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Invited Review

Use of dual-energy x-ray absorptiometry in non-ruminant nutrition research

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ABSTRACT - Precise body composition measurements are essential in animal nutrition studies because the impact of treatments is evaluated based on changes in body weight and composition. Various indirect techniques for animal compositional evaluation have been developed and evaluated for applicability in animal nutrition studies. A fast, accurate, minimally invasive method that requires little input is considered the ideal for providing information about the animal. Measurements obtained by dual-energy x-ray absorptiometry (DXA) are highly correlated with those obtained by chemical analysis and dissection. The algorithms of DXA software partition the six chemical components of the body (lipids, water, proteins, carbohydrates, non-bone mineral, and bone mineral) into three compartments (total body mineral content, fat mass, and lean mass). Questions have been raised about how this partitioning affects the precision of the DXA method. In addition, the relationship between the DXA measurements and dissected carcass tissues is nonrepresentational of the relationship between DXA and chemical analysis. Furthermore, since DXA devices and their software were developed primarily for human medicine, they may not be fully adequate for animal evaluation. Calibration is required to obtain true values. The DXA method has some advantages and disadvantages that should be identified and controlled before calibration. Nonetheless, DXA is a valuable tool that provides precise, repeatable body composition measurements of live monogastric animals and their carcasses.

Key Words: alternative method, birds, body composition, DXA, pig

Introduction

Animals of all species can vary considerably in terms of weight and body composition depending on their growth stage, nutritional background, genetic potential, and other factors. Precise body composition measurements of experimental animals are essential in nutrition studies because the effect of treatments is evaluated based on changes in body weight and composition (Mitchell et al., 1997a; Pomar et al., 2001; Ryan et al., 2011). Nutrition research traditionally uses slaughter techniques to measure body composition, but this destructive method is expensive and precludes the possibility of taking repeated measurements of the same animal. Methods based on indirect measurements allow repeated measurements of the same experimental animal, thus increasing accuracy and the ability to understand the growth and physiological processes involved in animal responses (Suster et al., 2006; Ryan et al., 2011). Indirect methods are preferred for determining animal nutritional requirements or for studying the effect of nutrition on animal growth. However, indirect methods require calibration to accurately estimate body composition (Szabo et al., 1999; Mitchell et al., 2003; Mercier et al., 2006).

Various indirect techniques for animal compositional evaluation have been developed and evaluated for applicability in animal nutrition studies. A fast, accurate, minimally invasive method that requires little input is considered the ideal for providing information about the animal (Mitchell et al., 2003). Methods currently used in nutrition research include linear body measurements (Maiwashe et al., 2002; Riva et al., 2004), ultrasonography for measuring backfat, muscle depth, and loin eye area (Liu and Stouffer, 1995; Oviedo-Rondón et al., 2007; Case et al., 2012), total body electrical conductivity (Berg et al., 1994; Dänicke et al., 2001; Fortun-Lamothe et al., 2002), video image analysis (McClure et al., 2003; Mollah et al., 2010; Craigie et al., 2012), dual-energy x-ray absorptiometry (DXA; Mitchell et al., 1997a; Marcoux et al., 2003;

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Mercier et al., 2006), computed tomography (CT; Font-i-Furnols et al., 2013; Milisits et al., 2013), and magnetic resonance imaging (MRI; Davenel et al., 2000; Kremer et al., 2012b). The advantages and disadvantages of these methods are inherent to the technology and the principles used. For example, linear measurements are taken at specific locations on the body (body length, circumference, etc.). Many of these measurements are obviously related to total body weight or carcass wholesale cut weight, but they are of limited value for determining compositional patterns (Pomar et al., 2001; Pomar et al., 2009). Ultrasonic measurements of backfat and loin depth are taken on the assumption that these depths are closely related to body (or carcass) lean and fat masses (McLaren et al., 1991). These ultrasonic measurements have to be taken at specific body locations by experienced operators (McLaren et al., 1991; Oviedo-Rondón et al., 2007; Case et al., 2012). Linear body and ultrasonic measurements can, however, lose their accuracy when applied to animals of different weights, shapes, genetic backgrounds, etc. (Houghton and Turlington, 1992; Hassen et al., 1999). These accuracy losses are especially important when changes occur at body locations not being evaluated by these methods. In addition, ultrasonography has the disadvantage of not generating a complete body image due to insufficient penetration power (Liu and Stouffer, 1995). Other indirect methods, such as total body electrical conductivity, are not addressed in this paper because they may not be precise, since they are affected by the measuring environment and other limiting factors (Berg et al., 1994).

