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Ingredients derived from the slaughter of bovines in dog food

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ABSTRACT: *This study evaluated the nutritional levels, apparent digestibility coefficients, and faecal characteristics of dogs fed with four by-products from bovine slaughter: testicles, residue sirloin steak, trachea, and liver. Ingredients were processed and packed in tins for heat treatment in autoclaves. For the digestibility and faeces quality, ingredients were mixed with a reference diet (commercial food) in the proportion of 30g kg^{-1} test ingredient and 70g kg^{-1} reference diet (as dry matter). Ten adult dogs were distributed in double Latin block squares (5x5) with five treatments and five periods, totalling ten repetitions per treatment. The residue sirloin steak presented the highest levels of essential (414.2g kg^{-1} of dry matter) and non-essential (399.0g kg^{-1} of dry matter) amino acids in tested ingredients. No differences (P>0.05) were observed in apparent digestibility coefficients of dry matter - ADCDM (907g kg^{-1}), ADCOM (930g kg^{-1}), ADCCP (841g kg^{-1}), ADCAEE (954g kg^{-1}) values, and DE (5069kcal kg^{-1}) and ME (4781kcal kg^{-1}) values between testicle, residue sirloin steak, and liver. The trachea presented lower digestibility and energy values (digestible and metabolizable) than the other ingredients. This lower trachea digestibility resulted in higher faecal volume for natural and dry matter (P<0.05). There was no difference (P>0.05) in faecal score between ingredients. Ingredients tested in this study can be used in feeds for adult dogs; however, their nutritional levels and digestibility values should be considered for correct diet balance.*
Key words: *foods, digestibility, energy, nutritional levels.*

Ingredientes provenientes do abate de bovinos em alimento para cães

RESUMO: *Foram avaliados os níveis nutricionais, coeficientes de digestibilidade aparente e características das fezes de cães alimentados com quatro ingredientes provenientes do abate de bovinos: testículo, apara de contra filé, traqueia e fígado. Os ingredientes foram processados e acondicionados em latas para tratamento térmico em autoclaves. Para o teste de digestibilidade e características das fezes os ingredientes foram misturados a uma dieta referência (alimento comercial) na proporção de 30g kg^{-1} do ingrediente teste e 70g kg^{-1} da dieta referência (base matéria seca). Dez cães adultos foram distribuídos em delineamento quadrado latino duplo (5x5), com cinco tratamentos e cinco períodos, totalizando dez repetições por tratamento. A apara de contra filé apresentou os maiores níveis de aminoácidos essenciais (414,2g kg^{-1} de matéria seca) e não essenciais (399,0g kg^{-1} de matéria seca) entre os alimentos avaliados. Não houve diferença (P>0,05) nos valores do coeficiente de digestibilidade aparente para matéria seca - MS (907g kg^{-1}), MO (930g kg^{-1}), PB (841g kg^{-1}), EEA (954g kg^{-1}), e os valores de ED (5069kcal kg^{-1}) e EM (4781kcal kg^{-1}) entre testículo, apara de contra filé e fígado. A traqueia apresentou os menores valores de digestibilidade e energia (digestível e metabolizável) entre os alimentos testados. A menor digestibilidade da traqueia resultou em maior volume de fezes na matéria natural e seca (P<0,05). Não houve diferença (P>0,05) no escore fecal entre os alimentos. Os ingredientes avaliados no presente estudo podem ser utilizados na alimentação de cães adultos, porém deve-se considerar os seus níveis nutricionais e os valores de digestibilidade para o correto balanceamento das rações.*
Palavras-chave: *alimentos, digestibilidade, energia, níveis nutricionais.*

INTRODUCTION

The nutritional needs of dogs and cats can be met by feeding them with complete extruded, semi-humid, and humid diets. These must satisfy daily nutritional needs in amino acids, carbohydrates, lipids, and macro and micro minerals in growth and maintenance phases and different physiological states. Ingredients which go into processed dog and cat foods can be of vegetable, animal, or mixed origin, seeing that nutritionists normally use ingredients considered conventional in these foods (broken rice, corn, soybean meal, corn gluten meal, meat and bone meal, and poultry meal) to provide a mixture which meets the needs of the animals (CARCIOFI, 2008). The use of alternative or non-conventional ingredients is limited due mainly to a lack of information on nutritional values.

