Dual-energy X-ray absorptiometry is a reliable non-invasive technique for determining whole body composition of chickens

S. Schallier ,[*] C. Li,[*] J. Lesuisse,[*] G. P. J. Janssens,[†] N. Everaert,[‡] and J. Buyse[*,1]

[*]*Department of Biosystems, Laboratory of Livestock physiology, KU Leuven, Kasteelpark Arenberg 30, 3001 Leuven, Belgium;* [†]*Department of Nutrition, Genetics and Ethology, Ghent University, Heidestraat 19, B-9820 Merelbeke, Belgium; and* [‡]*Gembloux Agro-Bio Tech, University of Liège, Passage des Déportés, 2, 5030 Gembloux, Belgium*

ABSTRACT In this study, a Lunar Prodigy dual-energy X-ray absorptiometry (**DEXA**) scanner was validated as a technique to estimate chicken body composition in a non-invasive way. Former research has emphasized the importance of validation of every scanner and software version. In a first trial, DEXA estimated body composition for broilers was correlated with chemical carcass analysis to develop prediction equations. As such, those equations can be used in later experiments with chickens to correct DEXA estimations to estimate body composition accurately by DEXA. DEXA estimated fat mass, lean tissue mass, bone mineral content (**BMC**) and total body mass, which is the sum of fat, lean mass and BMC, were compared to chemically analyzed crude fat, lean mass as the sum of protein and water and body ash content and scale body weight, respectively. Those regression equations were then used in a second trial to determine body composition based upon DEXA for breeders at different ages. In this experiment, fat and lean tissue determined by DEXA, were compared to dissection parameters commonly used for assessing carcass quality, namely breast muscle and abdominal fat. The first trial showed that DEXA provides high correlations for body mass ($\rho = 1$) and the individual tissue masses separately (ρ ranging between 0.98 and 1). These high correlations allow for accurate prediction of those components with the developed regression equations. Proportional fat and lean tissue were correlated with their chemical counterparts, however, to a lower extent than absolute values due to lower variation between the proportional weights. BMC percentage was not significantly correlated with ash percentage. Furthermore, in trial 2 high correlations were observed between dissection parameters and DEXA-corrected estimations. These correlations show that DEXA can assess carcass quality in breeders without sacrificing the birds. In conclusion, DEXA is a reliable technique to estimate breeder and broiler body composition in a non-invasive way, hence allowing for longitudinal studies over longer periods of time while avoiding sacrificing of birds.

Key words: chicken, dual-energy X-ray absorptiometry, body composition, validation, carcass quality

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INTRODUCTION

The assessment of body composition by non-invasive techniques in birds has been increasingly studied over the years. Moreover, in commercial birds such as meat type broiler chickens and laying hens, knowledge about their body composition is valuable for various areas in the poultry industry such as breeding and selection programmes, nutritional recommendations, and poultry management. Intensive selection for a fast and efficient body weight (**BW**) gain in commercial broiler chickens has yielded in a bird which reaches a slaughter weight of 2.5 kg in only 5 to 6 wk (Aviagen, 2014). This fast growth also resulted in some negative side effects, such as metabolic disorders, leg problems, and an increased fat deposition (Decuypere et al., 2003). However, this fat is nowadays undesired by people as they grow increasingly aware about their health and hence prefer lean meat. Body composition can be measured by both invasive techniques and non-invasive techniques. The most used and widely accepted invasive method is chemical carcass analysis (Nagy and Clair, 2000). However, since it is essential to select desirable birds for further breeding practices, it is crucial to use non-invasive techniques to avoid sacrificing the birds. Furthermore, non-invasive methods allow the researchers to study the animals in longitudinal studies over a longer period of time. One of those non-invasive methods that has gained vast interest in the last decades in both human and animal research, is "dual energy X-ray absorptiometry," mostly abbreviated as **DEXA** or **DXA**. In

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[1]Corresponding author: johan.buyse@kuleuven.be

humans, DEXA is commonly used in the determination of bone mineral content (**BMC**) and bone mineral density (**BMD**), often with applications in the identification of osteoporosis (Johnston et al., 1991; Massie et al., 1993; Grier et al., 1996; Blake and Fogelman, 1997; Blake and Fogelman, 2007; Lorente Ramos et al., 2011). Lately, this application has also proven useful in determining skeletal integrity of laying hens in a non-invasive way (Schreiweis et al., 2003; Hester et al., 2004; Schreiweis et al., 2005). DEXA scanners measure the attenuation of 2 X-ray beams with different energy levels by various tissues, hence allowing the quantification of total tissue, which is further separated in soft and hard tissue (Pietrobelli et al., 1996; Korine et al., 2004; Salas et al., 2012). The hard tissue comprises the bone mass, whereas the soft tissue can be divided in the fat and lean tissue mass. Previous studies on the use of DEXA in chickens, with different types of equipment, have proven to be good and reliable estimations of the body composition of these birds (Swennen et al., 2004; Salas et al., 2012). England et al. (2012) have even shown that the DEXA scanning can be used as a non-invasive technique to determine egg composition, hence allowing for further use of the embryo. Nevertheless, these studies also underline the importance of instrument validation and reestablishment of new regression equations for every tissue type (Mitchell et al., 1997; Swennen et al., 2004; Salas et al., 2012).

