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Use of bioelectrical impedance spectroscopy to provide a measure of body composition in sows

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ABSTRACT

The ability to accurately estimate fat mass and fat-free mass (**FFM**) has the potential to improve the way in which sow body condition can be managed in a breeding herd. Bioelectrical impedance spectroscopy (**BIS**) has been evaluated as a practical technique for assessment of body composition in several livestock species, but similar work is lacking in sows. Bioelectrical impedance uses population-specific algorithms that require values for the apparent resistivities of body fluids and body proportion factors. This study comprised three major aims: (i) to derive apparent resistivity coefficients for extracellular water (**ECW**) and intracellular water (**ICW**) required for validation of BIS predictions of total body water (**TBW**) in live sows against standard reference tracer dilution methods; (ii) to develop predictions of TBW to body composition prediction algorithms, namely FFM, by developing a body geometry correction factor (Kb) and (iii) to compare the BIS predictions of FFM against existing impedance predictors and published prediction equations for use in sows, based on physical measurements of back-fat depth and BW (P2-based predictors). Whole body impedance measurements and the determination of TBW by deuterium dilution and ECW by bromide dilution were performed on 40 Large White x Landrace sows. Mean apparent resistivity coefficients of body fluids were 431.1 Ω.cm for ECW and 1827.8 Ω.cm for ICW. Using these coefficients, TBW and ECW were over-estimated by 6.5 and 3.3%, respectively, compared to measured reference values, although these differences were not statistically different ($P > 0.05$). Mean Kb was 1.09 ± 0.14. Fat-free mass predictions were 194.9 kg, which equates to 60.9% of total sow weight, and 183.0 kg for BIS and the deuterium dilution method, respectively. Mean differences between the predicted and measured FFM values ranged from −8.2 to 32.7%, but were not statistically different ($P > 0.05$). Method validation (leave-one-out procedure) revealed that mean differences between predicted and measured values were not statistically significant ($P > 0.05$). Of the impedance-based predictors, equivalence testing revealed that BIS displayed the lowest test bias of 11.9 kg (8.2%), although the P2-based prediction equations exhibited the lowest bias and percentage equivalence, with narrow limits of agreement. Results indicate although differences between mean predicted and measured values were not significantly different, relatively wide limits of agreement suggest BIS as an impractical option for assessing body composition in individual sows compared to the use of existing prediction equations based on BW and back fat.

Implications

This study has provided apparent resistivity coefficients and a body geometry correction factor necessary for the assessment of body composition in sows using bioelectrical impedance spectroscopy. The bioelectrical impedance spectroscopy method using these factors was validated against reference tracer methods for measuring body composition. The significant finding of this study is that bioelectrical impedance spectroscopy, at present, with wide limits of agreement is not sufficiently accurate, compared to industry standard ultrasound measurements, for assessment of body composition in individual sows. Bioelectrical impedance spectroscopy does, however, allow assessment of hydration status and intra- and extracellular water ratios.

Introduction

Body mass can be considered to comprise two compartments, fat mass (**FM**) and fat-free mass (**FFM**); the two-compartment (2C) model of body composition (Hansard, 1964; Topel and Kauffman, 1988; Wang et al., 1999). Prediction models used for indicating changes

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in sow body composition have primarily focused on measuring body fat. Ultrasound measurement of back fat at the P2 position (P2; 65 mm from the dorsal midline at the level of the posterior edge of the head of the last rib) has long been used as an indicator of total fat (McMeekan, 1941; Mao et al., 1999). Most prediction equations for FM are based on back-fat depth at the P2 location and also incorporate other prediction variables such as BW and morphometric measurements including leg length (Smits et al., 2017). Although most equations are robust predictors of body composition (Lonergan et al., 2019), each equation must be fitted for specific genetic lines, populations of each herd, and account for short-term variations such as diet, pregnancy and lactation (McPhee and Daniels, 1991). Regardless, measuring sow body fat alone does not provide an indication of compositionally important changes that occur in the lactating sow as excessive tissue mobilisation can cause subsequent reproductive failure (Vinsky et al., 2006).

Fat-free mass includes muscle mass and other soft tissues, total body water (**TBW**) and bone mineral mass and can be accurately measured by measuring TBW using tracer dilution, such as deuterium dilution (Johnson and Coward-McKenzie, 2001). Therefore, any accurate method that can predict FM and FFM of a sow that might allow management interventions to prevent detrimental changes in body composition during lactation is desirable. Although tracer dilution has a long history (e.g. Hansard, 1964), it is an impractical technique for routine use.

