FULL ARTICLE

ISSN Online 1678-4456

Comparison of methodologies for fat determination and evaluation of calcium and phosphorus content in snacks for dogs*

Comparação de metodologias para determinação de gordura e avaliação do conteúdo de cálcio e fósforo em petiscos para cães

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ABSTRACT

In recent years, the relationship between humans and companion animals has tightened considerably and resulted in the expansion of the range of pet food industry products available in the market. In this context, snacks have gained greater popularity as pet owners seek to please their animals by providing such foods. Due to the growing importance of the snack segment, a need exists for accurate information on the nutritional composition of these products, such as fat concentration. No studies were found that evaluated the effectiveness of different methods applied for determining the content of this nutrient in dog snacks. In addition, too much mineral content can pose health risks. Thus, the objective of this study was to compare three methodologies for determining fat in pet snack products. The moisture, calcium and phosphorus content of each was also determined to compare the obtained results with each value stated on their product labels. Fat determination methods evaluated were ether extract (EE), ether extract after acid hydrolysis (EEHA), and fat content obtained from Ankom XT15 analyzer (ANKOM). Twenty-four snacks produced by 17 companies were evaluated. The results of the three methodologies were compared using the Tukey test. The comparison between the results of the laboratory analysis and the values stated on the labels was performed using descriptive statistics. There was no difference between the three methods evaluated ($p = 0.34$) regarding fat content. Regarding the nutritional compliance of the labels, 25% ($n = 6$) of the snacks presented higher moisture content than the declared amount, 50% ($n = 12$) presented lower fat content, 25% ($n = 6$) lower phosphorus content and, in 50% ($n = 12$), the calcium content was not within the minimum and maximum range stated on the label. Therefore, due to the absence of difference between the results, any of the three fat determination methodologies could be used. Regarding compliance of labels for calcium, phosphorus and fat content, greater control over the nutritional composition of these foods is required since most pet owners tend to supply large quantities of snacks to dogs, leading to excessive daily energy intake.

Keywords: Acid hydrolysis. Ankom. Ethereal extract. Label. Minerals.

RESUMO

Nos últimos anos a relação entre seres humanos e animais de companhia estreitou-se consideravelmente e houve uma expansão da gama de produtos da indústria *pet food* disponíveis no mercado. Nesse contexto, os petiscos ganharam maior popularidade, uma vez que os tutores buscam agradar seus *pets* com esse tipo de alimento. Devido à crescente importância do segmento de petiscos, há a necessidade de informações precisas sobre a composição nutricional desses produtos, como o teor de gordura, uma vez que não foram encontrados estudos que avaliassem a eficácia dos métodos de determinação do teor deste nutriente em petiscos destinados a cães e o excesso de minerais pode implicar em riscos para a saúde. Assim, o presente trabalho comparou três metodologias para determinação de gordura em petiscos para cães, e também determinou os seus respectivos teores de umidade, cálcio e fósforo, cujos resultados foram comparados aos valores declarados pelos fabricantes nos rótulos dos produtos. Os métodos de determinação da gordura avaliados foram: extrato etéreo (EE), extrato etéreo após hidrólise ácida (EEHA) e teor de gordura obtido em analisador Ankom XT15 (ANKOM). Vinte e quatro petiscos produzidos por 17 empresas foram avaliados. Os resultados das três metodologias de determinação da gordura foram comparados com o emprego do teste Tukey. A comparação entre os resultados das

*This article was adapted from the abstract published in the journal Archives of Veterinary Science. 2018; 23 (1):31-32, presented at the "IV Symposium on Nutrition of Company Animals".

análises laboratoriais e os valores declarados nos rótulos foi realizada por meio de estatística descritiva. Não houve diferença entre os três métodos avaliados (p = 0,34) em relação ao teor de gordura dos petiscos examinados. Em relação à conformidade nutricional dos rótulos, 25% (n = 6) dos petiscos apresentaram teor de umidade superior ao declarado, 50% (n = 12) apresentaram menor teor de gordura; 25% (n = 6) menor teor de fósforo e, em 50% (n = 12) deles, o teor de cálcio estava fora da faixa mínima e máxima declarada no rótulo. Portanto, devido à ausência de diferença entre os resultados, as três metodologias de determinação de gordura podem ser utilizadas. Quanto à conformidade dos rótulos em relação aos teores de cálcio, fósforo e gordura, é necessário maior controle sobre a composição nutricional desses alimentos, uma vez que a maioria dos tutores fornece petiscos em elevadas quantidades para os cães, que podem determinar excessivo consumo de energia.