Alternative ingredients can reduce food costs and also be important for correct nutritional balance; however, they depend on studies which indicate their nutritional values and levels used. KVAMME & PHILLIPS (2003) stated that the main ingredients of animal origin that can be used in cat and dog foods are liver, trachea, lungs, ground bone, spleen, stomach, reticulum (ruminants), tendons, tongue, carcass remnants, testicles, and kidneys. As

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well as nutritional values, there are other important considerations such as acceptability and processes to which they are submitted which can alter nutrient consumption and digestibility.

Based on this information we can observe that the evaluation of alternative ingredients is of great importance for correctly meeting animal nutritional requirements. Thus, the objective of this study was to evaluate the nutritional levels, digestibility coefficients, and faecal characteristics of dogs fed with alternative ingredients provided from bovine slaughter.

MATERIALS AND METHODS

Ten adult Brazilian Terrier dogs were used; they were aged 4.7±1.4 years with average weight of 9.1±1.0kg. During the digestibility test, the dogs were kept in stainless steel metabolic cages sized 110x70x100cm containing apparatus to separate faeces and urine.

We evaluated four ingredients (by-products) from bovine slaughter (testicles, residue sirloin steak - RSS, trachea, and liver) and a semi-humid commercial food (Dudog's®) which was considered as the reference diet for digestibility calculations (Table 1). Testicles, trachea, and liver were collected immediately after slaughter and RSS obtained by process of meat cutting (residues). After collection, the ingredients were frozen for later processing at BASA Alimentos, Brazil. During processing, the ingredients were defrosted and ground in a Cutter until a homogenous mass was obtained to which 1g kg^{-1} sodium chloride (common salt) was added to improve acceptability. Ingredients were then placed in 350g capacity cans for later capping. Full cans were taken to an autoclave for preheating to 85°C and sterilised at a temperature of 118-121°C for one hour.

Table 1 - Chemical composition analysis of reference diet (kg^{-1} of dry matter).

Ingredients listed on label: Fresh poultry meat, poultry liver, corn, yeast extract, sucrose, vegetable oil, common salt, citric acid, garlic, propylene glycol, calcium propionate, sodium phosphate, yucca extract, sodium nitrate, *Butylated hydroxytoluene* (BHT), Vitamin Mineral Premix[1]	
------------------Composition by chemical analysis----------------	
Dry matter, g	580
Crude protein, g	250
Fibre, g	18.1
Crude fat (acid ether extract), g	95.3
Minerals, g	48.4
Calcium, g	17.1
Phosphorous, g	8.2
Gross energy, kcal	4540

Additives (mg kg^{-1}): Copper 8.00mg; Iron 40.00mg; Manganese 10.00mg; Cobalt 0.50mg; Zinc 80.00mg; Iodine 1.00mg; Selenium 0.25mg; Folic acid 0.60mg; Pantothenic acid 12.50mg; Biotin 0.125mg; Choline 300.00mg; Niacin 15.00mg; Pyridoxine 2.00mg; Riboflavin 2.50mg; Thiamine 2.00mg; Vitamin A 8750.00UI; Vitamin B12 0.015mg; Vitamin D3 625.00UI; Vitamin E (min) 31.00mg; Vitamin K 1.25mg.

For the digestibility test, animals were distributed in double latin block squares (5x5), with five treatments (four test ingredients and one reference diet), and five repetitions/periods, totalling 10 repetitions per treatment. The test followed AAFCO (2015) recommendations with five days of adaptation to the diets and installations, and five days of total faeces collection per period. Experimental diets were prepared by mixing each test ingredient (testicles, RSS, trachea, and liver) with the reference diet in the proportion of 30g kg^{-1} test ingredient and 70g kg^{-1} reference diet (as dry matter).

The foods were mixed at feeding time to avoid fermentation and loss of nutritional quality. To mix the ingredients, commercial Dudog's® was manually ground and homogenized with the test ingredient. Homogenization was performed in such a way as to ensure that the animal could not separate out the experimental diet at feeding time. Animals were fed twice a day with a quantity sufficient to meet their metabolizable energy needs (MEN), according to NRC (2006) accepted equation: MEN (Kcal day^{-1}) = 130 x body $weight^{0.75}$. Water was supplied *ad libitum*.

