Technical Report

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Report Title: Report on the ability of a microwave device to predict rib fat and P8 fat depth in beef carcases

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Abstract

A portable ultra-wide band microwave system (MiS) was tested as a non-invasive objective measurement to predict beef carcase single site fat depth at commercial abattoirs. Experiment One used a laboratory calibration technique and tested the effectiveness of MiS coupled with either a Vivaldi Patch Antenna (VPA) or an open-ended coaxial probe (OCP). The VPA was used to predict hot carcase P8 (fat depth on the rump) across 4 slaughter groups (n=241). The VPA was also used to predict cold carcase rib fat (at the quartering site, 75% along the rib eye muscle) across 5 slaughter groups (n=598). The OCP measured hot carcase P8 across two slaughter groups (n=435). A machine learning stacking ensemble method was used to create the prediction equations. Datasets were grouped by prediction trait (P8 or ribfat) and probe/antenna then randomly divided into 5 groups based on tissue depth. Precision was greatest using OCP to predict P8 fat depth with a RMSEP of 2.47 mm and R2 of 0.70. The VPA precision was similar for the two tissue depths assessed, hot carcase P8 had an average RMSEP of 2.86 mm and R2 of 0.58 compared to cold carcase rib fat RMSEP of 2.60 mm and R2 of 0.55.

Experiment two tested the ability of a commercial MiS (C-MiS) device coupled to a VPA probe and on-site calibration to predict P8, rib fat depth and eye muscle area (EMA). The best prediction of hot carcase P8 (n=1650) was from C-MiS device 2 with an average RMSEP of 2.775 and R^2 0.81. Hot carcases (n=598) C-MiS scanned to predict cold rib fat had an average RMSEP of 3.712 and R^2 of 0.81. Hot carcases (n=598) C-MiS scanned to predict EMA had an average RMSEP of 10.267 and R^2 of 0.43.

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Executive Summary

Within the Australian beef industry, carcases are traded based upon their weight and the depth of subcutaneous fat at the P8 site. Fat depth is negatively correlated to carcase value (Polkinghorne, Philpott, Gee, Doljanin, & Innes, 2008), predominately due to increasing fatness decreasing saleable meat yield, and increased trimming costs to meet market specifications. These trimming specifications are linked to consumer preference for beef cuts with reduced visible subcutaneous fat (Steenkamp & van Trijp, 1996). Polkinghorne et al. (2008) estimated that a 1 mm increase in rib fat reduced carcase value by AUD$0.018/kg, thus carcase fatness is a key driver of beef carcase value. As such, Australian processors set narrow price grids based on hot carcase weight and subcutaneous fat depth specifications, and producers are paid on a per carcase basis based on these measurements. Accurate objective measurements of carcase fatness are is essential for carcase trading both in feedforward information to optimise the ability of boning rooms to deliver cuts that fit market specifications, and feedback to beef producers to improve on-farm production and reduced wastage of nutrition in unwanted fat.

Single site fat depth measurement of the Australian beef carcase is currently taken via an invasive technique, where a cutting knife or probe is inserted at the P8 site, or rib fat depth measured at the quartering site (Anonymous, 2005). Personnel performing cut measurements operate under the AUSMEAT accreditation and audit scheme, however these measurements can be imprecise and may be prone to operator error (Williams, Anderson, Siddell, Pethick, Hocking Edwards, & Gardner, 2017a). Non-invasive measurements which use electromagnetic or mechanical energies are rapidly becoming the preferred technique of assessing carcase composition (Scholz, Bünger, Kongsro, Baulain, & Mitchell, 2015). The advantages lie in improved precision and accuracy of the predictions, no destruction of the tissues, and enhanced producer confidence by removing the human element of the measurement. Any new carcase composition measurement must be able to operate at industry chain speeds while being safe, precise and accurate (Scholz et al., 2015).

A novel option for non-invasive measurement of fat depth is via the use of an ultrawide-band microwave system (MiS) (Hussain, 1998; Jafari, Liu, Hranilovic, & Deen, 2006; Marimuthu, Bialkowski, & Abbosh, 2016). A prototype portable MiS system using low power, non-ionizing electromagnetic waves has been developed at Murdoch University (Marimuthu & Gardner, 2019; Marimuthu, Hocking-Edwards, & Gardner, 2018). This technology takes advantage of the differing dielectric properties ($\varepsilon^* = \varepsilon' - j\sigma$, ε' = permittivity and σ = conductivity) of biological tissues, where an antenna transmits pulses into the tissues, resulting in a frequency-dependent diversion and scattering at the interface between differing tissues (Hussain, 1998;

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Jafari et al., 2006; Marimuthu et al., 2016). The backscattered array is collected by the same antenna and the signal analysed allowing measurement of the tissue layers (Jafari et al., 2006; Marimuthu et al., 2016). The low power frequencies of MiS is non-destructive thus poses no health risks to living or dead tissues (Zastrow, Davis, & Hagness, 2007). The measurement is instantaneous and requires no specific training of the operator apart from accurate placement of the antenna on the desired site of measurement. MiS for use in human medical imaging has been an active area of research over the last few decades, with numerous published reviews (Bolomey & Jofre, 2010; Chen, Liang, Wang, Wang, & Parini, 2008; Hussain, 1998; Jafari et al., 2006; Klemm, Craddock, Leendertz, Preece, & Benjamin, 2008). The research and application of microwave generated electromagnetic waves for the prediction of meat quality is limited (Damez & Clerjon, 2013). In the meat industry, microwave sensing coupled with OCP has been trialled in the laboratory to evaluate the changes in muscle characteristics during beef aging (Clerjon & Damez, 2009; Clerjon & Damez, 2007) and muscle moisture content in broiler meat (Jilani, Wen, Cheong, & Ur Rehman, 2016; Jilani, Wen, Rehman, Khan, & Cheong, 2016). To the authors knowledge this experiment is the first time MiS has been used as a carcase precision tool in a commercial setting.

Preliminary work has demonstrated the capacity of the prototype MiS to predict carcase single site fat depth in beef (Marimuthu & Gardner, 2019) and lamb (Marimuthu et al., 2018). In these studies, the precision for predicting hot carcase P8 fat depth had a root mean square error (RMSEP) varying from 1.63 – 3.78 mm and R-square (R2) from 0.53 to 0.69 (Marimuthu & Gardner, 2019). Building on the findings of the preliminary research, this study details the calibration and validation of the prototype MiS on a larger sample size of beef cattle with diverse genotypic and phenotypic traits, to assess the precision and accuracy of predicting single site carcase fatness in beef carcasses. We hypothesised that MiS can provide adequate precision and accuracy for predicting P8 and rib fat depth in beef carcasses.

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1 Project objectives

The overall objective of this work is testing and validation of a low cost, portable, MiS scanning device to predict fat depth in beef carcasses.

2 Experiment One: Laboratory calibration

2.1 Methods

2.1.1 Experimental design and slaughter protocol

Six slaughter groups of commercial Australian beef cattle were used across two experiments, to test the calibration and validation of the prototype MiS. Five groups of Bos Taurus (Angus, Hereford-Angus cross, or Hereford breed) cattle were sourced from the Beef Information Nucleus (BIN) project. Briefly, the BIN project is a collaboration between Meat Livestock Australia (MLA) and the major beef breed societies of Australia with the aim to improve genotypic and phenotypic data capture to accelerate genetic progress (Banks, 2011). One group of cattle were commercial Bos indicus Droughtmaster-Brahmin cross slaughter cattle not part of the BIN project. The slaughter groups and breeds are listed in Table 1.

The consignment of cattle and slaughter protocol was the same for both experiments. All cattle were grain-fed prior to slaughter for 100-105 days except for slaughter group 1 which were grass-fed. Cattle were processed at Meat Standards Australia (MSA) accredited commercial abattoirs, one located in Tasmania, one in New South Wales (NSW) and two in Queensland.

In line with Meat Standards Australia (MSA) protocols, all cattle were consigned direct from farm-gate to slaughter and processed within 48 hours from leaving the farm with no more than 12 hours in lairage prior to slaughter (MSA, 2016). Each slaughter group was processed on a different kill day, with the breakdown of groups, kill date and abattoir location listed in Table 1. Cattle were processed under standard commercial operating systems, identified with a carcase ticket and electrical stimulation and trimming performed to AUSMEAT standards (AUSMEAT, 2016).

Within one hour of slaughter, hot standard carcase weight was recorded, and manual measurement of the fat depth at the P8 site on the hot carcase was measured using the cut-and-measure technique. The P8 was measured by AUSMEAT accredited abattoir personnel with a metal ruler at the point defined by AUSMEAT (2016) “the point of intersection of a line from the dorsal tuberosity of the tripartite tuber ischia parallel with the chine, and a line at 90o to the sawn chine centred on the crest of the spinous process of the third sacral vertebrae”.

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Individual carcase grading was performed according to AUSMEAT chiller assessment measurements (Anonymous, 2005). BIN cattle (slaughter groups 1-4, 6) carcase assessment was performed by a single expert MSA grader at each abattoir, with a total of three different MSA graders used (one grader allocated per abattoir). The commercial cattle (slaughter group 5) carcase assessment was performed by a single in plant commercially accredited AUSMEAT grader. The left carcase was quartered at the 12/13th rib, cutting straight across the eye muscle. The depth of subcutaneous fat over the rib was measured using a metal ruler on the exposed cut surface, 75% across the dorsal surface of the rib eye muscle (Anonymous, 2005). Other carcase measurements included marbling, ossification, eye muscle area, ultimate pH and temperature of m. longissiumus thoracis (loin) (Anonymous, 2005).

2.1.2 MiS Carcase measurements

2.1.2.1 MiS coupled with VPA

With the MiS coupled with an Antipodal Vivaldi Antenna (VPA), the measurements were divided into two sub-experiments based on sampling time post mortem and carcase site measured; (a) hot carcase at the P8 site, (b) cold carcase at the rib site.