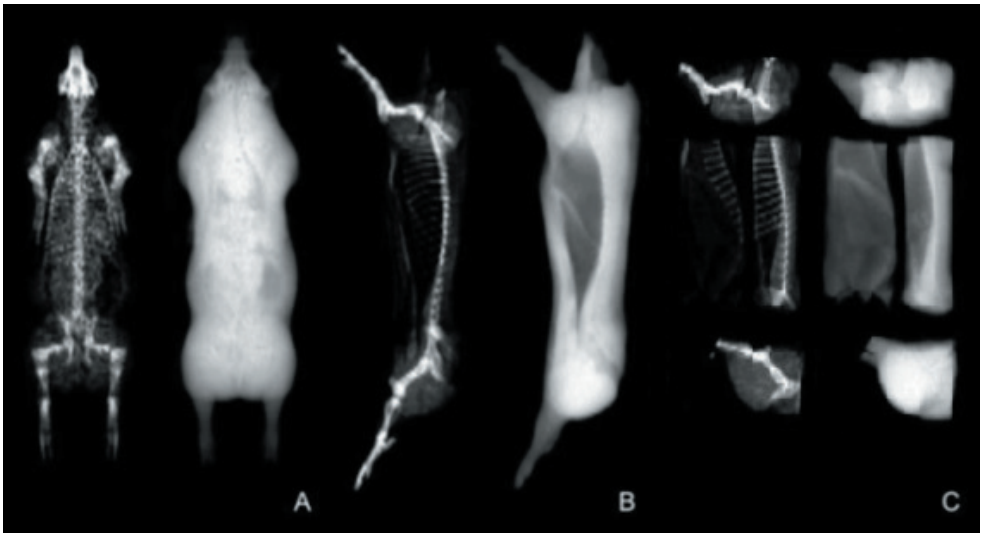
The DXA devices are more flexible than previous methods for estimating body composition in animals from different genetic lines (Mitchell et al., 1997b; Marcoux et al., 2005) or from different sexual categories because DXA evaluates the whole animal, not just parts of the body. Specific calibrations are, however, required for the evaluation of each animal type (i.e., pigs, sows, hens, poultry, etc.) or body parts (i.e., whole animal body, carcass, etc.). Other methods such as CT and MRI can also be used for whole-animal evaluation (Font i Furnols and Gispert, 2009; Barchia et al., 2010; Clarys et al., 2010; Kovner et al., 2010), but these methods are slow, expensive, and complex. However, the latter two technologies can be used to create methodologies for evaluating parts of the body to obtain an estimate for the whole animal (Kremer et al., 2012a; Kremer et al., 2012b). The purpose of this paper was to describe the use of DXA in non-ruminant nutrition research, highlighting some differences between DXA and the reference methods and identifying the advantages and disadvantages of this technology.

DXA as an alternative to *in vivo* body composition measurements

The value of DXA as a tool for evaluating the body chemical composition of live animals or the tissue composition of dissected carcasses has increased in recent years (Marcoux et al., 2005) because of its low instrumental and operating cost, high resolution, low radiation exposure, and rapid scan speed. Because this technique is non-invasive, live animals can be scanned at different production stages, thus improving research efficiency and reducing the number of animals required.

