Technical Report

Program and KPI:	Sub-program 1.1 KPI 2.6
Report Title:	Report on the validation of a prototype microwave device to measure fat depth at the rib and P8 sites on live cattle and validate against the corresponding ultrasound and abattoir measurements
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Date published:	28 February 2023

This project is supported by funding from the Australian Government Department of Agriculture, Fisheries and Forestry as part of its Rural R&D for Profit programme in partnership with Research & Development Corporations, Commercial Companies, State Departments & Universities.

Citation

Marimuthu J., Loudon K.M.W. and Gardner G.E (2023). Report on the validation of a prototype microwave device to measure fat depth at the rib and P8 sites on live cattle and validate against the corresponding ultrasound and abattoir measurements. February, 31 pp

Acknowledgements

This study was undertaken through the Advanced Livestock Measurement Technologies Project (ALMTech) and funded by the Department of Agriculture Rural Research and Development (R&D) for Profit program and Meat and Livestock Australia. Meat and Livestock Australia are thanked for their funding for data acquisition, and for access to commercial herds to compile the calibration datasets. The commercial partners NH Foods, John Dee and ACC are thanked for their collaboration in generating this data

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Abstract

Two experiments were performed to test the ability of a portable microwave system coupled with Vivaldi Patch Antenna (VPA) to objectively measure live cattle subcutaneous fatness to predict corresponding carcase traits. Experiment One was performed on-farm, where commercial feedlot slaughter cattle (n=517) were microwave scanned at the P8 (fat depth on the rump) and rib fat site (fat depth over the m. longissimus, between rib 12 & 13). Corresponding ultrasound measurements were taken (n=315) at the same time as microwave scanning. A machine learning stacking ensemble method was used to create the microwave prediction equations. Datasets were grouped by prediction trait (P8 or rib fat) and randomly divided into 5 groups based on tissue depth. Live animal microwave scanning had greater precision than ultrasound at predicting carcase P8 and rib fat depth. At the P8 site the average RMSEP was 2.61 mm, R^2 0.61, bias 0.179 and slope 0.07 mm, and at the rib fat site the average RMSEP was 2.16 mm, R^2 0.60, bias 0.301 mm and slope 0.10.

Experiment Two was performed in the abattoir, where commercial slaughter cattle were microwave scanned at the rib fat site immediately prior to entering the knocking box. Microwave scanning in the abattoir had poor prediction of corresponding carcase trait with an R^2 of 0.02, RMSEP 2.24, bias 0.019 and slope 0.757.

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Executive Summary

Within Australia beef carcases are traded based on carcase weight and a single-site measurement of subcutaneous fat depth at the P8 and/or rib fat site. However the ability of producers to consign cattle to slaughter to comply with carcase grid specifications for fatness currently relies on subjectively assessed fatness scoring. Carcase fatness can be objectively determined via ultrasound (Andersen, Busk, Chadwick, Cuthbertson, Fursey, Jones, Lewin, Miles, & Owen, 1983; Brethour, 1992; Faulkner, Parrett, McKeith, & Berger, 1990; Perkins, Green, & Hamlin, 1992; Perkins, Green, Hamlin, Shepard, & Miller, 1992; Waldner, Dikeman, Schalles, Olson, Houghton, Unruh, & Corah, 1992), though this technology is not currently used as a pre-slaughter objective measurement in the Australian system cattle as it is slow to use and requires an accredited and experienced operator. Thus there is a clear need in the Australian beef industry for portable live animal objective measurements that can operate with precision and accuracy, at race speeds, with no risk of safety to humans or livestock.

One technology demonstrating potential to objectively determine carcase fatness is a prototype, portable ultrawide-band Microwave System developed at Murdoch University. Microwave scanning uses low power, non-ionizing electromagnetic waves to determine tissue depth via the differing electromagnetic waves of biological tissue (Marimuthu, 2016). The microwave frequencies used in the system cause no pain or destruction to biological tissues thus are completely safe to operators without requiring shielding. The microwave system transmits and receives electromagnetic waves via the same antenna, with measurements collected instantaneously via the click of a button. No specialised operator training is required apart from correct identification of site to be measured.

