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## Determination of Chicken Body Composition Measured by Dual Energy X-Ray Absorptiometry

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**Abstract:** Traditionally, body composition data for poultry is determined by grinding /homogenizing the whole bird and obtaining a sample for wet chemistry analysis. The overall process is slow, requires a large amount of freezer space and the time-lag required for determining body composition reduces the opportunity to use data in real- time situations. Two studies were conducted to evaluate Dual-energy X-ray Absorptiometry (DEXA) as a means of measuring body composition in broilers and broiler breeders. In Trial 1, two hundred and forty Cobb 500 broilers were reared from day-old to 60 days of age. Broilers were extracted from the flock every 3 days during the 60 day grow-out in order to obtain a variety of body weights and body composition for developing the body composition equations. The birds were weighed and scanned using the small animal software mode of the DEXA scanner (LunarProdigy, GE®). DEXA provides measurements in grams of Bone Mineral Content (BMC), Fat Mass (FM) and Lean Mass (LM). It was assumed that the sum of BMC+FM+LM represented the total body mass. After the scan was performed, the carcasses were frozen for further chemical analysis. Prior to chemical analysis, the carcasses were thawed, autoclaved at 110°C with 1 atm pressure for 1-5 h depending upon Body Weight (BW) and homogenized in a heavy duty blender (Waring Laboratory, Blender LBC15, Model CB15). Samples of the homogenized carcasses were freeze dried, weighed, ground and analyzed for total ash, ether extract and crude protein. The measurements obtained from the DEXA scans were compared with the whole body chemical analysis for each broiler. Regression analysis of DEXA values (BMC, FM, LM) and chemical analysis (ash, ether extract and protein) were utilized to determine possible correlations. Prediction equations were then developed to adjust the original DEXA results to more accurately predict BMC, fat tissue and lean mass. The R2 values for the prediction equations using DEXA values were 0.999, 0.99, 0.96 and 0.99 for total mass, BMC, fat and lean mass (P<0.0001). In Trial 2, 156 Cobb 500 broiler breeder hens were scanned to validate the equations developed in Trial 1. The results indicate that the prediction equations were adequate and a reliable alternative for measuring body composition in broilers and broiler breeders. The high degree of correlation for all the variables indicates that with proper calibration the DEXA values can be used to predict body composition for these birds ( $R^2$  = 0.99, 9.99, 0.84 and 0.94 for total mass, BMC, FM and LM, respectively, p<0.001).

Key words: Body composition, meat yield, DEXA scanners

### INTRODUCTION

The meat yield and body composition of the modern broiler is continually being improved. Quick access to real-time body composition information about current broiler and broiler breeder flocks would provide valuable information to integrators, poultry management, meat processors, nutritionist and geneticists. Body composition changes of broilers or broiler breeders could be evaluated based on changes in the environment, body weight, age, feeding program and nutrient profile. Historically, the most common method to determine body composition in poultry has been to grind the whole bird and analyze carcass by proximate analysis. The overall process is slow, requires a large

amount of freezer space and the body composition data from a flock cannot be utilized for real-time studies. The current method of analyzing body composition becomes a rate-limiting step when evaluating a large population of birds.

The DEXA alternative to chemical analysis has the advantage of being a non-invasive technique. The same bird can be studied over an extended period of time without any detriment to its health or performance (the radiation dose per scan is very low). The amount of time needed per scan is short compared to the time invested in preparing a sample for chemical analysis. The ability to evaluate body composition with the DEXA gives the researcher more versatility and more birds can be

evaluated because less time is needed. DEXA is a technique developed for the measurement of bone mineralization and bone density in humans. The technology has the ability to determine body composition by measuring the attenuation of x-rays of two different energy levels by different materials, such as fat, lean and bone mineral mass. More recently, the utilization of this technology has been used to measure body composition in animals (Black et al., 2001; Elowsson et al., 1998; Mercier et al., 2006; Schreiweis et al., 2005). There are few reports in the literature on the use of DEXA for determining body composition in chickens. Validations of body composition equations are needed when using different DEXA scanners and software versions. This study was conducted with the objective of calibrating and validating the use of GE Lunar Prodigy DEXA for the measurement of body composition for broilers and broiler breeders.