The DXA scanners were developed primarily for humans but are also used in animal studies. Several types of DXA devices are available, but they all operate in the same manner. An x-ray radiation source is aimed at a radiation detector placed directly opposite the site to be measured. The subject is placed on the table in the path of the radiation beam. The computer software records and analyzes the collected data (Blake and Fogelman, 1997). As the radiation source and detectors move over the subject, the DXA scanner generates a projected two-dimensional image, similar to the traditional x-ray (Figure 1). This two-dimensional image is made up of hundreds of pixels, which are the smallest units of an image (Tothill, 1995). The pixel size depends on the software configuration set prior to the scan.

After the scan, individual pixels are classified into two distinct types: soft-tissue pixels in body regions without bone and pixels with bone (Mazess et al., 1989). The DXA technology differs from other x-ray technologies in that it involves the emission of x-ray at two energy levels (Pietrobelli et al., 1996). The energy of the emitted photons is known and the energy of the received photons is measured to estimate the energy lost (Goodsitt, 1992). Energy losses occur when a photon is blocked by a substance and interacts with the atomic orbital electrons of the substance (Kim, 2010). However, one of the most important data collected by DXA is the attenuation coefficient of the x-ray (R-value, meaning the ratio of attenuation between the high- and low-intensity x-ray). The DXA method uses this R-value to determine the fat content in soft-tissue pixels, while the lean content in those pixels is estimated as the difference between the total soft-tissue mass and estimated fat mass in the pixel. In bone pixels, the R-value is used to estimate bone and soft-tissue masses, while the composition of the soft-tissue mass in the bone pixels is estimated by attributing the same soft-tissue composition to these masses as in the surrounding soft-tissue pixels (Goodsitt, 1992; Pietrobelli et al., 1996). Based on all bone pixels, DXA estimates the



A: Image of a 90-kg live pig scanned in prone position. B: Image of a pig half-carcass. C: Image of pig primal cuts.

Figure 1 - Images obtained by DXA.

bone mineral content (BMC) and bone mineral density, the latter of which is estimated as the ratio between BMC and the total area of bone pixels. Total DXA fat and lean masses are estimated by adding the masses of these tissues calculated in bone and non-bone soft tissues.

Measurements provided by DXA are highly correlated with total ash, protein, and lipid chemical composition, as well as with dissected adipose and muscle tissues. However, DXA tissue compositional data may not be the same across DXA devices or correspond to the chemical or dissected composition of the animal body or carcass. In fact, DXA tissue estimates normally have to be calibrated using acrylic materials rather than human or animal tissues (Scholz et al., 2007). Consequently, dissected bone does not correlate well with DXA BMC or DXA lean mass, since bones contain significant amounts of fat, protein, and water (Nielsen, 1973). Nonetheless, linear regression can be used to convert DXA measurements to body or carcass compositional measurements, but these regressions are specific to each DXA device, animal type, and body part (Mitchell et al., 1997b). It is important to be aware of differences between the principles of the methodologies when using one procedure to calibrate another (Marcoux et al., 2003). Most of the lack of agreement or adjustment between two methods can be attributed to what the results actually mean. In the following sections, we will discuss the main differences between measurements obtained by chemical analysis or dissection and those obtained by DXA.

Body chemical components vs. DXA measurements

The algorithms of DXA software partition the six chemical components of the body (lipids, water, proteins, carbohydrates, non-bone mineral, and bone mineral) into three DXA compartments (BMC, fat mass, and lean mass) (Pietrobelli et al., 1996; St-Onge et al., 2004) (Table 1). In animal bodies, carbohydrates and soft-tissue minerals are generally very small (Nielsen, 1973) and can be assumed to be negligible. In pigs, for example, DXA lean and fat values obtained with a GE Lunar Prodigy (Lunar Corp., Madison, WI, USA) device can be converted into the chemical equivalents of protein and lipid masses, as suggested by Pomar and Rivest (1996) in the following manner:

$$\text{Total body protein (g)} = -1384 + 0.216 \times \text{DXA lean (g)}$$

$$\text{Total body fat (g)} = 2825 + 1.009 \times \text{DXA fat (g)}$$

Body phosphorus (P) and calcium (Ca) can be estimated as the sum of the P and Ca present in the bones, lean, and

