Accuracy of predicting chemical body composition of growing pigs using dual-

energy X-ray absorptiometry

C. Kasper [a], P. Schlegel [a], I. Ruiz-Ascacibar [a, 1], P. Stoll [a] and G. Bee [a]

[a] *Swine Research Unit, Agroscope, Tioleyre 4, 1725 Posieux, Switzerland*

[1] *Present address: Yara Iberian S.A.U., Infanta Mercedes St., E-28020 Madrid, Spain*

Corresponding author: Claudia Kasper. Email: claudia.kasper@agroscope.admin.ch

Short title: Body composition in pigs by DXA

Abstract

Studies in animal science assessing nutrient and energy efficiency or determining

nutrient requirements necessitate gathering exact measurements of body

composition or body nutrient contents. Wet chemical analysis methods or

standardized dissection are commonly applied, but both are destructive. Harnessing

human medical imaging techniques for animal science can enable repeated

measurements of individuals over time and reduce the number of individuals required

for research. Dual-energy X-ray absorptiometry (DXA) is particularly promising due to

its low acquisition and operating costs, low levels of radiation emission and simple

image processing. However, the measurements obtained with DXA do not perfectly

match dissections or chemical analyses, requiring the adjustment of the DXA via

calibration equations. In principle, DXA results should be independent of animal size

and body composition, because bone mineral content and the content of fat and lean

tissue are derived from the attenuation of X-rays transmitted through the body.

Several calibration regressions have been published, but comparative studies are

pending. Thus, it is currently not clear whether existing regression equations can be

directly used to convert DXA measurements into chemical values or whether each

individual DXA device will require its own calibration for the animal's breed, age,

class or sex. Our study builds prediction equations that relate body composition to

the content of single nutrients in growing entire male pigs (body weight range 20–100

kg) as determined by both DXA and chemical analyses, and we present the accuracy

of those predictions. Moreover, we show that the chemical composition of the empty

body can be satisfactorily determined by DXA scans of carcasses. This opens up

promising possibilities for the reduction of invasive procedures in the course of

nutritional studies. Finally, we compare existing prediction equations for pigs of a

similar range of body weights with the equations derived from our DXA

measurements and evaluate their fit with our chemical analyses data. We found that

existing equations for absolute contents that were built using the same DXA beam

technology predicted our data more precisely than equations based on different

technologies and percentages of fat and lean mass. This indicates that the creation

of generic regression equations that yield reliable estimates of body composition in

pigs of different growth stages, sexes and genetic breeds could be achievable in the

near future. DXA may be a promising tool for high-throughput phenotyping for genetic

studies, because it efficiently measures body composition in a large number and

wide array of animals.

Keywords: swine, bone mineral content, DXA, calibration, imaging techniques

Implications

The ability to determine body composition non-invasively opens opportunities for

improving studies of nutrition, nutrient balance and genetics. The present study

contains regression equations to estimate the nutrient composition, such as energy,

water, bone minerals (ash), total calcium, total phosphorus, total crude protein, total

nitrogen and total lipids) in the empty body of live pigs and in pig carcasses within a

body-weight range from 20 to 100 kg using DXA. We present regression equations to

estimate the empty body composition directly from the carcass. This rapid and non-

destructive method permits to reduce costs, time and number of animals needed for

research, since the same individuals can be scanned repeatedly.

Introduction

For studies in animal science that include measurements of nutrient and energy efficiency or the determination of protein, mineral and energy requirements, it is necessary to determine exact body composition. Two destructive methods are commonly used to determine body composition. Carcass dissection into fat, lean and bone tissue is used to determine carcass quality for nutrition and genetic selection studies. Wet chemistry analyses of the carcass are carried out to determine the nutrient content, and thus the nutrient and energy deposition rate and efficiency. The latter method certainly offers better accuracy and less operator bias, and it is commonly considered the gold standard. But both techniques are destructive, expensive and time-consuming, which precludes their application in studies requiring repeated measurements on individuals or large samples sizes. However, assessment of body composition or nutrient content *in vivo* could be used to evaluate the influence of feeding strategies, housing systems or environmental factors on the development of body composition or could facilitate monitoring nutrient efficiency. For genetics research, determining body composition and nutrient content with non-destructive methods could aid performance testing and the selection of parent individuals for breeding. Therefore, harnessing medical imaging techniques originally designed for humans, such as computed tomography (CT), magnetic resonance imaging (MRI) and dual-energy X-ray absorptiometry (DXA), is a promising area for animal science (Scholz et al., 2015). Imaging methods can be performed on carcasses or *in vivo* under anaesthesia, which enables repeated measurements of individuals through time, and can reduce the number of individuals required for research, as demanded by the 3R principles (National Centre for the Replacement, Refinement and Reduction of Animals in Research, 2019).

Medical imaging techniques such as DXA, CT and MRI not only enable repeated measurements of the same individual but also yield more reproducible results than dissections. Among medical imaging techniques, DXA has advantages that make it particularly attractive for application in animal science (Pomar et al., 2017), which mainly include financial and work security aspects. Compared to CT and MRI, DXA is easier to use, has lower instrument and operating costs, a more rapid scan speed, little to no operator bias and requires less image processing, consequently allowing for quick data analyses (Scholz et al., 2015). Moreover, ionizing radiation emitted by DXA is relatively low (Genton et al., 2002), making it a secure device for operators. The DXA provides information about lean mass, fat tissue mass and bone mineral content (BMC), the sum of which represents the total body mass. It also measures the bone surface, allowing for determination of bone mineral density (BMC divided by bone surface). However, because DXA is an indirect tool, it does not provide true values, although its values are precise and have, for example, less incidence of random error (Kipper et al., 2019a). Thus, the information from DXA devices does not fully correspond with the data from invasive techniques, and it is therefore necessary to adjust measurements to reference dissections or, ideally, chemical analyses via prediction equations (Mitchell et al., 1998b). Regression equations have been published for a range of breeds, sexes and weight classes of pigs, using different DXA beam technologies (Mitchell et al., 1998a; Mitchell et al., 1998c; Pomar et al., 2001; Mitchell et al., 2003; Suster et al., 2003; Pomar et al., 2017; Kipper et al., 2019b). The image-processing software on DXA devices used to compute tissue masses was developed for the human body. It is optimized for scanning humans in a supine position, and it might rely on human-specific assumptions about the

distribution of fat, lean tissue and bone tissue (Genton et al., 2002; Hunter et al.,

2011). Moreover, because algorithms are proprietary, these assumptions cannot be

checked or adapted to animal species.

