Assessment of energy content in dog foods

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SUMMARY

Animals can regulate food intake to meet their energy demands, so the nutritional composition of the diet should be balanced with its energy density to avoid over- or under-nutrition situations. The dog food market is registering significant growth, which is reflected in a broad portfolio of products with varied energy levels; however, true quantification of their energy value is unknown. Energy needs for dogs are commonly expressed as metabolizable energy, which is estimated with mathematical approaches (indirect estimation) or determined through digestibility and metabolism trials (direct estimation). This paper reviews the energy assessment of dog food, including common methodologies and experimental procedures.

Estimación del contenido energético en alimentos para perros: Revisión

RESUMEN

Los animales son capaces de regular la ingesta de alimento para satisfacer sus demandas energéticas, por lo tanto la composición nutricional de la dieta debe estar equilibrada con su densidad energética, para evitar situaciones de sobre o subnutrición. El mercado de alimentos para perros viene registrando un crecimiento significativo, el cual se refleja en un amplio portafolio de productos con diferentes valores energéticos; sin embargo, no se conoce la cantidad real de su contenido energético. Las necesidades de energía de los perros se expresan en unidades de energía metabolizable, la cual se estima a partir de aproximaciones matemáticas (estimación indirecta) o se determina mediante pruebas de digestiblidad y metabolismo (estimación directa). Este artículo revisa la estimación del contenido energético de los alimentos para perros, las metodologías para su estimación y los procedimientos experimentales disponibles para su cuantificación.

INTRODUCTION

Laboratory procedures allow fractioning food into its components, namely proteins, lipids, carbohydrates, minerals and vitamins. However, the assessment of energy requires a different approach (Pond *et al.*, 2005). The chemical energy contained in food is eventually transformed into heat, which can be measured (Case *et al.*, 2011). Animals obtain their energy by partial or complete oxidation of organic molecules absorbed from the diet and also from tissue catabolism. Energy transfer between chemical reactions occurs primarily through high-energy bonds in adenosine triphosphate (ATP) and related compounds (Pond *et al.*, 2005).

Determination of the energy content of foods is of great importance in animal nutrition considering that all metabolic processes involve energy transfer and expenditure. Energy is necessary to maintain and synthesize organic tissues and for physical activity and regulation of body temperature. Given its importance, it is not surprising that energy is usually the first requirement being satisfied by the diet. Regardless of the need that dogs have for essential amino acids or fatty acids, energy nutrients are firstly used to meet the demands of energy. Once this demand is satisfied, the remaining nutrients are used for other functions (Case *et al.*, 2011).

The increasing and widespread tendency to acquire dogs reflects the remarkable growth of the food market. From 1998 to 2010 the number of dogs in 50 countries increased 25% (Serisier *et al.*, 2013). This growth reflects a broad portfolio of available feed products,

which are segmented in the market by nutritional density and digestibility. According to the NRC (2006), the energy density of dog foods vary from 2800 to 4050 kcal metabolizable energy (ME)/kg depending on the processing, ingredients and additives. This paper aims to review the methodology to assess energy contents in dog food, as well as energy importance, fractioning, mathematical quantification, and available methods for energy determination.

Energy density

Nutritional value of food depends on its energy density, defined as the number of calories provided per unit weight. Energy density determines food consumption since the animal is able to regulate feed intake to meet its energy requirements, which depend on the breed, weight, age, sex, sexual condition (neutered, whole), housing characteristics and physical activity (Sallander *et al.*, 2010; Bermingham *et al.*, 2014). If energy density is too low, food consumption will be inhibited because of physical limitations of the gastrointestinal tract, which could lead to energy deficiency. On the other hand, a large number of very palatable products with high energy density are available in the pet market, which defies the ability of dogs to regulate their energy intake. This circumstance along with lack of physical activity is causing overweight and obesity in dogs (German, 2006; Sallander *et al.*, 2010) which has an average prevalence from 24 to 59% worldwide (Hodgkinson *et al.*, 2008; Larsson *et al.*, 2014). Additionally, owners tend to buy dog foods that are quickly consumed by the animal, ignoring that those foods are usually rich in energy. Ultimately, foods with high or low energy density can cause an energy imbalance resulting in impaired growth rate, weight, and body composition (Case *et al.*, 2011).