Experiment A

The MiS scanning of the hot carcase P8 site commenced at 40 min post slaughter on slaughter Groups 2 – 4 & 6 (Table 1). Determining the correct location for the probe took approximately 2 seconds and the signal transmission approximately 1 second.

Experiment B

Carcase sides were chilled overnight and the following morning, prior to MSA grading, cold carcase subcutaneous rib fat depth was measured using the prototype MiS coupled with an VPA antenna for slaughter groups 1, 2, and 4 – 6 (Table 1). The antenna was placed at the AUS-MEAT site for rib fat measurement, on the left side of the carcase at the 12th/13th rib, 75% along the dorsal surface of the rib eye muscle (Anonymous, 2005).

2.1.2.2 MiS Coupled with OCP

The prototype MiS coupled with an open-ended coaxial probe (OCP) scanned the hot carcase P8 site only, commencing 40 min post slaughter, on slaughter Groups 1 and 5 (Table 1).

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2.1.3 Description of MiS hardware and signal analysis

In both experiments, the portable MiS was designed as a single-port vector network analyser with built in computer modules, operating system and an automated python-based program utilising a R54 vector refractometer from Copper Mountain Technologies (Copper Mountain Technologies, Indianapolis, USA). The design and fabrication of MiS, OCP and VPA was conducted at Murdoch University, Western Australia.

The VPA was designed using a dual-elliptically tapered antipodal slot. VPA has a planar structure and was designed and fabricated on RO3010™ (Rogers Cooperation, Advanced Connectivity Solutions, USA) laminates of dielectric constant 10.2, dissipation factor 0.0022, thickness of 1.27 mm and copper cladding of 17μm top and bottom. The antenna was 95 mm in height, 110 mm in length, and 1.27 mm in width, embedded in a 4 mm Teflon casing, creating an overall dimension of 5.27 mm width and 99 mm height contact point with the carcase. A non-contact approach was used for the MiS system coupled with the VPA (Korostynska, Mason, & Al-Shamma'a, 2014; Marimuthu, 2016; Marimuthu et al., 2016; Mohammed, Abbosh, Mustafa, & Ireland, 2013), with the Teflon casing introduced to protect the antenna and maintain a set non-contact distance for precision. The narrow width of the VPA within Teflon creates good contact points on hot and cold carcases. The VPA has operating frequency from 100 MHz – 5.4 GHz with gain of 9.31dBi, directivity of 9.41dBi with main lobe direction at 90◦ of angular width (3dB) 65.9◦ at 3 GHz. (De Oliveira, Perotoni, Kofuji, & Justo, 2015; Fei, Jiao, Hu, & Zhang, 2011; Mohammed et al., 2013).

The OCP probe is a waveguide formed by two coaxial conducting cylinders (internal and external) (La Gioia, Porter, Merunka, Shahzad, Salahuddin, Jones, & O'Halloran, 2018; La Gioia, Salahuddin, O'Halloran, & Porter, 2018; Meaney, Gregory, Seppälä, & Lahtinen, 2016). The cylinders were constructed from aluminium with a conductivity of 3.77 x 107 S/m. The interior cylinder is the solid core of the probe with a 10 mm diameter while the hollow external probe has a 32 mm diameter. The space between the two cylinders is filled with Teflon, a dielectric material acting as an insulator. At the end of the waveguide, a flat disc (75 mm diameter, 3 mm thickness) around the fringe ensures minimal field diffraction. The designed OCP probe requires good probe-surface contact. It has 50 Ω characteristic impedance and operates at frequencies between 100 - 6 GHz. To connect the probe to the MiS, a SubMiniature version A (SMA) adapter was used. For adequate surface contact the OCP probe requires a semi-solid surface with homogenous properties hence it was used only on the hot carcase while the fat was in a semi-solid state.

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The MiS coupled with either OCP or VPA was set to transmit electromagnetic waves with 100 MHz to 5.4 GHz of microwave frequency at a power output of -10 dBm. The wave propagation is determined by the reflection coefficient (S11). The collection of reflected microwave signal S11(f) (f indicating frequency domain signals) were recorded at 10 MHz intervals from 100 MHz to 5.4 GHz, resulting in 531 frequency points (where j represents frequency points). With R indicating the raw signal, each collected S11(f)jR signal is composed of two components of data points: real (x(f)jR) and imaginary (y(f)jR) with the following equation:

$$S11(f)_{jR} = x(f)_{jR} + iy(f)_{jR} \quad j = 1, 2, \ldots..531$$

MiS calibration was performed at chiller temperatures using a “short, open and load” technique (Marimuthu, 2016). With ‘A’ indicating chiller ambient signals, the chiller ambient reflection coefficient $S11(f)j^{A}$ was recorded at 10 MHz intervals (100 MHz to 5.4 GHz -10 dBm) using the MiS coupled with probe/antenna in free space.

$$S11(f)_{jA} = x(f)_{jA} + iy(f)_{jA} \quad j = 1, 2, \ldots\ldots, 531$$

Processing of signals was performed whereby the chiller signal $S11(f)j^{A}$ was subtracted from the carcase signal in Matlab (R2019b)® (The Math Works Inc., Natick, MA, USA). Statistics and analysis was performed in WEKA® 3.9.4 (The University of Waikato, Hamilton, New Zealand).

$$S11(f)_j = S11(f)_{jR} - \ S11(f)_{jA}$$

$$S11(f)_j = (x(f)_{jR} - x(f)_{jA}) + \ i(y(f)_{jR} - \ y(f)_{jR})$$

$$S11(f)_j = x(f)_j + \ iy(f)_j \quad j = \ 1, 2, \ldots\ldots, 531$$

The prediction of P8 or rib fat depth was obtained by calculating the magnitude of the feature dataset from the real (x(f)j) and imaginary (y(f)j) of S11(f)j processed frequency domain using the following equation:

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$$Mag(S11(f)_j) = |S11(f)_j| = \sqrt{x(f)_j^{\,2} + y(f)_j^{\,2}}$$

2.1.4 Calibration of MiS device

Calibration of the prototype MiS was performed in the laboratory using “Short, Open and Load” techniques described in Marimuthu (2016). Prior to capturing carcase measurements in the abattoir, an ambient temperature free space measurement was captured, where the device was pointed into free space, ensuring no objects were within 2 meters. The free space measurement was used in data processing. Data pre-processing, calculation and prediction of tissue depth was conducted in Matlab (R2019b)®(The Math Works Inc., Natick, MA, USA) by the methodology described in Marimuthu *et al.* (2020,2021).

2.1.5 Statistical analysis

The statistical method for constructing the prediction equations for both VPA and OCP measurements were the same. For the VPA, the estimation for each site (Experiment (a) P8 or Experiment (b) rib fat depth) was derived separately. The P8 or rib fat depth was derived by calculating the magnitude of the calibrated and processed frequency domain signals (|S11(f)j|k (j = 1,2, ….., 531) where k represents the individual carcase). The statistical method for constructing the prediction equations was by a machine learning ensemble stacking method in WEKA® 3.9.4 (The University of Waikato, Hamilton, New Zealand). The ensemble stacked generalisation approach layered two different prediction models to create a meta-algorithm (Elshazly, Elkorany, Hassanien, & Azar, 2013; Ribeiro & dos Santos Coelho, 2020). The models chosen to construct the layers were based on the fact that the behaviour between microwave signals and biological tissues is non-linear. The first prediction model layer was composed of algorithms derived from Support Vector Machine and Random Forest which are non-linear models suited to multivariate applications (Cui & Fearn, 2017). The second layer used Partial Least Squares Regression to construct a two-component model. The second layer is used to optimally combine the model predictions in a linear fashion (Sill, Takács, Mackey, & Lin, 2009), with Partial Least Squares Regression well suited to linear modelling (Cui & Fearn, 2017). The Ensemble method was chosen over independent models (Support Vector Machine, Random Forrest or Partial Least Squares Regression) as by combining them the accuracy is improved though the averaging of error in divergent models (Güneş, Wolfinger, & Tan, 2017).

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For VPA Experiment A, the estimations for P8 site (groups 2-4 and 6) were pooled then randomly divided into 5 groups balanced for P8 fat depth. A k-fold cross validation (k=5) technique was performed, where training was performed on 80% of the data (4 groups) and validated on the remaining 20% (the 5th group), resulting in a total of 5 validation groups. The P8 validation predictions for Experiment A are reported in Table 2

For VPA Experiment B rib fat datasets (groups 1,2 and 4-6), the same training and validation procedures were applied. All data was again pooled and randomly divided into 5 groups balanced for rib fat depth prior to performing the a k-fold cross validation (k=5) technique using ensemble machine learning (Table 3).

For OCP measurements, the same methodology was applied, Slaughter group 1 and 5 OCP estimations were pooled then randomly divided into 5 groups balanced for P8 fat depth before the k-fold cross validation (k=5) training and validation. The validation predictions for Experiment 2 are reported in Table 4

For all results, the precision between actual and MiS predicted values is expressed as R-square (R2) and root mean square error of the prediction (RMSEP). Within the text, the R2 values for the model are expressed as the percentage (%) of the variation that the model describes. Accuracy of the prediction model is expressed via bias and slope. The slope is the difference between the actual and predicted slopes. The bias was calculated by taking the difference between the predicted and actual values at the mean of the dataset. The average slope represents the mean of the deviation of each slope estimate from 1.

2.2 Results

Descriptive statistics are provided in Table 1, demonstrating the range in HCWT, P8 fat depth and rib fat depth from the 6 slaughter groups.

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Table 1 Descriptive statistics including animal numbers (n), antenna OCP or VPA, and mean ± standard deviation, minimum and maximum for hot standard carcase weight (kg), P8 fat depth (mm) and Rib fat depth (mm).