The results of imaging techniques should, in principle, be independent of animal size

and body composition, and therefore, regression equations might not require

adjustment for different growth stages, sexes and genetic breeds (Mitchell et al.,

1998a; Marcoux et al., 2005; Soladoye et al., 2016). To date, no comparative study

has evaluated the fit of previously published regression equations to a new set of

data. Thus, it is unclear whether existing regression equations can be directly used to

convert DXA measurements into chemical values or whether each individual DXA

device requires its own calibration. The objectives of this study on pigs of 20 to 100

kg were 1) to estimate the chemical composition of the EB from values determined

by DXA on the live animal; 2) to estimate the chemical composition of the carcass

from the values determined by DXA on carcass; 3) to estimate the chemical

composition of the EB from the carcass values determined by DXA, over the

complete range of body weights. Finally, we compared existing prediction equations

with the equations derived from our data and evaluated their fit with our data.

Materials and methods

Animals

We used 68 entire males originating from 17 litters in two farrowing series of the

Agroscope Large White pig dam line. The details of housing, feeding and slaughter

procedures were the same as described by Ruiz-Ascacibar et al. (2017). In brief, pigs

with a mean BW of 8.9 kg (± 2.1 kg SD) were selected at weaning and and kept in

17.35 m^2 pens in groups of a maximum of 14. The pigs had *ad libitum* access to a

diet based on BW, with the pigs having < 20 kg BW receiving a weaning diet, those

between 20 and 60 kg BW getting one of two grower diets and pigs with > 60 kg BW

(20–60 kg BW) being fed one of two finisher diets. The grower and finisher diets

differed by 20% in their CP content (165 vs 139 g CP per kg grower diet and 140 vs

117 g CP per kg finisher diet). Both diets were formulated to contain the same

amount of digestible energy (13.2 MJ/kg feed). Pigs were either slaughtered at a live

BW of 20 kg ($N = 6$) or kept on feed until they reached a target BW of 60 kg ($N = 18$)

or 100 kg ($N = 37$ for EB, and $N = 44$ for carcass), as determined by weekly weighing

(Grüter SST-WA-03, Eschenbach, Switzerland; ± 100 g). Three days prior to

slaughter (-3), the pigs were fasted for at least 16h and then sedated using isoflurane

(Attane, Piramal Critical Care, Inc., Bethlehem, PA, USA) prior to DXA scanning.

Sedation was conducted to prevent movement during the scanning procedure. The

pigs were scanned on the DXA and subsequently their BW was determined using a

scale (BW$_{-3}$). The awakened pigs were brought back to their respective pens. The

live DXA scans under anaesthesia were carried out three days before slaughter in

order to meet the required withdrawal period for isoflurane before the sale of

carcasses for human consumption. A technical problem occurred on the DXA that

disabled scanning for a period of two weeks. Thus, live scans of seven 100 kg

category pigs could not be conducted. After 16 h of feed withdrawal, the pigs were

slaughtered using the Agroscope experimental abattoir for exsanguination after CO_2

stunning. Pigs were weighed (Berkel IWS IT 6000, Berkel-Obrecht, Spreitenbach,

Switzerland; ±200 g) before and after exsanguination to determine blood mass. The

BW prior to exsanguination corresponds to the BW at slaughter (BW$_{slaughter}$).

Immediately after exsanguination, hair and claws were removed and collected.

Animals were then eviscerated and the carcass and head were sawed into two

halves. The two half carcasses including the head without brain were weighed for

correspondence with the warm carcass weight. Then, they were chilled at 2°C and a

cold carcass weight was determined 24 h *post mortem*. The left carcass halves

(including halved head without brain) were then processed into primal cuts (head,

neck, shoulder, ham, loin, tail and feet) and scanned using DXA. Subsequently, the

cuts were frozen for further processing to determine the carcass chemical

composition as described by Ruiz-Ascacibar et al. (2017). To prevent thawing and re-

freezing, the carcass halves were divided into primal cuts before scanning because

grinding prior to chemical analysis is always performed in a frozen state. The carcass

cuts of the seven pigs slaughtered during the DXA failure were exceptions in being

thawed for the DXA scanning and then re-frozen. The gall bladder, bladder, stomach,

intestine and hind gut were emptied and rinsed with clean water to remove any

remaining bile, urine and digesta. The eviscerated organs and brain were then

pooled and homogenized for further processing in order to determine the chemical

composition of the EB as described by Ruiz-Ascacibar et al. (2017).

DXA image acquisition

A GE Lunar DXA (i-DXA, GE Healthcare Switzerland, Glattbrugg, Switzerland) with a

narrow-angle fan beam (Collimator Model 42129) was used to scan the live pigs and

carcass halves. The DXA has a 100 kV generator and K-edge filtering, resulting in

approximately 39 keV and 71 keV X-ray effective energies. The calibration was

checked and passed before each scanning session by scanning a calibration

phantom according to the manufacturer's instructions. Scans were conducted using

the 'total body thick' mode (0.188 mA, scan speed 80mm/s according to Carver et al.,

2013) with enCORE software (version 16). It has to be noted that, according to the

manufacturer, this mode is not recommended for pigs of smaller sizes. In this study,

the mode with the highest level of radiation was used for all individuals regardless of

size. This was done in order to avoid switching between modes, which could

introduce additional variation between the measurements of pigs of different BW

classes. The sedated pigs were placed on the DXA table in a standardized way in a

prone position with the hind legs extended while the front legs were positioned along

the side but kept away from the body by two wedges of foam plastic (Figure 1a). The

left carcass cuts were placed next to each other on the DXA table with the skin side

facing downwards. The cuts were arranged in a standardized manner to reflect an

uncut half carcass (Figure 1b). All scans started from the head.