Bioelectrical impedance analysis (**BIA**) is a non-invasive, inexpensive and portable method of measuring body composition, which can be used repeatedly to monitor growth and development of a single animal (Kyle et al., 2004b). The underlying principle of BIA is that the opposition (impedance, Z) to the flow of an alternating electrical current (typically at a single fixed frequency of 50 kHz) through a cylindrical conductor is proportional to the conductive volume of the cylinder. In its application to animals *in vivo*, the animal is assumed to represent a cylinder or series of inter-connected cylinders, i.e., legs and trunk, with the conductive volume being that of TBW. Since TBW is exclusively located in the FFM compartment, measurement of whole body impedance can be used to quantitate FFM and, by difference with BW, FM (Cox-Reijven, 2002). Bioelectrical impedance spectroscopy (**BIS**), a derivative of BIA, measures impedance and its components, resistance (R) and reactance (Xc), typically in the range 3 to 1 000 kHz and uses biophysical models to predict body composition. It is considered to provide more accurate predictions of body composition, that are not population specific, allowing quantification of TBW and FFM as well as extracellular water (**ECW**), providing a tool for study of water balance in the live animal. It has been used to measure hydration status and body composition in humans for many years (Kyle et al., 2004a and 2004b), whilst studies in livestock have found BIA measurements to successfully provide a rapid and inexpensive measurement of body composition in lambs (Berg and Marchello, 1994), steers (Marchello et al., 1999b), horses (Ward et al., 2015) and pigs (Swantek et al., 1999; Marchello et al., 1999a; Daza et al., 2006).

The aims of this study were: (i) to derive apparent resistivity coefficients required for validation of BIS predictions of TBW and ECW in non-reproductive sows against standard reference tracer dilution methods, (ii) to develop a body geometry correction factor specific for use of BIS in sows required for body composition algorithms to predict FFM and (iii) to compare the prediction of FFM using BIS with prediction equations in the literature based on BW and back-fat depth at the P2 site.

Material and methods

Setting

This study was undertaken at the SunPork Research Facility (SunPork Farms, Queensland (QLD)) in November 2016.

Animals

A total of 40 (non-pregnant) Large White X Landrace multiparous sows (parity 1.7 ± 0.6; mean ± SD), weighing 227.2 ± 26.3 kg, were held in free-access stall housing and offered water *ad libitum* and 2.3 kg/sow/d of a standard sow diet (12.8 MJ digestible energy/kg, 0.42 g standardized ileal digestible lysine/MJ digestible energy). The final data set included 37 animals; three animals had incomplete dilution data.

Impedance measurements

The BIS measurements were taken using an ImpediVet SFB7 bioimpedance spectrometer (ImpediMed Ltd., Pinkenba, Queensland). The SFB7 is a portable battery-operated impedance device that measures resistance and reactance at 256 discrete logarithmically spaced frequencies in the range 3 to 1 000 kHz. A measurement scan takes <1 s. It is a tetrapolar device applying the alternating current (200 μA) via two distal current electrodes and measuring impedance via two proximally placed (10 cm separation) voltage sense electrodes. In the present study, electrodes were fabricated as brass discs (5 mm diameter) mounted in a handle at 10-cm inter-electrode spacing. The reference point for one current drive electrode was the middle carpal joint with the voltage sense electrode placed 10 cm cranial from that point. The second current drive electrode was located at the caudal reference point of the hock joint with the voltage sense electrode placed 10 cm cranial from that point (Fig. 1). All measurements were made on the left side of the animal. Sows were restrained standing in stall housing, while the electrode discs were coated with conductivity gel and pressed into direct contact with the skin removing the need for shaving the area. Five consecutive replicate impedance scans were obtained, and care was taken to ensure that sows remained calm, and still, for readings. If movement was observed, measurements were repeated. Since impedance varies with distance between the voltage sense electrodes, impedance measurements were normalized for the inter-electrode length. However, since the precise electrical current path is unknown, a surrogate measurement of length (L, cm) spanning the body which can be reliably measured was used, i.e. from the base of the head to the base of the tail, a procedure similar to that used in humans where height is used as an index for inter-electrode distance.

Tracer dilution methods for measurement of total body water and extracellular water

Total body water was determined by the deuterium dilution technique (Houseman et al., 1973) and ECW from bromide dilution (Mørkeberg et al., 1991) according to the following protocol. Fourteen days before the experiment commenced, sows were weighed using livestock scales (Iconix FX Series, A1 Weighing & Equipment, Qld) before being transported to the research facility. The tracer dilution procedure followed was that used previously in horses (Ward et al., 2016). Sodium bromide (NaBr) [(ACS (AR); ACE Chemical Company, Camden Park, SA)] was added to deuterated water (0.75 M, 99.9% purity; Novachem, Melbourne, Victoria), and the solution was administered at a dose rate of deuterated water 1 g/kg BW and NaBr 0.75 mmol/kg BW. As the procedure was delayed 2 weeks from the time of the sows arrival, BW was predicted using a prediction equation ($R^2 = 0.93$) developed from a group of sows of similar age, weight and feeding allocation ($n = 91$) from the original herd (Supplementary Figure S1). The individual solution was drawn into pre-weighed 50-ml syringes ready for administration by oral intubation using orogastric tubing (13 mm GVP; Provet, Brisbane, Qld). Full syringes were weighed prior to intubation and again post-intubation to allow for calculation of the exact weight of tracer infused (to 0.01 g). Sows were restrained in a closed stall and held using a commercial short hog holder (DHA Rural, Qld).