Kill Date	Slaughter group	Abattoir	Breed	n	Sex	Antennae hot carcase measurement	Antennae cold carcase measurement	Hot standard carcase weight (kg)	Hot P8 fat depth (mm)	Cold rib fat depth (mm)
19 Jul 2018	1	JBS Longford, Tasmania	Hereford-Angus Cross	156	Steers	OCP	VPA	306.6 ± 28.7 (229.8 – 365.4)	8.13 ± 2.98 (4 – 20)	6.82 ± 2.55 (2 – 17)
17 Dec 2018	2	NH Foods Wingham, NSW	Hereford	45	Heifer	VPA	VPA	253.0 ± 20.6 (218.5 – 299.5)	17.0± 3.42 (9 - 25)	7.45 ± 2.27 (3 – 13)
06 Feb 2019	3	NH foods Wingham, NSW	Hereford	78	Steers	VPA	-	352.1 ± 25.9 (269.0 - 400.0)	20.6 ± 4.20 (13 - 34)	11.10 ± 3.60 (5 - 23)
01 Apr 2019	4	John Dee Warwick, Queensland	Angus	48	Steers	VPA	VPA	288.8 ± 28.7 (213.5 - 338.0)	13.7 ± 3.70 (5 - 22)	9.13 ± 3.27 (3 - 20)
16 Apr 2019	5	ACC, Brisbane, Queensland	Droughtmaster-Brahmin Cross	279	Mixed	OCP	VPA	257.9 ± 17.9 (202.6 – 308.7)	13.4 ± 4.07 (5 - 28)	7.25 ± 3.25 (3 - 20)
05 Jun 2019	6	NH foods Wingham, NSW	Herefords	70	Steers	VPA	VPA	351.3 ± 37.5 (284.5 – 467.5)	21.1 ± 4.51 (13 - 40)	14.0 ± 5.46 (5 - 28)

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2.2.1 MiS coupled to VPA

2.2.1.1 Experiment A

The precision of the VPA on the hot carcase P8 site had an average RMSEP of 2.86 mm, with the R2 on average explained 58% of the variation (Table 2). The range in values across the 5 validation tests for RMSEP was 0.67 mm and R2 varied by 0.25 units. The maximum bias was 0.187 mm and at most the slope deviated 0.09 mm from 1. The association between actual and predicted hot carcase P8 fat depth using the VPA is depicted in Fig 1.

Table 2 VPA Experiment A. Precision and accuracy estimates for k-fold (k=5) cross validation of models predicting hot carcase P8 fat depth using a VPA (slaughter group 2, 3, 4 and 6). Groups have been balanced for P8 fat depth. Precision estimates include R2 and root mean square error of the predicted (RMSEP). Accuracy estimates include slope which is the difference between the actual and predicted slopes, expressed as a deviation from 1, and bias which represents the difference between the actual minus predicted value calculated at the mean of P8 fat depth. P8 fat depth (mm) and hot standard carcase weight (kg) values reported are mean ± standard deviation (minimum, maximum) of the raw values for each of the 5 validation groups.

Validation Group	N in validation	R2	RMSEP (mm)	Bias (mm)	Slope	Hot standard carcase weight (kg)	Hot P8 fat depth (mm)
1	49	0.61	2.85	+0.061	-0.10	312.3 ± 51.2 (221.5 – 467.5)	18.12 ± 4.54 (10 - 33)
2	48	0.47	3.12	-0.011	0.09	305.9 ± 41.6 (223.0 – 401.5)	17.67 ± 4.69 (5 - 30)
3	48	0.46	3.17	+0.036	0.08	305.8 ± 46.7 (218.5 – 417.5)	17.69 ± 4.54 (8 - 30)
4	48	0.71	2.50	+0.187	-0.09	297.0 ± 44.2 (223.0 – 411.5)	17.64 ± 4.52 (8 - 29)
5	48	0.64	2.64	-0.139	0.00	303.0 ± 49.4 (213.5 – 435.0)	17.62 ± 4.37 (9 - 27)
	Average	**0.58**	**2.86**	**0.087***	**0.07***	304.8 ± 46.6**	17.75 ± 4.49**

*mean of the absolute values, **value represents the pooled mean ± SD of all animals

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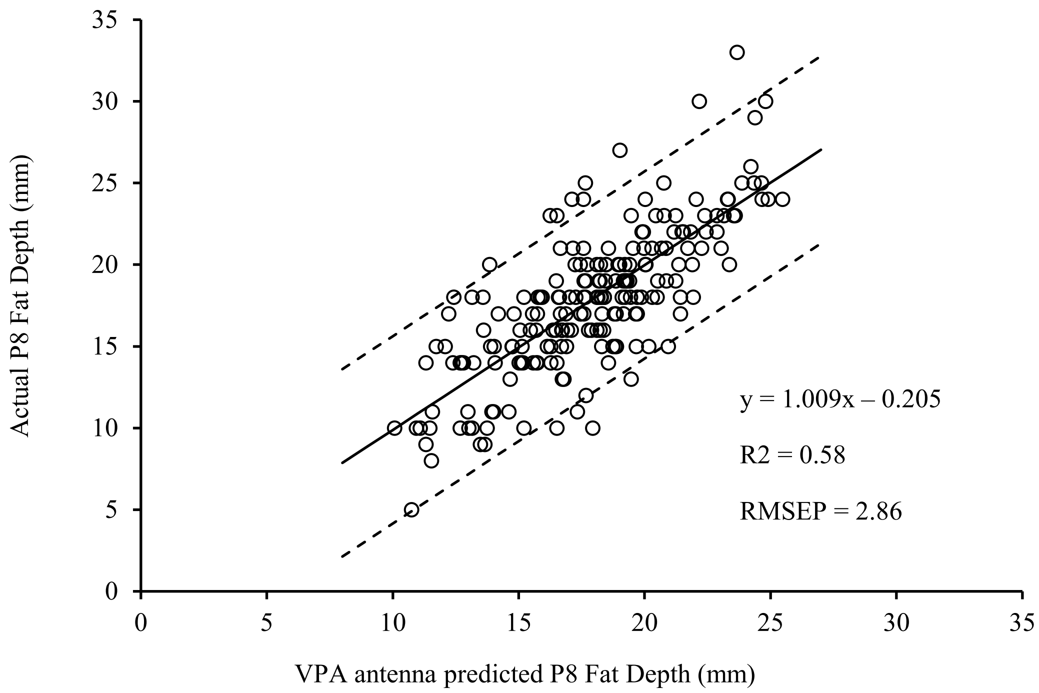

Figure 1. VPA Experiment A. The association between actual and microwave predicted hot carcase P8 fat depth (mm) using a VPA antenna. The predictions are derived from the validation tests detailed in Table 2 (n=241). The actual tissue depths were then regressed against the predictions. Solid line represents the relationship between predicted and actual measurements Dashed lines represent ± 2 x RMSEP on the Y axis.

2.2.1.2 Experiment B

The average precision of the VPA to predict cold carcase rib fat (Table 3) had an average RMSEP of 2.60 mm, with the R2 explaining on average 55% of the variation. The RMSEP range across the 5 validation tests was 0.32 mm and R2 varied by 0.09 units. The maximum bias was 0.117 mm and the maximum slope deviated 0.07 mm from 1. The association between actual and predicted rib fat depth for cold carcase VPA is depicted in Figure 2.

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Table 3 Experiment B. Precision and accuracy estimates for k-fold (k=5) cross validation of models predicting cold carcase rib fat depth using a VPA (slaughter groups 1, 2, 4 - 6). Precision estimates include R2 and root mean square error of the predicted (RMSEP). Accuracy estimates include slope which is the difference between the actual and predicted slopes, expressed as a deviation from 1, and bias which represents the difference between the actual minus predicted value calculated at the mean of rib fat depth. Rib fat depth (mm) and hot standard carcase weight (kg) values reported are mean ± standard deviation (minimum, maximum) of the raw values for each of the 5 validation groups.

Validation Group	N in validation	R2	RMSEP (mm)	Bias (mm)	Slope	Hot standard carcase weight (kg)	Cold rib fat depth (mm)
1	120	0.55	2.66	-0.117	-0.03	290.4 ± 41.5 (222.1 – 467.5)	8.06 ± 3.97 (2 – 27)
2	120	0.56	2.62	+0.064	0.01	297.3 ± 40.4 (224.0 – 392.0)	8.03 ± 3.95 (2 - 27)
3	120	0.55	2.60	+0.099	0.04	287.5 ± 40.6 (202.6 – 411.5)	8.03 ± 3.87 (3 - 26)
4	119	0.50	2.71	+0.117	0.00	290.6 ± 42.3 (216.5 – 401.5)	8.02 ± 3.84 (3 - 25)
5	119	0.59	2.39	-0.077	-0.07	290.4 ± 40.0 (228.8 – 435.0)	8.00 ± 3.74 (3 - 22)
	Average	**0.55**	**2.60**	**0.095***	**0.03***	291.2 ± 40.9**	8.03 ± 3.86**

*mean of the absolute values, **value represents the pooled mean ± SD of all animals