Figure 1 (a) Positioning of live animals on the DXA table for scanning under anaesthesia. **w:** Plastic
foam wedge used to keep the front legs away from the body. **m:** Mask for the continuous
administration of isoflurane. **(b)** Positioning of cut carcass on the DXA table.
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DXA image processing

Scan images from live animals were processed to remove artefacts (i.e. regions not

belonging to the animal, such as the mask and tube on the sedating apparatus and

the ear tag) and to position the regions of interest (ROI). The ROI were placed in

order to be similar to the human ROI according to the supplier's guidelines. This was

done by defining reference points for the pig (Supplementary Material Section 1,

Figure S1). The ROI in the scans of carcasses were set on "right arm" for all primal

cuts. The variables 'total mass', 'BMC' (bone mineral content), 'lean' and 'fat' of the

live whole body and of each left carcass half (listed in the upper part of Table 1) were

exported from the software and used for further analysis.

Laboratory analyses and calculations

Chemical analyses of energy, water, ash, Ca, P, N and lipid content (listed in the

lower part of Table 1) were conducted as described in Ruiz-Ascacibar et al. (2017;

2019). In brief, all fractions of the $EB_{slaughter}$, which were 1) the pooled left carcass

cuts, including the left half of the head without brain, 2) the pooled empty digestive

tract, the organs and brain, 3) the hair and claws and 4) the blood, were ground,

homogenised and dried separately. For the $EB_{slaughter}$, all calculations of contents

were based on warm carcass weight (to closely mimic the situation in the live

animal), and for the carcass, they were based on cold carcass weight. The contents

of energy, water, ash, Ca, P, N and lipids in the left carcass half were combined with

those of the right carcass half to obtain values for the full carcass. The CP content

was calculated by multiplying the N content determined by wet chemistry by 6.25.

Analyses were conducted in duplicate, but if results differed by more than 5%, up to

four replicates were analysed. Since the live pigs were scanned by DXA three days

prior to slaughter, the chemical composition of the EB at that time (EB_{-3}) was

obtained individually from the $EB_{slaughter}$ values as follows: The chemical composition

of $EB_{slaughter}$ was divided by $EBW_{slaughter}$ and multiplied by EBW_{-3}. The EBW_{-3}

corresponded to the BW_{-3} multiplied by 95.3%, 96.2% or 95.6% (the average ratio of

EBW slaughter to BW slaughter for the 20, 60 and 100 kg category pigs,

respectively).

Statistical analysis of results

The plausibility of the data was checked before the statistical analysis

(Supplementary Material Section 2), including a check of the differences between the

total levels of raw ash, CP, lipids and water and the $EBW_{slaughter}$ and cold carcass

weight obtained by scales. Histograms of the raw data obtained by chemical

analyses (Figure S2) were visually checked. In all analyses, the experimental unit

was the individual pig. The code on which statistical analyses were based in R

(version 3.6.3; R Core Team, 2020) can be found in the Supplementary Material. For

the comparison of chemical and DXA-derived values, we conducted Welch's two-

sample t-test for unequal variances. To compute the difference, we subtracted the

mean of the respective chemical value from the DXA value and expressed it as a

percentage of the chemical value. Regression equations were computed with the lm()

command, and figures were created using the package ggplot2 (version 3.2.1;

Wickham, 2016). BW_{DXA}, $energy_{DXA}$, $water_{DXA}$, ash_{DXA}, Ca_{DXA}, P_{DXA}, CP_{DXA}, N_{DXA} and

$lipid_{DXA}$ were used to denote the values predicted by the regression equations. To

facilitate interpretation, we kept variables on their original scale in all models. The

models for predicting EBW_{-3} and carcass weight contained total mass by DXA as the

sole predictor variable. A stepwise reduction of linear models was used to select

estimates of body composition provided by DXA. The full models for the prediction of

all chemical parameters included BMC, lean tissue and fat tissue as estimated by

DXA. In case more than one of the predictors of the full model was not significant, we

first omitted the variable from which, from a biological point of view, we expected the

weakest relationship to the estimated chemical content. Because the data stem from

an experiment with different dietary treatments, we examined whether dietary

treatment had an effect on the slopes and intercepts of the regression equations

(Supplementary Material Section 3). The effect of dietary treatment was never

significant and the inclusion of the dietary treatment changed slopes and intercepts

only marginally (Table S1); thus, we present models without dietary treatments.

Adjusted R^2 as a measure of goodness-of-fit was extracted from the R model object,

and the root mean square error (RMSE) was calculated as $\sqrt{\frac{\sum_{i=1}^{n}(measured - predicted)^2}{n}}$,

where *measured* is the value determined by chemical analysis, *predicted* is the value

estimated from the DXA measurement via the regression equation and *n* is the

sample size. To facilitate comparison among variables with a wide range of contents

in the body, we computed the residual coefficient of variation (rCV), which is the

percentage of the RMSE to the chemically measured value, as a standardized

metric. We performed cross-validation of the regression equations via a jack-knife

approach by iteratively omitting one data point from the data set and computing the

prediction error, using the leave-one-out method in the caret package (version 6.0-

86; Kuhn, 2020). The models did not fully comply with the assumptions for linear

least squares regressions (Supplementary Material Section 4), which could possibly

lead to a misestimation of the parameters. Thus, using the leave-one-out cross-

validation procedure, we checked whether the estimates of intercepts and slopes

were robust or if they deviated from our estimates due to a disproportionate leverage

of certain data points. This also allowed us to derive non-parametric confidence

intervals for slopes and intercepts (for details, see Supplementary Material Section

4). Even though our main goal was to build regression equations over the whole

range of slaughter weights, we also present specific (regional) regressions for each

target slaughter-weight category (20, 60 and 100 kg, see Supplementary Material

Section 5). This was done to compare the slopes and intercepts of regional

regressions with the ones of the global regression. However, these are probably less

reliable since they are based on only a subset of the data, and the number of

observations for each regression is low, in particular for the 20 kg category.