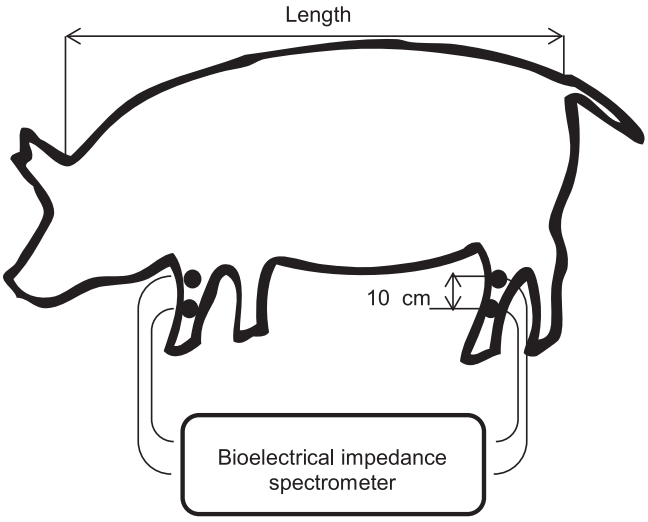

Fig. 1. Electrode locations (●) and length measurement (Length) on the live sow during a bioelectrical impedance spectroscopy measurement.

A drinking water mouth gag (Bainbridge; Qld) was placed in the left side of the mouth separating the top and lower jaw. Stomach tubing was then lubricated using canola oil and passed down the oesophagus into the stomach. The dose was administered, followed by 100 ml of water and 50 ml of air to ensure the tube was clear of fluid and total dosing. Using jugular venepuncture, 10 ml samples of blood (lithium-heparin BD vacutainer; Provet, Qld) were collected at time 0 (before dosing) and at 3 and 4 h post-intubation of the tracer dilution. An 18 g vacutainer needle was used to collect the sample into 2 × 5 ml EDTA blood tubes. Samples were centrifuged at 3000 × *g* for 15 min within 4 h of collection, and plasma was used for analysis of deuterium and bromide concentrations.

Morphometric measurements

Unlike single-frequency BIA which assumes a simple cylindrical body geometry and empirically derived population-specific prediction equations, BIS uses fundamental mixture theory modelling that corrects for non-cylindrical body shape by applying a body geometry correction factor referred to as Kb (De Lorenzo et al., 1997). A population mean Kb was calculated from morphometric measurements (lengths and girths) of the body segments between the two voltage sense electrodes in a sample of sows of the same genotype and parity from two herds ($n = 20$). The Kb was calculated using the following equation adapted from validation of BIS in horses (Ward et al., 2016):

$$\mathrm{Kb} = \left(1|(\mathrm{BL}+\mathrm{FL}+\mathrm{RL})^2\right)*\left(\mathrm{BL}|\mathrm{Girth}^2\right) + \left(\mathrm{FL}|\mathrm{fp}^2\right) + \left(\mathrm{RL}|\mathrm{rp}^2\right)*\left(\mathrm{BL}*\mathrm{Girth}^2\right) + \left(\mathrm{FL}*\mathrm{fp}^2\right) + \left(\mathrm{RL}*\mathrm{rp}^2\right)$$

where:

BL = body length (linear distance, shoulder–hip vertically above electrodes, cm); Girth = girth circumference (measured immediately behind the front legs, cm); FL = fore leg length (from the sense electrode to the junction of the leg and trunk, cm); RL = rear leg length, (from the sense electrode to the junction of the leg and trunk, cm); fp = fore leg perimeter (mean of circumference at the sense electrode and the top of the leg, cm) and rp = rear leg perimeter (mean of circumference at the sense electrode and the top of the leg, cm).

Ultrasound measurements of back fat

Measurement of back-fat depth at the P2 site (P2) was taken using a Sonoscape A6 Ultrasound Machine (Sonoscape Medical Corp., Shenzhen, China). Back-fat depth was measured by taking measurements 65 mm from the midline, directly above the last rib on the left and right sides of the sow until measurements differ by <1 mm when the average of the measurements was used (Hoving et al., 2011).

Laboratory analyses

Deuterium analysis

Deuterium concentration in the plasma was analyzed by Fourier transform IR spectroscopy using an IRAffinity-1 FTIR spectrometer (Shimadzu Corp., Kyoto, Japan), which was fitted with an ATR sample attachment with reference to a standard curve prepared from deuterium dissolved in pooled control sow plasma. Plasma samples were thawed at room temperature before samples were vortexed and centrifuged at 300 × *g* for 15 min in a bench centrifuge. Aliquots of the supernatant were used for analysis as described by Collins et al. (2013). CV for inter-analysis was <2.0%.