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1 Milestone description

KPI: 3.7 The commercial utilisation of a prototype microwave device to measure fat depth at the rib and P8 sites on cattle

2 Project objectives

The overall objective of this work is the testing and validation of a low cost, portable, prototype microwave scanning device for predicting P8 and rib fat depth in live cattle and carcase.

3 Experiment One: On Farm Microwave scanning

3.1 Methods (Experiment One: On Farm Microwave scanning)

3.1.1 Experimental design and slaughter protocol

Five slaughter groups of commercial Australian feedlot beef cattle were used to test the calibration and validation of the prototype ultrawide-band microwave system (MiS). Four groups of *Bos Taurus* (Angus or Hereford) were sourced from the Beef Information Nucleus project. The BIN herds represent the major beef breeds within Australia aiming to improve phenotypic and genotypic data capture to accelerate genetic progress (Banks, 2011). One group of slaughter cattle were commercially sourced which consisted of a mix of *Bos Taurus* and *Bos Indicus.* The slaughter groups and breeds are listed in *Table 1*.

Live animal measurements were taken at the feedlot, 0 – 13 days prior to slaughter. Immediately prior to cattle entering the crush, microwave scanning was performed on cattle from all slaughter groups as they stood unrestrained in the race. The centre of the antenna was placed in contact with the P8 site and over the rib (m. longissimus thoracis between the 12th and 13th rib to correspond with the carcase quartering site) (AUSMEAT, 2016). Cattle were moved into the crush, liveweight was recorded, then animals were restrained in a head-bail for ultrasound measurements. Ultrasound scanning for subcutaneous fat depth at the P8 and rib site was performed on groups 1 – 4 by the same BreedPlan™ accredited ultrasound scanning technician (BreedPlan Agricultural Business Research Institute, Armidale, NSW Australia). The measurements were made using an Esaote Aquilla portable ultrasound with a 3.5 MHz transducer (180mm linear probe).

The consignment of cattle and slaughter protocol was the same for all groups. Cattle were processed at Meat Standards Australia (MSA) accredited commercial abattoirs in New South Wales and Queensland. Keeping with MSA slaughter cattle standards, all cattle were consigned direct from farm-gate to slaughter and processed within 48 h from leaving the farm with no more than 12 h in lairage prior to slaughter (MSA, 2016). Each slaughter group was processed on a different kill day, with the breakdown of slaughter groups, kill date, and abattoir location listed in *Table 1*. Processing of cattle was conducted under standard commercial

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operating systems, animals were 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”. Immediately after the ruler P8 measurement, microwave scanning was performed at the P8 site, where the antenna placed in full contact with the carcase with the antenna centre positioned directly over the P8 site. The carcases continued along the abattoir chain into the chiller.

Approximately 24 hours after slaughter the left side of the carcase was quartered at the 12/13th rib, cutting straight across the eye muscle. A manual measurement of subcutaneous fat depth over the rib eye muscle was measured using a metal ruler, the site corresponding to 75% across the dorsal surface of the rib eye muscle (AUSMEAT, 2016). Microwave scanning of the rib site was performed with the antenna positioned just below the quartering site.

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Table 1 Descriptive statistics including animal numbers (n), live animal measurement date, kill date, and mean ± standard deviation, minimum and maximum for liveweight (kg), ultrasound measured P8 and rib fat (mm), hot standard carcase weight (kg), hot carcase P8 fat depth (mm) and cold carcase rib fat depth (mm)