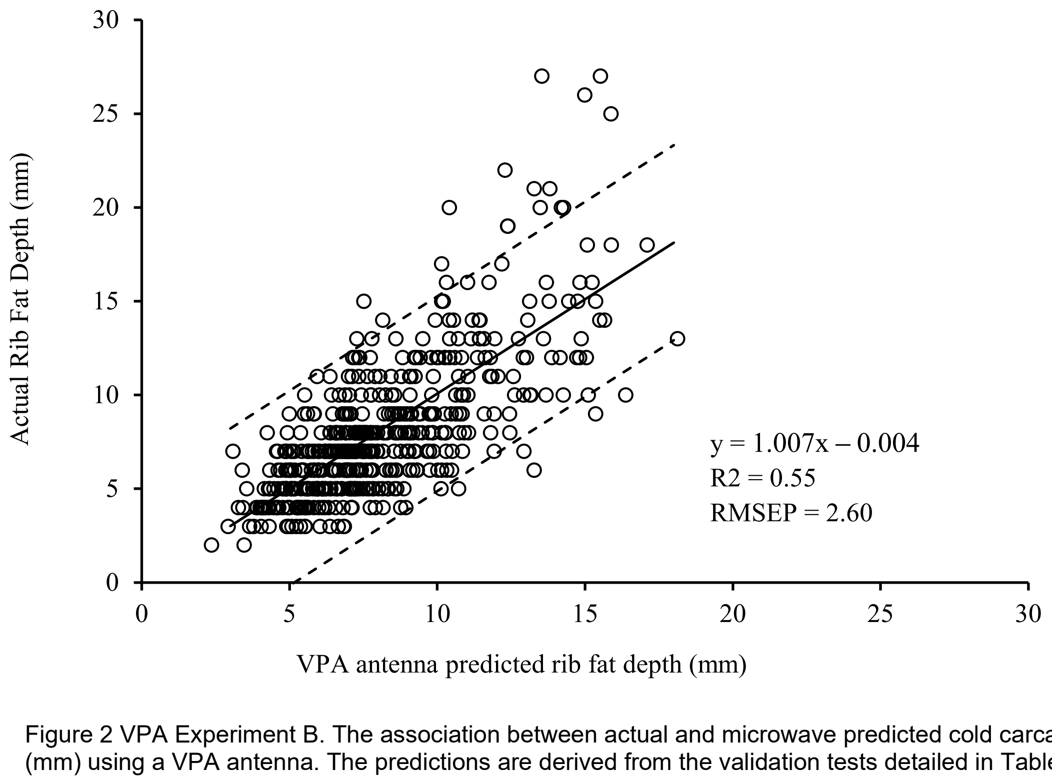

Figure 2 VPA Experiment B. The association between actual and microwave predicted cold carcase rib fat depth (mm) using a VPA antenna. The predictions are derived from the validation tests detailed in Table 3 (n=598). The actual tissue depths were then regressed against the predictions. Solid line represents the relationship between predicted and actual measurements. Dashed lines represent ± 2 x RMSEP on the Y axis.

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2.2.2 MiS coupled to OCP

The precision of OCP to predict hot carcase P8 fat depth had an average RMSEP of 2.47 mm, with the R2 explaining 70% of the variation (Table 4). There was a 0.27 mm range in RMSEP over the 5 validation groups, and the R2 varied by 0.06 units. The maximum bias was 0.239 mm with the maximum slope deviating 0.9 mm from 1. The association between actual and predicted hot carcase P8 fat depth using the OCP is depicted in Fig. 3.

Table 4 Precision and accuracy estimates for k-fold (k=5) cross validation of models predicting hot carcase P8 fat depth using an OCP probe (slaughter group 1 and 5). Groups have been balanced for P8 fat depth. Precision estimates include R2 and root mean square error of the predicted (RMSEP). Accuracy estimates include slope which is the difference between the actual and predicted slopes, expressed as a deviation from 1, and bias which represents the difference between the actual minus predicted value calculated at the mean of P8 fat depth. P8 fat depth (mm) and hot standard carcase weight (kg) values reported are mean ± standard deviation (minimum, maximum) of the raw values for each of the 5 validation groups.

Validation Group	N in validation	R2	RMSEP (mm)	Bias (mm)	Slope	Hot standard carcase weight (kg)	Hot P8 fat depth (mm)
1	87	0.72	2.45	-0.062	0.00	277.3 ± 31.5 (228.6 – 353.0)	11.51 ± 4.69 (4 - 28)
2	87	0.72	2.42	+0.022	-0.01	275.3 ± 32.8 (207.3 – 350.9)	11.48 ± 4.62 (4 - 27)
3	87	0.73	2.34	+0.162	-0.02	278.4 ± 34.3 (212.2 – 365.4)	11.42 ± 4.52 (4 - 24)
4	87	0.68	2.53	+0.179	-0.03	277.0 ± 33.3 (202.6 – 362.8)	11.32 ± 4.34 (4 - 21)
5	87	0.67	2.61	-0.239	+0.9	269.6 ± 29.2 (216.5 – 365.2)	11.54 ± 4.61 (4 - 22)
	Average	**0.70**	**2.47**	0.133*	**0.03***	275.5 ± 32.3**	11.45 ± 4.54**

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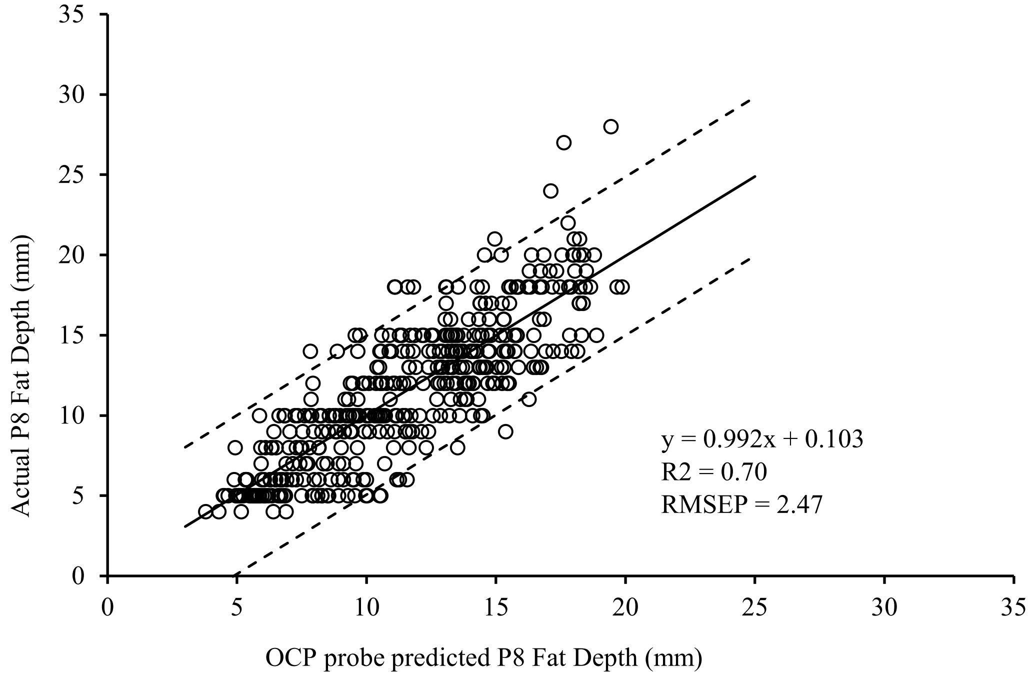

igure 3 The association between actual and microwave predicted hot carcase P8 fat depth (mm) using a OCP probe. The predictions are derived from the validation tests detailed in Table 4 (n=435). The actual tissue depths were then regressed against the predictions. Solid line represents the relationship between predicted and actual measurements. Dashed lines represent ± 2 x RMSEP on the Y axis.

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2.3 Discussion

Supporting our hypothesis, the prototype MiS had good precision and accuracy in predicting beef carcase fat depth. The precision indicators were the greatest when MiS was coupled with OCP to predict hot carcase P8. This precision was improved on previous MiS OCP prediction, where average RMSEP for predicting hot P8 ranged from 1.63 to 3.78 mm and R2 from 0.53 to 0.69 between validation tests (Marimuthu & Gardner, 2019). All bias values were ≤0.23 mm which was approximately one third of the bias previously reported (Marimuthu & Gardner, 2019), demonstrating the ability of this system to accurately predict fat depth in the beef carcase. A direct comparison cannot be made between the OCP and VPA probe on the hot carcase P8 as they utilised different animals, however the authors suggest that one reason for the apparently greater precision of the OCP may in part be due to the larger area of electromagnetic wave/field interaction in comparison to the VPA (Meaney et al., 2016). A major challenge in deploying dielectric probes/antennas is maintaining even surface contact between the device and material under test. The cylindrical design of OCP allows for excellent contact on a semi-solid surface while the probe remains at a perpendicular angle. The planar design of the VPA with a 5.27 mm wide contact point may not always be perfectly perpendicular to the tissue surface thus distorting the field measured.

The precision of MiS coupled with VPA to predict cold carcase rib fat depths in Experiment (a) was similar to that previously reported (Marimuthu & Gardner, 2019). Furthermore, within Experiment (b), the VPA predicted cold carcase rib fat with similar precision and accuracy as it did for hot carcase P8. Although direct comparisons cannot be made, as the measurements were taken at different time points and at different sites, this does suggest the potential for this device to perform on a hot or cold carcase at variable times post mortem. Predicting cold carcase fat depth is a clear advantage over ultrasound (Hoskins, Martin, & Thrush, 2019), however further testing and validation at series of temperature thresholds on the same tissue site is required. There was less variation in the ranges of R2 and RMSEP for the cold carcase VPA predictions compared to the hot carcase VPA predictions which was unexpected as on a cold carcase, subcutaneous fat hardening into an irregular shape impedes even contact, resulting in a probe to air-fat interface, refracting the electromagnetic wave/field away from the antenna and carcase. Another factor to consider in the difference between hot versus cold precision is potential variability in tissue hydration. Animals slaughtered may be at varying stages of dehydration which may add to the imprecision of the hot measurements by altering the dielectric properties of the tissues (Vijay, Jain, & Sharma, 2015). At the time of cold carcase measurement the bulk of dehydration and carcase shrinkage would have occurred (Greer, Jones, Dilts, & Robertson, 1990; Jones, Murray, & Robertson, 1988) thus stabilising any water content differences of the tissues.