Bromide analysis

Bromide ion concentration in plasma samples was determined using a HPLC (Shimadzu Corp., Kyoto, Japan). Plasma samples were thawed at room temperature and de-proteinized with ice-cold acetonitrile (100 μl plasma added to 200 μl acetonitrile). Samples were centrifuged at 300 × *g* for 10 min in a micro-centrifuge. Aliquots of 20 μl supernatant were transferred, using a syringe, to an auto sampler vial for bulk analysis using the method of Miller et al. (1989), as described by Collins et al. (2013). Bromide ion concentrations were determined by reference to standards prepared in an identical manner from a pooled sample of control sow plasma. CV for inter-analysis was <2.0%.

Data analysis

Bioelectrical impedance spectroscopy

The BIS data were analyzed using Bioimp software (v4 18.0.0; ImpediMed Ltd., Pinkenba, Queensland, Australia). Resistance and

reactance for each recorded scan were fitted to a Cole model (Cornish et al., 1996). Resistance at zero frequency (R_0, i.e. that of ECW) and infinite frequency (R_{inf}, i.e. that of TBW) was determined as described by Cornish et al. (1993) using software default settings. Goodness of curve fitting was assessed as the %SE of the estimate and was <0.5%. The resistance (R_i) of intracellular water (**ICW**) was calculated from R_0 and R_{inf} as

$$R_i = \left(\frac{R_0 * R_{inf}}{R_0 * R_{inf}}\right)$$

Mean values for R_0, R_{inf} and R_i from the five replicate scans for each sow were used in subsequent analyses. The SD between replicates was <3.5%.

Apparent resistivity coefficients for ICW (ρ_{ICW}) and ECW (ρ_{ECW}) were computed from R_0, R_i, BW, length, TBW and ECW, as measured by dilution, using the resistivity module of the Bioimp software (v4 15.0.0; ImpediMed Ltd., Pinkenba, Queensland, Australia). Body composition was predicted from these parameters using the body composition module of the Bioimp software which implements Hanai mixture theory algorithms (Ward et al., 2016). Default coefficients for body density (1.05 mg/ml) (Ward et al., 2016), FFM hydration (0.757 ml/g, Patience, 2012), resistivity coefficients (ρ_{ICW} and ρ_{ECW}) and Kb were determined as described above. Fat-free mass is calculated as TBW/FFM hydration with FM calculated as BW − FFM.

Dilution analysis – total body water

Plasma deuterium concentration was calculated from the maximum concentration at time 3 or 4 h corrected for the background concentration at baseline. Total body water was then calculated according to:

$$TBW = \left(\frac{D_2O \text{ dose}}{D_2O \text{ conc.}}\right) * 0.937 * 0.96 * 1.04$$

where 0.937 is the correction factor for the fraction of water in plasma, 0.96 is a correction factor for deuterium binding to non-exchangeable sites and 1.04 is the correction for the deuterium space difference that is that of H_2O (Cornish et al., 1996).

Dilution analysis – extracellular water

Bromide concentrations at 3 and 4 h were corrected for background bromide present in baseline samples and the mean values used. The ECW pool was assumed to be equivalent to the bromide space calculated according to:

$$ECW = \left(\frac{NaBr \text{ dose}}{NaBr \text{ conc.}}\right) * 0.9 * 0.95 * 0.94$$

where 0.9 is the correction factor for the distribution of bromide in the non-extracellular sites, 0.95 is the correction factor for the Donnan equilibrium and 0.94 is the plasma water fraction (Cornish et al., 1996).

Cross-validation of body composition predicted from bioelectrical impedance spectroscopy resistivity coefficients

A cross-validation (leave-one-out validation, LOOV) procedure (Secor and Nagy, 2003) was used to validate predictions of body composition by BIS. Each sow was removed one at a time, and resistivity coefficients were calculated based on the remaining sows. This process was repeated for all sows. The calculated ρ_{ICW} and ρ_{ECW} were then used to predict TBW, FFM and FM for each sow and cross-validated against the respective TBW, FFM and FM measured by deuterium dilution. Significance of differences between measured reference and predicted body composition values from the LOOV procedure was assessed using paired sample *t*-tests.

Prediction of body composition

Fat mass was predicted from sow BW and P2 measurements using each of the following published equations:

King et al. (1986)

$$FM = (0.117 + 0.00804 * P2) * BW$$

Dourmad et al. (1997)

$$FM = -26.4 + 0.221 * (BW * 0.96) + 1.331 * P2$$

Gill (2006)

$$FM = -8.14 + 0.167 * BW + 0.883 * P2$$

Smits et al. (2017)

$$FM = (0.2696 * BW) + (1.398 * P2) - 33.9$$

Fat-free mass was then calculated as BW − FM.