Palavras-chave: Hidrólise ácida. Ankom. Extrato etéreo. Rótulo. Minerais.

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Received: July 04, 2019
Approved: April 14, 2020

How to cite: Zafalon RVA, Rodrigues RBA, Conti RMC, Perini MP, Rentas MF, Vendramini THA, Risolia LW, Macedo HT, Brunetto MA. Comparison of methodologies for fat determination and evaluation of calcium and phosphorus content in snacks for dogs. Braz J Vet Res Anim Sci. 2020;57(2):e159691. https://doi.org/10.11606/issn.1678-4456.bjvras.2020.159691

Introduction

Recent societal changes, such as a reduction in the number of children per family, verticalization of housing, and population aging boosted growth of the world population of companion animals. With 74.3 million dogs and cats (Associação Brasileira da Indústria de Produtos para Animais de Estimação, 2016), Brazil is second in this ranking. Furthermore, concern about providing complete and balanced food for companion animals has also increased. Pet owners seek to please their animals and reward them with other types of food, such as snacks, which have become more popular and represent significant market opportunity for pet food companies (National Research Council, 2006; Schuch, 2009).

According to Brazilian legislation (Brasil, 2009), foods destined for dogs and cats have category ratings. Pet food is classified as complete when it is able to fully meet the nutritional requirements and may have specific or functional properties. Snacks are presented in two categories. One refers to specific food for the purpose of pleasure or reward and is not characterized as a complete food and may have specific properties. The other category is a chewable product based on by-products of animal origin and it may contain ingredients of vegetable origin. Those are used for fun and have negligible nutritional value (Brasil, 2009). There are few studies on this type of food in the scientific literature, although several classifications and empirical information on this segment are published. The snacks differ in their type and are available in the market as biscuits, bones and natural parts dehydrated, and products based on digestible leather, among others.

The main objective of pet food snacks is to please or reward. Thus, the fat content of these products becomes critical since this macronutrient increases palatability (Bauer, 2006). In addition, fat raises the energy density of food and its content in snacks is of great importance, since such foods, if provided indiscriminately, can contribute to the development of overweight and obesity. Therefore, accurate determination of fat is essential for proper nutritional labeling and to transmit clear information to the owners (Nielsen, 2010).

The fat content present in foods can be determined by different methodologies. Soxhlet is the most widely used method and consists of the quantification of fat through a Soxhlet extractor and nonpolar solvents such as petroleum ether. Another method is a filtration system developed by Ankom Technology Inc. (Macedon, NY, USA) (Liu, 2011) which was approved by the AOAC in 2005. It is a closed system, which also uses petroleum ether, but the time required for fat extraction is less. Depending on the type of sample to be analyzed, there may be changes in the Soxhlet methodology. In extruded dry foods, for example, the formation of starch-lipid complexes occurs during processing, which makes it difficult for ether to extract the fat (Lumley & Colwell, 1991; National Research Council, 2006) and may result in underestimated results. Therefore, it is necessary to perform acid hydrolysis prior to preparation of the samples, to allow the hydrolysis of these complexes,

and to allow the quantification of this component (Nielsen, 2010). Thus, in this type of food, the standard method for fat determination is ethereal extract with acid hydrolysis.

Regarding snacks, there are no published studies that evaluated effectiveness of methods for determination of fat content in companion animal food. The objective of this study was to (i) compare three methodologies for determination of fat content in snacks for dogs; and (ii) compare the results of the evaluated content of fat, calcium, phosphorus, and moisture with the values declared on the product labels.

Material and Methods

Experimental groups

Twenty-four brands of snacks for adult dogs in maintenance, manufactured by 17 companies, were purchased at pet food stores in the city of Pirassununga-SP. The samples were analyzed by three methodologies for fat determination: ether extract (EE), fat extraction by acid hydrolysis (EEHA) and Ankom XT15 fat analyzer (ANKOM). The contents of calcium, phosphorus, ethereal extract by Ankom XT15 method and percentage of humidity were also analyzed to perform the comparison of the values declared on the label with those obtained in the laboratory.

The snacks obtained for the study were considered complementary, as they cannot be characterized as complete food according to Brazilian legislation and this information should be clear on the label (Brasil, 2009). All snacks were specific foods for pleasure or reward and may have specific properties.

Collection and processing of material

The snacks were listed in alphabetical order, so the name of the manufacturer was omitted.