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Future validation needs to test the transportability of these equations on actual kill-group datasets as well as pooled data. Balancing the groups for tissue depth normalises any differences that may influence model coefficients such as environment or calibration factors. However transporting equations across actual kill group data sampled at different sites is required to ensure the predictions are not affected by chiller or day factors. Chiller factors to take into consideration include carcase arrangement and spray chilling. Tight carcase alignment can affect operator ability to obtain perfect antenna placement and the use of spray chilling resulting in a wet surface will reduce electromagnetic precision as water has its own dielectric properties thus will alter microwave transmission and reflection (Vijay et al., 2015).

The value in improved technology lies in its ability to be easily adopted into commercial industry (Toohey, van de Ven, & Hopkins, 2018). Barriers to commercial adoption include accuracy, cost, space, and ease of use (Toohey et al., 2018). Industry accredited beef carcase objective single site fat scoring technologies exist, such as the optical Hennessy Grading Probe™ (HGP) (Auckland, New Zealand). Initial experiments demonstrated excellent precision of the HGP to predict P8 fatness in beef. Across two abattoir sites utilising 828 carcasses, Phillips et al (1987) demonstrated on the hot carcase P8, using a quadratic equation that HGP prediction R2 ranged from 0.88 – 0.94 and residual standard deviation (a similar statistic to RMSEP when sample size is large) from 1.29 to 1.84 mm. Using one abattoir site and 1850 carcasses sampled over 12 months Hopkins (1989) precision of HGP using a linear equation gave an R2 of 0.94 and residual standard deviation from 1.99 to 2.00 across two data subsets. While HGP has been AUSMEAT accredited for decades, the commercial adoption of this portable device is low, with a 2015 survey of Australian processors finding 75% of processors were aware of HGP, however only 32% implemented it into their grading system (Toohey et al., 2018). Interestingly the survey identified that processor perceived accuracy of HGP was questionable (Toohey et al., 2018) which highlights the importance of repeat commercial validation studies with targeted education of new devices for industry. While in this study, the prototype MiS does not have as high a precision as HGP, further investigation directly comparing different antenna designs is required. Identifying highly responsive frequency's may offer greater precision and accuracy as well as exploring varying frequency time domains.

The long term industry aim when developing new objective carcase technologies is the ability is to be able to move away from single site measurements, as these will have reduced predictive power when compared to whole carcase composition technologies (Anderson, Williams, Pannier, Pethick, & Gardner, 2015; Williams et al., 2017a). A potential enhancement for MiS lies in its ability to integrate into an array structure for multi-site determination of

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carcase fatness, similar in design to technologies like the ultrasound-based AutoFom system (Busk, Olsen, & Brøndum, 1999). Although there is no reported evidence of AutoFom used in beef, single site ultrasound has been trialled to predict carcase fat depth and composition in beef (Andersen, Busk, Chadwick, Cuthbertson, Fursey, Jones, Lewin, Miles, & Owen, 1983; May, Mies, Edwards, Harris, Morgan, Garrett, Williams, Wise, Cross, & Savell, 2000; Miles, Fisher, Fursey, & Page, 1987) and sheep (Miles, Fursey, Fisher, & Page, 1991). However, to the authors knowledge ultrasound has not been applied within these industries due to industrial limitations such as air-bubbles on the surface of the subcutaneous fat impeding the ultrasound signal, and variable surface contact. In the pork industry the AutoFom is located immediately after dehairing when the carcase has a wet, smooth surface, with skin-on, and therefore no air-bubbles resulting in good probe contact and signal penetration. In contrast, the MiS coupled with VPA technology used in this study was based on a non-contact approach (Korostynska et al., 2014; Marimuthu, 2016; Marimuthu et al., 2016; Mohammed et al., 2013). The Teflon casing was introduced to protect the antennae and maintain a set non-contact distance to maintain precision. In future, the authors predict that a set distance between antennae and carcass may be able to be achievable via automation using various sensors (Marimuthu, 2016; Marimuthu et al., 2016; Mohammed et al., 2013).

As MiS is non-destructive, thus completely safe for human operators without requiring shielding (Zastrow et al., 2007), this will also enable MiS to be tested as an objective measurement technology in the live animal. As MiS is instantaneous requiring no specialised training for use apart from correct probe placement, this technology may offer great advantages over the current techniques of ultrasound determined fat depth in the live animal. Subcutaneous fat depth underpins carcase boning decisions and value-based trading thus technologies that improve the precision and accuracy of this measurement will greatly enhance beef industry productivity and profitability. The low cost and portability of MiS offers the opportunity to establish an objective carcase measurement solution for smaller operators distinct from other technologies which are typically focused on large-scale enterprises.

3 Experiment Two: On-site calibration block

3.1 Methods (Experiment Two: On-site calibration block)

3.1.1 Experimental design and slaughter protocol

Fourteen slaughter groups of commercial Australian beef cattle were used to test the calibration and validation of a commercial MiS (C-MiS). All cattle were grain fed prior to slaughter and processed at the same MSA accredited commercial abattoir in Queensland. As

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per MSA protocols, all cattle were consigned direct from farm-gate to slaughter and processed within 48 hours from leaving the farm with no more than 12 hours in lairage prior to slaughter (MSA, 2016). The breakdown of groups and kill date are listed in Table 5. Cattle were processed under standard commercial operating systems, identified with a carcase ticket and electrical stimulation and trimming performed to AUSMEAT standards (AUSMEAT, 2016).

Within one hour of slaughter the carcase measurements were obtained as per the methodology in Section 2.1.1. and 2.1.2.1.

3.1.1.1 P8 fat depth

Microwave scanning of the P8 site was performed immediately after the manual measurement of P8 by an AUSMEAT accredited abattoir personnel measurement. For groups 1 – 8 (Table 5) C-MiS device 2 (C-MiS2) was used (n=1303). For groups 9-14 (Table 5) C-MiS device 4 (C-MiS4) was used (n=678). The centre of the VPA antenna was positioned over the P8 site for measurement.

3.1.1.2 Rib fat depth and eye muscle area

Microwave scanning of hot carcase rib fat and eye muscle area (EMA) was obtained on the chain approximately 40 minutes after slaughter prior to entry. A subset of carcases from those listed in Table 5 were scanned. C-MiS device 1 was used for measurements, with the centre of the antenna positioned over the 12 – 13th rib, approximately 10 cm from midline (Figure 4). Carcases scanned for rib fat and EMA were graded by MSA graders (n=568) and another group (n=2,405) scanned for rib fat only were graded by in plant graders.

Figure 4: Microwave scanning of hot carcase rib fat site on the chain

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3.1.2 Description of MiS hardware and signal analysis

The commercial device used the same software and signal analysis as detailed in Section 2.1.3. There was a change in hardware in the construction of the commercial device, with the external casing constructed from high grade, heat resistant and food safe plastic. The commercial device design had an updated antenna connection. Previously, the antenna was unplugged from the system when not in use. The commercial device antenna is permanently fixed to the system within the plastic casing. The commercial device contains a 9000mH lithium battery attached directly to the handle, with a UBC charging port. The dimensions of the C-MiS are 15 cm length x 11 cm x 30 cm depth (*Figure 5(a)*). During measurements The C-MiS was attached via a hook to a pully system mounted on the chain.

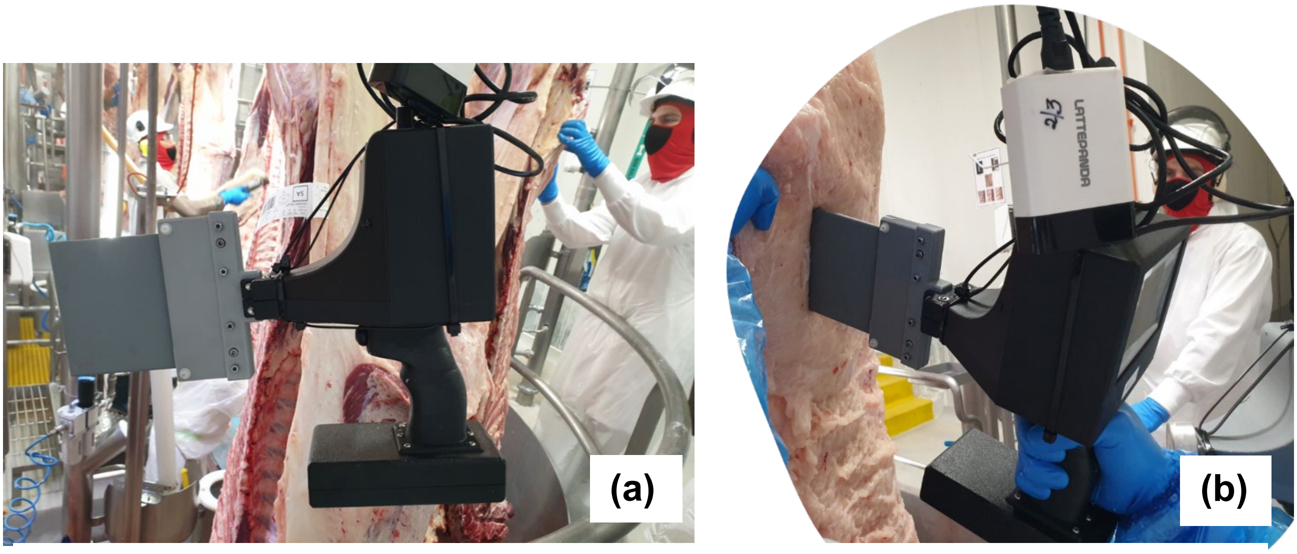

Figure 5: Microwave System (a) C-MiS2 at Kill floor at commercial abattoir attached to pully for the hot carcase measurements. (b) C-MiS2 Microwave scanning of hot P8

3.1.3 Calibration of MiS device

The within device calibration was performed using the “Open, Short and Load” calibration technique within the abattoir chiller (Marimuthu, 2016). Open calibration used a free space measurement, which was captured by pointing the device into free space, ensuring no objects were within 2 meters of the device. Short calibration used the calibration block, where the antenna contacted a metal plate embedded within the block (Figure 6). Load calibration used the calibration block where the antenna contacted Teflon (Figure 6). Based on the calibration measurements, each C-MiS device had a 3 term error coefficient calculated.