Group Number	Live animal measurement date	Breed and feedlot location	n	Live Weight (kg)	Ultrasound measured P8 (mm)	Ultrasound measured Rib Fat (mm)	Kill Date	Abattoir	Hot standard carcase weight (kg)	Hot carcase P8 fat depth (mm)	Cold carcase Rib Fat depth (mm)
1	13/12/2018	Herefords (Tamworth)	45	485.73 ± 37.51 (413 – 562)	15.16 ± 2.79 (10 – 20)	8.50 ± 1.37 (6 – 12)	17/12/2018	NH Foods Wingham, NSW	253.72 ± 20.24 (219 – 300)	17.02 ± 3.43 (9 – 25)	7.50 ± 2.29 (3 – 13)
2	01/02/2019	Herefords (Tullimba)	50	554.88 ± 43.21 (478 – 666)	14.45 ± 3.14 (8 – 21)	9.23 ± 1.55 (6 – 13)	6/2/2019	NH Foods Wingham, NSW	301.27 ± 24.44 (254 – 369)	17.84 ± 2.82 (9 – 24)	9.65 ± 2.57 (6 – 16)
3	19/03/2019	Angus (Tullimba)	150	545.97 ± 50.38 (412 – 664)	12.98 ± 2.33 (7 – 19)	9.53 ± 1.70 (5 – 14)	1/04/2019	John Dee Warwick, Qld	301.43 ± 30.33 (214 – 379)	13.78 ± 3.35 (5 – 22)	9.61 ± 2.39 (3 – 16)
4	22/05/2019	Herefords (Tullimba)	70	626.15 ± 60.79 (530 – 816)	15.73 ± 2.66 (11 – 25)	13.59 ± 3.00 (9 – 22)	5/06/2019	NH Foods Wingham, NSW	352.19 ± 37.12 (295 – 468)	20.49 ± 3.31 (13 – 30)	14.32 ± 3.19 (9 – 22)
5	16/04/2019	Mix Breed (Brisbane Valley)	202	(486.88 ± 38.88) (384 – 590)	-	-	16/04/2019	ACC, Brisbane, Qld	256.51 ± 18.52) (187 – 306)	(13.55 ± 4.13) (5 – 28)	(7.28 ± 3.24) (3 – 20)

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3.1.2 Description of microwave hardware and signal analysis

The microwave hardware, antenna design and signal analysis is described in full in Marimuthu *et al.* (2020, 2021). In brief, a vector analyser was constructed using a Copper Mountain Technologies® R54 vector reflectometer (Copper Mountain Technologies, Indianapolis, USA) with inbuilt, automated, operating system and python-based programs for data acquisition and signal processing. The vector analyser was coupled with a single broadband Vivaldi patch antenna (VPA) designed and fabricated at Murdoch University (Perth, Western Australia). The VPA is a planar antenna, 95 mm height, 110 mm length, 1.27 mm thick, encased in 4 mm Teflon covering, with electromagnetic waves emitted in an arc from point of contact, approximately 120 mm in length and 80 mm width. The VPA frequency range was operating between 300 – 6.5 GHz and transmission gain 10 dB with main lobe angular width (3 dB) 85^{o} at 4.0 GHz. The reflected signals were collected back by the antenna, termed the reflection coefficient, S11(*f*), where *f* indicates the frequency domain signal. The S11(*f*) were recorded at 10 MHz intervals from 100 MHz – 5.4 GHz, resulting in 531 frequency points. Where j represents frequency points, and R indicates the raw signal, each $S11(f)_{jR}$ signal collected consists of two component point; real ($x(f)_{jR)}$) and imaginary ($y(f)_{jR)}$) with the following equation;

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

Calibration of MiS was conducted at ambient temperature prior to measurement using “Short, Open and Load” techniques (Marimuthu, 2016). 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).

3.1.3 Statistical analysis

The prediction equations were constructed using a machine learning ensemble stacking method in WEKA® 3.9.4 (The University of Waikato, Hamilton, New Zealand) and detailed in Marimuthu et al., (2020, 2021). In brief, the stacking method consisted of layering two prediction models to create a meta-algorithm (Elshazly, Elkorany, Hassanien, & Azar, 2013; Ribeiro & dos Santos Coelho, 2020). Layer one was composed from Support Vector Machine and Random Forest, and layer two used a Partial Least Squares Regression two component model.

To establish the ability of live microwave scanning to predict ultrasound derived P8 and rib fat depth, the estimations from groups 1 – 4 were pooled and randomly divided into 5 groups balanced for the site to be predicted (P8/Rib). Models were firstly run without liveweight included in the model and secondly with liveweight included. A leave-one-group-out cross validation methodology was used to test the prediction of each measurement type (P8/ Rib), where 80% of the data (4 groups) were used for training, and validated on the remaining 20% (5th group). This was repeated such that every group was validated against, resulting in a total of 5 validation groups.