Finally, we compared the prediction equations we obtained from our data set with

those published in the literature (Mitchell et al., 1998a; Mitchell et al., 1998c; Pomar

et al., 2001; Mitchell et al., 2003; Pomar et al., 2017; Kipper et al., 2019b). To do this,

we applied the published equation to the DXA values of our data set and computed

the RMSE of the values predicted using the published equations and the chemically

measured values from our study.

Results and discussion

Correspondence of wet chemistry and DXA measurements

Table 1 shows the descriptive statistics of DXA values from the live pigs three days

prior to slaughter and from the carcass, the scale weight of the EB_{-3} and carcass and

the wet-chemistry values from the EB_{-3} and the carcass, respectively. Table 2 shows

the results of testing for significant differences between DXA and chemical values.

Scale weight and DXA weights of the EB and carcass were comparable ($P > 0.05$),

with a mean difference of 1% in both EB and carcass. As noted previously, the exact

correspondence of body weight measured by DXA and weigh scales does not

necessarily mean that the body composition determined by the two measuring

methods is equally accurate (Mitchell et al., 1998c). In contrast to body weight, the

values for ash, CP and lipids obtained from chemical analyses differed ($P < 0.01$)

from the BMC, lean tissue and fat mass obtained by DXA, except for the values of

lipids and fat in the carcass ($P > 0.05$). Such observations were reported previously

(Suster et al., 2003). The DXA readout does not provide information on water

content. Therefore, the water content in the fat-free mass (sum of CP, ash and

water), which was determined by chemical methods, must be taken into account. It

represented 76% (± 1.46% SD; CV=1.91%) in the EB and 74% (± 1.47% SD;

CV=2.00%) in the carcass. This content is in line with the reported 73% to 77% water

in fat-free mass (Speakman, 1997; Bocquier et al., 1999; Lerch et al., 2015).

Because the major proportion of body water is in the fat-free mass and because DXA

derives lean tissue mass indirectly via body water (Hunter et al., 2011), the water

may be attributed with CP to DXA lean. The sum of CP and water mass was

comparable ($P > 0.05$) with lean mass. The higher concordance between lean mass

and CP with water in the carcass in comparison to the EB could be explained by gut

water content and/or a lipid-to-protein ratio in the intestines and organs that differs

from that in the carcass.

The BMC was 34% and 21% lower ($P < 0.01$) than the ash content of the EB and

carcass, respectively. The skeleton is rich in minerals and contains a high proportion

of certain minerals, such as Ca and P. However, other minerals found in body ash

are mainly present in body tissues and fluids, so they are not considered as part of

BMC. Thus, BMC is expected to be lower than body ash. When comparing BMC with

ash in single bones, the correspondence of the values was greatly improved

(Schlegel and Gutzwiller, 2020). Fat tissue mass was 24% lower ($P < 0.05$) than lipid

content in the EB but comparable ($P > 0.05$) in the carcass. The fat content of the

gastrointestinal tract, the organs and the visceral adipose tissue (see Weigand et al.,

2020) may have caused this discrepancy.

Table 1. *Body and carcass composition of growing pigs determined at 20, 60 and 100 kg body weight*

	BW category	Empty body				Total carcass			
		Mean	SD	Min[a]	Max[b]	Mean	SD	Min[a]	Max[b]
DXA	**Total mass (g)**								
	20	18756	884	17819	20350	13438	808	12667	14981
	60	57979	2410	54005	62604	44229	2178	40386	49095
	100	99963	3437	92085	106175	75916	2614	70892	81680
	BMC[c] (g)								
	20	414	12	395	427	450	21	420	474
	60	1378	94	1194	1569	1497	109	1279	1696
	100	2357	222	1874	2790	2522	265	1895	3057
	Lean (g)								
	20	16957	781	16083	18365	11535	691	10828	12725
	60	50452	2219	47393	54702	35904	2003	32395	39253
	100	84050	3836	78359	91771	59948	2707	54925	67860
	Fat (g)								
	20	1384	133	1216	1558	1454	294	991	1786
	60	6149	804	4991	7876	6828	920	4939	8793
	100	13556	2134	9013	16746	13446	2000	9087	17357
Wet Chemistry	**EBW-3 or carcass weight (g)**								
	20	18400	876	17500	20000	13520	800	12809	15055
	60	57117	2320	53250	61600	43971	2144	40225	48886
	100	98619	3391	90650	104800	75409	2551	70474	81081
	Energy content (MJ)								
	20	120	12	108	141	105	13	94	128
	60	535	38	479	627	476	39	420	576
	100	1028	78	858	1192	899	69	752	1048
	Water (g)								
	20	12761	504	12182	13664	9333	455	8882	10160
	60	35737	1628	33925	39583	26909	1343	24533	29328
	100	58585	2762	54251	64877	44203	2066	40703	50787
	Ash (g)								
	20	556	36	488	586	542	40	473	583
	60	1844	130	1562	2066	1799	137	1538	1987
	100	3156	469	2043	4261	3054	451	1941	4118
	Calcium (g)								
	20	156	13	137	172	166	15	147	186
	60	564	68	463	755	582	70	475	766
	100	955	179	477	1397	974	175	487	1413
	Phosphorus (g)								
	20	95	10	77	103	94	10	76	103
	60	317	33	272	402	313	35	258	394
	100	545	75	345	715	532	75	333	701
	CP (N×6.25, g)								
	20	2709	104	2585	2880	2200	114	2084	2409
	60	9264	644	8231	10343	7745	644	6742	9003
	100	16079	909	14210	18218	13279	844	11487	15595
	Nitrogen (g)								
	20	433	17	414	461	352	18	333	385
	60	1482	103	1317	1655	1239	103	1079	1440
	100	2573	145	2274	2915	2125	135	1838	2495
	Lipids (g)								
	20	1454	245	1170	1829	1394	255	1132	1806
	60	8142	989	5951	10226	7554	972	5486	9768
	100	16567	2269	10910	20750	14964	2013	10016	18912