Faeces were collected at least twice a day, weighed, stored in individual plastic bags, identified, and frozen at -20ºC for later preparation and laboratory analysis. In the laboratory, faecal samples from each dog were separately defrosted and homogenized, forming a composite sample for each animal. Then an aliquot from each faecal, ingredient, and experimental diet sample was taken for pre-drying in a forced ventilation drying oven at 55°C for 72 hours. These were then ground in a ball mill.

Ingredients, experimental diets, and faeces were analysed for dry matter (DM), crude protein (CP), gross energy (GE), fat – acid ether extract (AEE), and mineral matter (MM) according to the method described by SILVA & QUEIROZ (2002). Amino acid composition of the ingredients was performed by high performance liquid chromatography (HPLC) at the *Ajinomoto do Brasil* company laboratory. Gross energy (GE) of faeces, ingredients, and diets, was determined in a bomb calorimeter. Laboratory results were used to calculate digestible energy (DE), metabolizable energy (ME), apparent digestibility coefficients (ADC) of dry matter and nutrients (CP, GE, AEE) of each ingredient by the substitution method, using the equation proposed by MATTERSON et al. (1965). A correction factor was added to the ME determination equation to estimate energy loss from urine according to NRC (2006).

Faecal score evaluation used an adapted classification described by MAIA et al. (2010), where scores varied from 1 to 5, in which 1 = liquid faeces which could not be collected (diarrhoea); 2 = soft malformed faeces, with a consistency unsuitable for collection; 3 = soft faeces, formed and wet, but maintaining a collectable consistency; 4 = well-formed faeces, with adequate form and firm consistency, leaving no mark on the floor when collected; and 5 = hard small dry faeces. After faecal score evaluation, faeces were collected and weighed daily to quantify the volume of faeces in natural and dry matter.

Data were analysed for normality (Shapiro-Wilk), and when meeting this premise were submitted to analysis of variance using the PROC GLM procedure in statistical analysis system SAS (2009) software. Comparison between means was performed using the Student-Newman-Keuls (SNK) test at 0.05 probability. Faecal score was analysed by the Kruskal-Wallis test considering a 0.05 probability.

RESULTS AND DISCUSSION

From the ingredients studied (Table 2), the residue sirloin steak presented the highest quantity of CP (820.9g kg^{-1} of dry matter), while trachea and liver presented the highest AEE (226.3 and 217.6kg^{-1} of dry matter, respectively) and testicles the highest MM (68.9kg^{-1} of dry matter) value. Gross energy values obtained for residue sirloin steak and liver were similar (5401 and 5504kcal kg^{-1} of dry matter, respectively). The lowest gross energy value was obtained with trachea (5007kcal kg^{-1} of dry matter).

Comparing the ingredients evaluated in this study with available publications revealed differences in relation to values described in NRC (2006) and TACO (2011). Crude protein values in NRC (2006) for mechanically separated raw bovine meat (MSM) and liver were 369.4 and 645.1g kg^{-1} of dry matter, respectively. In TACO (2011), crude protein values for bovine lean steak and liver were 779.6 and 721.3g kg^{-1} of dry matter, respectively. These differences between crude protein values can be explained by the variations between products of animal origin, mainly different processing techniques and/or a lack of standardisation for cuts of meat (VIEITES et al., 2000). Specifically with meat, we can get different cuts with different chemical compositions in bovine, mainly in relation to fat quantity. The liver does not suffer much variation in relation to cut and constitution, and thus presents the smallest variations in chemical composition. Nutritional information on the testicles and trachea are scarce in literature.