Single-frequency bioimpedance analysis

Resistance at 50 kHz (X_c50) was extracted from the BIS data files using the Bioimp software. This was then used to predict FFM according to the following prediction equations:

Kraetzl et al. (1995)

$$FFM = \left(7.126 + 0.389 * BW + 0.039 * girth + 0.129 * \left(\frac{L^2}{R_{inf}}\right) - 0.285 * X_c50\right) / 0.757$$

Swantek et al. (1999)

$$FFM = 0.486 * BW - 0.881 * (R50 - 40.7) + 0.48 * (L - 40) + 0.86 * (X_c50 - 6.2) + 7.959$$

where:

L = body length (linear distance, shoulder–hip vertically above electrodes, cm).

R_{inf} = resistance at infinite frequency (i.e. that of TBW).

X_c50 = reactance at 50 kHz.

R50 = resistance at 50 kHz.

Statistical analyses

Statistical analysis followed procedures recommended for impedance validation studies (Guo et al., 1996; Tronstad and Pripp, 2014). Statistical analysis was performed using either SPSS Statistics (IBM SPSS® version 25.0; IBM, Chicago IL, USA) or Medcalc (version 19.1, MedCalc Software bv, Ostend, Belgium). Data were checked for normal distribution using an Anderson–Darling test. Data are presented as mean ± SD. Relationships between predicted body composition parameters (TBW, FFM and FM) and the measured values using the reference dilution methods were assessed by Passing and Bablok regression with the level of agreement being assessed by Pearson's correlation coefficient (r_p) and Lin's concordance correlation coefficient (r_c). Agreement between methods was determined by paired samples *t*-test, 2SD limits of agreement (**LOAs)** analysis (Bland and Altman, 1986), and absolute percentage error expressed as the median absolute percentage error (**MAPE**). A value of $P < 0.05$ was considered statistically significant in all tests.

Results

Characteristics of the sows are presented in Table 1. Total body water was 60.9% of BW and comprised 26% ECW and 74% ICW. The calculated Kb value was 1.09 ± 0.14 (Table 2).

Impedance parameters and computed apparent resistivity values for multi-frequency BIA and single-frequency BIA measurements are

Table 1
Subject characteristics of sows. Data presented as mean ± SD and range ($n = 40$).

Parameter	Mean ± SD	Range
BW, kg	227.2 ± 26.3	167.0–278.0
Length,[1] cm	147.0 ± 8.4	123.0–167.5
Back-fat depth at P2, mm	17.2 ± 3.8	10.1–27.2
Total body water,[2] TBW, L	138.5 ± 20.4	83.6–175.1
Extracellular water,[3] ECW, L	36.0 ± 12.4	11.7–62.8
Intracellular water,[4] ICW, L	102.5 ± 18.5	49.8–139.1
ECW:ICW	0.37 ± 0.15	0.11–0.68

[1] Measurement of crown to rump.
[2] Determined by deuterium dilution.
[3] Determined by bromide dilution.
[4] Determined as TBW-ECW.

Table 2
Morphometric measurements of sows for calculation of body geometry correction factor (Kb). Data presented as mean ± SD and range.

Segment	Measurement (cm)	Mean	SD	Range
Trunk	Length[1]	137.4	6.8	130.0–150.0
Trunk	Girth circumference[2]	146.5	8.5	135.5–160.0
Foreleg	Length[3]	5.6	2.2	2.0–10.0
Foreleg	Circumference[4]	41.4	2.1	39.2–46.0
Rear leg	Length[5]	5.3	1.7	4.0–9.0
Rear leg	Circumference[6]	49.6	3.0	45.5–54.0
	Kb	1.09	0.14	0.85–1.31

[1] Measurement of crown to rump.
[2] Measured immediately behind the front legs.
[3] Point of elbow to knee.
[4] Average of circumference at point of elbow and knee.
[5] Point of stifle to hock.
[6] Average of circumference at point of stifle and hock.

Table 3
Whole body resistances (R_{50}, R_0, R_∞ and R_i) and apparent resistivities (ρ_{ICW} and ρ_{ECW}) of intra- and extracellular water of sows. Data presented as mean ± SD and range.

Parameter	Mean ± SD	Range
Resistance at 50 kHz, R_{50}, ohm	61.4 ± 12.7	40.6–85.2
Resistance at zero frequency, R_0, ohm	85.2 ± 15.0	59.5–111.6
Resistance at infinite frequency, R_∞, ohm	42.6 ± 11.5	24.3–64.2
Intracellular resistance, R_i, ohm	88.1 ± 34.7	39.3–163.3
Apparent intracellular resistivity, ρ_{ICW}, ohm.cm	1827.8 ± 836.4	497.5–3437.8
Apparent extracellular resistivity, ρ_{ECW}, ohm.cm	431.1 ± 291.0	28.3–1177.8

presented in Table 3. The data show that apparent resistivities varied between sows with a mean 45.7% SD for ρ_{icw} and 67.5%SD for ρ_{ecw}.