Considering that feed intake is controlled by the total energy intake, the contents of other nutrients should be balanced with respect to energy density. That is, energy density determines the proportions in which other nutrients (such as amino acids, carbohydrates, fatty acids, minerals and vitamins) must be present to meet the requirements. Therefore, it is more appropriate to express levels of energy nutrients in terms of energy concentration rather than as a percentage of weight in dry food. This would allow making comparisons between different types of foods regardless of water, nutrient or energy content (Case *et al.*, 2011).

A proper assessment of the energy content in pet food allows food companies to determine more accurately the proportions of ingredients in the formulation and the percentage of nutrients that matches the level of activity and health of the animal. Additionally, owners can be better informed of the amount of food to offer depending on the type of product. Ignoring the energy density of food can lead to under or overestimation of the ration. This was confirmed in the study by Hodgkinson *et al.* (2008). They found that depending on the size of the dogs, up to 80% of the brands recommended quantities of dogfood that would not supply the correct amount of ME according to the requirement, resulting in animals with over or underweight. A proper knowledge of energy content would give food

products an added value and help owners to select the proper food in a market where costs are high and the offer of domestic and international products is rapidly increasing.

Energy fractions

Energy contents are usually expressed in terms of gross, digestible, metabolizable or net energy.

Gross energy (GE). It is the maximum amount of energy that is potentially available to the animal. The GE concentration depends on the proportion of carbohydrates, fats and proteins. GE can be determined directly by subjecting the feed sample to combustion into a calorimeter. It can also be determined indirectly knowing the feed composition and the energy density of the nutrients -values that vary depending on the amount of carbon, hydrogen, and oxygen in the molecule (NRC, 2006). The heat of combustion for nonstarch polysaccharides (pectin, cellulose gum, galacto-oligosaccharides and inulin) and starch is close to 4.0 kcal/g. GE values of egg protein (albumin), milk protein (casein, lacto-albumin), connective tissue, gluten and soy are near 5.73 kcal/g. The heat of combustion for tallow, fish oil and sunflower oil ranges from 9.39 to 9.46 kcal/g. Refined palm oil has a lower heat of combustion (9.08 kcal/g) due to its content of shorter chain fatty acids (Kienzle, 2002).

Digestible energy (DE). Animals are unable to use all of the GE present in food. Digestible energy (DE) density is calculated by deducting fecal energy losses from GE. This fraction corresponds to the energy absorbed through the gut (Case *et al.*, 2011). According to Malca *et al.* (2006), pet food digestibility should be equal to or greater than 80%, and values below 75% are not recommended. Castrillo *et al.* (2005) reported that *average content of GE was 5.2 Mcal/kg in extruded dog food* (ranging from 4.7 to 5.7 Mcal/kg) and 84.9% GE digestibility (ranging from 68.76 to 91.05%). Accordingly, the DE content was 4.4 Mcal/kg, ranging from 3.3 to 5.2 Mcal/kg. Regarding energy calculations, direct and indirect methodologies have been proposed to estimate DE in dog food.

Direct estimation. It involves quantifying nutrients consumed and excreted via feces. Fecal output is measured through a direct method referred as Total Collection (TC) of feces. The TC is the standard or reference method to assess nutrient digestibility. It involves confining the animal into a metabolic cage (Dobenecker *et al.*, 2010), which allows collecting feces separate from urine, preventing coprophagy and having greater control of environmental factors (Sabchuk *et al.*, 2012). This method involves a period of adaptation -to both the diet and the cage- which fluctuates from three to seven days, followed by a period of fecal collection lasting four to six days (Adeola, 2001). Nott *et al.* (1994) suggested that short-term assays (three days of adaptation and four days of collection) do not compromise accuracy. Hervera *et al.* (2008) proposed a 10-day adaptation period followed by seven days of collection. However, protocols by AAFCO (2016) and FEDIAF (2014) recommended five days of adaptation followed by five days of collection.