As such, the first purpose of this research aimed to plot appropriate prediction equations and then validate the technique against dissection results, using the Lunar Prodigy DEXA fan beam scanner. To achieve this research goal, broiler chickens were scanned after which true body composition was determined by performing a chemical carcass analysis on every bird. Furthermore, Korine et al., (2004) have shown that feathers are perceived as fat by DEXA scanners because of their relatively low density. Experimental setup was based on previously well executed and documented trials from Swennen et al., (2004) and Salas et al., (2012). Since longitudinal studies on live birds are one of the main advantages of this equipment and prediction equations are expected to correct for this discrepancy, whole birds with feathers are scanned and analyzed chemically.

Thereafter, dissection is still a widely used method in both research and industrial environments to assess carcass quality and determine body composition for various purposes. However, this is a costly and time consuming method with main disadvantage of killing and thus losing further purpose of the animal. As a result, the second objective of this study was to compare weights of breast muscle and abdominal fat, as the sum of leaf and gizzard fat, to DEXA whole body results. Particularly in broiler breeders, extensive knowledge about whole body composition and correlation with these specific tissue amounts is lacking. However, breast muscle yield and abdominal fat are still important factors in broiler breeder selection procedures (Thiruvenkadan et al., 2011). Hence, proving DEXA as a good and non-invasive alternative for measuring body composition in these broiler breeders, would provide DEXA scanning as an extremely helpful tool in future selection practices.

MATERIALS AND METHODS

Ethics

The present research was approved by the ethical commission for experimental use of animals of the KU Leuven under accession number P187/2013.

DEXA Scanning Methodology

Chicken body composition was measured using the Lunar Prodigy DEXA scanner (GE Healthcare, Madison, WI USA) with enCORE software version 12.30. A complete body scan was performed and analyzed in the small animal body mode. At the start of each scanning day, a quality assurance (**QA**) program was performed using a phantom standard to ensure accurate calibration of the scanner. Euthanized chickens were placed in dorsal position with spread wings and stretched legs to avoid extensive overlap of the body parts. Afterwards, the lines defining the "regions of interest" (**ROI's**) were corrected for the appropriate body parts. Since these lines are fixed at specific intersections, a calculated compromise was consistently applied. An example is depicted in Figure 1. Based on the attenuation of the 2 X-ray beams by different absorbing materials, the software calculated the estimated values for total tissue, lean and fat tissue, BMC, and fat percentage. These values are obtained for every ROI and the whole body region. It should be noted that, even though for both trial 1 and trial 2 (explained below) only total body composition is evaluated, different ROI's need to be defined in a correct way to ensure correct determination of whole body composition.

Trial 1. Development of the Regression Equations

Forty Ross 308 broiler chickens (male and female) ranging from 3 to 8 wk of age were scanned with DEXA. These chickens were reared on floor pens under standard temperature and light conditions and were fed ad libitum on either a control or a reduced balanced protein (**RP**) diet. In the RP diet, both the dietary crude protein and amino acids were reduced by 10% compared to the control diet with energy levels remaining the same between the two groups for starter, grower, and finisher feeding phases. These age, gender, and diet variations were applied to induce a wide range of BWs and compositions which is essential for the setup of proper regression equations. Indeed, BWs of selected chickens ranged from 597 to 5,661 g. Before the

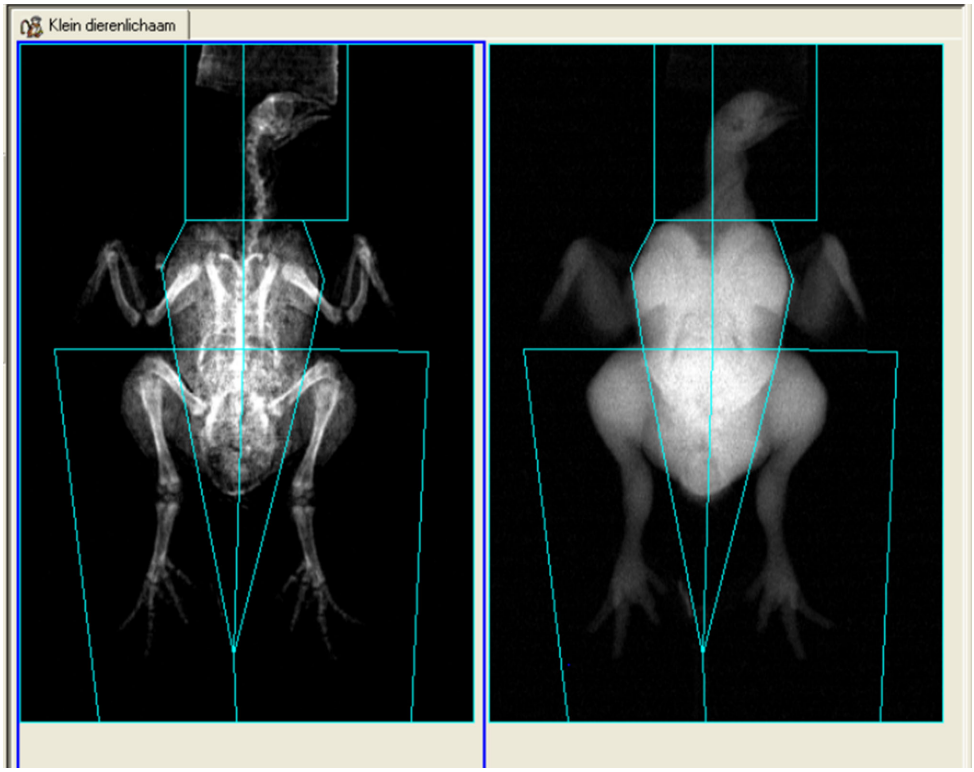

Figure 1. DEXA scan images of a chicken. Lines define the various regions of interest (ROI's) applied in a consistent way for all birds.

scanning procedure, selected chickens were fasted 24 h then anesthetized via electronarcosis and then euthanized with intravenous injection of T61. After scanning, all chickens were frozen and, based on their body composition estimated by DEXA scanning, 20 of them were selected for chemical carcass analysis. This selection process aimed to obtain a wide range of BWs combined with large variations in lean and fat tissue.