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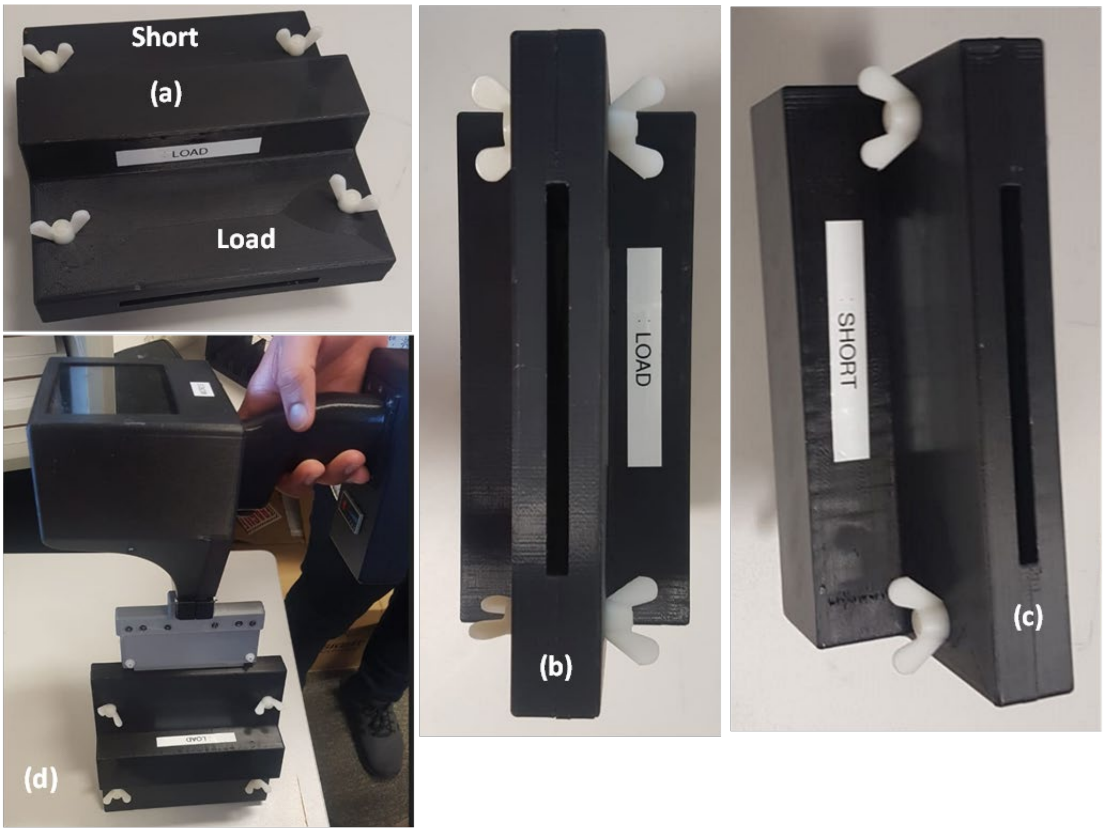

Figure 6 (a) Calibration block unit (b) antenna slot for load calibration (c) antenna slot for short calibration (d) antenna in situ demonstrating how calibration is taken

3.1.4 Statistical analysis

The method for constructing prediction equations was via a machine learning ensemble stacking method in WEKA® 3.9.4 as described in Section 2.1.5.

For each carcase trait (P8, rib fat, EMA), the estimations were pooled, randomly divided into 5 groups balanced for its trait. A k-fold (k=5) cross validation technique was performed.

As per Section 2.1.5 only validation data is reported.

To evaluate the precision predictors for P8 fat depth with regards to AUS-MEAT accreditation, the C-MiS P8 fat depth predictions were compared to the ruler measured P8 fat depth. The error rate was calculated as per the AUS-MEAT national accreditation standards (AUS-MEAT, 2020), where predictions must be within ± 1mm for P8 fat depths up to and including 5mm, within ± 2mm for P8 fat depths over 5 mm and including 10 mm, and within ± 3 mm for P8 fat depths greater than 10 mm. Predictions must be made with 90% accuracy.

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3.2 Results (Experiment Two:)

Descriptive statistics are presented in *Table 5* demonstrating the range in HCWT, fat depths and EMA.

Table 5 Descriptive statistics including animal numbers (n), and mean ± standard deviation, minimum and maximum for hot standard carcase weight (kg), P8 fat depth (mm) and Rib fat depth (mm).

Kill Group	Kill Date	Type	n	P8 Device	HCWT (kg)	Hot P8 (mm)	Cold Rib Fat (mm)	EMA (kg)
1	27 May 2022	RV Long Fed	330	C-MiS2	452.54 ± 46.96 (196.60 – 548.60)	19.55 ± 5.62 (8.00 – 35.00)	17.68 ± 5.80 (4.00 – 35.00)	93.46 ± 6.91 (78.00 – 120.00)
2	27 May 2022	JC Black Angus	49	C-MiS2	439.63 ± 42.52 (341.40 – 513.60)	20.08 ± 5.99 (10.00 – 35.00)	21.84 ± 2.63 (15.00 – 25.00)	71.51 ± 3.79 (63.00 – 77.00)
3	27 May 2022	JC Black Angus	146	C-MiS2	405.47 ± 25.77 (329.60 – 480.00)	21.16 ± 4.93 (5.00 – 35.00)	20.81 ± 3.15 (12.00 – 26.00)	72.25 ± 4.78 (62.00 – 85.00)
4	27 May 2022	JC Wagyu	127	C-MiS2	402.80 ± 39.71 (264.00 – 498.60)	19.21 ± 6.34 (6.00 – 35.00)	18.16 ± 3.31 (10.00 – 30.00)	81.60 ± 5.25 (65.00 – 94.00)
5	30 May 2022	RV Mid Fed	177	C-MiS2	392.83 ± 26.40 (240.40 – 451.60)	15.82±3.93 (8.00 – 27.00)	13.15 ± 4.29 (3.00 – 29.00)	94.77 ± 7.53 (75.00 – 120.00)
6	30 May 2022	Wool worths	303	C-MiS2	263.44 ± 20.29 (194.40 – 305.80)	11.90 ± 4.28 (3.00 – 27.00)	5.84 ± 2.62 (3.00 – 15.00)	80.90 ± 5.83 (54.00 – 96.00)
7	1 June 2022	WW Export	57	C-MiS2	305.51 ± 12.82 (279.20 – 333.60)	11.70 ± 4.24 (3.00 – 25.00)	13.11 ± 1.65 (5.00 – 16.00)	66.84 ± 8.39 (52.00 – 82.00)
8	1 June 2022	Arcadian	432	C-MiS2	343.63 ± 26.46 (156.60 – 433.00)	9.73 ± 4.58 (2.00 – 35.00)	6.20 ± 2.84 (3.00 – 33.00)	79.68 ± 5.41 (65.00 – 98.00)
9	3 June 2022	Stockyard Wagyu	71	C-MiS4	421.10 ± 49.99 (211.80 – 524.80)	13.37 ± 5.85 (3.00 – 30.00)	14.80 ± 4.72 (9.00 – 32.00)	106.44 ± 11.70 (85.00 – 130.00)
10	3 June 2022	Wool worths	193	C-MiS4	471.59 ± 38.15 (248.40 – 555.80)	20.07 ± 5.41 (5.00 – 35.00)	17.33 ± 5.75 (9.00 – 33.00)	104.67 ± 11.89 (80.00 – 128.00)
11	3 June 2022	RV Long Fed	226	C-MiS4	286.10 ± 16.44 (247.20 – 337.80)	10.85 ± 3.75 (2.00 – 25.00)	5.08 ± 2.25 (3.00 – 12.00)	82.57 ± 6.09 (65.00 – 109.00)
12	3 June 2022	JC Black Angus	123	C-MiS4	418.82 ± 45.19 (255.80 – 526.80)	22.02 ± 5.09 (11.00 – 35.00)	17.94 ± 4.46 (12.00 – 31.00)	78.80 ± 6.35 (65.00 – 97.00)
13	3 June 2022	JC F1	123	C-MiS4	452.80 ± 46.95 (330.20 – 572.40)	29.68 ± 8.77 (12.00 – 48.00)	25.22 ± 7.09 (13.00 – 46.00)	86.63 ± 4.90 (76.00 – 97.00)
14	6 June 2022	Stockyard Angus	206	C-MiS4	399.30 ± 31.81 (200.60 – 466.20)	18.31 ± 4.86 (10.00 – 30.00)	14.23 ± 5.96 (3.00 – 34.00)	101.90 ± 9.44 (86.00 – 130.00)

3.2.1 Hot P8 fat depth

C-MiS2 predicted Hot P8 fat depth with an average RMSEP of 2.775 mm with an R^2 explaining 81% of the variation (*Table 6*(a)). Across the 5 validation groups RMSEP varied by 0.529 mm and R^2 varied by 0.06 units. The largest bias across all validation groups was 0.219 mm and the greatest slope deviation was 0.034 from 1. The association between actual and predicted hot carcase P8 fat depth using the C-MiS2 is depicted in Figure 6(a).

C-MiS4 predicted Hot P8 fat depth with an average RMSEP of 3.094 with an R^2 explaining 85% of the variation (*Table 6*(a)). Across the 5 validation groups RMSEP varied by 0.222 mm

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and R^2 varied by 0.03 units. The largest bias across all validation groups was 0.450 mm and the greatest slope deviation was 0.041 from 1. The association between actual and predicted hot carcase P8 fat depth using the C-MiS4 is depicted in Figure 6(b).