Table I. NRC (2006) equations to predict gross energy (GE), digestible energy (DE) and metabolizable energy (ME) in dog food (Ecuaciones del NRC (2006) para predecir energía bruta (EB), energía digestible (ED) y energía metabolizable (EM) en alimentos para perros).

Step 1. Determination of GE by calorimetry or using the following equation: GE_{pred} (kcal/kg) = $(5.7 \cdot CP) + (9.4 \cdot fat) + (4.1 \cdot (NFE + CF))$
Step 2. Estimation of GE digestibility: %GE dig_{pred} (kcal/kg) = $91.2 - (1.43 \cdot \%CF$ in DM)
Step 3. DE content: DE_{pred} (kcal/kg) = $GE_{pred} \cdot \%GE\ dig_{pred}/100$
Step 4. Prediction of energy losses in the urine (E_u): $E_u = 1.04 \cdot g\ CP$ $E_u = 1,25 \cdot g\ DP$
Step 5. Prediction of ME: ME_{pred} (kcal/kg) = $DE_{pred} - E_u$
NFE: nitrogen free extract, CF: crude fiber, CP: crude protein, DP: digestible protein

Identifying the stools corresponding to the food consumed within the evaluation period is a technical problem in TC trials. This is solved by adding a marker to the diet to visually determine when to start and stop collecting feces. A marker is a non-absorbable substance that stains the stool and is added to a meal at the beginning and at the end of the collection period. Collection begins with the appearance of the first colored stools. Marked feces are the first feces collected, which are saved for later processing and laboratory analysis along with the following non-colored feces produced in the next days (in the absence of the marker, feces return to its usual color). The collection period ends by adding the marker again to a meal. Collection stops when colored feces start to appear; so, in this occasion marked feces are not collected. Some dyes, such as indigo carmine and red carmine, are commonly used as markers, at levels ranging from 0.25 to 0.5% of the diet (Sands *et al.*, 2001; Lindemann *et al.*, 2010; Stein *et al.*, 2011). The apparent digestibility by TC is calculated with the following equation: Digestibility = [(amount of nutrient consumed - amount of nutrient in the feces)/amount of nutrient consumed] x 100 (Lima *et al.*, 2014).

According to Kawauchi *et al.* (2011), direct estimation of digestibility and energy content can also be calculated for specific dietary ingredients (ing) with difference and regression methods, widely used in pig and poultry studies. Digestibility assessment of an ingredient by the difference method involves feeding a reference diet (rd) without the ingredient of interest (test ingredient), and also a test diet (td) with the ingredient included. Separate digestibility tests are performed with both diets and then the following equation is used: $ADC_{ing} = ADC_{rd} + [ADC_{td} - ADC_{rd}] / [$Inclusion level of the ingredient in the td (g/kg)/100], where ADC corresponds to the coefficient of apparent digestibility. On the other hand, the regression method consist on feeding a basal diet without the test ingredient and also other diets with increasing levels of the test ingredient. The ADC of the diets is adjusted to a linear regression model where ADC_{ing} is estimated extrapolating to 100% inclusion of the test ingredient.

Index method. The Index or Indicator Method (IM) is an alternative method that does not require total

collection of feces or to keep the animals in metabolic cages (Schneider and Flatt, 1975). Some researchers refer to the indicator as an indirect method when they want to compare it to the TC (Schneider and Flatt, 1975; Ly *et al.*, 2002; Osorio *et al.*, 2012). Fecal samples can be collected from dogs kept in regular kennels. It involves administering an inert substance named *external indicator* in the diet and later collecting a representative sample of feces. A suitable indicator should meet the following characteristics: be inert, non-toxic, nondigestible, fully recovered in the feces, easily mixed in the food, and easy to be chemically analyzed (Adeola, 2001). Once the concentration of the indicator and the nutrient in food and feces is known, apparent digestibility can be calculated using the following equation: Digestibility = 100- (100 · (% indicator in food/% indicator in feces) · (% nutrient in feces/% nutrient in food)).