Samples were ground in a 1 mm sieve mill. The snacks that presented maximum declared humidity above 14% were previously dehydrated in a forced ventilation oven at 55 °C for 72 h. For all samples, dry matter (DM) analysis was performed according to the methodology described by Silva & Queiroz (2002), in an oven at 105 °C.

Evaluation of different methodologies for fat determination

The ethereal extract (EE) was determined by Soxhlet system according to Silva & Queiroz (2002). A 2g aliquot from the sample was used and maintained for 7 h in the Soxhlet apparatus with petroleum ether used as solvent. After ether recovery, the flasks were kept in a laboratory oven at 105 °C for 12 h. Subsequently, they were cooled in the desiccator at room temperature and weighed by gravimetry to determine the fat content.

The recommendations of Horwitz & Latimer (2005) were followed to determine the fat extraction by acid hydrolysis (EEHA). The samples were pre-digested with 40% hydrochloric acid (40% HCL) for 45 min after boiling at a constant temperature of 250 °C. A 50 mL volume of 40% HCL was added in 3 g of sample for digestion. After this procedure, the samples were filtered with funnel and filter paper. The filter paper was taken to the Soxhlet apparatus for 7 h. Then, the flask was placed in a laboratory oven at 105 °C for 12 h, and then cooled at room temperature in a desiccator for further cool weighting. The fat content was determined gravimetrically and expressed in percentage.

Finally, to determine the fat content using the Ankom XT15 analyzer, approximately 1.8 g of sample were placed in a filter bag, weighed, and sealed to decrease transfer contamination. The sealed bags were dried in a laboratory oven at 105 °C for 30 min, and then cooled in a desiccator for later extraction. After the extraction process, the sample was dried at 105 °C for 3 h, cooled in the desiccator and weighed. The solvent used was petroleum ether.

Evaluation of calcium and phosphorus contents

For the evaluation of the calcium and phosphorus contents, the dry mineral solution described by Association of Official Analytical Chemists (1995) was initially prepared. About 2 g of the sample was weighed in a porcelain crucible and incinerated until light ash was obtained. About 20 mL of HCL (1:1) were added and after 20 min of silica dehydration and mineral solubilization, the samples were filtered with standard filter paper, analytical funnel and 100 mL volumetric flask with distilled water.

The phosphorus content was determined by the colorimetric method (Association of Official Analytical Chemists, 1995), and the following reagents and solutions were used: basic bismuth carbonate, concentrated sulfuric acid, ammonium molybdate, vitamin C and standard solution of phosphorus. To perform the procedure, a mineral solution aliquot was transferred to a 50 mL Florence flask. A 0.1 mL volume of the mineral solution was pipetted into the flask. Approximately 20 mL of distilled water, 5 mL of acidic ammonium molybdate solution and 2 mL of vitamin C were also added to the flask. The volume was filled with distilled water and after 5 min the absorbance (0) and transmittance (100) were measured.

Titration with EDTA method (Association of Official Analytical Chemists, 1995) was used for calcium determination. A 50 mL aliquot of the mineral solution was volumetrically

pipetted and transferred to a 250 mL beaker. A 20 mL volume of 5% ammonium molybdate and 1 mL of 10% hydrochloric acid were added, after which the beaker was brought to a hot plate at 80 °C for complete precipitation. After cooling, filtration was performed with Whatman No. 40 filter paper. The filtrate was collected in a 500 mL Erlenmeyer flask and successive washes were performed with distilled water until complete extraction. Then, 20 mL of 4N sodium hydroxide was added to the filtrate and, by raising the pH of the solution, color changed from yellow to crystalline white. After this procedure, 5 mL of 0.1N potassium cyanide and 5 mL of 20% triethanolamine were added and the volume was raised to 300mL with distilled water. A pinch of the chalcone mixture was added and titration was performed with EDTA 0.02 M until the reddish-pink color turned to deep blue.

Statistical analysis

The results were analyzed by the Shapiro-Wilk test to verify the normality of the residues (PROC UNIVARIATE) and the homogeneity of the variances was done by the F-test. Command PROC GLM was used to analyze variance and means were compared by the Tukey test at the level of 5% of significance using Statistical Analysis System (SAS version 9.4). Descriptive statistics were used to compare the values obtained in the laboratory and those declared on snack labels.

Results and Conclusion

The results of fat content of snack foods using the three methodologies are shown in Table 1. There was no difference ($p = 0.34$) of fat results among the methodologies evaluated.