When assessing the ability of MiS prediction of P8 fat depth under the AUS-MEAT National Accreditation standards for C-MiS 2 there were n=329 predictions outside the tolerance ranges (total n=1303), thus the AUS-MEAT error rate was 25.25%. When assessing C-MiS 4 there were n=198 predictions outside the tolerance limits (total n=678), thus the error rate was 29.20%.

Table 6 Precision and accuracy estimates for k-fold (k=5) cross validation of models predicting hot carcase P8 fat depth using (a) C-MiS device 2 (kill group 1 – 8) or (b) C-MiS device 4 (kill group 9-14). Groups have been balanced for P8 fat depth. Precision estimates include R2 and root mean square error of the predicted (RMSEP). Accuracy estimates include slope which is the difference between the actual and predicted slopes, expressed as a deviation from 1, and bias which represents the difference between the actual minus predicted value calculated at the mean of P8 fat depth. P8 fat depth (mm) and hot standard carcase weight (kg) values reported are mean ± standard deviation (minimum, maximum) of the raw values for each of the 5 validation groups.

Validation Group	N in validation	R^2	RMSEP (mm)	Bias (mm)	Slope (mm)	Ruler measured Hot P8 (mm)
(a) C-MiS device 2						
1	261	0.82	2.672	-0.219	-0.022	15.46 ± 6.37 (4.00 - 33.00)
2	261	0.80	2.783	-0.136	0.003	14.78 ± 6.26 (3.00 - 32.00)
3	261	0.81	2.807	0.155	0.016	14.80 ± 6.42 (2.00 - 35.00)
4	260	0.84	2.543	0.087	-0.034	14.38 ± 6.28 (3.00 - 35.00)
5	260	0.78	3.072	-0.014	-0.022	15.38 ± 6.61 (2.00 - 35.00)
	Average	0.81	2.775	0.122	0.019	14.96 ± 6.39 (2.00 - 35.00)
(b) C-MiS device 4						
1	136	0.84	3.091	-0.450	0.066	18.90 ± 7.45 (3.00 - 42.00)
2	136	0.87	2.977	-0.332	0.041	19.40 ± 7.79 (5.00 - 45.00)
3	136	0.85	3.199	-0.136	-0.030	17.66 ± 8.23 (5.00 - 45.00)
4	135	0.84	3.128	0.124	0.013	18.78 ± 7.80 (5.00 - 48.00)
5	135	0.87	3.072	-0.026	-0.010	18.79 ± 8.35 (3.00 - 43.00)
	Average	0.85	3.094	0.214	0.032	18.69 ± 7.93 (3.00 - 48.00)

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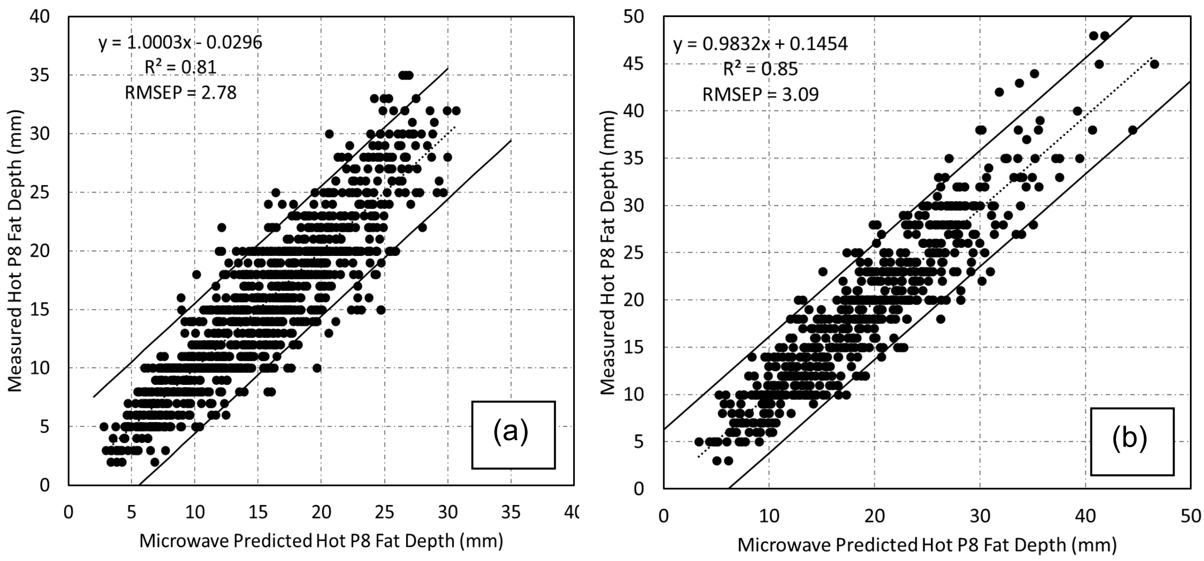

Figure 7 The association between actual and microwave predicted hot carcase P8 fat depth (mm) using (a) C-MiS device 2 or (b) C-MiS device 4. The predictions are derived from the validation tests detailed in Table 6. The actual tissue depths were then regressed against the predictions. Solid line represents the relationship between predicted and actual measurements Dashed lines represent ± 2 x RMSEP on the Y axis.

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The individual signal components of microwave demonstrated good association up to 30 mm of P8 fat depth (Figure 8). When real and imaginary signal components were plotted against each other they show a complex interaction which may not be well described by our linear modelling techniques (Figure 9). This represents an area for on-going investigation to better model this data.

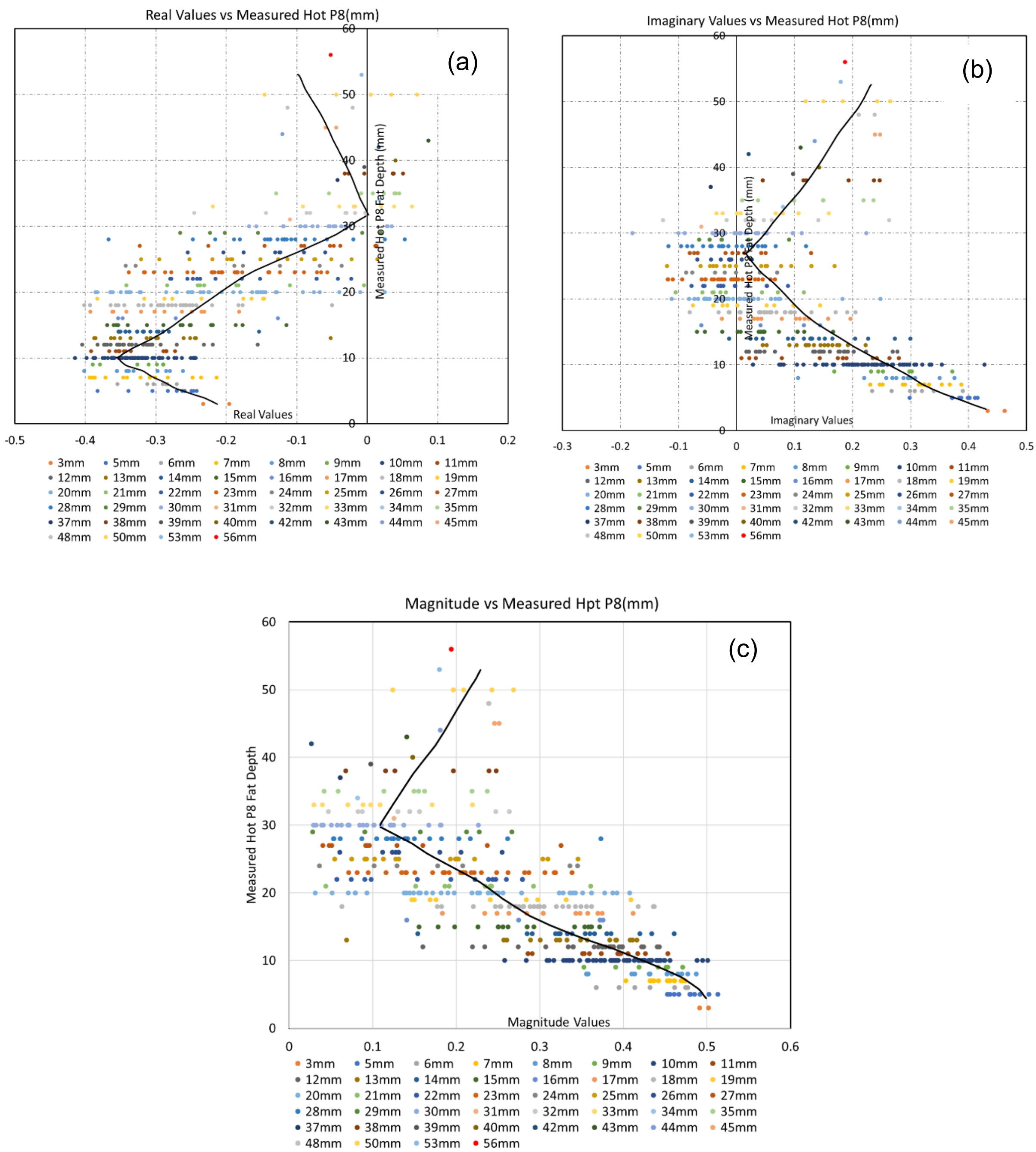

Figure 8. The association between measured P8 fat depth and (a) Real signal component (b) imaginary signal component (c) magnitude signal component.