Table 1 - Partitioning of the six basic components into the chemical analysis and DXA compartments

Main chemical component of the body	Chemical analysis compartment	DXA compartment
Lipids	Ether extract	Fat mass
Water	Water	Lean mass
Proteins	Nitrogen	Lean mass
Carbohydrates	-	-
Non-bone minerals	-	-
Bone minerals	Ash	Bone mineral content

DXA - dual-energy x-ray absorptiometry.
Adapted from Pietrobelli et al. (1996) and St-Onge et al. (2004).

fat tissues of the body based on the DXA body measurements, assuming that P and Ca in bone accounts for 18% and 36% of BMC, respectively, that body proteins are associated with lean tissue containing 1.04% P and 0.042% Ca, and that body lipids are associated with fat tissue containing 0.05% P and 0.005% Ca (Nielsen, 1973; Letourneau-Montminy et al., 2015).

Some assumptions used by DXA in relation to bones should also be emphasized, since the technology is not designed to evaluate internal bone composition. Bone marrow fat is assumed to be constant between 4 and 5% (Mitchell et al., 1997a; Mercier et al., 2006), except in the skull, where the composition is assumed to be 17% due to the high lipid content of the brain (Hologic, 1996). However, these values depend on the brand of commercial DXA device used. The assumed values may constitute an important bias when comparing methods, since chemical analysis considers the actual lipid content, whereas the DXA method merely extrapolates the content to humans.

Lastly, although the dimensions of DXA equipment allow for pigs, dogs, and cats, among other non-ruminant animals, to be assessed, DXA software was developed solely to evaluate humans (except for some modes that allow small animals, such as rats, to be scanned). Consequently, any adjustments made to the software to better estimate body composition in humans (usually information considered confidential) may add a new source of bias in animal evaluation (Kipper et al., 2015).

Dissected carcass tissues vs. DXA measurements

The relationship between DXA measurements and dissected carcass tissues is somewhat nonrepresentational, as tissues obtained by dissection include several chemical components (Marcoux et al., 2003). The DXA method also identifies the various components within each dissected tissue (Table 2). Adipose and muscle tissues correspond to the respective content of lean and fat masses, while bone tissue corresponds to these two components plus BMC (Pietrobelli et al., 1996). The reason dissected tissues can be related to DXA measurements is that dissected adipose tissue has a strong correlation with DXA fat mass, dissected muscle tissues are correlated with DXA lean mass, and dissected bone tissues are correlated with BMC and lean mass (Marcoux et al., 2005). This is because fat is rich in lipids, the reference material of DXA fat mass; similarly, dissected muscle tissues are correlated with DXA lean mass, because lean beef is used as the reference for DXA lean mass (Pietrobelli et al., 1996). Bone tissue is correlated with BMC because DXA calibration can be carried out with artificial parts that simulate bones and its correlation with lean mass is due to the strong positive relationship between muscle and bone (Pietrobelli et al., 1996; Schoenau, 2005). Therefore, it is necessary to take into consideration the fact that the manner in which the dissection method classifies the tissues will affect the model used to estimate their values using DXA measurements (Marcoux et al., 2003).

In some cases, due to practical or commercial issues, artificial assumptions are made to relate dissected carcass tissues and DXA measurements. Examples include the cartilage and skin, which can be treated as bone and adipose tissues, respectively, during dissection (Marcoux, 2001). The treatment of cartilage represents one of the greatest contrasts: it is often treated as bone during dissection, while DXA treats it as soft tissue. As a result, the bias in the DXA algorithms used to estimate dissected bone increases as the ratio of cartilage in the sample increases, especially in ribs (Marcoux et al., 2003; Mercier et al., 2006). Another point worth noting is that the entire foot can be classified as a bone in dissection, whereas the skin and foot tendons are considered to be part of the soft tissue compartment in DXA, thus generating additional bias. The skin can be treated as adipose tissue during dissection; however, skin has a lower lipid content than adipose tissue does. This chemical difference is also detected by DXA: adipose tissue (without skin) exhibits a fat mass of approximately 58%, while skin exhibits only 28% (unpublished data). A similar issue occurs in jowl classification: this meat cut can be treated as adipose tissue in dissection, but a small portion of muscle tissue is present. Different methods for estimating dissected tissues in pig and lamb carcasses and cuts can be found in Marcoux et al. (2005) and Mercier et al. (2006).