Chromium sesquioxide (Cr_2O_3) is the most commonly used external indicator (Jang, 2014), at levels ranging from 0.2 to 0.3% of the diet (Gajda *et al.*, 2005; Faber *et al.*, 2011). Other indicators, such as acid-insoluble ash, indigestible dry matter, indigestible neutral detergent fiber, indigestible acid detergent fiber and acid-detergent lignin are natural components of food, so they are regarded as internal indicators (Sales *et al.*, 2004; Pinto *et al.*, 2013).

As mentioned, TC of feces is not required for the IM. This method relies on a technique known as *grab sampling* in which fecal samples are directly taken from the rectum or from recent stools. However, IM does not have a uniform methodology for fecal sampling or agreement upon the minimum number of samples or collection days required for representative sampling. Agudelo *et al.* (2010) reported that a composite fecal sample of several days is required to achieve representativeness for less digestible nutrients, while a single sample taken when chromium excretion has stabilized could be enough for more digestible components such as dry matter (DM) and energy. Jang *et al.* (2014) reported that apparent digestibility and fecal chromium concentration in pigs stabilized five days after a steady supply of diets containing this indicator. They also found that a composited sample of at least two days is required to achieve greater precision and less variation

Table II. Predicted digestible and metabolizable energy (DE and ME, respectively) for dogs by the NRC (2006) equation compared with other mathematical approaches (Predicción de la energía digestible y metabolizable (DE y ME, respectivamente) para perros mediante la ecuación del NRC (2006) comparado con otras aproximaciones matemáticas).

Type of energy		Type of food (market segment)						
		Super premium		Premium		Economical		Cheap
		Puppies	Adults	Puppies	Adults	Puppies	Adults	Adults
		(n=17)	(n=34)	(n=12)	(n=17)	(n=8)	(n=18)	(n=14)
DE	Eq. 1	4621±259[a]	4433±295[a]	4242±168[a]	4083±296[a]	4106±119[a]	3928±255[a]	3710±221[a]
	Eq. 2	4594±287[b]	4382±314[b]	4152±175[b]	3979±315[b]	4024±128[b]	3811±288[b]	3573±242[b]
	Eq. 3	4501±375[b]	4237±512[b]	4011±274[b]	3803±519[b]	3813±193[b]	3618±362[b]	3267±401[b]
	Eq. 4	4380±375[b]	4115±554[b]	4016±299[b]	3747±544[b]	3726±209[b]	3517±355[b]	3161±421[b]
	Eq. 5	3999±213[b]	3895±220[b]	3652±134[b]	3582±238[b]	3558±81[b]	3418±210[b]	3298±197[b]
ME	Eq. 6	4278±236[a]	4135±277[a]	3936±164[a]	3818±292[a]	3799±105[a]	3686±233[a]	3474±225[a]
	Eq. 7	4512±221[b]	4399±232[b]	4155±142[b]	4072±253[b]	4048±88[b]	3958±223[b]	3764±208[b]
	Eq. 8	4065±217[b]	3952±224[b]	3711±134[b]	3633±238[b]	3617±83[b]	3527±213[b]	3344±195[b]