Although most of the snacks analyzed in this study have a high carbohydrate content (according to the nutritional composition declared on the respective labels), the different processing types of these snacks may explain the lack of difference of fat content results. In dry foods for dogs and cats, during the extrusion process, in the presence of water, heat, shear and pressure, gelatinization of the starch occurs, which is fundamental to increase the digestibility of this carbohydrate (Murray et al., 2001). In the extrusion process, the gelatinized starch, especially the gelatinized amylose chains, forms complexes with the fat present in these foods, which is called amylose-lipid complexes (Lumley & Colwell, 1991; National Research Council, 2006). According to Tran et al. (2008), starch-protein complexes can also occur. Therefore, to determine fat in this type of food, the ethereal extract method is recommended using acid hydrolysis (Murray et al., 2001). However, since most of the snacks evaluated in this study may have been cooked or baked, and did not go through the extrusion process, it can be assumed that no formation of amido-lipid complex occurred during processing, as there was no difference between the methods used.

Table 1 – Fat contents (%) obtained by different methodologies in snacks for dogs (n=24), according to the kind of determination method. Pirassununga, Brazil, 2020

Methods	ANKOM[1]	EEHA[2]	EE[3]	P value
Means	8.83	10.49	9.19	0.342

[1]ANKOM: fat content obtained with Ankom XT15 analyzer. [2]EEHA: ethereal extract after acid hydrolysis. [3]EE: ethereal extract determined by Soxhlet system.

Urrego et al. (2017) compared these three methodologies for fat determination in moist foods for dogs and cats, and also did not observe differences in fat content. It was suggested that the type of processing used in these foods (cooking and / or pasteurization), the small carbohydrate content, and probably a higher amount of free lipids could justify the results found.

Regarding compliance with the guarantee level of the labeled products, according to the laboratory analyses, 25.0% (n = 6) of the snacks had a moisture content higher than declared; 48.5% (n = 11) presented lower fat content; 70.8% (n = 17) higher phosphorus content and, in 48.5% (n = 11), the calcium content found was outside the minimum and maximum range declared on the label (Tables 2 and 3).

Previous studies carried out in Brazil also evaluated label compliance in pet food industry products. Carciofi et al. (2006), for example, demonstrated that in extruded dry food for dogs, there was non-compliance of the label for several nutrients such as crude protein, ethereal extract, crude fiber and calcium in a considerable portion of the analyzed foods of various categories (economic, standard, super premium). Urrego et al. (2017) had also observed in moist foods that crude protein and ethereal extract contents were lower than that declared on the label in some foods analyzed. These results indicated the need for greater supervision, since according to Brazilian legislation (Brasil, 2003), these differences between declared values on labels and results obtained in the laboratory are not allowed.

Comparing the results of this study with the European Pet Food Industry Federation (2018) recommendations for complete and balanced foods, three snacks (B, E and H) exceeded the established limit for calcium (2.5% MS) at 200, 130 and 25.2%, respectively (Table 3). According to the guidelines of the World Small Animal Veterinary Association, it is recommended that snacks should not exceed 10.0% of the animal's daily energy intake (Freeman et al., 2011; Villa Verde & Abad, 2015). However, many owners have no control over the energy intake of their pets and end up providing

Table 2 – Comparison of moisture content and ethereal extract analyzed and described in the labels, according to the snack code. Pirassununga, Brazil, 2020

Items	Moisture[1]	Moisture[2]	EE[3]	EE[4]
Snack A	0.82	23.61	7.17	10.50
Snack B	14.11	19.00	10.65	12.35
Snack C	22.47	10.00	10.85	8.89
Snack D	28.60	19.00	7.73	11.11
Snack E	17.00	22.00	8.56	10.26
Snack F	21.95	14.00	1.82	3.72
Snack G	24.03	26.00	3.97	3.38
Snack H	16.59	30.00	4.76	10.00
Snack I	20.54	30.00	12.05	11.43
Snack J	18.45	18.00	10.62	15.85
Snack K	8.35	10.00	8.51	5.00
Snack L	12.00	12.00	7.50	4.55
Snack M	7.02	10.00	8.12	9.33
Snack N	8.27	10.00	8.02	5.56
Snack O	9.04	10.00	9.09	13.33
Snack P	7.50	10.00	3.90	6.11
Snack Q	9.85	12.00	11.87	4.55
Snack R	23.46	30.00	12.27	7.14
Snack S	9.15	12.00	8.06	6.82
Snack T	17.37	22.00	2.65	2.82
Snack U	17.17	24.00	14.95	10.53
Snack V	7.76	10.00	13.17	6.11
Snack X	9.02	8.30	3.42	5.79
Snack Z	16.09	14.00	0.37	0.70
Mean	15.28	16.91	7.92	7.74
SEM[5]	6.39	7.38	3.84	3.76

[1]Moisture: moisture analyzed. [2]Moisture: moisture declared on label. [3]EE: ethereal extract analyzed. [4]EE: ethereal extract declared on label. [5]SEM: standard error of the mean.