In relation to amino acid evaluation (Table 2), the trachea presented the lowest essential amino acid (EAA) values (199.8g kg^{-1} of dry matter). Residue sirloin steak presented the highest EAA values (414.2g kg^{-1} of dry matter), highlighting lysine and leucine levels as 74.4 and 72.0g kg^{-1} of dry matter, respectively. This higher lysine concentration obtained in residue sirloin steak may be due to its high demand in muscle tissue formation. According to KNABE (1996) dietary lysine is considered the most influential nutrient in protein deposition. This is due to its constant presence in body protein and its preferred metabolic destination for muscle tissue deposition. The liver and testicles presented intermediate EAA values (283.9 and 291.9g kg^{-1} of dry matter, respectively). Non-essential amino acid (NEAA) values were close together for all tested ingredients (Table 2); however, trachea glycine gave the highest concentration (127.2g kg^{-1} of dry matter).

The trachea is formed from cartilaginous tissue which is rich in collagen fibres. According to TORRE et al. (2005) collagen fibres present a reduced quantity of essential amino acid in their composition. As a consequence, ingredients with elevated collagen fibre content are rich in non-essential amino acid, mainly glycine. The trachea amino acid composition obtained in this experiment corroborated information described by TORRE et al. (2005), as glycine was the highest of all amino acid levels in the trachea.

Knowledge of amino acid levels in ingredients is important in correct nutritional balance for animals. Essential amino acids are important for synthesising many proteins in the body. However, those considered non-essential make nitrogen and carbon available for the synthesis of other amino acids as well as compounds essential for life, such as: purines, pyrimidines, neurotransmitters, etc. (NRC, 2006). According to PESTI (2009), the EAA:NEAA ratio in foods is an indicator of their protein biological value. Residue sirloin steak and liver presented a better EAA:NEAA ratio (51:49).

There were no leftovers or regurgitations during the evaluation period. Results showed that there were no differences (P>0.05) in apparent digestibility coefficient between testicle, residue sirloin steak and liver (Table 3). MURRAY et al.

Table 2 - Chemical composition analysis of bovine co-products (g kg-1 of dry matter)*.

Nutrients	Testicles	Residue sirloin steak	Trachea	Liver
Dry matter	185.6	317.7	273.4	332.3
Crude protein	761.3	820.9	725.3	669.6
Fat (acid ether extract)	206.4	180.0	226.3	217.6
Minerals matter	68.9	61.4	66.2	45.7
Gross energy (kcal)	5220	5401	5007	5504
-----Essential amino acid-----				
Lysin	45.2	74.4	24.9	38.4
Threonine	27.3	39.7	19.3	28.2
Methionine	12.0	22.1	7.8	13.2
Arginine	47.9	55.5	48.7	33.3
Histidine	15.0	27.9	7.8	17.0
Isoleucine	25.6	40.6	14.5	27.7
Leucine	53.6	72.0	32.7	56.5
Phenylalanine	30.5	38.1	20.2	33.5
Valine	34.8	43.9	23.9	36.1
∑EAA	291.9	414.2	199.8	283.9
-----Non-essential amino acids-----				
Cystine	9.20	8.2	3.6	5.8
Alanine	45.6	53.3	53.1	35.5
Aspartic acid	59.9	82.0	44.8	56.4
Glutamic acid	96.4	139.8	78.6	79.2
Glycine	66.3	49.1	127.2	37.3
Serine	32.0	35.3	24.2	28.8
Tyrosine	23.1	31.3	11.9	24.7
∑NEAA	332.5	399.0	343.4	267.7
EAA:NEAA ratio	47:53	51:49	37:63	51:49

*Analysis performed in triplicate; ∑EAA – Sum of essential amino acids; ∑NEAA – Sum of non-essential amino acids.

(1998) evaluating the digestibility in dogs of different protein sources, fresh bovine meat, and fresh chicken meat, also described no differences between apparent digestibility for DM, OM, CP, AEE, and GE.

The trachea presented the lowest ($P<0.05$) apparent digestibility coefficient of DM, OM, and CP values. NEIRINCK et al. (1991) evaluating amino acid composition and digestibility of poultry viscera meal, bovine lung, and raw mince as dog foods, obtained the lowest ingredient digestibility value (898.8g kg^{-1}) for crude lung protein. According to the authors, this was due to the low digestibility of connective tissue in the lung. This information corroborates results in our study where the trachea presented a high quantity of cartilage tissue which is rich in collagen. According to NEIRINCK et al. (1991) the quality of a protein is dependent on the concentration and availability of amino acids.