[a,b]lowercase letters in the same column indicate significant difference between means obtained by NRC (2006) (Eq. 1 and Eq. 6 for DE and ME, respectively) and the other equations (p<0.05).
Eq. 1: DE (kcal/kg DM) = GE * ((91.2 - 1.43 · CF) /100) (NRC, 2006) and GE (kcal/kg DM) = (5.7 · CP) + (9.4 · Fat) + (4.1 · (NFE+ CF)).
Eq. 2: DE (kcal/kg DM) = 5.11 · CP + 8.94 · Fat + 3.49 · NFE - 2.87 · CF (Kienzle *et al.*, 1998a).
Eq. 3: DE (kcal/kg DM) = (3.58 + 7.21 · Fat - 15.45 · CF) · 1000 (Castrillo *et al.*, 2001).
Eq. 4: DE (kcal/kg DM) = GE * ((94.0 - 4.04 · CF) /100) (Castrillo *et al.*, 2001).
Eq. 5: DE (kcal/kg DM) = (3.3 · CP) + (8.5 · fat) + (3.5 · NFE) (NRC, 1985).
Eq. 6: ME (kcal/kg DM) = GE · ((91.2 - 1.43 · CF) /100) - (1.04 · CP) (NRC, 2006).
Eq. 7: ME (kcal/kg DM) = (4 · CP) + (9 · fat) + (4 · NFE) (Atwater, 1902).
Eq. 8: ME (kcal/kg DM) = (3.5 · CP) + (8.5 · fat) + (3.5 · NFE) (Atwater, 1910).

compared with a single grab sample randomly taken. Mroz e*t al*. (1996) found that digestibility by the IM was lower compared with TC, which is explained by the incomplete recovery of the indicator in the feces.

The type of accommodation (kennel) is one of the advantages of the IM. Kennels provide more space for animals than metabolic cages, and the sense of wellbeing allows them to express their natural behavior (Spangenberg, 2007). However, greater care is required to ensure that the indicator is not recycled by coprophagy and to avoid contamination of feces with environmental factors such as rain and dust (Sabchuk *et al.*, 2012).

Indirect estimation. It is based on the chemical composition of the diet, particularly on its fiber content. Although dogs belong to the order Carnivora, they are omnivorous regarding their eating habits and digestive capacity, so they should consume dietary fiber. The pet food industry is very interested in dietary fiber because of the beneficial effects of fermentable and soluble fibers on health. These effects include increased viscosity of digesta, delayed gastric emptying, longer sensation of satiety, reduced glucose uptake, lower blood cholesterol, and enhanced commensal bacteria in the gut (Faber *et al.*, 2011; Godoy *et al.*, 2013). Conversely, non-fermentable fibers increase digesta flow, dilute the energy density of the diet, and increase fecal bulk and moisture as well as laxation (Silvio *et al.*, 2000; Case *et al.*, 2011). Beet pulp and cellulose are commonly used as a source of dietary fiber in pet foods. Beet pulp contains soluble fiber and insoluble components in a desirable ratio, while cellulose is mostly regarded as insoluble and poorly fermentable (Godoy *et al.*, 2013).

Kienzle *et al*. (1998a) used a database including 128 digestibility studies to propose the following regression model, which includes crude protein (CP), fat, nitrogen free extract (NFE) and crude fiber (CF) contents as independent variables for estimating DE: DE (kcal/kg) = 5.11 · CP + 8.94 · fat + 3.49 · NFE - 2.87 · CF (chemical composition expressed in g/kg). They found a negative linear relationship between CF and DE. Similarly, Castrillo *et al*. (2001) used fat and CF contents of 38 dog foods to develop the following equation: DE (Mcal/kg DM) = 3.58 + 7.21 · Fat - 15.45 · CF (chemical composition expressed in kg/kg DM) which explains 93.1% of the variation in the data evaluated. That study concluded that CF content might be a good predictor of DE.