Table 3 – Declared and observed levels of calcium and phosphorus, as percentage of dry matter in snacks for adult dogs, according to the snack code. Pirassununga, Brazil, 2020

Items	P[1]	P[2]	Ca[3]	Ca Min[4]	Ca Max[5]
Snack A	0.63	0.63	0.26	0.18	0.00
Snack B	4.03	1.85	7.51	1.85	5.56
Snack C	0.49	0.78	0.23	1.11	2.22
Snack D	0.58	2.47	0.56	2.47	7.41
Snack E	3.46	1.28	5.75	1.28	2.56
Snack F	0.41	0.00	0.74	0.58	1.34
Snack G	0.55	0.27	0.40	0.27	1.08
Snack H	0.95	0.57	3.13	0.21	2.86
Snack I	0.94	0.43	0.94	1.43	0.00
Snack J	0.45	1.10	0.33	1.83	3.66
Snack K	0.62	0.22	0.22	0.22	1.11
Snack L	0.45	0.11	0.58	0.23	2.84
Snack M	0.30	0.22	0.12	0.06	0.06
Snack N	0.36	1.67	0.78	0.44	1.22
Snack O	0.52	1.33	1.02	2.00	2.22
Snack P	0.49	0.11	0.53	0.22	1.67
Snack Q	0.58	0.11	0.79	0.23	2.84
Snack R	0.77	0.11	0.89	0.11	2.86
Snack S	0.26	0.25	0.24	0.15	0.16
Snack T	0.72	0.38	0.20	1.03	1.54
Snack U	0.26	0.39	0.73	0.66	1.32
Snack V	1.18	0.11	1.75	0.89	3.11
Snack X	0.33	0.29	0.06	0.09	0.00
Snack Z	0.84	0.00	0.88	0.00	11.63
Mean	0.61	0.84	1.19	2.47	0.73
SEM[6]	0.66	0.93	1.81	2.63	0.73

[1]P: phosphorus analyzed. [2]P: phosphorus declared on label. [3]Ca: calcium analyzed. [4]Ca Min: minimum calcium declared on label. [5]Ca Max: maximum calcium. [6]SEM: standard error of the mean.

excessive amounts of these products. Consequently, in such cases, animals supplied large quantities of snacks with high calcium content would ingest a high amount of calcium, which could result in risks to the health of these animals.

This excessive calcium consumption may be related to lower zinc absorption, due to the fact that these two minerals compete for the same absorption site, which can result in zinc deficiency (Scott et al., 1986). Furthermore, Schoenmakers et al. (1999) and Lauten et al. (2002) found that diets with high calcium levels were associated with the occurrence of growth-related osteoarticular disorders in large dog breeds.

Regarding phosphorus concentrations, we observed that 70.8% (n=17) of samples had values above than those declared on the respective labels and two snacks (B and E) presented levels above the maximum limit (European Pet Food Industry Federation, 2018) of 1.6% MS for complete and balanced foods, exceeding 152.0% and 116.0% respectively. These snacks, if provided in large quantities (10.0% above recommendations) could imply a risk to the health of these animals, in a way similar to high calcium intake. The high consumption of phosphorus may predispose animals to develop struvite uroliths, or even hamper phosphorus dissolution (Abdullahi et al., 1984; Osborne et al., 2009). Besides, high phosphorus consumption is associated with negative effects on renal function variables in healthy cats (Dobenecker et al., 2018) and is also associated with a higher risk of developing chronic kidney disease (CKD) (Böswald et al., 2018).

Due to these risks, it is worth emphasizing how important it is that accurate information stated on product labels regarding the concentrations of certain nutrients is in accordance with the results obtained in the laboratory, since most pet owners tend to supply large quantities of snacks frequently (Schuch, 2009).