Advantages of DXA

Non-destructive methods like DXA allow for accurate, repeated measurements of total body composition, resulting in accurate evaluations of the effect of the experimental treatments used in non-ruminant nutrition research. With

Table 2 - Relationship of DXA measurements to dissection tissues

Dissection	DXA
Adipose tissue	Fat mass
	Lean mass
Muscle	Fat mass
	Lean mass
Bone	Fat mass
	Lean mass
	Bone mineral content

DXA - dual-energy x-ray absorptiometry.
Adapted from Goodsitt (1992), Gotfredsen et al. (1997), and Tothill and Hannan (2002).

this technology, mathematical growth models can be calibrated using data obtained repeatedly over time for a given individual. The DXA method is less expensive than comparable slaughter techniques and reduces the error due to individual variability (Suster et al., 2006). Furthermore, DXA provides high reproducibility and minimizes operator effect (Raffan et al., 2006; Suster et al., 2006; Kipper et al., 2015). In addition, operator bias is usually high in the dissection of small (young) pigs, but this effect is not observed with DXA, in which accuracy is independent of animal size (Mitchell et al., 1998).

The DXA method has other advantages that are not directly related to theoretical concepts. Since slaughter is not necessary, some logistical issues are easier to manage, especially given that the trial requires fewer animals. Furthermore, DXA technology makes it possible to test a larger number of treatments in an experiment without increasing the number of animals. This method is extremely fast and the results are available immediately after image acquisition. Lastly, unlike actual destructive methods, the use of DXA in animal genetic selection could save individuals that exhibit advantageous characteristics or high genetic and economic value from being slaughtered.

Disadvantages of DXA

One of the major difficulties in using this technology is related to the hydration of the sample (i.e. the level of water in fat-free tissues [FFT]) (Emmans and Kyriazakis, 1997; St-Onge et al., 2004). Water content varies from one animal to another, being high in young and low in mature individuals (Emmans and Kyriazakis, 1995). After maturity is reached, the water:FFT ratio remains constant, around 0.73 in mammals (Wang et al., 1999). For example, in pigs, the ratio is approximately 0.87 at 1.5 kg body weight (BW), but this value falls to 0.76 at 54 kg and to approximately 0.73 at 145 kg BW (Shields et al., 1983). The water:FFT ratio is considered to be constant in DXA and can be deemed a bias in some situations (Roubenoff et al., 1993). This issue is so important that it has been extensively studied in juvenile and adult humans with disorders/diseases that alter the water equilibrium in the body (Snead et al., 1993; Georgiou et al., 1997). The water:FFT ratio bias must be taken into account in studies in which changes in BW of animals are an important factor.

Since the invention of DXA, thickness has been acknowledged as one of its most limiting factors (Goodsitt, 1992). The deeper the tissue, the lower the precision of consecutive measurements and, consequently, the less true the value (Jebb et al., 1995). In thick samples, the low-energy x-ray is more attenuated than the high-energy x-ray, an effect known as the beam hardening effect (Seibert and Boone, 2005). Although the data provided by DXA are related to the values of the chemical methods, they are not identical, which means that some adjustments are required. Another point to be taken into account when making adjustments is that DXA devices and software were developed for use in human medicine and may not always be suitable for animal studies.

Accuracy, repeatability and reproducibility of DXA devices

Evaluating the accuracy of an instrument means determining the closeness between its measurements and the accepted reference values in terms of trueness and precision. The trueness of a measurement refers to the closeness of the agreement between the average value obtained from a large series of test results and an accepted reference value, while precision refers to the degree of internal agreement between independent measurements taken under specific conditions. A device is said to be accurate when it is true, i.e., when its measurements are close to the true values, and precise when there is no spread around the true value (International Organization for Standardization, 1993).