Frozen birds were transferred to aluminum containers, autoclaved for 16 h at 120°C and then reweighed. Carcasses were then homogenized and frozen. Before taking samples for proximate analysis, samples were slowly thawed and then heated to 60°C. Water content was determined by drying the samples at 103°C until a constant weight was obtained. Crude protein content, calculated as N x 6.25, was measured by Kjeldahl method (ISO, 2005), crude fat content was determined by Soxhlet method (ISO 1443, 1973). Ash content was measured by overnight combustion at 550°C.

All obtained chemical values were corrected for weight changes during autoclaving because little moisture gain or loss can occur during this process. Hence, mass of the carcass is affected and should be considered when calculating dry matter mass. Chemically determined lean mass was calculated as the sum of crude protein and water. Thereafter, chemical values were compared to the DEXA counterparts and linear regression equations were fitted between the chemical carcass and DEXA data. Proportional tissue masses for DEXA values are calculated based on the total tissue mass determined by DEXA. Chemically analyzed proportional tissue masses are based on the total body scale weight.

Trial 2. Dissection of Female Breeders

This experiment was conducted to compare DEXA prediction equations against dissection results. A total of 336 female broiler breeders were used in this trial. All breeders were reared under recommended management guidelines for light, temperature, and stocking density (Aviagen, 2013a). During the rearing period, chickens were divided in 4 dietary treatments to obtain birds with different BW and body composition profiles. The 4 diets consisted of a standard diet, a low protein (called body fat) diet (10% reduction in AA and crude protein) on either a standard BW curve and a 20% increased BW curve and a 10% diluted diet (10% reduction in energy and protein). Dietary protein and energy levels of the different diets for these 4 groups are presented in Table 1. In the laying period, all breeders received a standard recommended breeder diet (Aviagen, 2013b). At 7 different ages, namely 5, 10, 15, 19, 23, 27, and 31 wk of age, 12 breeders per group (48 per age, 336 in total), were randomly selected for DEXA scanning and dissection procedures. Selected birds were first euthanized with CO_2 inhalation, frozen, and later defrosted for DEXA scanning according to the aforementioned method. After each scanning, the breeder was dissected to obtain leaf and gizzard fat, together referred to as abdominal fat (as described by Tzeng and Becker, 1981 and Becker et al., 1984) and whole breast

Table 1. Dietary protein and energy levels of the different diets in the four groups.

Diet	Group	Control	Body fat[1]	Body fat + 20% Heavier[2]	Diluted[3]
Starter 1 (0 to 14 d)	Energy (Kcal)[4]			2,800	
	CP (%)			19	
Starter 2 (15 to 35 d)	Energy (Kcal)	2,800	2,800	2,800	2,520
	CP (%)	17	15.3	15.3	15.3
Grower (36 to 105 d)	Energy (Kcal)	2,600	2,600	2,600	2,340
	CP (%)	14	12.6	12.6	12.6
Pre-breeder (105 to 154 d)	Energy (Kcal)	2,700	2,700	2,700	2,430
	CP (%)	14.5	13	13	13
Pre-breeder (154 d–5%)	Energy (Kcal)	2,700			
	CP (%)			14.5	
Breeder (5%– 35 wk)	Energy (Kcal)			2,800	
	CP (%)			15	

[1]Body fat diets are low protein diets with a 10% reduction in CP and AA.
[2]Low protein diets with a 10% reduction in CP and AA but on a 20% heavier body weight curve.
[3]Diluted diets with a 10% reduction in both CP, AA, and energy levels.
[4]Metabolizable energy in Kcal according to WPSA for poultry.

muscle weight. A Pearson correlation analysis was then performed between these tissues weights on the one hand and DEXA values that are corrected for the prediction equations developed in trial 1 on the other hand. Leaf and gizzard fat, and their sum, were compared with total body fat by DEXA and breast muscle was related to whole body lean tissue mass obtained by DEXA analysis.

Statistics

The associations between DEXA and chemical carcass values in trial 1 and tissue and DEXA-corrected values in trial 2 were determined by least-squares linear regression analysis. Chemical body composition values were compared with their DEXA estimated counterparts with a paired *t*-test. All analyses were performed using the JMP Pro 13 software package (SAS Institute Inc., Cary, North Carolina, USA). In all tests, results were considered significant when $P < 0.05$. Data are presented as mean ± standard error of the mean (SEM).

RESULTS

Trial 1. Development of the Regression Equations

Mean values ± SEM for measured tissues by DEXA and chemical carcass analysis and differences between the methods are presented in Table 2. The degree of correlation, indicated by ρ as the correlation coefficient and the root mean square error (**RMSE**) of the regression are also depicted in this table. Figure 2 contains relevant regression equations and corresponding coefficients of determination R^2 (R squared).