According to the NRC (1994) an ideal balance between essential amino acid and no-essential amino acid concentrations (EAA:NEAA) is important for efficient protein use in chickens. Our study demonstrated that the relationship between essential and non-essential amino acids in the trachea (Table 2) was lower than other ingredients (37:63), a factor which probably contributed to its lower digestibility.

In relation to digestible and metabolizable energy values, there were no differences ($P>0.05$) between values for testicles, residue sirloin steak, and liver. The trachea presented the lowest ($P<0.05$) digestible and metabolizable energy values of all evaluated ingredients (Table 3). The trachea also had the lowest apparent digestible CP value (734.0g kg^{-1}), which could have promoted a negative effect ($P<0.05$) on the metabolisation coefficient. According to D'MELLO (2003), consuming an unbalanced diet alters amino acid concentrations in plasma and tissues, which in turn will be deaminized and excreted in the form of urea, increasing energy loss and possibly reducing the utilization of energy. TAVARES et al. (2010) evaluating DE and ME in both raw and cooked poultry co-products (neck, dorsum, and feet) observed process differences in

Table 3 - Apparent digestibility coefficients (g kg^{-1} of dry matter), digestible and metabolizable energy, and faecal characteristics of dogs fed bovine co-products.

Parameter	Testicles	Residue sirloin steak	Liver	Trachea	SEM[1]	P value
Digestibility coefficients and energy values						
Dry matter[2],g kg^{-1}	900^a	909^a	913^a	790^b	1.51	0.001
Organic matter[2],g kg^{-1}	933^a	928^a	928^a	800^b	1.48	0.001
Crude protein[2], g kg^{-1}	836^a	827^a	860^a	734^b	1.50	0.020
Acid ether extract[2], g kg^{-1}	950^a	962^a	951^a	917^b	0.66	0.001
Digestability energy[2], Kcal kg^{-1}	5048^a	5061^a	5097^a	4717^b	37.5	0.023
Metabolizable energy[2], Kcal kg^{-1}	4745^a	4808^a	4791^a	4245^b	39.0	0.001
Intake dry matter, faecal score and volume						
Intake, g d^{-1}	162	157	156	163	0.71	0.235
Faecal score	4.2	4.0	4.0	3.8	0.15	0.075
Volume						
Natural matter[2], g d^{-1}	63.6^a	63.8^a	64.2^a	75.3^b	1.15	0.001
Dry matter[2], g kg^{-1}	299^a	285^a	287^a	378^b	2.10	0.001

[1]SEM: Standard error of the mean; [2]Different superscript letters within the same line indicate significant differences by the SNK test ($P<0.05$).

energy use, in that the cooked foods presented higher energy utilisation. This effect cannot be ignored in our study, since the digestible (4717 to 5097kcal kg^{-1}) and metabolizable (4245 to 4808kcal kg^{-1}) energy of ingredients processed were high (Table 3).

In this study, we observed that dogs consuming diets containing testicles, residue sirloin steak, and liver had lower ($P<0.05$) faecal quantities (natural and dry matter) than those fed diets containing trachea, despite faecal scores not varying ($P>0.05$; Table 3). ZANATTA et al. (2013) evaluating faecal characteristics in dogs fed with diets containing poultry viscera meal or soybean meal as the main sources of protein reported higher faeces production and faecal wateriness in dogs fed with the diet containing soybean meal. Despite our study only having used products of animal origin, we were able to observe that the trachea worsened faecal characteristics. This is due to its lower organic matter digestibility which increases faecal volume, and also to its lower apparent digestibility coefficient of CP which probably increases intraluminal osmotic pressure resulting in considerable water secretion from the lumen to the colon which leads to increased faecal water content.

CONCLUSION

Ingredients such as testicles, residue sirloin steak, liver and the trachea can be used as alternatives to conventional ingredients in dog foods; however, the trachea should be used with moderation due to its lower digestibility and energy values.

BIOETHICS AND BIOSSECURITY COMMITTE APPROVAL

All experimental procedures were previously approved by the Ethics and Wellbeing Committee of Universidade Vila Velha (UVV), decree 136/10.

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