329   [a]Minimum
330   [b]Maximum
331   [c]Bone mineral content

	Item DXA	Item Chemical	DXA [g]	Chemical [g]	Δ%[a]	t[b]	df	*P*
Empty Body	Total mass	EBW$_{-3}$	79587 ± 27838	78482 ± 27504	1	0.22	119.98	0.826
	BMC[c]	Ash	1877 ± 680	2513 ± 954	−34	−4.22	108.47	<0.001
	Lean	CP	67537 ± 22844	12753 ± 4602	81	18.36	64.86	<0.001
	Lean	CP + water	67537 ± 22844	60089 ± 20181	11	1.91	118.20	0.059
	Fat	Lipid	10173 ± 4749	12594 ± 5609	−24	−2.57	116.83	0.011
Carcass	Total mass	Carcass weight	62016 ± 20708	61626 ± 20521	1	0.11	133.99	0.913
	BMC[c]	Ash	2068 ± 711	2500 ± 901	−21	−3.1	127.11	0.002
	Lean	CP	49312 ± 16005	10836 ± 3705	78	19.31	74.16	<0.001
	Lean	CP + water	49312 ± 16005	47385 ± 15215	4	0.72	133.66	0.473
	Fat	Lipid	10636 ± 4407	11806 ± 4895	−11	−1.46	132.55	0.146

Given the expected discrepancies between the values obtained by DXA and wet

chemistry, the conversion of DXA estimates of body composition via prediction

equations was necessary to obtain quantitative estimates of body composition. The

prediction equations had similar high precision and accuracy for EB and carcass. The

non-parametric confidence intervals show that estimates of slopes and intercepts

were robust (Table 3) even though model diagnostics were not optimal (see

Supplementary Material Section 4).

Weight

The EBW and carcass weight could be predicted by the regression equations using

DXA total mass with the highest R^2 and lowest rCV (Table 3, Figure 2, Figure 3).This

was expected given the non-significant difference between the weights obtained by

scales and DXA (Table 2). The RMSEs in the EB and the carcass were very similar,

and we obtained an excellent fit (R^2 = 1 after rounding) with both EB and carcass.

The intercept of the prediction equation was slightly shifted below zero for the EB,

and above zero for the carcass. The slope was almost 1 in the EB and slightly below

1 in the carcass. This shows that DXA can estimate the BW with very high reliability.

The coefficients of regional regressions for each target slaughter-weight category

were very similar to the global regression and all obtained very high R^2 (Table S2,

Figures S3 and S4).

CP, N, water and lipids

For energy$_{DXA}$, CP$_{DXA}$, N$_{DXA}$ and water$_{DXA}$, we obtained excellent precision and

accuracy in terms of R^2 (> 0.99) (Figure 2 and 3) and error (rCV < 3.6%) when we

back-predicted the chemical values using the equations presented in Table 3.

Lipid$_{DXA}$ was slightly less precise (R^2 > 0.97). Accuracy was better in the EB than in

the carcass (rCV = 5.4% and 7%, respectively). The DXA values for fat were

consistently lower than chemically measured lipid values for the pigs in the 20-kg

slaughter category, which was more pronounced in the empty body than in the

carcass. The same phenomenon was observed in a study comparing DXA and

chemical analyses in small pigs (5 to 27 kg live weight; (Mitchell et al., 1998b). It

appears that DXA overestimates the fat content of pigs with a high percentage of

body fat (> 20%) and underestimates the fat content in lean pigs (Mitchell et al.,

1998b). Similar findings have also been reported in humans (Genton et al., 2006).

Because CP in the present study was directly calculated from N, the precision and

error of the prediction equations for N$_{DXA}$ are analogous to the ones for CP$_{DXA}$ from

lean mass. The ease with which we could predict water content from lean tissue by

DXA (water$_{DXA}$) can be related to the fact that lean tissue mass is not measured

directly by DXA but is instead derived from the water content and is based on specific

assumptions, such as a fixed ratio of lean tissue mass to water content (Hunter et al.,

2011; but note that they used a different DXA device and software). Regional

regression lines overlapped with the global one and obtained similar R^2 and error,

except for CP and N, which had reduced R^2 (Table S2, Figures S3 and S4).

Minerals

Predictions of ash_{DXA}, Ca_{DXA} and P_{DXA} were less precise (R^2 between 0.80 and 0.89)

and subject to major error (rCV between 12.0 and 16.5%; Table 3). Ca and P

contents are certainly strongly correlated with the ash content, and one must expect

that these predictions cannot be better than those of the ash content. Whereas Ca_{DXA}

solely depended on BMC, P_{DXA} also included lean mass, which is in line with the

knowledge that body Ca and P are found at about 99% and 80%, respectively, in the

bones (Suttle, 2010). A similar lower prediction accuracy for ash has been reported in

other studies (Suster et al., 2003). The slopes of regional regressions for each target

slaughter-weight category differed importantly for ash and P, but were rather similar

for Ca (Table S2, Figures S3 and S4). Fitting regional regressions did not only result

in reduced prediction errors, but also reduced R^2 compared to the global regression.