Total mass, determined by DEXA scanner, is the sum of lean tissue, fat, and BMC determined by the scanner software. This DEXA estimated total mass was lower (2217 ± 316 g) than the scale weight (2259 ± 321 g) with an average difference of 42 ± 6 g or 1.87 ± 0.12% ($P < 0.0001$). A high positive correlation between both values was observed (Table 2), with regression equation and R^2 shown in Figure 2A (correlation coefficient (ρ) = 1, $R^2 = 1$, $P < 0.0001$). Based on the results, the following equation was developed to estimate total BW based on DEXA values for total mass:

$$\text{Total BW (g)} = 2.525\ (\pm 4.615) + 1.018\ (\pm 0.002) * \text{Total mass DEXA (g)}$$

Fat mass and percentage were generally overestimated by DEXA compared to chemical values ($P < 0.0001$). For absolute total body fat, a strong positive correlation was again observed with $\rho = 0.98$ and $R^2 = 0.95$ ($P < 0.0001$) (Table 2, regression shown in Figure 2B). Percentage of fat was also positively correlated between DEXA and chemical values, however, with lower strength than absolute fat mass ($\rho = 0.77$, $P < 0.0001$) (Table 2). Corresponding regression is depicted in Figure 2C. As a result, a lower proportion of the chemical fat percentage variance is explained by the DEXA fat percentage ($R^2 = 0.59$). DEXA overestimated fat percentage by 43% on average. Based on the results, following regression equations can be used to predict fat mass and fat percentage based on DEXA fat values.

$$\text{Total Body Fat (g)} = -35.98\ (\pm 20.64) + 0.872\ (\pm 0.045) * \text{Fat DEXA (g)}$$

$$\text{Total Body Fat percentage (\%)} = -1.288\ (\pm 2.597) + 0.806\ (\pm 0.159) * \text{Fat percentage DEXA (\%)}$$

As depicted in Table 2 and regression in Figure 2D, a high positive correlation was present between DEXA lean mass and chemical lean tissue values ($\rho = 1$, $R^2 = 1$, $P < 0.0001$). Lean tissue mass was underestimated by DEXA with an average difference of 114 ± 17 g or

Table 2. Comparison of DEXA estimated body mass and absolute and proportional tissue weights with the corresponding scale weight and chemically determined tissue weights. Differences between the methods are presented as both absolute and proportional values. Correlation coefficient (ρ) and the root mean square error (RMSE) are presented as well. The coefficient of determination (R squared, R^2) of the comparisons can be found in Figure 2 (n = 20).

Tissue	Chemical analysis	DEXA	Absolute difference (g)	Percentage difference (%)	ρ	RMSE
Body mass (g)	2259 ± 321[1]	2217 ± 316***	42 ± 6	1.87 ± 0.12	1**	10.87
Fat mass (g)	285 ± 55	368 ± 62***	−83 ± 14	−40.99 ± 6.99	0.98**	54.51
Fat %	11.52 ± 0.87	15.89 ± 0.83***	−4.37 ± 0.58	−43.68 ± 7.14	0.77**	2.57
Lean mass (g)[2]	1926 ± 266	1812 ± 254***	114 ± 17	6.25 ± 0.66	1**	56.05
Protein (g)	450 ± 63				0.99**	38.29
Water (g)	1476 ± 204				1**	49.59
Lean %[2]	86.30 ± 0.88	82.40 ± 0.83***	3.89 ± 0.60	4.46 ± 0.68	0.76*	2.65
Protein	20.27 ± 0.34				0.17[NS]	1.56
Water	66.02 ± 0.85				0.72*	2.74
BMC (g)[3]	55.95 ± 7.98	36.53 ± 4.82***	19.42 ± 3.35	33.39 ± 1.82	0.98**	6.60
BMC %[3]	2.53 ± 0.06	1.71 ± 0.05***	0.83 ± 0.06	32.14 ± 1.81	0.44[T]	0.25

[1]Body mass determined by scale is compared with DEXA total body mass.
[2]Chemical lean tissue (protein + water), protein and water are compared with DEXA lean tissue.
[3]Bone mineral content (BMC) mass and percentage as DEXA parameter to compare with chemical ash content.
***Means differ significantly with $P < 0.0001$ by paired t-test.
**Correlation is significant with $P < 0.0001$.
*Correlation is significant with $P < 0.001$.
[NS]Correlation is not significant.
[T]Correlation showed a trend with $P = 0.0545$.

6.25 ± 0.66% ($P < 0.0001$). Similarly to fat percentage, lean tissue percentage (sum of water and protein percentage), was also positively correlated with DEXA lean tissue, but again to a lesser extent than absolute lean values ($\rho = 0.76$, $P = 0.0001$) (regression is shown in Figure 2E). The total lean tissue mass and percentage can be calculated from DEXA estimates by following equations:

$$\text{Total Body Lean tissue (g)} = 28.84\ (\pm 24.03) + 1.047\ (\pm 0.011) * \text{Lean DEXA (g)}$$

$$\text{Total Body Lean percentage (\%)} = 19.95\ (\pm 13.47) + 0.805\ (\pm 0.163) * \text{Lean percentage DEXA (\%)}$$