To establish the ability of ultrasound to predict carcase P8 and rib fat depth, two analyses were run on the ultrasound estimations from groups 1 – 4. The first method was a direct linear comparison of ultrasound to abattoir P8 or rib fat depth. The second method was based on a

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linear regression performed in WEKA. Predictions from groups 1-4 were pooled and divided into 5 groups based on prediction site (P8/Rib). Liveweight was not included in the model.

To establish the ability of live microwave scanning to predict carcase P8 and rib fat depth, the WEKA ensemble stacking technique was applied. Two models were run using the same methodology. The first model included data only from groups 1 – 4 which had corresponding live animal ultrasound measurement. The second model included data from all slaughter groups, 1 – 5. The method of constructing the models was to pool the estimations for P8/Rib and randomly divided into 5 groups balanced for either P8 or rib site. A leave-one-group-out cross validation methodology was used to test the prediction of each measurement type (P8/ Rib), where 80% of the data (4 groups) were used for training, and validated on the remaining 20% (5th group). This was repeated such that every group was validated against, resulting in a total of 5 validation groups.

For all results, the precision of condition score or MiS measurements to predict either ultrasound derived, or carcase derived measurements are expressed as root mean square error of the prediction (RMSEP) and R-square (R^2), with R^2 expressed within the text as the percentage (%) of the variation that the model describes. Bias and slope estimates represent the accuracy of the prediction model. The bias is the difference between the predicted and the actual values at the mean of the dataset, while the slope is the deviation of the slope of the relationship from 1. For average slope and bias calculations across the five validation groups, the absolute values |x| were used.

3.2 Results (Experiment One: On Farm Microwave scanning)

3.2.1 Live animal microwave scanning to predict ultrasound measured subcutaneous fat depth

3.2.1.1 Prediction of live animal P8 site subcutaneous fat depth

The prediction of ultrasound P8 site fat depth using microwave demonstrated very similar precision and accuracy indicators whether liveweight was or was not included in the model (*Table 2(a)*).

When liveweight was not included in the model, the average precision of microwave to predict ultrasound P8 was slightly better, with an RMSEP of 2.14 mm, 0.02 mm higher than the average RMSEP of 2.16 mm when liveweight was included in the model (*Table 2(a)*). However, the range in values across the 5 validation groups for RMSEP was slightly greater when liveweight was not included in the model at 59 mm compared to 40 mm when liveweight was included in the model (*Table 2(a)*). Without liveweight in the model the average R^2 explained 45% of the variation compared to an average of 43% with liveweight (*Table 2(a)*). Again, across the 5 validation groups the variation in R^2 was slightly greater for the models without liveweight included, with these values varying by 12 units, compared to variation in R^2 of 8 units for the models without liveweight (*Table 2(a)*).

The accuracy indicators were slightly improved when liveweight was included in the model with an average bias of 0.174 mm compared to 0.205 mm without liveweight (*Table 2(a)*). With liveweight included in the model the range in bias values across the 5 validation groups was less at 0.587 mm compared to 0.741 mm without liveweight (*Table 2(a)*). The average slopes

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were very similar, when liveweight was included in the model it was 0.03 mm lower at 0.09 mm (*Table 2(a)*). Across the 5 validation groups the maximum slope deviated 0.28 mm from 1 when liveweight was not included in the model, or 0.17 mm from 1 when liveweight was included in the model (*Table 2(a)*).

The association between actual and microwave predicted ultrasound P8 fat depth without liveweight included in the model is depicted in *Figure 1(a)*.

3.2.1.1 Prediction of live animal Rib site subcutaneous fat depth

The prediction of ultrasound rib site fat depth using microwave demonstrated very similar precision and accuracy indicators whether liveweight was or was not included in the model (*Table 2(b)*).

When liveweight was included in the model, the average precision of microwave to predict ultrasound rib was slightly better, with the average RMSEP of 1.79 mm being 0.07 mm lower than with no liveweight included (*Table 2(b)*). The RMSEP range across the validation groups for models containing liveweight to not containing liveweight differed by only 0.01mm (*Table 2(b)*). The average R^2 was 3 units higher when liveweight was included in the model, explaining 58% of the variation (*Table 2(b)*). The range of R^2 across the 5 validation groups when liveweight was included was 23 units compared to 22 units without liveweight included (*Table 2(b)*).