#### **MATERIALS AND METHODS**

Trial 1 equation development and calibration: A total of 240 Cobb 500 broilers were utilized for this experiment. The birds ranged from day-old to 60 days of age in order to obtain an ample variety of body weights and body composition. The birds were reared in floor pens and fed ad libitum. This study complied with the provisions of the Institute Animal Care and Use Committee as specified by the Animal and Plant Health Inspection Service, USDA in 9 CFR Part 1(1-91) for use of poultry in research.

Starting with day-old chicks, birds were extracted from the flock every 3 days until 60 days of age for DEXA and wet chemistry analysis (12 birds per scan). The birds were not fasted before they were sacrificed; consequently the body composition results (DEXA and wet chemistry) include the contents of the gastrointestinal tract.

**DEXA scans:** The birds extracted from the flock were humanely sacrificed using carbon dioxide inhalation. After euthanization, the birds were placed face down on the DEXA scanner and scanned utilizing the small animal software module (Lunar Prodigy from  $GE^{\otimes}$ , encore software version 12.20.023) as shown in Fig. 1. Defining the whole bird as a Region of Interest (ROI), the DEXA provided measurements in g of Bone Mineral Content (BMC), g fat content and g lean mass for each bird. The sum of the DEXA measurements was assumed to be g Total Body Mass.

Chemical analysis of carcasses: After scanning the broilers with the DEXA, the birds were frozen for further analysis at a later time. Prior to chemical analysis, the carcasses were thawed and transferred to individual aluminum tubs, weighed and a small amount of water

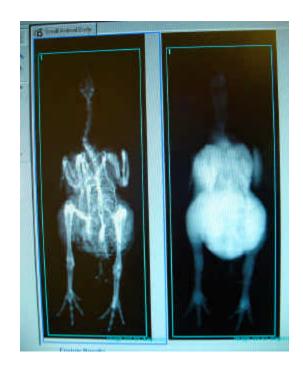


Fig. 1: DEXA scan of broiler

was added. The tubs were covered with aluminum foil and autoclaved at 110°C with 1 atmosphere of pressure from 1 to 5 hours depending on the size of the bird (adapted from Sibbald and Fortin, 1982).

After autoclaving, the tubs containing the autoclaved broilers were cooled to room temperature and reweighed. Any weight loss during this process was assumed to be water. The autoclaved carcasses (including feathers) were transferred into a heavy duty blender (Waring Laboratory, Blender LBC15, Model CB15) for homogenization. Once homogenized, a sample of approximately 150 g was obtained and freeze dried. After freeze drying, samples were reweighed and ground for further analysis.

The total ash, ether extract and crude protein of the carcasses were determined according to AOAC approved methods (AOAC, 1990, 1995). As suggested by Barker and Sell (1994), the carcass dry matter was calculated from the weight of the broiler and recorded amounts of additions and losses of water during autoclaving and after freeze drying. The quantitative amount of total ash, ether extract and crude protein of the broiler carcasses were converted into as is body composition (with water) data to compare to the DEXA scan results.

**Statistical analysis:** All the variables were converted to log-normal values prior to statistical analysis. The relationship between the log-normalized DEXA values

and log-normal wet chemical analysis was determined by regression analysis. The analyses were performed with the statistical package JMP<sup>®</sup> 8.0 of SAS Institute (2008) and the level of significance was P<0.05.

Calibration of DEXA measurements: If regression analysis showed a high correlation between the lognormal DEXA values and log-normal wet chemistry values, prediction equations were developed to correct the DEXA results to more closely reflect the values found with wet chemistry. These equations were utilized to obtain a more accurate measurement of body composition.

Trial 2 equation validation: A second experiment was conducted to evaluate the accuracy of the prediction equations developed in Trial 1. A total of 156 Cobb 500 breeder hens were utilized in experiment 2. The birds ranged from 22 to 65 weeks of age. The hens had been reared on 3 different planes of nutrition to obtain birds with light, standard and heavy body weights relative to the primary breeder guidelines. Birds from all the feeding regimes were extracted from the flock at 22 weeks of age, first egg, peak of production and 65 weeks of age to measure body composition (13 birds per plane of nutrition, 39 birds total per scan). Methods used for scanning and wet chemistry were the same as described for Trial 1.