The accuracy, trueness, and precision of measurements obtained by DXA in pig half-carcasses and primal cuts were recently studied by our group (Kipper et al., 2015). Briefly, a trial was conducted to investigate the repeatability and reproducibility of DXA measurements taken in pig half-carcasses. The repeatability measures the error inherent to DXA readings. The repeatability conditions were created by scanning each carcass ten times in the same position. The reproducibility includes sources of variation in repeatability. The reproducibility was therefore related to the variation inherent to the positioning of the carcass on the DXA table. The reproducibility conditions were created by scanning each carcass once in each of ten different positions. After the scans, all images obtained under both repeatability and reproducibility conditions were analyzed either by using a custom rectangular region of interest (ROI) or by placing the carcass within the head, trunk, leg, and arm regions of interest of the standard grid for the human body. Coefficients of variation (CV) were computed for each carcass individually and then combined, assuming a normal variance distribution (Glüer et al., 1995).

The reproducibility CV values were always greater than repeatability CV values (Table 3). This means that DXA achieved lower variability in the results obtained under repeatability conditions. This finding is consistent with the proposed experimental design and demonstrates that the

technology was well calibrated. Methods with inadequate reproducibility can be easily adjusted and improved, but methods with inadequate repeatability require major structural changes (Burdick et al., 2005). The repeatability was generally lower than 1% and reproducibility was lower than 3%. Soft tissue was predicted with greater precision among the ROI. The custom ROI did not provide precise estimates of BMC, but it produced acceptable values for bone mineral density. The trunk ROI did not provide precise estimates of fat. This suggests that appropriate positioning of the half-carcass on the DXA table is required to avoid variability in the results.

The repeatability and the reproducibility of DXA devices have been studied in limited number of research projects. For example, Nielsen et al. (2004) described a measurement protocol suitable for use in postmortem specimens of low economic value (tarsus and carpus distally) from a large number of commercial pigs to quantify the precision of that protocol and to estimate accuracy of the technique for measuring BMC in this region. To achieve these objectives, these authors used the feet and tails of the pigs to evaluate bone mineralization. Scanning precision (i.e., repeatability) for each ROI was determined with data derived from ten separate scans from one pig. The reproducibility was evaluated with ten repeated scans performed as above, except that the specimen was removed from the table and repositioned before each scan. As in our study, reproducibility was always greater than repeatability, but an interesting finding was that small ROI presented higher CV and, therefore, ROI size needs to be established in accordance with the coveted precision of the measurement. Raffan et al. (2006) scanned ten dogs six times each, alternating between dorsal and lateral recumbency to determine the precision of body composition measurements in dogs. This study demonstrated that DXA is a precise method of body composition analysis in dogs. Nevertheless, because differences were found between body positions and between operators, the protocols need to be properly standardized to ensure proper measurement repeatability.

Repeatability is related to the technology and cannot be easily corrected, while reproducibility is related to the measurement protocol and can therefore be adjusted to the required precision of the measurements.

Our team has recently published a study (Kipper et al., 2015) about how the thickness of meat samples can affect DXA measurements. Briefly, belly samples with a constant mass (and constant composition) were scanned, but between consecutive scans, the bellies were cut in half and stacked to increase the thickness. The samples were scanned using three software configurations. The scanning mode used was a total body, with one scan for each of the following options: thin (for samples less than 16 cm thick), standard (for samples of 16 to 25 cm thick), and thick (for samples more than 25 cm thick).