When chemically determined water and protein mass are individually correlated with DEXA lean mass, high positive correlations were also observed for both parameters ($P < 0.0001$). As a result, absolute water and protein content can also be predicted by regression equations below. However, if proportions of water and proteins are correlated with proportional DEXA lean tissue, only water percentage correlated significantly with this lean percentage ($\rho = 0.72$, $P = 0.0004$) (Table 2). Therefore, only percentage of water can properly be estimated based on the DEXA lean values in the regression equation below.

$$\text{Total Protein (g)} = 8.492\ (\pm 16.411) + 0.244\ (\pm 0.008) * \text{Lean DEXA (g)}$$

$$\text{Total Water (g)} = 20.35\ (\pm 21.26) + 0.803\ (\pm 0.010) * \text{Lean DEXA (g)}$$

$$\text{Total Water percentage (\%)} = 5.535\ (\pm 13.897)\ 0.734\ (\pm 0.168)\ \text{Lean percentage DEXA (\%)}$$

Figure 2F presents the relation between DEXA BMC (g) and chemically determined ash (g) contents. Again, a highly positive correlation was observed ($P < 0.0001$), with DEXA BMC underestimating the total body ash content (36.53 ± 4.82 g BMC vs. 55.95 ± 7.98 g ash) ($P < 0.0001$). The equation, predicting total body ash by DEXA BMC is presented below. In contrast, percentage of ash was not significantly correlated with BMC percentage, no regression equation is thus useful ($\rho = 0.44$, $P = 0.0545$).

$$\text{Total Body Ash (g)} = -3.538\ (\pm 2.962) + 1.629\ (\pm 0.070) * \text{BMC (g)}$$

All established regression equations above had a significant slope ($P < 0.05$) and non-significant intercept different from 0.

Trial 2. Dissection of Female Breeders

DEXA values of lean tissue and fat mass were corrected according to the regression equations developed in trial 1. The results from trial 2 are shown in Table 3 which comprises the absolute weights (± SEM), correlation coefficient ρ, coefficient of determination R^2, the RMSE, and the significance of correlation between the dissection and DEXA results. Breast muscle mass is correlated with DEXA corrected whole body lean tissue mass, leaf fat, gizzard fat, and abdominal fat (leaf fat + gizzard fat) are correlated with DEXA-corrected whole body fat tissue mass.