Kienzle *et al*. (1998b) and Castrillo *et al*. (2001) proposed equations to estimate apparent digestibility of energy based on the FC content of food: %GE digestibility = 91.2 - (1.43 · %CF) (CF expressed on a DM basis) (Kienzle *et al*. (1998b), %GE digestibility = 94.0 - (4.04 · %CF) (CF expressed on a DM basis) (Castrillo *et al*., 2001). According to Hervera *et al*. (2007), CF is not a good predictor of the actual fiber content of a food, therefore, this mathematical approach can overestimate energy density in fiber-rich foods. Van Soest (1973) indicated that CF amounts for only 0 to 80% of cellulose, 10 to 50% of lignin and 20% of hemicellulose. Bartges and Anderson (1997) also indicated that CF quantifies only from 5 to 20% of the total fiber. Kienzle *et al*. (2006) found a more accurate equation based on total dietary fiber content (TDF) compared with the equation based on CF content (r = 0.94 and 0.87, respectively). The method to determine CF is well known, easy and inexpensive, but it underestimates fermentable fiber

content (Bartges and Anderson, 1997). The method by Prosky *et al.* (1985) to quantify TDF is more complex and expensive, but it does a better estimation of the actual content of fermentable and non-fermentable fiber. Finally, there is also the NRC (1985) approach, which uses the modified Atwater factors, so that ED (kcal/kg) = CP · 3.3 + fat · 8.5 + NFE · 3.5), where chemical composition is expressed in g/kg. Notably, the use of the above methods is not suitable for identifying factors related to fiber digestibility, processing (Castrillo *et al.*, 2009) and the effects of enzyme additives on energy digestibility for dogs (Case *et al.*, 2011). Commercially, the fiber content of foods is highly variable, ranging from 0.61 to 9.40% (Hervera *et al.*, 2007) and it is the nutrient that most reduces digestibility and energy content of food. However, the effect of fiber on digestibility depends on the fiber source used, as indicated Godoy *et al.* (2013), who reported that corn fiber is an efficacious fiber source for pets, showing no detrimental effects on nutrient digestibility or palatability.

The DE content of foods can also be predicted by near-infrared spectroscopy (NIRS) (Castrillo *et al.*, 2005) and *in vitro* digestion techniques (Hervera *et al.*, 2007). The NIRS allows rapid nutritional assessment of foods, as long as there are sufficient *in vivo* data to conduct a robust calibration process. Castrillo *et al.* (2005) used NIRS to predict the DE content of commercial extruded dog foods and obtained determination coefficient and standard error (both from cross-validation) of 0.93 and 0.11 Mcal/kg DM, respectively.

The *in vitro* method is intended to simulate the digestive process in the stomach and small intestine through two stages of multienzyme incubation. The first stage lasts two hours with pepsin (10 mg/g food) in acidic pH, and the second lasts four hours with pancreatin (100 mg/g feed). After filtration, the indigestible residue is incinerated to obtain organic matter digestibility. Following this procedure, Hervera *et al.* (2007) predicted *in vivo* apparent digestibility of organic matter (OMd) and energy (Ed), and DE content in 54 commercial extruded dog foods (*in vivo* values previously obtained by digestibility trials). They observed a linear relationship between *in vitro* and *in vivo* OMd (R^2= 0.92) and between *in vitro* OMd and *in vivo* Ed (R^2= 0.92). Prediction accuracy of DE content by the *in vitro* method (R^2= 0.97) was higher than that by the NRC (1985) equation (R^2= 0.87) and slightly higher than that proposed by NRC (2006) (R^2= 0.95). According to these results, the *in vitro* method provides an accurate prediction of DE and can be used as a simple and reproducible alternative to avoid the use of experimental animals in digestion trials.

Metabolizable energy (ME). The next stage of energy fractioning involves the so-called metabolicity. Energy losses occur as a result of gas production and urea excretion. As gas production in dogs and cats is minimal (Castrillo *et al.*, 2009; Wichert *et al.*, 2014) only urinary losses are taken into account to determine metabolizable energy (ME). The ME is commonly used to express the energy density of foods for dogs. Hodgkinson *et al.* (2008) determined the ME content of 15 commercial brands of dry food for growing and adult dogs. The ME in each case varied form 3507 to 4584 kcal/kg (mean: 4022 kcal/kg), and from 3178 to 4405 kcal/kg (mean: 3871 kcal/kg), respectively. These values correspond to 79.3% metabolicity (ME/GE) in both cases. Similarly to DE, the ME can be determined directly or indirectly.