Neither the scanning mode options nor their interaction with the thickness had an effect on the DXA measurements. Thickness affected the percentage of fat, soft tissue, and lean mass, but did not affect the fat mass (Figure 2). The DXA measurements exhibited several different distribution patterns in relation to the thickness. The soft tissue and lean mass exhibited a quadratic pattern, while the percentage of fat was sigmoidal and the fat mass was not affected. There was a 43% variation between the lean masses estimated for the smallest and largest thicknesses. The percentage of fat increased up to a thickness of approximately 10 cm was similar between 10 and 20 cm and increased again beyond 20 cm. Despite their effect on the fat percentage, the fat mass values did not change (Figure 2). Thus, the variation in the fat percentage may be due to the soft tissue and its relation to fat mass. The results suggest that the most stable range of thicknesses is approximately 17 to 23 cm.

Table 3 - Coefficient of variation of DXA measurements obtained in pig half-carcasses using different regions of interest (ROI) under repeatability and reproducibility conditions[1]

	Custom ROI	Head ROI	Trunk ROI	Arm ROI	Leg ROI
Repeatability condition					
BMD (g/cm^2)	0.61	0.52	0.57	0.53	0.56
BMC (g)	0.68	0.56	0.68	0.56	0.55
Percentage of fat (%)	0.81	0.65	4.20	0.78	0.81
Soft tissue (kg)	0.07	0.04	0.10	0.04	0.04
Reproducibility condition					
BMD (g/cm^2)	0.64	0.64	0.67	0.67	0.62
BMC (g)	3.59	0.59	2.20	0.52	0.60
Percentage of fat (%)	2.30	1.71	21.16	2.64	2.69
Soft tissue (kg)	0.24	0.32	0.78	0.32	0.22

DXA - dual-energy x-ray absorptiometry; BMD - bone mineral density; BMC - bone mineral content.
[1] Coefficient of variation adjusted to account for the normal distribution of variance between carcasses.
Adapted from Kipper et al. (2015).

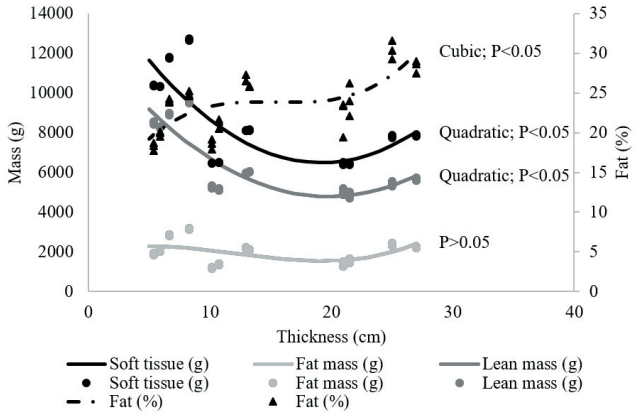


Figure 2 - Variation in DXA measurements in relation to the thickness of samples of constant mass. The solid and dotted lines represent the regressions; the circles and triangles represent the observations. Adapted from Kipper et al. (2015).

The differences observed in the DXA measurements may be due to a physical phenomenon called beam hardening, which is characterized by more pronounced attenuation of the low-energy x-ray (Seibert and Boone, 2005). This effect reduces R-values and consequently increases estimates of fat content. In addition, the effect of beam hardening increases in substances with higher R-values (Goodsitt, 1992). Thus, the greater the lean percentage, the more it will be underestimated due to this effect (Jebb et al., 1995). However, there were no differences in the magnitude of the effect when different scanning modes were compared. Since the configurations were designed to account for this effect, it could be deduced that these configurations do not work properly under the study conditions. However, more knowledge is required on this subject and this effect should be further examined in future research.

Conclusions

The DXA method is a valuable tool for providing accurate, repeatable, and reproducible body composition measurements of live monogastric animals and their carcasses. This non-destructive method is less expensive than the traditional slaughter techniques, allows repeated measures, reduces the errors due to individual weight or compositional variation, and removes operator biases. Subjects can be scanned quickly and compositional measurements are available soon after the scan. However, calibration is required to convert DXA values to the true chemical or dissected values. The factors that can affect the precision of measurements and the disadvantages of this technology must be known and controlled to avoid potential bias. The subject hydration and thickness are between the most important ones.

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