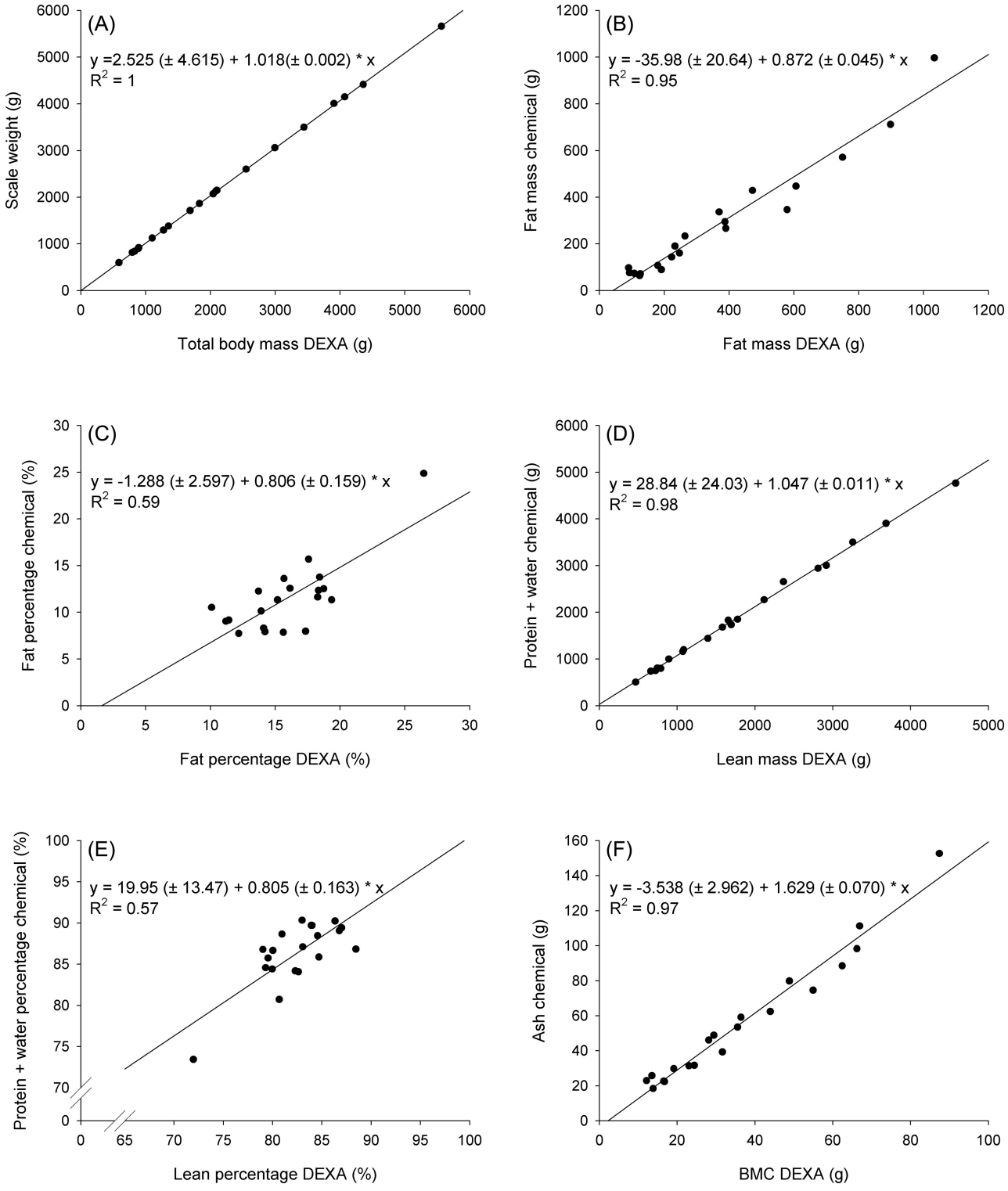

Figure 2. Least-squares regressions between DEXA estimates on *x*-axis and corresponding chemical analysis values on *y*-axis. Regression equations and coefficients of determination (R squared, R^2) are depicted on every figure. A: Total body mass, B: Fat mass, C: Fat percentage, D: Lean mass (chemical = protein + water), E: Lean percentage (chemical = protein + water), F: DEXA bone mineral content (BMC) vs. chemical ash.

Negative fat mass values obtained by DEXA were considered to be 0 because of the non-relevance of negative mass. For these birds, lean tissue mass was excluded from the analysis since lean tissue is overestimated because of negative fat values (lean tissue mass = total tissue mass—BMC—fat mass). Furthermore, fat mass regression equation in trial 1 showed a general overestimation of fat by DEXA, resulting in some negative fat weights after correcting for this excess. Negative corrected fat mass values were likewise considered to be 0.

Table 3. Comparison between dissection results and corresponding DEXA values. Correlation coefficient (ρ) and the coefficient of determination (R^2) with the root mean square error (RMSE) between dissected breast muscle and DEXA lean tissue mass and between dissected leaf fat, gizzard fat, abdominal fat, and DEXA fat mass are shown (n = 336).

Tissue comparison	Dissection	DEXA	ρ	R^2	RMSE
Breast muscle vs. DEXA lean mass (g)	396.6 ± 12.7	2116.9 ± 52.7	0.98***	0.96	194.13
Leaf fat vs. DEXA fat mass (g)	13.65 ± 0.75	85.71 ± 4.85	0.96***	0.92	30.08
Gizzard fat vs. DEXA fat mass (g)	2.93 ± 0.16	85.71 ± 4.85	0.89***	0.79	49.84
Abdominal fat[1] vs. DEXA fat mass (g)	16.57 ± 0.94	85.71 ± 4.85	0.96***	0.93	28.61

[1]Abdominal fat = Leaf fat + Gizzard fat.
***Correlation is significant with $P < 0.0001$.