The results of the cross-validation are presented in Table 4. Mean differences between the predicted and measured values for all body composition parameters ranged from −8.2 (FFM) to 32.7% (FM). Paired *t*-tests revealed, however, that these differences were not statistically significant ($P > 0.05$). Limits of agreement plots are presented as supplementary data (Supplementary Figure S2). For all measures other than FM, significant positive proportional bias existed with the difference between measured and predicted values increasing with animal size.

Fat-free mass values predicted from either published equations or impedance measurements are presented in Table 5. The prediction equations of FFM were statistically ($P < 0.001$) highly correlated (r_p) with deuterium dilution-measured FFM. However, there was less agreement, i.e., deviating from the line of identity and exhibiting much lower concordance correlation values. Impedance-based predictions exhibited larger biases. Limits of agreement (2 SD) analysis for prediction equations were approximately ±28 kg or 15%; by comparison, impedance-based LOA were approximately ±50 kg (27%). The Dourmad et al. (1997) predictor showed the lowest percentage equivalence, followed by Gill (2006).

Discussion

Bioelectrical impedance analysis is based on the principle of differential conductance of an electrical current through body tissues/fluids. Conductance is high in electrolyte-rich body water and very much lower, or non-existent, in lipids and bone material. Conversely, impedance (resistance) is highest in fat-containing tissues but lowest in water-containing tissues. Impedance varies inversely but quantitatively with tissue water volume. As body water is located primarily in FFM, that in turn is predominately protein in muscle (Avril et al., 2013), measurement of TBW allows prediction of FFM and hence FM. The present study set out to validate the BIS technique for the prediction of TBW and FFM in non-pregnant sows. This was a two-stage process, firstly the derivation of apparent coefficients necessary for the transformation of measured impedance to a prediction of TBW, and secondly, validation of body composition predictions using these coefficients. In addition, the study compared prediction of FFM using BIS with body composition predicted from back fat at the P2 position and BW measurements showing the BIS predictions had wide LOAs compared to prediction equations of Dourmad et al. (1997), Gill (2006) and Smits et al. (2017) based on equivalence.

Measured impedance is determined by the inherent resistivities of ICW and ECW and the geometry (length and cross-sectional area) of the conductive volume. However, body conformation varies greatly between animals; hence, resistivities determined from BIS *in vivo* are apparent resistivities (Ward et al., 2015) compared to those determined *in vitro* (Geddes and Baker, 1967; Stuchly and Stuchly, 1980). Consequently, it is not possible to compare the resistivities determined here with published values for other animal species. Although impedance measurements have been performed in a variety of farm animals including sheep (Hegarty et al., 1998), cattle (Thomson et al., 1997; Schäff et al., 2017), horses (Ward et al., 2016) and pigs (Swantek et al., 1992 and 1999; Kraetzl et al., 1995), there is no consensus on electrode locations (Schäff et al., 2017) which makes comparison of impedance data within the same species difficult. Published resistance data for live pigs only exist for single-frequency BIA measurements. Swantek et al. (1999) found R_{50} values ranging from 34 to 43 Ω, while Kraetzl et al. (1995) published values ranging from 89 to 127 Ω, compared to 40.6 to 85.2 Ω in the current study. These differences reflect variance in electrode positions and animal size between studies. Swantek et al. (1999) evaluated impedance on pigs weighing 49.4 to 129.3 kg with electrodes placed at the anterior baseline of the ears and at the third sacral vertebrae. Kraetzl et al. (1995) used similar electrode positions to those in the current study, but sows in that study weighed on average 178 kg compared to 227.2 kg in this study. Since as noted above absolute values for resistivity coefficients are dependent upon body geometry, this makes comparison difficult where body size and shape is markedly different.

The BIS technique attempts to account for variation in body conformation from the theoretical model of an homogeneous cylinder that underpins BIA by incorporating in the algorithms a correction factor for body shape (Matthie, 2008). The impedance method is predicated on the assumption of a notional cylindrical conductive volume. The Kb correction factor is designed to account for this geometric arrangement. A Kb of 1.09 closely approximates a Kb of 1 which applies to a simple single cylinder. This reflects electrode locations used in the present study that are close to the trunk such that the volume of limb sections being measured is small in proportion to the volume of the trunk. This is unlike humans and horses where the electrical volume of the legs and arms (in humans) is large relative to the trunk. The body proportion factor, Kb, varied between individual sows by 12.8% reflecting inter-animal variation in shape. It should also be noted that morphometric measurements taken for the derivation of the body proportion factor were performed in a separate group of sows albeit of a similar age, weight and

Table 4
Cross-validation of prediction of body water compartment volumes and body composition in sows by bioimpedance spectroscopy with measures from reference tracer dilution methods. Data presented as mean ± SD.