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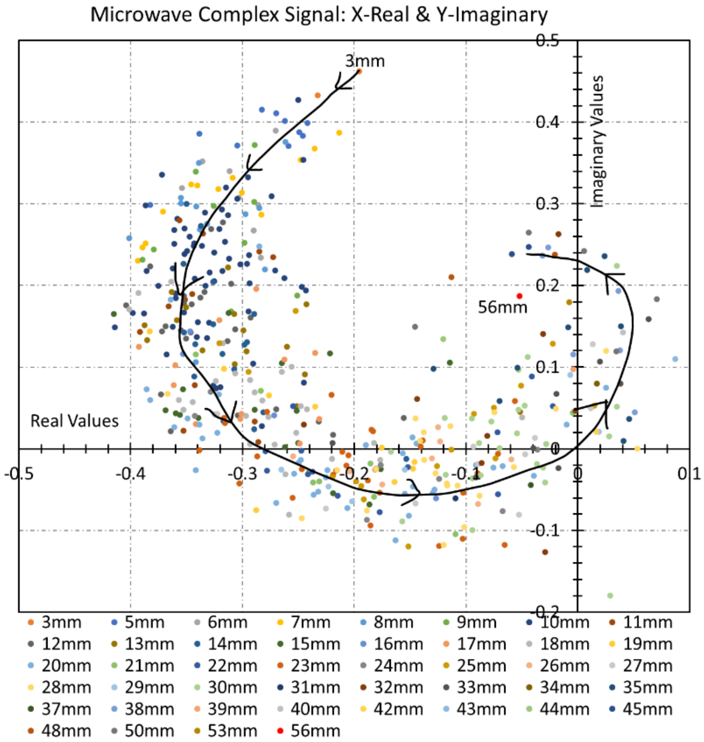

Figure 9. The association between real and imaginary signal components in the measurement of P8 fat depth.

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3.2.2 Hot rib fat depth

Carcases graded by MSA graders

The prediction of rib fat had an average RMSEP of 3.712 mm with an R^2 explaining 81% of the variation (*Table 7*). Across the 5 validation groups the variation in RMSEP was 0.622 mm and R^2 varied by 0.09 units. The average bias was small, <0.3 mm, and the maximum bias across the 5-validation groups 0.698 mm. The average slope was < 0.1, and the across the 5-validation group the most the slope deviated was 0.158 from 1.

The prediction of EMA had an average RMSEP of 10.267 cm^2 with an R^2 explaining 43% of the variation. Across the 5 validation groups RMSEP varied by 1.599 and R^2 by 0.14 units. The average bias was < 0.5 cm^2 and the greatest bias across the 5 validation groups was 0.846 cm^2. The average slope deviated 0.108 cm^2 from 1, and the maximum deviation across the 5 validation groups was 0.208 cm^2.

Table 7 Precision and accuracy estimates for k-fold (k=5) cross validation of models predicting (a) rib fat depth or (b) EMA. Groups have been balanced for each trait. Precision estimates include R2 and root mean square error of the predicted (RMSEP). Accuracy estimates include slope which is the difference between the actual and predicted slopes, expressed as a deviation from 1, and bias which represents the difference between the actual minus predicted value calculated at the mean of trait. Rib fat depth (mm), EMA and hot standard carcase weight (kg) values reported are mean ± standard deviation (minimum, maximum) of the raw values for each of the 5 validation groups.

Validation Group	N in validation	R^2	RMSEP	Bias (mm)	Slope (mm)	Ruler measured rib fat depth (mm)	Ruler measured EMA (mm)
(a) prediction of rib fat							
1	114	0.76	3.766	0.024	0.080	13.18 ± 7.67 (1.00 - 38.00)	
2	114	0.85	3.729	0.473	-0.158	13.46 ± 9.11 (1.00 - 38.00)	
3	114	0.85	3.273	0.248	-0.079	12.04 ± 8.32 (1.00 - 39.00)	
4	113	0.81	3.895	-0.698	0.018	12.11 ± 8.82 (0.00 - 44.00)	
5	113	0.78	3.895	-0.031	0.062	12.89 ± 8.29 (1.00 - 38.00)	
	Average	0.81	3.712	0.295	0.079	12.75 ± 8.45 (1.00 - 44.00	
(b) prediction of EMA							
1	114	0.38	9.587	-0.657	0.208		91.30 ± 11.89 (58.00 - 123.00)
2	114	0.49	9.930	0.256	-0.148		91.83 ± 13.80 (63.00 - 131.00)
3	114	0.35	11.186	0.426	-0.031		92.52 ± 13.94 (62.00 - 121.00)
4	113	0.47	9.884	0.846	-0.104		92.21 ± 13.58 (59.00 - 132.00)
5	113	0.48	10.752	-0.214	-0.050		93.30 ± 14.98 (63.00 - 133.00)
	Average	0.43	10.267	0.480	0.108		92.23 ± 13.64 (58.00 - 133.00)

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Carcases graded by in plant graders

The prediction of rib fat had an average RMSEP of 3.822 mm with R^2 explaining 71% of the variation. Across all 5 validation groups the RMSEP varied by 0.388 mm and R^2 by 0.08 units. The average bias was small, <0.2 mm and the maximum bias across all validation groups was < 0.25 mm. The average slope was small, 0.025 mm from 1, and the most the deviated across all 5 validation groups was 0.055 mm.

Table 8 Precision and accuracy estimates for k-fold (k=5) cross validation of models predicting rib fat depth graded by in plant graders. Groups have been balanced for rib fat depth. Precision estimates include R2 and root mean square error of the predicted (RMSEP). Accuracy estimates include slope which is the difference between the actual and predicted slopes, expressed as a deviation from 1, and bias which represents the difference between the actual minus predicted value calculated at the mean of rib fat depth. Rib fat depth (mm) and hot standard carcase weight (kg) values reported are mean ± standard deviation (minimum, maximum) of the raw values for each of the 5 validation groups.

Validation Group	N in validation	R^2	RMSEP (mm)	Bias (mm)	Slope (mm)	Ruler measured rib fat depth (mm)
(a) prediction of rib fat						
1	481	0.70	3.871	0.221	0.008	12.94 ± 7.07 (3.00 - 36.00)
2	481	0.77	3.535	-0.221	-0.055	12.27 ± 7.32 (3.00 - 36.00)
3	481	0.71	3.891	-0.154	0.013	12.42 ± 7.24 (3.00 - 35.00)
4	481	0.70	3.923	0.107	0.044	12.95 ± 7.16 (3.00 - 38.00)
5	481	0.69	3.885	0.075	-0.005	12.72 ± 7.00 (3.00 - 34.00)
	Average	0.71	3.822	0.156	0.025	12.66 ± 7.16 (3.00 - 38.00)

3.3 Discussion (Experiment Two)

The commercial MiS device with VPA antenna could predict hot carcase P8 fat depth with similar precision and accuracy to that reported in Experiment One (Table *2*). While the two different devices used in Experiment Two cannot be compared directly, as the measurements were taken on different carcases, the predictions from C-MiS device 2 were closer to those reported from the prototype device with VPA antenna used in Experiment One (Table *2*). C-MiS device 2 prediction of P8 had an average RMSEP 0.085 mm lower than that reported in Experiment One of 2.860 mm. The accuracy indicators were very similar between C-MiS device 2 and Experiment One, the bias varying by only 0.035 mm and slope by 0.012 mm. The C-MiS device 4 predictions had an average RMSEP 0.234 mm higher than that reported in Experiment One. The C-MiS device 4 average bias was 2.5 x higher, and average slope 4.5x higher than Experiment One. This experiment has demonstrated that the commercial device with VPA antenna has not improved prediction of P8 fat depth. The inherent precision

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and accuracy across both experiments is the similar, however the predictions did not meet AUS-MEAT national accreditation error tolerance limits. This is in contrast to MiS scanning on the hot lamb carcase, where predictions are meeting AUSMEAT accreditation. It is unclear why there is such difference between the species. A factor that has not been explored is if differences in chemical composition of fatty acids (Wood, Richardson, Nute, Fisher, Campo, Kasapidou, Sheard, & Enser, 2004) impact dielectric properties. A factor to always consider when calibrating against manual human measurements is that this technique can be prone to 'human error' (Williams et al., 2017a; Williams, Jose, McGilchrist, Walmsley, McPhee, Greenwood, & Gardner, 2017b). Ideally emerging measurement technologies should be calibrated against computed tomography, which is the most accurate available technology to predict body lean and fat (Young, Lewis, McLean, Robson, Fraser, Fitzsimons, Donbavand & Sim, 1999 as cited in (Jones, Lewis, Young, & Simm, 2004). Finally, the VPA antenna may not have the best electromagnetic wave/field interaction to measure P8. Experiment One demonstrated that the OCP had very good precision and accuracy of prediction. A direct comparison between OCP and VPA prediction of beef carcase fat depth cannot be made as no experiments have directly compared the two. The decision to use VPA for the commercial device was to create one device compatible across species, with VPA working well in lamb. However future experiments could focus on directly comparing various probes/antenna to see if it improves beef single site fat depth prediction.

The C-MiS could predict rib fat with average precision. The rib fat average RMSEP for both the MSA grader and in plant grader validations measuring a hot carcase was 1 mm greater than that reported in Experiment One when a cold carcase rib fat was scanned. The aim of this experiment was to determine if C-MiS could offer hot carcase measurements to replace the current cold fat ruler technique. Factors which may have impacted precision and accuracy include incorrect anatomical location of measurement and dehydration and shrinkage of fat in a cold carcase. Furthermore, the challenge with objectively measuring rib fat is the AUSMEAT and MSA guidelines request for a seam fat measurement, rather than total fat depth over the eye muscle. Finally, as above, calibrating against a human ruler measurement of rib fat can be prone to error.

The C-MiS could predict EMA with average precision and accuracy. This is the first experiment testing the ability of MiS to predict EMA. As the measurement was taken on an unsplit hot carcase, there may have been error measuring at the correct anatomical location. Further work comparing different probes/antennas is required to see if predictive ability can be improved.

4 Conclusion

Overall, the results demonstrate that a portable MiS system can estimate single site fatness in beef cattle, across different timepoints, abattoirs and herds. However when applied to the AUS-MEAT National Accreditation Standards the MiS prediction do not fit within the error tolerance range.

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