Direct estimation. It requires total urine collection and determination of energy losses in the form of urinary nitrogen (Adeola, 2001). Urine collection usually begins some hours after the feces collection period has started and it is completed after the end of that period (Agudelo *et al.*, 2007). Urine is collected in a container located underneath the metabolic cage. To stop microbial growth and nitrogen loss (ammonia volatilization) the container is usually added with an inorganic acid (ej: sulphuric acid) (Kawauchi *et al.*, 2011). Urine volume produced is measured daily and then an aliquot is stored frozen for later analysis. Finally, ME consumption is determined by the difference between DE consumption and energy losses through urine. Metabolicity studies are not generally conducted in pens or kennels due to the difficulties to collect the urine.

Indirect estimation. ME assessment can be costly and time consuming. Therefore, Atwater (1902 and 1910) proposed factors for estimating ME from the proximal analysis of foods. The first Atwater factors (Atwater, 1902) were 4, 9 and 4 kcal/g to estimate ME from CP, fat, and NFE, respectively. This equation does not take fiber content into consideration. Atwater (1902) factors established digestibility of proteins, fats and carbohydrates as 90, 96, and 98%, respectively (NRC, 2006). These factors assume that metabolic losses of protein were constant (1.04 kcal/g CP). Highly digestible ingredients such as meat, offal, chicken, fish, and highly purified starchy and dairy products were used to calculate those factors. Digestibility of nutrients in dry pet foods is usually lower than 90% (Castrillo *et al.*, 2005) with some exceptions for fat (Dobenecker *et al.*, 2010). Consequently, the Atwater (1902) factors overestimate ME. Kienzle (2002) used a dog food database (n= 124) to compare predicted ME (kcal/g) by Atwater's equation (1902) with results determined experimentally. Results showed that predicted ME overestimated foods containing less than 4 kcal/g, which represents the largest amount of pet foods commercially available (NRC, 2006).

Some years after his first equation, Atwater (1910) proposed the so called *modified Atwater factors* (3.5, 8.5 and 3.5 kcal/g for CP, fat and NFE, respectively) which provide a better estimate of ME compared to Atwater (1902) factors. The modified Atwater factors consider lower digestibilities: 80% for protein, 90% for fats and 85% for the NFE. This method assumes that CF does not generate energy. Although modified Atwater factors are accepted by AAFCO (2016) and FEDIAF (2014), they underestimate ME of low-fiber, highly-digestible foods, while overestimate ME of foods high in CF or very low digestible protein, fat and carbohydrates (Hand *et al.*, 2000; Castrillo *et al.*, 2009). Kienzle (2002) used a database of foods for cats (n= 83) to associate predicted ME (kcal/g) using the modified Atwater factors (1910) with ME determined experimentally. Results were similar to those obtained with dog foods: predicted ME underestimates foods having more than

3.7 Kcal/g and overestimates those with less than 3.7 kcal/g. Irrefutably, an equation that assumes fixed digestibility for nutrients cannot accurately cover the full range of products on the market since it ignores any differences between dietary ingredients and processing methods (Hervera *et al.*, 2007). Not surprisingly, prediction is inaccurate for products with low or high digestibility, such as diets for weight reduction or nursing animals, respectively (Kienzle *et al.*, 1998b).

Regarding the Atwater factors, several human nutrition researchers have questioned the potential contribution of dietary fiber to energy, which is not considered in the equation. Dietary fiber is a generic term that includes substrates with unique traits such as chemical structure, physical properties and physiological effects (Kritchevsky, 1988). This complex group comprises: a) non-starch polysaccharides (cellulose, hemicellulose, pectin, gums and mucilages), b) functional fiber: resistant starch, fructans, fructooligosaccharides and lactulose (Hand et al., 2000). The microbial population in the gastrointestinal tract can ferment most fiber compounds generating short-chain fatty acids that can be absorbed (Hand *et al.*, 2000; Godoy *et al.*, 2013). Cummings (1983) and Miles (1992) suggested that non-starch polysaccharides present in human diets could represent 3 kcal/g, turning it into a significant source of energy for people consuming high fiber diets.