It is also important to highlight that, in relation to the amount of ether extract, around 48.5% of the snacks declared a lower amount of fat than that found in the laboratory, which may result in weight gain, or difficulty losing weight, in the case of dogs in weight loss programs. Generally, the amount of snack provided is controlled according to its energy content, which is estimated from the guarantee levels stated on the labels by a veterinary nutritionist, and of which fat is of utmost importance, so that the maximum

daily amount does not exceed 10.0% of the animal's daily energy requirement (Freeman et al., 2011; Villa Verde & Abad, 2015).

Table 4 shows the metabolizable energy values, calculated from the ether extract declared on the label, and also from the ether extract analyzed by the Ankon method. Both calculations were performed using the most recent equation for estimating the metabolizable energy, published in the National Research Council (2006). We observed that some snacks had a 10% higher energy density, when the value of the analyzed ether extract was considered, compared to the calculation, in which the values of ether extract declared on the labels were considered. This may result in excess calorie consumption coming from the snack, even in situations where the amount of snack was calculated by a professional with knowledge of dogs' energy requirement, since the information stated on the label may underestimate the energy of the snack.

This higher consumption of snacks may result in dogs becoming overweight or obese, if an excessive intake of these foods are often given to the animal. Several published studies observed a strong correlation between snacking and the development of overweight and obesity in dogs, noting the importance of the number and frequency of supply of these products (Colliard et al., 2006; Courcier et al., 2010; Diez & Nguyen, 2006; Edney & Smith, 1986; Kienzle et al., 1998). In the study by Courcier et al. (2010), the relationship between socioeconomic and environmental factors with canine obesity were assessed and the authors concluded that the supply of commercial snacks is considered an important risk factor for canine obesity.

Table 4 – Metabolizable energy values estimated according to the National Research Council (2006), based ether extract values analyzed and declared on label, according to the snack code. Pirassununga, Brazil, 2020

Items	EM[1]	EM[2]
Snack A	2.80	2.87
Snack B	3.05	3.04
Snack C	3.29	2.99
Snack D	2.84	3.00
Snack E	2.88	2.88
Snack F	3.00	3.07
Snack G	2.52	2.47
Snack H	2.38	2.50
Snack I	2.78	2.64
Snack J	2.99	3.14
Snack K	3.56	3.41
Snack L	2.68	2.58
Snack M	3.66	3.52
Snack N	3.20	3.10
Snack O	3.30	3.46
Snack P	3.09	3.18
Snack Q	3.61	3.29
Snack R	2.59	2.33
Snack S	3.60	3.53
Snack T	2.47	2.46
Snack U	3.02	2.82
Snack V	3.37	3.07
Snack X	0.69	0.79
Snack Z	2.80	2.94
Mean	2.88	2.92
SEM[3]	0.37	0.39

[1]EM: Energy content analyzed (kcal/g). [2]EM: energy content declared on label (kcal/g). [3]SEM: standard error of the mean.

In veterinary medicine, obesity is the most common nutritional and metabolic disorder (German, 2006) and its frequency in dogs has been reported in several studies, varying from 20 to 40% in the populations studied (Crane, 1991; Edney & Smith, 1986; Gossellin et al., 2007; Hand et al., 1989; Lund et al., 2006; McGreevy et al., 2005). This condition is very worrying, since it can lead to numerous animal health risks such as increased incidence of endocrinopathies, locomotor disorders (ruptured ligaments and discopathy), impaired immune response, skin diseases (pyodermatitis and seborrhea), reproductive disorders and dyslipidemias (German, 2006).

Our study had the limitation of not describing the processing methods of the evaluated snacks, since the type of processing was not declared on the label. However, this study presents important information about the snack segment, which is still little studied, and also highlights the need for correct information dissemination about the composition of pet snacks.

Conclusion

Regarding the different methodologies for fat determination, since there was no difference of fat results, the selection of the methodology could be based on ease to perform or cost. Regarding label nutritional compliance, a considerable portion of commercial dog snacks showed nonconformity in relation to composition. This justifies the pet owner taking greater care of the quantity of snacks offered to his dog, especially if the animal already has predisposing risks to some illness. It is important to note that the influence of the processing types of each snack was not evaluated in this study and the suggestion that new work considering the processing could be considered.

Conflict of Interest

There is no conflict of interest among the authors.

Ethics Statement

This is a study to compare methodologies and evaluate the content of commercial products for dogs, but only with laboratory evaluations, without involvement of animals, so the study did not need approval from the ethics committee.

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Financial Support: None.