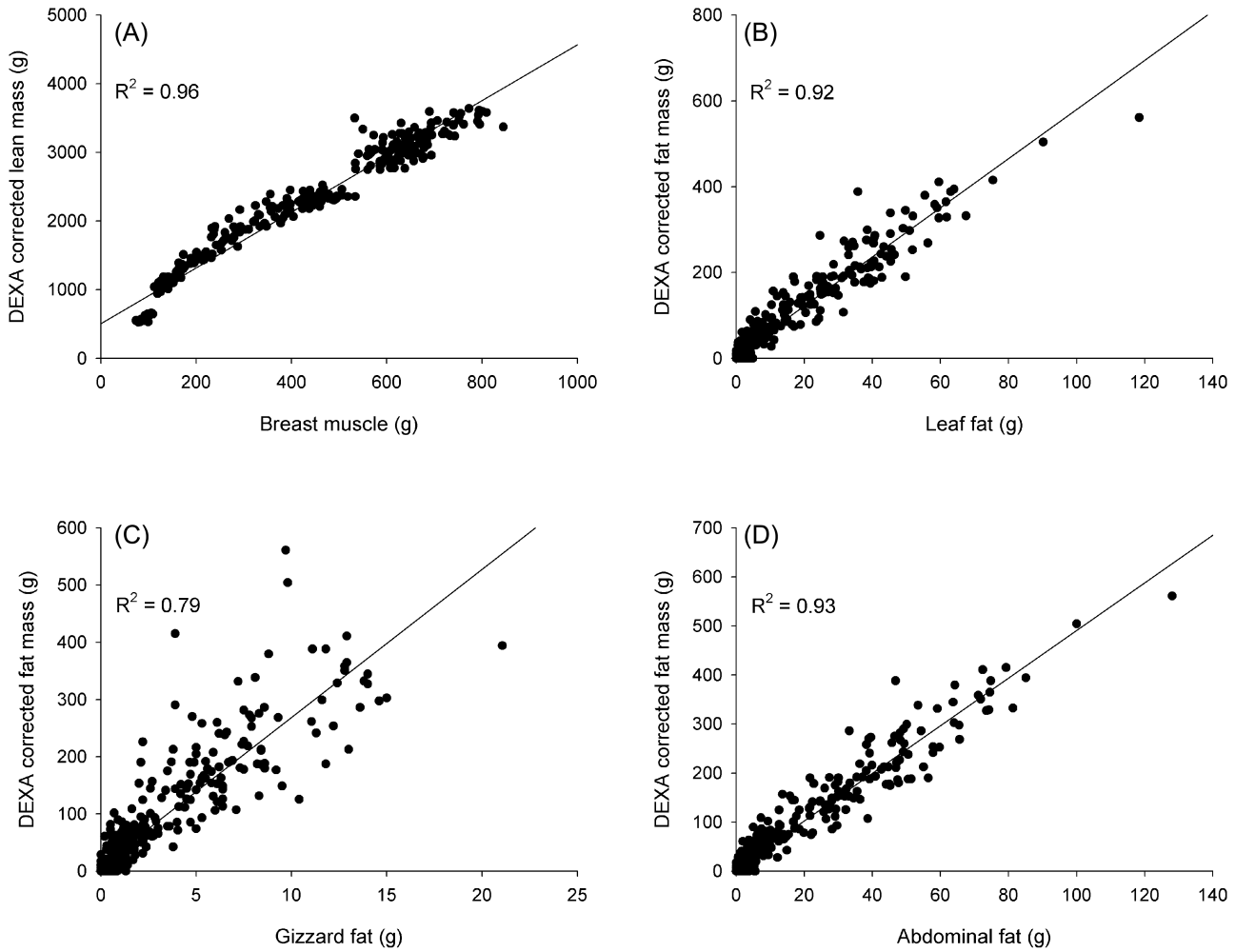

Figure 3. Least-squares regressions between dissection results on x-axis and corresponding DEXA values on y-axis. DEXA estimates are corrected based on the regression equations established in trial 1, also shown in Figure 2. Abdominal fat is the sum of leaf fat and gizzard fat. A: Breast muscle vs. DEXA-corrected lean tissue, B: Leaf fat vs. DEXA-corrected total fat, C: Gizzard fat vs. DEXA-corrected total fat, D: Abdominal fat vs. DEXA-corrected total fat.

A high positive correlation was present between breast muscle mass and DEXA-corrected lean tissue mass ($\rho = 0.98$, $P < 0.0001$) (Table 3, regression is depicted in Figure 3A). Leaf fat showed a high positive correlation with DEXA corrected fat tissue mass ($\rho = 0.96$, $P < 0.0001$) as well as gizzard fat, however, to a lesser extent than leaf fat ($\rho = 0.89$, $P < 0.0001$). Regression equations for these 2 tissues are depicted in Figure 3B and C. The sum of leaf and gizzard fat, denoted as abdominal fat, presented the best correlation with DEXA corrected fat, with $\rho = 0.96$ ($P < 0.0001$). Corresponding regression is shown in Figure 3D.

DISCUSSION

Trial 1 aimed to calibrate a Lunar Prodigy DEXA scanner against chemical carcass analyses to establish reliable predictive regression equations for broiler chickens. Hence, DEXA accuracy can be determined since chemical analysis is still the "gold standard" for measuring body composition. In addition, trial 2

investigated the correlation of DEXA whole body scans, corrected for equations developed in trial 1, and commonly used dissected tissues in determination of body composition and carcass quality. Importantly, since placement of the different ROI's can affect reproducibility and calculation of the body tissue amounts (Grier et al., 1996; Nagy and Clair, 2000), these ROI's were always defined in a consistent way by one researcher.

The results of the first trial demonstrated that dual energy X-ray absorptiometry is a reliable non-invasive tool for predicting the different body tissue masses. A nearly perfect correlation was found between DEXA estimated body mass and BW determined by a scale. DEXA slightly underestimated this BW, as can be seen in the 1.018 slope of the corresponding equation with an average difference of 1.87% (±0.12%). This underestimation of total body mass was the result of overestimating fat, while underestimating both lean tissue and ash, since the latter is composed of more tissue than only this BMC. Even though previous research with DEXA in chickens also reported high positive correlations in total body mass, they reported a small overestimation of this BW by DEXA (Mitchell et al., 1997; Korine et al., 2004; Swennen et al., 2004; Salas et al., 2012). In pigs, however, Mitchell et al. (1998a,b) reported a small underestimation of BW by DEXA as well. This discrepancy, between studies, however, small, underlines once more the importance of calibration for every specific instrument and software version. Research in other species also observed high correlations for total BW (Brunton et al., 1993; Mitchell et al. 1998a, Mitchell et al., 1998b; Speakman et al., 2001). This agreement with scale weight already indicates that DEXA can estimate total tissue as the sum of lean tissue, fat mas, and BMC in an accurate way.

The high correlation of fat mass was in good agreement with previous reports of Swennen et al. (2004) and Salas et al. (2012) with R^2 of 0.913 and 0.96 in chickens, respectively. This was also observed by Korine et al. (2004) in small birds, Bertin et al. (1998) in rats and Speakman et al. (2001) in dogs and cats. Mitchell et al. (1997), however, observed lower correlations for fat mass between chemical and DEXA analysis. This low correlation was mainly caused by larger discrepancies for birds weighing less than 2,000 g and was also highly dependent on the DEXA scan mode. Even though a high correlation was observed in the present study, DEXA overestimated fat by approximately 41%. This overestimation, however, to different extents, was also demonstrated in previous studies in various species (Brunton et al., 1993; Bertin et al., 1998; Nagy and Clair, 2000; Speakman et al., 2001; Korine et al., 2004; Swennen et al., 2004, Salas et al., 2012). Pietrobelli et al. (1998) and Speakman et al. (2001) attribute fat mass estimation errors to the variations in soft tissue hydration. Korine et al. (2004) also provides several possible explanations for different tissue mass overestimations in birds regarding DEXA phantom calibration and tissue estimation but also chemical carcass methodologies. Moreover, they observed a larger deviation for fat mass estimations in feathered birds compared to plucked birds, implying that DEXA mistakes feathers for fat because of the lower density of feathers. However, feathers consist over 90% protein in the form of keratin and contain only a small percentage of lipids (around 1%) (Saravanan and Dhurai, 2012). Since chickens were scanned and analyzed with feathers, a correction was made through the developed regression equation. It is thus possible to correct for this error in longitudinal studies on live chickens since plucking of the birds is not an option. Fat percentage was also positively correlated, however, to a lesser extent than fat mass (ρ = 0.77). This is in accordance with Swennen et al. (2004) who observed a correlation of $\rho = 0.593$, attributing this to the smaller range of values compared to the absolute data points.