Methodologies that estimate DE are incomplete, considering that energy requirements of dogs are commonly expressed as ME. In this regard, the NRC (2006) suggests that a correction factor for energy losses in urine can be used to predict ME. This correction factor considers urinary losses as 1.04 kcal/g CP or 1.25 kcal/g of digestible protein (DP), assuming 83.5% digestibility (NRC, 1985). The equations proposed by the NRC (2006) to estimate GE, DE and ME are shown in **table I**.

Net energy (NE). The ME comprises net energy (NE) and dietary thermogenesis (energy needed for digestion and absorption of nutrients). Part of NE is used to support functions associated with body maintenance (net energy for maintenance, NE_m), resulting in additional heat production. Retained net energy (NE_r) is obtained by subtracting total heat production from ME. The NE_r accounts for the energy ultimately used to perform physical work, growth, pregnancy and lactation (Case *et al.*, 2011). Heat loss has to be measured to calculate the NE of food. Heat production can be measured directly using an animal calorimeter or indirectly from the exchange of oxygen and carbon dioxide (Hand *et al.*, 2000; Larsson *et al.*, 2014). However, this way of expressing energy content is not commonly used in pet food (Castrillo *et al.*, 2009).

COMPARISON OF ENERGY PREDICTION EQUATIONS IN DOG FOODS

Prediction equations described in this review were applied to a database of 120 commercial foods for dogs **(table II)**. The foods were classified according to market segments (super premium, premium, economic and cheap) and targeted animals (puppies and adults). The DE and ME predicted by the NRC (2006) were used as reference values based on the findings by Hervera

et al. (2008) and contrasted with the remaining equations through a paired t-test ($\alpha= 5\%$). Hervera *et al.* (2008) compared the potential of NRC (2006), *in vitro* digestion method, NIRS technology, and NRC (1985) to predict DE of commercial dog foods obtained in vivo (4.62 Mcal/kg DM). The authors demonstrated that the first three methods presented better accuracy ($R^2=$ from 0.93 to 0.99) than that proposed by the NRC (1985) ($R^2= 0.90$).

The DE predicted by NRC (2006) was higher than that obtained with the other approaches. Furthermore, the estimated ME by NRC (2006) was intermediate to that obtained with the Atwater (1902 and 1910) factors. Results show that the chemical composition of the diet is not sufficient to predict its DE content, even using the same independent variables, as with eq. 1 and 4, which use only CF to estimate GE digestibility. In the case of Atwater factors, constant digestibility values for nutrients are assumed regardless of the composition of ingredients used for the formulation and thermal treatments of the food, which in turn determine their market segment. Not all equations include fiber content to estimate energy density of the food (Atwater factors) and when they do, their magnitude is variable. Knowing that fiber is the nutrient that most negatively impacts DE and –therefore- ME, it is necessary to assess the effect of different sources and fiber levels by performing in vivo digestibility and metabolicity tests to validate the predictive value of the existing equations.

CONCLUSIONS

The nutritional value of food depends on its energy density, which in turn determines the amount of food and nutrients consumed by the animal, and therefore its weight and body composition. The ME is the most common way of expressing energy density in dog food. To date, ME calculation has not considered the contribution that dietary fiber makes to energy metabolism. Research efforts should address the effects of different fiber sources on ME, since fiber has a great impact on digestibility and metabolicity. Different fiber sources (soluble and insoluble fibers) may contribute differently to ME as their digestibilities vary according with their chemical composition. Furthermore, chemical assessment of fiber in dog foods should be comprehensive and rigorous, going beyond traditional analysis (crude fiber). Finally, more digestion and metabolism studies are required to establish accurate equations that match specific fiber contents in current dog foods and validate the ones in use.

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