Using the prediction equations developed in Trial 1, the corrected DEXA values and wet chemical measurements were subjected to regression analysis. The analyses were performed with the statistical package JMP<sup>®</sup> 8.0 of SAS Institute (2008) and level of significance was determined at P<0.05.

#### **RESULTS AND DISCUSSION**

The chemical analysis results for ash, ether extract and crude protein were utilized to calculate total body mineral content, total fat mass and total lean mass on an as is basis, in order to compare wet chemistry body composition data to DEXA values. Additionally, the chemical lean mass was calculated by adding the moisture content of the carcass to the obtained crude protein content (as determined by chemical analysis). This was done under the assumption that lean body mass has more than 70% water (Brommage, 2003) and that the lean mass measurement reported by DEXA includes all components of the soft tissue excluding the fat tissue. High correlations have been reported in the literature (Mitchell et al., 1997) between lean body mass measured by DEXA and total body water.

**Total body mass:** DEXA total body mass measurements, in grams, were obtained by adding BMC

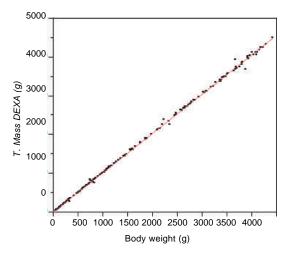


Fig. 2: Relationship between Total Body Mass determined by DEXA scan (small animal mode) and scale weight. Total Body Mass (g) = e (0.099 + 0.984 \* Ln(Total Mass (g))) R<sup>2</sup> = 0.999, n = 240, P<0.0001

and tissue mass (fat and lean tissues). In general, DEXA measurements of birds above 100 grams of body weight were higher than the scale measurements. Overestimation of scale body mass by DEXA has been reported in chickens (Swennen et al. 2004; Mitchell et al., relationship between 1997). measurement of body weight and DEXA total body mass are shown in Fig. 2. There was a high positive correlation between the DEXA and chemical analysis measurements ( $R^2 = 0.999$ , P<0.0001) for total body mass. DEXA technology can accurately measure total body mass. Brunton et al. (1993) also reported a high correlation between total body weight measured by scale and DEXA.

Based on the results, the following prediction equation was developed to adjust the Total Body Mass in grams utilizing the DEXA Total Body Mass measurement (BMC, fat tissue and lean tissue added together):

Total Body Mass (g) = 
$$e^{(0.099 + 0.984 + Ln(Total Mass (g)))} R^2 = 0.999$$
, n = 240, P < 0.0001

Bone mineral content: DEXA has been reported to be accurate in determining bone mineral content and bone density in humans, therefore the measurement of bone minerals in broilers was also expected to be accurate. The relationship between DEXA bone mineral content and total body ash content is presented in Fig. 3. There was a high positive correlation between the DEXA BMC and total body ash (R² = 0.99, P<0.0001). In agreement with the present findings, positive correlations between DEXA BMC and chemical ash content have been reported for chickens, 6 kg pigs, cats, dogs and chicken

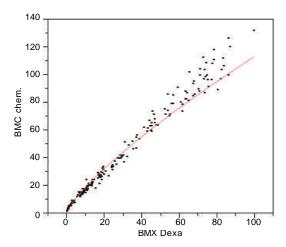


Fig. 3: Relationship between bone mineral content determined by DEXA scan (small animal mode) and ash chemical analysis. BMC (g) = e<sup>(1.13 + 0.782</sup> \*Ln (BMC Dexa (g) )) R<sup>2</sup> = 0.99, n = 227, P = <0.0001

legs ex vivo (Brunton et al., 1993; Speakman et al., 2001; Swennen et al., 2004; Baird et al., 2008). For white Leghorns (Schreiweis et al., 2005), the bone mineral content of the legs measured by DEXA in live birds was correlated with excised bone ash ( $R^2 = 0.73$ , P<0.0001). Onyango et al. (2003) indicated that BMC, Bone Mineral Density (BMD) (measured by dual x-ray absorptiometry) and tibia ash are more sensitive as indicators of dietary Ca and P concentrations than shear force. The same authors concluded that BMD as measured by dual energy x-ray absorptiometry may be used to predict the percent bone ash for tibia. Additionally, Schreiweis et al. (2003) also reported that densitometric scans were comparable to those obtained from other bone tests commonly used for White Leghorns fed diets with different Ca concentrations. They reported that bone breaking force was correlated with BMD (r = 0.65, P<0.001) and bone ash weight (r = 0.77, P<0.001). The authors concluded that densitometry accurately measures differences in BMD and BMC in live birds fed varying concentrations of dietary calcium.