This suggests that it is difficult to fit reliable regional calibration models for ash, Ca

and P with the present dataset, and that more data and more work is needed.

Figure 2 Predicted values against measured chemical values in the empty body of growing pigs.
Predicted values were obtained by applying the regression equations to the DXA measurements.
Dots represent individual data points.

Figure 3 Predicted values against measured chemical values in the carcass of growing pigs.
Predicted values were obtained by applying the regression equations to the DXA measurements. Dots
represent individual data points.

Prediction of empty body composition at slaughter from carcass

The use of carcass DXA scans to predict the ash and CP content of the EB produced

levels of accuracy and low error that were equivalent to those obtained by predicting

EB ash and CP content from live scans (Table 3, Figure 4). The prediction of lipid

content of the EB using carcass fat content determined by DXA was marginally less

precise and had greater error (R^2 = 0.96 vs 0.97, rCV = 8.5% vs 5.4%) than

predictions from live scans (Table 3, Figure 4). With increasing BW, the regression

line of the prediction models for CP_{DXA} in the EB based on carcass scans deviated

from those with the live scans. The reason for this might be that a considerable

amount of lipids and CP are contained in the organs and thus missing from the

carcass. Depending on the nature of the problem (e.g. when repeated measurements

are not necessary) and the required degree of accuracy and prediction, invasive

scanning of live animals requiring anaesthesia could, therefore, be replaced by

carcass scans.

Table 3. *Models to estimate chemical composition of the empty body and the carcass from DXA values*

	Chemical variable (predicted variable)	Term (DXA variable)	Estimate[a]	P	R^2	RMSE[b]	rCV[b]
Empty body	**Empty body weight** (BW_{DXA})	Intercept	−149.059 (-157.219, -142.729)	0.015	1.000	150.08 g	0.20%
		Total mass	0.988 (0.988, 0.988)	< 0.001		154.12 g	0.20%
	Energy content ($energy_{DXA}$)	Intercept	-34.94 (-35.915, -33.029)	0.001		22.90 MJ	2.90%
		Lean	0.007 (0.006, 0.007)	< 0.001	0.995		
		Fat	0.038 (0.037, 0.038)	< 0.001		24.22 MJ	3.10%
	Water ($water_{DXA}$)	Intercept	1254.428 (1216.314, 1287.547)	< 0.001		578.90 g	1.20%
		Lean	0.682 (0.682, 0.683)	< 0.001	0.999	594.95 g	1.30%
	Ash (ash_{DXA})	Intercept	32.3 (18.894, 44.955)	0.792		316.73 g	12.60%
		BMC	1.322 (1.313, 1.333)	< 0.001	0.886	325.01 g	12.90%
	Ca (Ca_{DXA})	Intercept	-2.807 (-7.461, 1.962)	0.953		123.37 g	16.20%
		BMC	0.407 (0.404, 0.411)	< 0.001	0.829	126.50 g	16.60%
	P (P_{DXA})	Intercept	-9.819 (-12.744, -7.637)	0.653		52.20 g	12%
		BMC	0.145 (0.131, 0.156)	< 0.001	0.893		
		Lean	0.003 (0.002, 0.003)	0.033		55.49 g	12.80%
	CP (CP_{DXA})	Intercept	-784.767 (-805.418, -760.985)	< 0.001		454.08 g	3.60%
		Lean	0.2 (0.2, 0.201)	< 0.001	0.99	466.43 g	3.70%
	N (N_{DXA})	Intercept	-125.563 (-128.867, -121.758)	< 0.001		72.65 g	3.60%
		Lean	0.032 (0.032, 0.032)	< 0.001	0.99	74.63 g	3.70%
	Lipids ($lipid_{DXA}$)	Intercept	-294.21 (-342.958, -230.694)	0.336		690.75 g	5.50%
		Fat	0.986 (0.974, 0.996)	< 0.001	0.984		
		Lean	0.042 (0.04, 0.045)	< 0.001		730.25 g	5.80%
Carcass	**Carcass weight** (BW_{DXA})	Intercept	172.744 (164.49, 180.989)	0.005		151.36 g	0.20%
		Total mass	0.991 (0.991, 0.991)	< 0.001	1.000	154.90 g	0.30%
	Energy content ($energy_{DXA}$)	Intercept	-36.077 (-36.933, -34.427)	0.002		27.10 MJ	3.80%
		Lean	0.007 (0.007, 0.008)	< 0.001	0.990		
		Fat	0.036 (0.036, 0.037)	< 0.001		28.57 MJ	4.00%