The accuracy indicators were slightly improved when liveweight was included in the model, with an average bias of 0.280 mm compared to 0.344 mm without liveweight (*Table 2(b)*). The range of bias across the 5 validation groups was less than 1 mm when liveweight was included in the model and 1.284 mm without liveweight (*Table 2(b)*). The slopes were very similar, with the maximum slope without liveweight included in the model deviating 0.27 mm from 1 compared to 0.26 mm with liveweight included (*Table 2(b)*).

The association between actual and microwave predicted ultrasound P8 fat depth without liveweight included in the model is depicted in *Figure 1(b)*.

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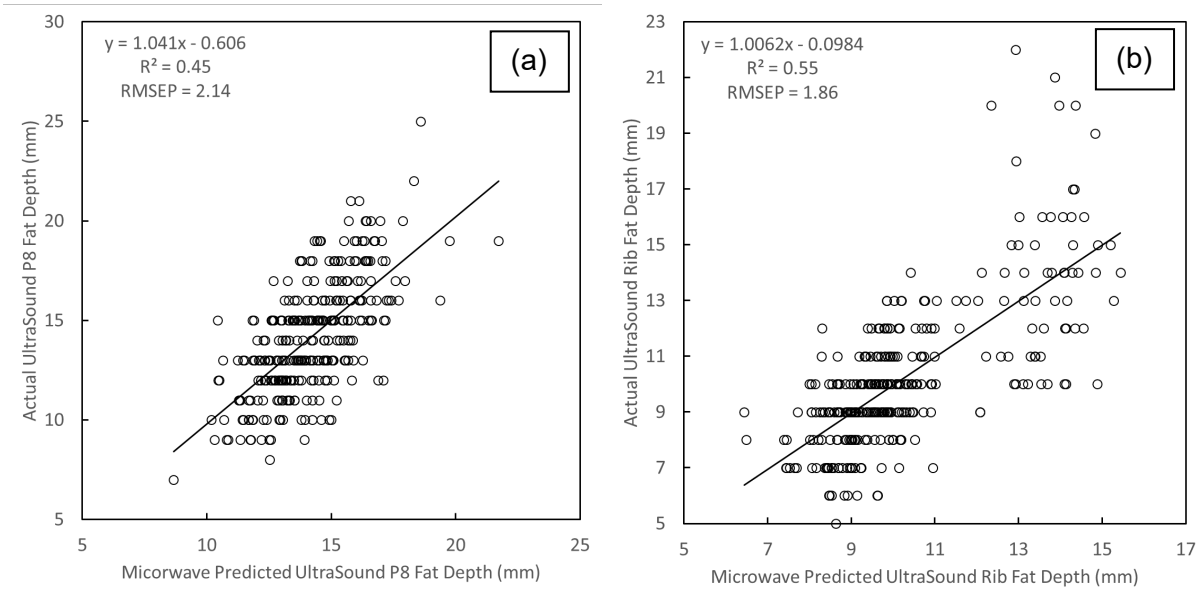

Figure 1 The association between actual and live animal microwave predicted ultrasound (a) P8 site (b) Rib eye fat depth. The predictions are derived from the validation tests detailed in Table 2. The actual tissue depths were regressed against the predictions. Solid line represents the relationship between predicted and actual measurements.