In contrast with our results, Mitchell *et al.* (1997) found a low correlation between DEXA BMC and total body ash. The authors could not explain the lack of correlation. The authors utilized different modes of scan and pooled all the information, when they separated the modes of scan they obtained a higher correlation with total body weight. Other authors also reports low correlations between DEXA BMC and total body ash in pigs (Brunton *et al.*, 1993; Mitchell *et al.*, 1996) and rhesus monkeys (Black *et al.*, 2001). Baird *et al.* (2008) reported that bone mineral content determined by ashing was significantly different by 9.2% (P = 0.0001) from BMC determined in

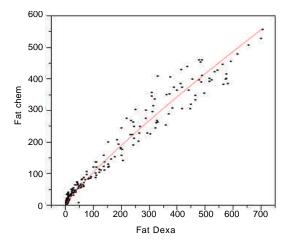


Fig. 4: Relationship between Fat content determined by DEXA scan (small animal mode) and fat chemical analysis. Body Fat (g) = e<sup>(0.65 + 0.867 \* Ln (Fat Dexa (g)))</sup> R<sup>2</sup> = 0.96, n = 232, P = <0.0001

vivo by DEXA and indicated that GE Lunar Prodigy DEXA instrument significantly underestimated the in vivo BMC in chickens.

Based on the results, the following prediction equation was developed to adjust the total BMC in grams utilizing the DEXA body bone mineral content measurement:

BMC (g) = 
$$e^{(1.13 + 0.782 * Ln (BMC Dexa (g)))} R^2 = 0.99$$
, n = 227, P = < 0.0001

Fat tissue: Similar to BMC, the results indicate a high correlation between the DEXA fat tissue and ether extract results ( $R^2$  = 0.96, P<0.0001, Fig. 4) with a general overestimation of fat tissue by the scanner. In agreement with our observations, Swennen *et al.* (2004) found a high correlation coefficient between DEXA fat tissue and chemical measurements of fat ( $R^2$  = 0.913, P<0.0001) and no effect of chicken body size on the DEXA measurement. Brommage (2003) reported that DEXA overestimated body fat percentage in mice but presented a linear relationship between DEXA ratio and fat carcass analysis. High correlation between DEXA and chemical fat mass has also been reported for rhesus monkeys (Black *et al.*, 2001).

Mitchell *et al.* (1997) found low correlations between DEXA and chemical measurement of body fat percentage for birds smaller than 2000 g but a better correlation for birds weighing more than 2000 g. For those body weights, the authors report a lower  $R^2$  than the one found for fat composition in this experiment ( $R^2 = 0.62$  vs.  $R^2 = 0.96$ ) and recommended the need of additional measurements to obtain a reliable equations. Overestimations of body fat and low correlations with

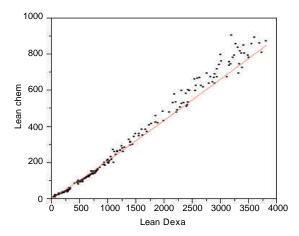


Fig. 5: Relationship between Lean Mass content determined by DEXA scan (small animal mode) and protein chemical analysis plus carcass water. Lean Body Mass (g) = e<sup>(-0.18 + 1.018 \* Ln (Lean Dexa (g) ))</sup> R<sup>2</sup> = 0.999 n = 216, P = <0.0001

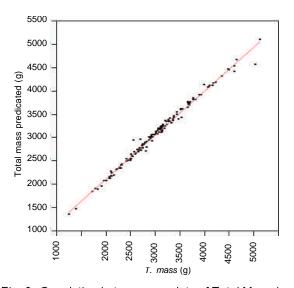


Fig. 6: Correlation between raw data of Total Mass (sum of BMC, Lean mass and fat mass) measured with DEXA and Total Mass calculated using the equation of Experiment 1. R<sup>2</sup> = 0.99, P<0.0001

chemical determination have been also previously observed in young piglets with better results with larger animals (Brunton *et al.*, 1993).