Chemical values for lean tissue mass can be calculated as the sum of the protein and water content with water accounting for the largest part in this lean tissue mass. The high positive correlation between chemical and DEXA lean tissue mass was in accordance with previous research on various species (Mitchell et al., 1997; Nagy and Clair, 2000; Speakman et al., 2001; Swennen et al., 2004; Salas et al., 2012). However, Swennen et al. (2004) observed an average overestimation of the lean tissue by DEXA of 14.9% (±5.4%) whereas an average underestimation of 6.25% (±0.66%) was observed in the present study, again emphasizing the need for proper calibration of every unique scanner and software. The observed underestimation of lean tissue can partially be attributed to the aforementioned feather measuring discrepancy in which it is believed that feathers, mainly composed of protein, are misperceived as fat. However, established regression equation with $R^2 > 0.99$ allows for proper lean tissue prediction based on DEXA estimations. Similarly to fat percentage, lean tissue percentage was positively correlated but to a smaller extent than the absolute values (ρ = 0.76). As a consequence, it is realized that it will be more difficult to accurately discriminate between smaller differences in fat and lean percentage in a small range of tissue weights.

The high correlation between DEXA BMC and body ash was in accordance with previous reports from Swennen et al. (2004) and Salas et al. (2012). Mitchell et al. (1997), again observed a low correlation (R^2 = 0.46) between the 2 values. The relatively large difference, on average 33.39% (±1.82 %), between the 2 parameters was in agreement with other studies on chickens, pigs, and cats and dogs (Brunton et al., 1993; Speakman et al., 2001; Swennen et al., 2004; Salas et al., 2012). This underestimation is mainly caused by the fact that ash is comprised of minerals in both soft tissue and bones. As such, bones make up a large, but not complete part of the total body ash content. In addition, Schreiweis et al. (2003; 2005) reported a high

correlation between DEXA BMD and BMC with bone ash content in laying hens. Baird et al. (2008) also observed a high correlation between DEXA in vivo BMC and both DEXA ex vivo BMC and ash content of the tibia. However, in their study DEXA also underestimated tibia BMC in vivo compared to both ex vivo and ash contents. This indicates that possibly not only the soft tissue mineral content causes the DEXA BMC underestimation in our study, but that surrounding tissue when measuring bones in vivo can also affect the results. The high correlation in our study still allows for good estimation of total body ash by DEXA BMC when correcting with the developed regression equation. In general and regarding all examined tissues, discrepancies between studies can partially be due to the use of different machines with various versions of software. Importantly, Salas et al. (2012) used a more recently developed scanner, similar to the one used in this study.

To assess carcass quality in terms of fat and lean tissue contents in chickens, dissection is the most commonly used method. In research, however, this does not allow for longitudinal studies on the same animals and increases the number of experimental animals needed. In that regard, non-invasive, easy to use methods can be a good alternative to overcome these limitations. Especially in breeder research and selection programmes, it would be convenient to use favorable animals to reproduce and create the ideal offspring. Indeed, determining body condition would not mean killing and thus losing the animal. As such, sib-testing and selection can be avoided, lowering the costs and animals needed. Moreover, DEXA is less prone to sampling bias that can otherwise occur during body composition determination. Hence, we compared dissection results of abdominal fat and breast muscle to DEXA whole body tissue amounts. DEXA tissue estimations were corrected based on the regression equations from trial 1 before comparing to dissection parameters.

Breast muscle yield is a good estimate for total body lean tissue amounts as can be seen by the high correlation between the parameters ($\rho = 0.98$). The same observations were made for fat contents with the highest correlation between abdominal fat (leaf and gizzard fat) and total DEXA fat ($\rho = 0.96$). These findings are in accordance with older research from Becker et al. (1979) and Sonaiya (1985) that indicate that abdominal fat is a very good estimate for total body fat. Even though the measurement of abdominal fat by dissection is far less expensive and time consuming than determining whole body fat contents by chemical analysis, the method still requires killing of animals. This can be a challenging factor especially for research purposes with longitudinal studies and limited amount of birds. Here, we demonstrate that DEXA can be a reliable, non-invasive and easy to use method to estimate total body composition in chickens.

CONCLUSION

In this study, we demonstrated that DEXA is a reliable non-invasive technique for estimating broiler chicken body composition with proper regression equations. More specifically, all absolute tissue values obtained by dissection showed high correlation with DEXA estimates. Moreover, high correlations were also observed for breeder dissection results with DEXA whole body tissue amounts. As such, DEXA is a good alternative for determining body composition compared to some invasive, time-consuming, and costly techniques, allowing for longitudinal studies on the animals.

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