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Table 2 Precision and accuracy estimates for leave-one-group-out cross validation of models of microwave to predict ultrasound measured (a) P8 site and (b) rib fat depth. Precision estimates include R^2 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 the predicted site. Ultrasound measured fat depth (mm) and live 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	Live weight (kg)	Ultrasound measured fat depth (mm)	Live Weight not included				Live Weight included			
				R^2	RMSEP (mm)	Bias (mm)	Slope (mm)	R^2	RMSEP (mm)	Bias (mm)	Slope (mm)
(a) Prediction of ultrasound measured P8 site fat depth using Microwave Scanner											
1	63	556.34 ± 72.76 (412 – 774)	14.38 ± 2.80 (7.0 – 22.0)	0.51	1.95	+0.095	+0.09	0.38	2.22	-0.053	+0.17
2	63	555.72 ± 57.30 (440 – 770)	14.13 ± 3.20 (9.0 – 25.0)	0.36	2.54	+0.058	+0.03	0.45	2.36	-0.182	-0.05
3	63	554.80 ± 74.04 (413 – 816)	14.41 ± 2.83 (10.0 – 21.0)	0.41	2.16	-0.131	-0.06	0.46	2.08	+0.195	-0.14
4	63	546.22 ± 63.36 (426 – 708)	14.13 ± 2.90 (9.0 – 20.0)	0.53	2.02	+0.285	-0.28	0.41	2.21	+0.046	+0.04
5	63	560.70 ± 58.22 (420 – 686)	13.62 ± 2.62 (8.0 – 21.0)	0.42	2.04	-0.456	-0.16	0.45	1.96	-0.396	-0.03
	Average	554.77 ± 65.28 (412 – 816)	14.14 ± 2.87 (7.0 – 25.0)	**0.45**	**2.14**	**0.205****	**0.12***	**0.43**	**2.16**	**0.174****	**0.09****
(b) Prediction of ultrasound measured Rib fat depth using Microwave Scanner											
1	63	556.31 ± 65.72 (431 – 740)	10.11 ± 2.94 (6.0 – 22.0)	0.51	2.10	-0.007	-0.27	0.55	2.01	-0.067	-0.24
2	63	556.46 ± 66.56 (440 – 774)	10.13 ± 2.38 (6.0 – 20.0)	0.47	1.79	-0.124	+0.23	0.49	1.76	-0.138	+0.22
3	63	553.68 ± 74.00 (412 – 816)	10.06 ± 2.56 (6.0 – 17.0)	0.69	1.46	-0.307	+0.04	0.72	1.38	-0.248	+0.08
4	63	551.03 ± 63.87 (413 – 708)	10.08 ± 2.88 (5.0 – 21.0)	0.54	1.98	-0.420	-0.09	0.57	1.89	-0.218	-0.10
5	63	565.57 ± 63.21 (420 – 736)	10.79 ± 2.75 (7.0 – 20.0)	0.55	1.99	+0.864	-0.23	0.58	1.90	+0.731	-0.26
	Average	556.61 ± 66.61 (412 – 816)	10.24 ± 2.70 (5.0 – 22.0)	**0.55**	**1.86**	**0.344****	**0.17****	**0.58**	**1.79**	**0.280****	**0.18****

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

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3.2.2 Live animal ultrasound scanning to predict corresponding carcase measurements

3.2.2.1 Ultrasound measured P8 site fat depth to predict carcase trait

The ability of live animal ultrasound scanning at the P8 site to predict the carcase measurement had improved precision and accuracy indicators when the WEKA linear regression model was used compared to the direct linear comparison model (*Table 3*). The average RMSEP was 0.75 mm lower with the WEKA model at 2.82 mm (*Table 3*). The range across the 5 validation groups was 0.56 mm for the WEKA model and 0.43 mm for the direct comparison (*Table 3*). The average R^2 for both models was the same, explaining 54% of the variation, however the range across the 5 validation groups was 7 units smaller with the direct comparison model at 17 units compared to 24 units of the WEKA model (*Table 3*).

The average bias was approximately 13x smaller with the WEKA linear regression model at 0.176 mm, and the average range across the 5 validation groups of the WEKA model was 0.472 mm, approximately half the range in bias of the direct comparison (*Table 3*). The average and maximum slope deviation from 1 was smaller with the WEKA linear regression model (*Table 3*).

3.2.2.2 Ultrasound measured Rib site fat depth to predict carcase trait

In contrast to the P8 prediction, the precision indicators were only slightly improved with the WEKA linear regression model versus the direct comparison to predict rib fat depth. The average RMSEP of the WEKA model was only 0.01 mm less at 2.53 mm and the range across the 5 validation groups of the WEKA model to direct comparison differed by only 0.2 mm less (*Table 4*). The average R^2 for the WEKA model explained 47% of the variation compared to 46% for the direct comparison, however there was a greater range across the 5 validation groups for the WEKA model at 24 units compared to 12 (*Table 4*).

The accuracy indicators were very similar, with the average bias slightly less for the direct comparison at 0.180 mm, and a lower range across the 5 validation groups at 0.517 mm compared to 0.755 for the WEKA linear model (*Table 4*). The average bias was the same for both models and maximum bias only 0.01 mm higher for the WEKA linear model at 0.21 mm (*Table 4*).

