between starch gelatinization (SG) degree (%) and the coefficient of total tract apparent digestibility (CTTAD) of nutrients (%) of prepared diets on database of University of Zaragoza. Organic matter (CTTAD OM = -208.0 + 6.99SG – 0.04 $SG^2$; $R^2$ = 0.34; P = 0.001), crude protein (CTTAD CP = -229.9 + 7.27SG – 0.041$SG^2$; $R^2$ = 0.55; P<0.001), and nitrogen-free extract (CTTAD NFE = -165.3 + 6,08SG - 0.036$SG^2$; $R^2$ = 0.26; P=0.009)

## DISCUSSION

In the present study it was observed that the degree of grinding and the application of specific mechanical energy during processing resulted in differences quite remarkable in gelatinization index. The finely ground and extruded with more application of specific mechanical energy exhibited high gelatinization of the starch content (99.9%), the extruded food 1.8mm with lower mechanical energy application had gelatinization index of 76.7%, the food with grinding of 2.0mm reduced to 62.6% and the pelleted with low use of specific mechanical energy and grinding of 1.8mm produced only 26.3% of gelatinization. The influence of raw material particle size over the extent of

cooking was observed by BAZOLLI et al. (2015). In their study the particle size of cereals (rice, sorghum and maize) affected starch gelatinization during the process of extrusion. The correlation between cooking and milling has also been demonstrated by ROBERTI-FILHO (2013). In their study it was found that the extruder setting affect gelatinization of the starch and the implementation of specific mechanical energy decreased linearly with the increase in size of corn particles. This can be explained because there is a consequent reduction in viscosity at lower gelatinization of grossly ground corn, reducing the mass of the flow resistance within the extruder.

Starch gelatinization and the degree of grinding of the ingredients influenced the digestibility of nutrients. For other species, food processing is known to influence nutrient digestibility (KIENZLE, 1993; HEALY et al., 1994; AMERAH et al., 2007). ROBERTI-FILHO (2013) also confirmed this influence and attributed the decrease in digestibility to the reduction in starch gelatinization. In this study, diets with a lower gelatinization index had lower digestibility coefficients. The lower digestibility can be explained by the unavailability of whole grains, grossly milled with increased particle size resulting in limitation of contact between the enzymes and nutrients in the digestive tract with less use of diet (AMERAH et al., 2007, BAZOLLI et al., 2015). Crude protein had lower digestibility for the diet with the lowest level of SG and increased linearly for the higher gelatinization. This increase can be explained by the denaturation and realignment of chains of amino acids introduced by extrusion which favor the hydrolysis and intestinal absorption. And the greater digestibility of fat could also be explained by increased exposure of the cell content, favoring the formation of micelles and absorption of fats.

When the same ingredient mixture was processed in order to obtain increasing levels of starch gelatinization in the controlled conditions studies, more accurate correlation between SG and nutrients digestibility was found. Significant correlations between starch gelatinization and nutrients digestibility were found in the database studies, even considering a range on chemical composition and nutrients digestibility, consequence of a natural variation of commercial diets formulation.

In the controlled and database studies, variation of the digestibility of nutrients were explained by starch gelatinization. OM, CP, Fat, Starch and GE of the controlled study increased quadratically and optimal amount of SG could be deducted for each nutrient. It seems that a SG of 95.5% results in maximum CTTAD of OM, 86.5% of SG for the CTTAD of CP, 85.5% of SG for the CTTAD of Fat, 82.8% of SG for the CTTAD of Starch and 86.4% of SG for the CTTAD of GE. For the first database fat digestibility adjusted to a quadratic equation, and a SG of 95.7% could maximize its digestibility; for the second database a SG of 84.9% could result in maximum CTTAD of OM, 86.7% of SG for the CTTAD of CP, and 84.4% of SG for the CTTAD of NFE.

In general the controlled studies highlighted the importance of a proper SG for a consistent CP and OM digestibility. Surprisingly, in both kind of studies (controlled and database), SG affected CP more than OM digestibility. At least two hypotheses can explain the higher CP digestibility for the diets with higher SG. First, with the better OM digestibility, less fermentation occurred in the large intestine, resulting in less fecal excretion of microbial nitrogen explaining the higher apparent CP digestibility. Second, a more intense extrusion may results in higher SG but also a better protein denaturation and reorientation, favoring protein hydrolysis and amino acid absorption on the gut. Starch gelatinization is an important parameter to evaluate the extrusion processing efficiency, been related to organic matter and protein digestibility.

In this study it was found that the diet with a lower gelatinization index produced wetter feces with a lower pH. The increased production of feces in natural matter can be explained by the lower coefficient of dry matter digestibility. The pH reduction is explained by the increased formation of SCFA and lactic acid. According to CUMMINGS & ENGLYST (1995), although the starch is almost completely digested in the small intestine, a part can escape from enzymatic digestion and ends up being fermented in the large intestine. Such fermentation will lead to the production of volatile fatty acids and lactic acid, reduction of fecal pH and increasing the osmotic pressure in the lumen (CAMPBELL & FAHEY, 1997; FAO, 1998; KIENZLE et al., 2001). Diets with gross grinding and lower starch gelatinization also produced more fermentation and pH reduction of dog feces in the ROBERTI-FILHO study (2013). In other

studies, increasing the particle size of the ingredients have also found a greater production of total SCFA, increased fecal concentration of butyrate and propionate in the feces of dogs (BAZOLLI, 2007), mice (FERGUSON et al 2000;. HENNINGSSON et al. 2003), pigs (HEDEMANN & KNUDSEN., 1997; Bird et al, 2007) and humans (RAMAKRISHNA et al 2000;. RABBANI et al, 2001), reduction of ammonia and fecal pH linear increase of propionic acid. and quadratic butyrate of feces in dogs (ROBERTI-FILHO, 2013).

The increase of SCFA concentrations, in particular butyrate is considered beneficial for improving the health of the mucosa and intestinal health by stimulating proliferation, maturation and differentiation of colonocytes crypts, facilitating the secretory functions and normal colon absorption (TOPPING, CLIFTON, 2001) and are considered intestinal health indicators (SWANSON et al., 2002; HAENEM et al, 2013;. BELOSHAPKA et al, 2014).The diet with a lower gelatinization index produced very high amounts of lactate. It is known that in normal individuals a few species of bacteria convert lactate acetate and butyrate (LOUIS et al., 2007) and in vitro lactate can be converted into butyrate and propionate (BOURRIAUD et al., 2005), thus lactate also may contribute to intestinal health.

## CONCLUSION

Starch gelatinization is dependent on raw material particle size and energy application during the food processing. The apparent digestibility of crude protein seems to be the nutrient most influenced by starch gelatinization, as it digestibility increased on all evaluated experiments with the increase on starch gelatinization. The influence of starch gelatinization on the apparent digestibility of starch (or nitrogen-free extract) and fat is less clear, but also significant for some diets. Starch gelatinization from 83% to 87% seems be able to maximize the apparent digestibility of nutrients, although for some diets a linear response was described. The intake of less cooked foods results in higher fecal production, but do not alter feces quality of beagle dogs. The less gelatinized starch is more fermented on colon, increasing the fecal

concentration of lactate and most short-chain fatty acids, including butyrate, aspect that can be explored as a nutritional intervention to promote gut health.

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