	Analyte	Parameter	Coefficient (CI)	p-value		RMSE	rCV
	Water (water$_{DXA}$)	Intercept	1058.187 (1037.353, 1083.199)	< 0.001	0.998	545.64 g	1.50%
		Lean	0.720 (0.719, 0.720)	< 0.001		558.48 g	1.50%
	Ash (ash$_{DXA}$)	Intercept	-33.611 (-46.826, -19.201)	0.791	0.875	311.89 g	12.50%
		BMC	0.807 (0.766, 0.856)	< 0.001		325.87 g	13.00%
		Lean	0.018 (0.016, 0.019)	0.026			
	Ca (Ca$_{DXA}$)	Intercept	19.401 (13.755, 25.059)	0.701	0.799	132.05 g	16.50%
		BMC	0.377 (0.374, 0.381)	< 0.001		135.06 g	16.90%
	P (P$_{DXA}$)	Intercept	-6.388 (-9.128, -3.897)	0.781	0.863	56.53 g	13.00%
		BMC	0.109 (0.103, 0.121)	0.001		59.43 g	13.70%
		Lean	0.004 (0.004, 0.005)	0.002			
	CP (CP$_{DXA}$)	Intercept	-482.745 (-500.385, -462.708)	0.014	0.983	476.85 g	4.40%
		Lean	0.230 (0.229, 0.230)	< 0.001		487.59 g	4.50%
	N (N$_{DXA}$)	Intercept	-77.239 (-80.062, -74.033)	0.014	0.983	76.30 g	4.40%
		Lean	0.037 (0.037, 0.037)	< 0.001		78.01 g	4.50%
	Lipids (lipid$_{DXA}$)	Intercept	-538.43 (-568.553, -483.998)	0.121	0.970	828.34 g	7.00%
		Fat	0.933 (0.916, 0.948)	< 0.001		870.83 g	7.40%
		Lean	0.049 (0.045, 0.052)	0.001			
Empty body from carcass	Empty body ash (ash$_{DXA}$)	Intercept	65.76 (52.137, 79.908)	0.595	0.887	317.47 g	12.30%
		BMC carcass	1.252 (1.245, 1.263)	< 0.001		325.73 g	12.60%
	Empty body CP (CP$_{DXA}$)	Intercept	-349.157 (-372.694, -321.981)	0.131	0.985	564.13 g	4.30%
		Lean carcass	0.28 (0.279, 0.28)	< 0.001		579.08 g	4.40%
	Empty body lipid (lipid$_{DXA}$)	Intercept	-688.199 (-727.719, -628.837)	0.106	0.967	999.45 g	7.70%
		Fat carcass	1.067 (1.048, 1.088)	< 0.001		1058.83 g	8.20%
		Lean carcass	0.055 (0.05, 0.059)	0.004			

424 [a]Including their non-parametric confidence intervals, CI. For empty body: 93% CI, for carcass: 94% CI, empty body from carcass: 93% CI (see Supplementary
425 Material Section 4).
426 [b]Note: Upper value: RMSE and rCV from back-prediction of chemical values using the regression equations, lower value: RMSE and rCV from cross-
427 validation (leave-one-out method)

Comparison with published regression equations

Empty body

Our results are in line with published regression equations for the EB, which were

built from wet chemistry analyses and live scans of pigs ranging from 5 to 97 kg live

BW (Mitchell et al., 1998a), pigs with a mean live BW of 105.6 kg (Kipper et al.,

2019b) and a mean warm carcass weight of 85 kg (Pomar et al., 2001, as cited in

Pomar et al., 2017). Figure 5 shows the data points with regressions derived from

this study and the regression lines from the literature for the EB (or live animal) for

CP, water, fat and ash in the EB (or live animal). Concerning CP, the regression

equations by Kipper et al. (2019b) (Figure 5, 2nd row) and Pomar et al. (2017) (Figure

5, 3rd row fit our data quite well, whereas the one by Mitchell et al. (1998a) (Figure 5,

1st row) had a slightly worse fit. The intercepts of published equations and our study

differ by 109 g to 784 g CP, which is a rather small amount given the total protein

mass of the EB. Slopes were almost identical to our study (Figure 5). Using the

regression by Mitchell et al. (1998a) to predict data from our study led to an

overestimation of CP content in the EB throughout all weight categories. A possible

explanation for the slightly worse fit of the regression equations from Mitchell et al.

(1998a) than those from Kipper et al. (2019b) and Pomar et al. (2017) could be the

differences in the DXA technology used. As in the present study, Kipper et al.

(2019b) and Pomar et al. (2017) used a DXA scanner with a narrow fan beam,

whereas Mitchell et al. (1998a) used the pencil-beam technology. It has been shown

previously that different devices, and particularly different beam technologies, yield

considerably variable results in terms of total BW (compared to scales), along with fat

and lean mass in human subjects (Genton et al., 2002) and livestock (Scholz et al.,

2015). It is conceivable that in the case of extreme proportions of body fat, such as,

for example, piglets versus finishing pigs, or pigs versus small ruminants or other

livestock species, the absolute values obtained by DXA could be inaccurate

compared to the 'standard' body composition (Hunter et al., 2011). The DXA

technologies and their software might differ in the way they deal with these issues,

leading to the observed discrepancies. Concerning water, the published equations

from Mitchell et al. (1998a) (Figure 5, 1st row) and Kipper et al. (2019b) (Figure 5, 3rd

row) fit our data well (RMSE = 7.3% and 1.7%, respectively). The slopes of the lines

from the data in this study and from both published equations were similar. Given the

large amount of water in the body, differences in intercepts of approximately 776 g to

1 kg are not relevant, and the regression lines were very close to each other (Figure

5). However, for pigs with higher BW, Mitchell's regressions led to a slight

overestimation of water content in the EB. Concerning lipids, the regression equation

by Pomar et al. (2017) had a relatively good fit to our data (Figure 5, 2nd row), with a

similar slope, and it differed by 2 kg at the intercept. Thus, while this regression

predicted the lipid content of pigs between 60–100 kg well, it overestimated the lipid

content of piglets. Regression equations for lipids and ash by Kipper et al. (2019b)

(Figure 5, 4th row) were similar to ours but consistently predicted higher values than

our equation did. Kipper et al.'s (2019b) slope for lipids was similar to ours; however,

their intercept was higher (by 2.7 kg), leading to a regression line almost parallel but

slightly shifted upwards as compared to ours. Thus, using Kipper's equation on our

data reflected well the differences between individuals in fat content by DXA, but it

overestimated the actual values.