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Table 3 Precision and accuracy estimates for the leave-one-group-out cross validation models, direct linear versus WEKA linear regression, of ultrasound measured P8 site to predict abattoir P8 site. Groups have been balanced for Carcase P8 fat depth (mm). Precision estimates include R^2 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 the predicted site. Ultrasound measured fat depth (mm), live weight (kg), hot standard carcase weight (kg) and carcase P8 fat depth values reported are mean ± standard deviation (minimum, maximum) of the raw values for each of the 5 validation groups.

						Direct linear comparison				WEKA linear regression model			
Validation Group	N in validation	Live weight (kg)	Ultrasound measured P8 fat depth (mm)	Hot standard carcase weight (kg)	Carcase P8 fat depth (mm)	R^2	RMSEP (mm)	Bias (mm)	Slope (mm)	R^2	RMSEP (mm)	Bias (mm)	Slope (mm)
1	63	542.46 ± 65.87 (420 – 774)	14.17 ± 2.82 (9 – 22)	297.41 ± 40.15 (219 – 435)	16.15 ± 4.16 (5 – 24)	0.47	3.73	+2.282	-0.04	0.52	2.88	-0.245	+0.04
2	63	561.25 ± 70.72 (412 – 816)	13.76 ± 2.67 (7 – 21)	307.75 ± 46.30 (214 – 468)	16.19 ± 4.12 (7 – 25)	0.51	3.30	+1.910	-0.14	0.46	3.02	-0.332	+0.05
3	63	560.34 ± 55.50 (462 – 736)	14.10 ± 2.62 (8 – 19)	306.36 ± 33.40 (234 – 418)	16.24 ± 4.10 (8 – 25)	0.60	3.56	+2.821	-0.15	0.48	2.93	-0.050	+0.01
4	63	555.75 ± 67.67 (431 – 740)	14.25 ± 3.16 (10 – 25)	305.59 ± 42.68 (226 – 418)	16.58 ± 4.37 (9 – 30)	0.64	3.68	+2.891	-0.12	0.70	2.46	-0.114	-0.09
5	63	551.19 ± 65.14 (413 – 696)	14.37 ± 2.88 (9 – 20)	303.05 ± 43.66 (222 – 412)	16.51 ± 4.16 (9 – 25)	0.50	3.59	+2.134	-0.07	0.55	2.83	+0.140	+0.01
	Average	**553.87 ± 65.09* (412 – 816)**	**14.10 ± 2.85* (7 – 25)**	**303.87 ± 41.37* (214 – 468)**	**16.28 ± 4.20* (5 – 30)**	**0.54**	**3.57**	**2.408****	**0.10****	**0.54**	**2.82**	**0.176****	**0.04****

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

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Table 4 Precision and accuracy estimates for the leave-one-group-out cross validation models, direct linear versus WEKA linear regression, of ultrasound measured Rib site fat depth to predict abattoir Rib site fat depth. Groups have been balanced for Carcase Rib fat depth (mm). Precision estimates include R^2 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 the predicted site. Ultrasound measured fat depth (mm), live weight (kg), hot standard carcase weight (kg) and carcase Rib fat depth values reported are mean ± standard deviation (minimum, maximum) of the raw values for each of the 5 validation groups.