Parameter	Value		Cross-validation			
	Measured	Predicted	Difference (L or kg)	Difference (% measured)	LOA (% measured)	*P* value
Total body water, l	138.5 ± 20.4	147.6 ± 34.8	−9.1 ± 37.0	−8.2 ± 28.2	−63.5 to 47.0	0.211
Extracellular water, l	36.0 ± 12.4	37.2 ± 5.2	−1.2 ± 12.5	−17.6 ± 53.2	−121.8 to 86.6	0.564
Intracellular water, l	102.5 ± 18.5	110.3 ± 30.9	−7.8 ± 34.7	−10.7 ± 35.3	−79.8 to 58.5	0.177
Fat-free mass, kg	183.0 ± 27.0	194.9 ± 46.0	−11.9 ± 48.9	−8.2 ± 28.2	−63.5 to 47.0	0.146
Fat mass, kg	42.7 ± 16.8	30.7 ± 50.9	11.9 ± 48.9	32.7 ± 127.8	−217.8 to 283.1	0.146

LOA = 2SD limits of agreement.

Table 5
Fat-free mass (FFM) in sows determined by deuterium dilution method and compared with prediction equations from BW and back-fat depth (P2-based predictors) or bioelectrical impedance spectroscopy.

Prediction equation	Mean ± SD (kg)	Bias kg (%)	r_p	r_c	SEE (kg)	LOA (kg)	MAPE[1]	% Equivalence[2]
Deuterium dilution	183.0 ± 27.0							
P2-based predictors								
King et al. (1986)	167.6 ± 17.4	−15.7 (8.5)	0.886	0.614	12.8	−12.3 to 43.7	8.91	14.0
Dourmad et al. (1997)	179.3 ± 18.9	−2.0 (1.1)	0.869	0.036	13.6	−25.9 to 29.9	4.07	6.1
Smits et al. (2017)	174.7 ± 17.5	−8.6 (4.7)	0.874	0.386	13.4	−20.0 to 37.3	5.76	9.8
Gill (2006)	181.0 ± 21.0	−2.3 (1.2)	0.850	0.059	14.5	−26.1 to 30.7	4.11	6.5
BIS prediction equations								
Kraetzl et al. (1995)	219.9 ± 28.9	36.6 (20.0)	0.470	0.895	24.3	−93.3 to 20.1	14.6	23.5
Swantek et al. (1999)	156.7 ± 16.9	−26.6 (14.5)	0.730	0.614	18.8	−10.2 to 63.4	14.5	20.9
BIS[3]	194.9 ± 46.0	11.9 (8.2)	0.190	0.150	25.4	−83.9 to 107.8	21.1	13.5

Bias = measured – predicted values; r_p = Pearson correlation coefficient; r_c = concordance correlation; SEE = SE of estimate; LOA = 2SD limits of agreement (measured-predicted).
[1] Median absolute percentage error.
[2] Two one-sided t test (TOST).
[3] Bioelectrical impedance spectroscopy.

back-fat depth at the P2 location but potentially adding to measurement error. Ideally, as noted by de Lorenzo et al. (1997), a Kb value should be determined for each individual, this is generally impractical and use of a population mean value is necessary. The proportional bias observed with increasing body size (supplementary data) may be a reflection of the inadequacy of this approach, however.

The BIS approach for prediction of TBW or other body composition measures was assessed by comparison of predicted TBW values with those determined by a reference technique, deuterium dilution. Deuterium dilution has been used previously to estimate TBW in pigs (Hansard, 1964), but studies have been limited to pigs weighing less than 150 kg compared to an average BW of 227.2 kg for sows in the present study. Total body water, measured using deuterated water, was found to be 44.8% of BW in a 98 kg pig, 58.3% in a 109 kg pig (Rozeboom et al., 1994) and 43% in a 145 kg pig (Shields et al., 1983). Ferrell and Cornelius (1984) found obese pigs (46.5% fat) to have a 21% decrease in TBW compared to pigs that were composed of 25.4% fat. This observation is supported by the data of Lee et al. (1989), who observed that as fatness decreased (indicated by back fat at the P2 location reducing from 18 to 12 mm), body lipid content reduced by 25%, while water content increased by 20%. In the present study, TBW averaged 61.7% for a sow of mean BW of 227.2 kg. This equates to a mean FFM of 183.8 kg and a mean FM of 43.3 kg, a value similar to other published studies on Large White X Landrace pigs in Australia. Smits et al. (2017), using parity 2 sows, reported an estimated average FM of 43.8 kg based on back-fat measurements at the P2 location which is similar to results of Lewis and Bunter (2011) that recorded body fat to be 40.3 kg in non-pregnant sows. These data lend confidence to the soundness of our determinations of TBW using the dilution method and hence FFM and FM.