Figure 5 Comparison of own and published regression lines for DXA measurement on
chemical value in empty body (live DXA scans) of growing pigs. The solid black lines are
regression lines obtained from the data in this study, and the dashed red lines are published
regression lines. Equations and rCV in black pertain to the present study; the equations in
red are previously published equations. rCV in red gives the prediction error of the published
equation applied to the DXA variable obtained in the present study. 1st row: lean tissue by
DXA on chemically determined CP and water in grams (Mitchell et al., 1998a). 2nd row: lean
and fat tissue by DXA on chemically determined CP and lipid in grams (Pomar et al., 2017).
3rd row: Lean tissue by DXA on chemically determined CP and lean tissue by DXA on
chemically determined water. 4th row: fat tissue by DXA on chemically determined lipids and
BMC by DXA on chemically determined ash in kilograms (Kipper et al., 2019b).
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Carcass

The published equations for CP and water in half carcasses using absolute values

(Mitchell et al., 1998c) built from wet chemistry analyses and carcass scans of pigs

slaughtered at a live BW of 30 to 120 kg fit our data well, with generally lower rCVs

than for the EB (Figure 6, upper row). The slopes were almost identical and the

intercepts differed by 640 g for CP and less than 400 g for water. Thus, the prediction

errors when using the equations by Mitchell et al. (1998c) were only slightly higher

than using our own regression equations. Comparing the regression equations for

percentages of lean mass and water with the ones from Mitchell et al. (2003),

obtained from half carcasses with a mean weight of 43 kg, proved to be more

difficult. To create our prediction equations, we calculated the percentage of lean

tissue (sum of CP and water) or fat in the carcass half as the proportion of the

chemical value of the sum of all chemical components (see Supplementary Material

Section 6, Table S3). The equations from Mitchell et al. (2003) were dramatically

different from ours, with a difference in the intercept of 13% for lean tissue and 33%

for fat (Figure 6). The slopes were also very different. The resulting regression lines

clearly missed our data points. This means that the equations published by Mitchell

et al. (2003) are in no way suitable for predicting the chemical values for the

percentage of lean mass and lipid from the present study. The reason for these

striking differences could be that when the absolute values are transformed to

percentages, an important amount of variation and also co-variation between DXA

and wet chemistry values is lost. Consequently, fitting a regression line through data

points that have such a reduced variation is difficult, and the resulting parameters of

the equations might not be as robust as the ones derived from regressions on the

absolute values. The pigs in this study were within a rather narrow weight range

(Mitchell et al., 2003), comparable to our 100 kg BW category. Thus, these equations

might not be suitable for predicting the proportion of lean tissue and lipid in the

smaller pigs in our study because those differ in the proportion of lean tissue and

lipids from larger pigs (Figure 6 lower row). As for lipids, Mitchell et al.'s (2003) and

our regression lines seemed to converge for pigs with a lipid content of around 30%,

which was outside the range in our study.

Figure 6 Comparison of this study and other published regression lines for DXA
measurement of chemical values in carcasses (live DXA scans) of growing pigs. Solid black
lines are regression lines obtained from data in this study, while dashed red lines show
published regression lines. Equations, rCV and R^2 in black pertain to the present study; the
equations in red are previously published equations. rCV in red gives the prediction error of
the published equation applied to the DXA variable obtained in the present study. Upper row:
lean tissue DXA on chemically determined CP and water in grams (Mitchell et al., 1998c).
Lower row: percentage of lean (CP + water) and fat tissue DXA on chemically determined
percentage of lean and fat mass (sum of EP and water; Mitchell et al., 2003). Different
categories of live weight at slaughter are illustrated by shading of the data points: light grey =
20 kg, dark grey = 60 kg, black = 100 kg.

Conclusion

In this work, we show that although calibration regressions are usually done for each

DXA device and/or working group, there is considerable agreement between the

parameters of published and our own regressions in pigs. Therefore, in the near

future, it might be quite realistic to create generic regression equations for body

composition in pigs that yield reliable estimates for pigs of different growth stages,

sexes and genetic breeds. To achieve this goal, all published results should ideally

be summarized within a meta-analysis or, even better, a joint analysis. This may

allow for a broader application of DXA, including for research groups that do not have

the possibility to generate their own calibration equations. This endeavour would be

greatly facilitated by data sharing among laboratories or, better, the existence of

open data sets providing both chemical and DXA values. We illustrate the possibility

of predicting the chemical content of individual nutrients such as P and Ca from DXA

values. This prospect is especially important for the emerging field of precision

feeding, in which the nutrient demands of single individuals have to be closely

monitored. We have also shown that body composition of the EB can be inferred with

rather good precision and accuracy from scanning carcass halves. Replacing the

invasive, time-consuming and labour-intensive scanning of live pigs with the

scanning of carcasses has great potential to improve animal welfare standards and

could facilitate nutritional studies that investigate overall nutrient deposition efficiency

using a large number of animals. Another promising application of DXA for

measuring body composition in a relatively efficient and low-cost manner are genetic

studies. Currently, the effort associated with chemical analysis certainly represents a

bottleneck for those types of studies in which traits have to be measured in hundreds

to thousands of individuals. DXA could be applied for high-throughput phenotyping,

enabling the generation of large amounts of data for many individuals.

Ethics approval

This experimental procedure was approved by the Office for Food Safety and

Veterinary Affairs (2015_37_FR) and all procedures were conducted in accordance

with the Ordinance on Animal Protection and the Ordinance on Animal

Experimentation.

Data and model availability statement

The data that support the findings of this study are publicly available in Zenodo

(Kasper *et al.*, 2020). The code used for models and the statistical analyses are listed

in the Supplementary Material (Section 7).

Author ORCIDs

C.K.: 0000-0001-7305-3996

P.S.: 0000-0001-5095-0889

G.B.: 0000-0002-6397-7543

Author contributions

Conceptualization: C.K. and P.S. Data Curation: C.K. and P.St. Formal Analysis:

C.K. Investigation: I.R.A., P.S., P.St. and G.B. Methodology: C.K., P.S. and G.B.

Software: C.K. and P.St. Supervision: P.S. and G.B. Visualization: C.K. Writing –

Original Draft Preparation: C.K. Writing – Review & Editing: C.K., P.S., I.R.A., P.St.

and G.B.

Declaration of interest

The authors report no conflicts of interest with any of the data presented.

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