Based on the present results, a prediction equation was developed to adjust the DEXA body fat measurement in grams. The total body fat content in grams can be calculated as follows:

Body Fat (g) = 
$$e^{(0.65 + 0.867 * Ln (Fat Dexa (g)))} R^2 = 0.96$$
, n = 232,  
P = < 0.0001

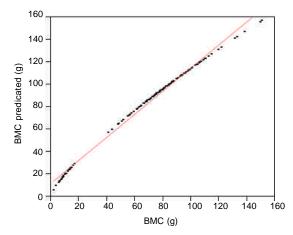


Fig. 7: Correlation between raw data of bone mineral content measured with DEXA and bone mineral content calculated using the equation of Experiment 1. R<sup>2</sup> = 0.99, P<0.0001

Body lean mass: The correlation between DEXA lean mass and chemically determined lean (chemically analyzed crude protein content plus carcass water content) is shown in Fig. 5. There is a positive correlation between the DEXA determined lean mass and the chemically determined lean mass ( $R^2 = 0.999$ , P>0.0001). In agreement with this experiment, high correlations between lean body mass and body protein analysis have been reported in pigs, rhesus monkeys. cats and dogs (Mitchell et al., 1998; Brunton et al., 1993; Black et al., 2001; Speakma et al., 2001). Swennen et al. (2004) report overestimation of chicken lean mass by DEXA and a high correlation between DEXA lean tissue and chemical lean tissue mass, protein and water mass

The following prediction equation was developed to adjust the total Lean mass in grams utilizing the DEXA body lean mass measurement:

Lean Body Mass (g) = 
$$e^{(-0.19 + 1.019 + Ln (Lean Dexa (g)))} R^2 = 0.999 n = 216, P = < 0.0001$$

**Equation validation:** Trial 2 utilized broiler breeder hens to validate the prediction equations developed in Trial 1. Significant linear correlations were found between the body composition components as determined by DEXA scanning and the corresponding wet chemistry results (R² = 0.99, 9.99, 0.84 and 0.94 for total mass, BMC, fat and lean tissue, respectively, P>0.001) (Fig. 6 to 9). The results indicate that the prediction equations developed in Trial 1 for using DEXA to measure body composition are a reliable alternative for measuring body composition in broilers and broiler breeders. The high degree of correlation for all the variables indicates that

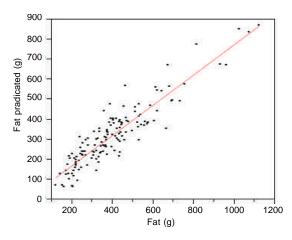


Fig. 8: Correlation between raw data of fat mass content measured with DEXA and fat mass content calculated using the equation of Experiment 1. R<sup>2</sup> = 0.84, P<0.0001

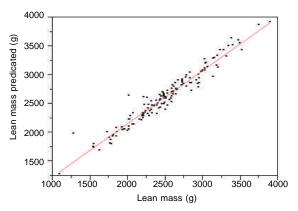


Fig. 9: Correlation between raw data of Lean Mass content measured with DEXA and lean mass content calculated using the equation of Experiment 1. R<sup>2</sup> = 0.94, P<0.0001

with proper calibration the DEXA values can be used to predict body composition for these birds.

Conclusions: This study demonstrates that dual-energy X-ray absorptiometry (DEXA) is a reliable alternative for evaluating body composition in chickens. The body composition data for broilers in the present study were consistent with previously reported results. The development of prediction equations from DEXA scans of individual broilers and their wet chemistry allowed an accurate prediction (based on high correlation coefficients) of body composition of broiler breeders during the validation phase. DEXA evaluation of body composition of poultry may not be sufficiently accurate without appropriate equation development and validation. The ability to predict body composition is important because it allows the researcher, nutritionist

or geneticist to obtain valuable information quickly that can influence the decision making process. The use of DEXA to determine body composition is a superior method because it is a non-invasive technique that allows accurate determination *in vivo* with short scanning times.

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