In the absence of an independent sample of sows, in which BIS-based prediction of TBW could be compared to dilution measurements, cross-validation within the existing sample was necessitated. Cross-validation is commonly undertaken by splitting the sample into prediction and cross-validation samples (Tronstad and Pripp, 2014). However, the small number of animals precluded this approach and the alternative LOOV procedure was adopted (Sokal and Rohlf, 1995). Although not significant (although sample size limited statistical power), differences between predicted and measured body composition measures, e.g. of up to 19.7% for ECW, were observed. The differences for TBW and FFM were smaller (6.7%), a value consistent with that observed in validation studies in other species, including humans (Kyle et al., 2004a). The reasons for these differences are unclear. BIS is an indirect predictive method that applies population mean coefficients to individuals. A large inter-animal variation in resistivity coefficients was observed, as observed in humans (Ward et al., 2015) inducing increasing prediction error in animals further from the population mean. Impedance in the live animal is also dependent on physiological confounders including hydration state influenced by feeding and water loss through defecation and urination (Ward et al., 2016). In the present study, water consumption leading up to baseline sampling of TBW and urinary loss during the dilutional method was not accounted for. Furthermore, FFM is calculated by assuming a constant hydration of FFM for TBW, which presumes an equivalent overall hydration of FFM between individuals with different intra- to extracellular distributions of water. Additional variation is inherent in the measurement of length, probe placement and posture in the live, mobile animal. It is also important to mention that sows were weighed 2 weeks before the deuterium dilution studies with BW on the day estimated, a further potential source of error. It was noted by Ward et al. (2016) when using the identical device and probe placement in horses as in the current study, differences in probe placement between repeated measures translate to measurement error. These sources of variation are further propagated in the final prediction of body composition since they are also inherent in the calculation of the body proportion factor, i.e., circumference and length of each cylinder used in the equation. For example, if each measurement taken was in error by as little as 0.5 cm (approximately 2%), the body proportion factor exhibits a 9.7% change.

Currently, prediction of body composition in sows is based on BW and P2 measurements. The King (King et al., 1986), Dourmad (Dourmad et al., 1997), Smits (Smits et al., 2017) and Gill (Gill, 2006) equations predicted FFM using estimated FM from BW. In contrast, impedance-based methods first predict FFM and then FM is calculated by the difference between FFM and BW. Despite this difference in approach, it may be expected that if prediction equations were generated in a similar population of sows, then FFM predictions would not vary markedly between prediction methods and ideally be close to reference values. Equivalence testing provides a measure of predictive power at the population level. In contrast, LOA analysis allows an assessment of predictive power for an individual animal. The LOAs for the prediction equations were of similar magnitude and much smaller than those observed for the impedance-based methods. The observation that impedance performs poorly when predicting body composition in an individual animal is consistent with observations in humans (De Lorenzo et al., 1997). Mean LOA for the prediction equations was approximately ±25 kg. This equates to ±13.7% for 95% confidence when predicting FFM in an individual sow; this compares to ±40.2% (95% confidence) for the best performing impedance-based prediction (Swantek et al., 1999). Clear differences in absolute values (bias) were observed for all predictions when compared to reference values. Similarly, there was a large variation in both precision and accuracy of the correlation, as indicated by r_c, between predicted and measured FFMs. Nevertheless, the SE of the estimate for the correlations was largely similar for the prediction equations but approximately 50% larger for BIS methods. This variation in statistical measures of predictive power between methods makes it difficult to identify an optimal predictor. This analysis indicates that prediction equations perform better than those based on impedance, although for BIS, this advantage is not large as mean equivalence for the prediction equations was 9.1% compared to 13.8% for BIS. Overall, the Dourmad et al. (1997) predictor was the best performing prediction equation with BIS the best performing impedance-based predictor, 6.1 and 13.5% equivalence, respectively. The lowest MAPE exhibited for the Dourmad et al. (1997) prediction equation supports these observations.

In conclusion, this study has not demonstrated that BIS has a clear advantage over the current published prediction equations for assessment of body composition in sows. BIS performed acceptably when predicting FFM at a population level but particularly poorly in individual animals. This was unexpected since BIS has found wide acceptance for measuring body composition in other species, notably humans (Ward, 2019). Reasons for this reflect BIS being a predictive method applying population-derived mean values for resistivities and body proportion (Kb) to individual animals. Consequently, if there is wide biological variation in these parameters in a population, then this will translate to relatively poor predictive power. Certainly, wide inter-animal variation in resistivities was observed, although this is unlikely to reflect true differences in actual values which are determined by tightly controlled acid–base and water physiological mechanisms. This variation is more likely a reflection of methodological variability associated with dilutional measurement of ECW and TBW and variability in physical (dimensional) measurements required for BIS which are inherent in studies in the live animal.

Supplementary materials

Supplementary data to this article can be found online at https://doi.org/10.1016/j.animal.2020.100156.

Ethics approval

The CHM Alliance Animal Ethics Committee approved the protocol for this experiment (CHM PP 91/16) in accordance with the Australian Code for the Care and Use of Animals for Scientific Purposes (National Health and Medical Research Council, 2013).

Data and model availability statement

None of the data were deposited in an official repository.

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Declaration of interest

The authors declare no conflict of interest.

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