						Direct linear comparison				WEKA linear regression model			
Validation Group	N in validation	Live weight (kg)	Ultrasound measured Rib eye fat depth (mm)	Hot standard carcase weight (kg)	Carcase Rib fat depth (mm)	R^2	RMSEP (mm)	Bias (mm)	Slope (mm)	R^2	RMSEP (mm)	Bias (mm)	Slope (mm)
1	63	553.69 ± 70.79 (413 – 816)	10.00 ± 2.26 (6 – 16)	307.05 ± 45.42 (223 – 468)	10.32 ± 3.42 (3 – 20)	0.41	3.00	+0.061	+0.07	0.43	2.59	+0.232	-0.12
2	63	552.72 ± 63.56 (412 – 774)	9.92 ± 2.63 (5 – 20)	303.15 ± 38.96 (214 – 435)	10.36 ± 3.50 (3 – 21)	0.52	2.44	-0.060	+0.09	0.41	2.68	+0.339	+0.01
3	63	549.02 ± 60.44 (434 – 708)	10.08 ± 2.72 (6 – 21)	299.33 ± 40.84 (223 – 402)	10.36 ± 3.45 (4 – 21)	0.48	2.30	-0.151	+0.16	0.54	2.34	+0.180	-0.12
4	63	564.07 ± 68.78 (454 – 736)	10.72 ± 2.88 (6 – 20)	310.98 ± 41.64 (240 – 418)	10.38 ± 3.44 (4 – 21)	0.40	2.60	+0.366	+0.20	0.36	2.84	-0.416	+0.21
5	63	563.34 ± 67.27 (431 – 740)	10.47 ± 2.92 (6 – 22)	308.70 ± 43.92 (226 – 418)	10.39 ± 3.45 (5 – 22)	0.51	2.36	+0.264	+0.05	0.60	2.18	-0.230	-0.09
	Average	**556.59 ± 66.13 (412 – 816)**	**10.24 ± 2.71 (5 – 22)**	**305.84 ± 42.15 (214 – 468)**	**10.36 ± 3.43 (3 – 22)**	**0.46**	**2.54**	**0.180****	**0.11****	**0.47**	**2.53**	**0.279****	**0.11****

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

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3.2.3 Live animal microwave scanning to predict corresponding carcase measurements

3.2.3.1 Live animal microwave P8 site fat depth to predict carcase trait

The ability of live animal microwave scanning to predict carcase P8 fat depth (*Table 5(a)* / *Table 6(b)*) had improved precision compared to the ability of live animal ultrasound to predict carcase P8 (*Table 3*). Comparing the same data set as the ultrasound animals, slaughter groups 1 – 4 (*Table 5(a)*), the precision of microwave was improved in comparison to both the ultrasound models, with the average microwave RMSEP of 2.75 mm less than Ultrasound direct comparison and WEKA linear regression (*Table 3*). The average R^2 of live animal microwave to predict carcase P8 fat depth explained 57% of the variation, 3 units higher than the ultrasound models (*Table 3*). With the increased data set of slaughter groups 1 – 5 (*Table 6(a)*) the precision indicators improved, with an average RMSEP of 2.61 mm and the R^2 explaining 61% of the variation.

The accuracy indicators of live microwave, using groups 1 – 4 (*Table 5(a))* were very similar to the WEKA linear model ultrasound prediction ((*Table 3*), with a difference of only 0.002 mm for bias and 0.02 mm for the slope deviation from 1. The bias and slope both reduced with the increased dataset of groups 1 – 5 (*Table 6(a)*), however across both datasets the microwave the maximum bias was small, at 0.55 mm and the maximum slope deviated 0.15 mm from 1.

The association between actual and microwave predicted carcase Rib fat depth without liveweight included in the model is depicted in *Figure 2(a).*

3.2.3.2 Live animal microwave Rib fat site fat depth to predict carcase trait

The ability of live animal microwave scanning to predict carcase Rib fat depth (*Table 5(a)* / *Table 6(b)*) had improved precision and accuracy compared to the ability of live animal ultrasound to predict carcase traits. Data from slaughter groups 1 – 4 (*Table 5(b))* had an average RMSEP of 2.43 mm, 0.10mm lower than ultrasound (*Table 4*) and the average microwave R^2 explained 51% of the variation, 4 units higher than ultrasound. Again using an increased dataset (*Table 6(a)*) the precision improved, with an average RMSEP of 2.16 mm and R^2 explaining 60% of the variation.

The accuracy indicators for live microwave predictions using slaughter groups 1 – 4 (*Table 5(b))* were very similar to the ultrasound predictions (*Table 4*), with the average bias 0.63 mm lower than the WEKA regression model and 26 mm higher than the direct linear comparison. The average microwave slope deviated only 0.01 mm more from 1 (*Table 5(b))* than the ultrasound predictions (*Table 4*). Using all slaughter groups, 1 – 5, the bias and slope also improved (*Table 6(b)*), however again the microwave bias across the two datasets was small, with a maximum bias of 0.499 mm and maximum slope deviating 0.18 mm from 1.

The association between actual and microwave predicted carcase Rib fat depth without liveweight included in the model is depicted in *Figure 2(b)*.

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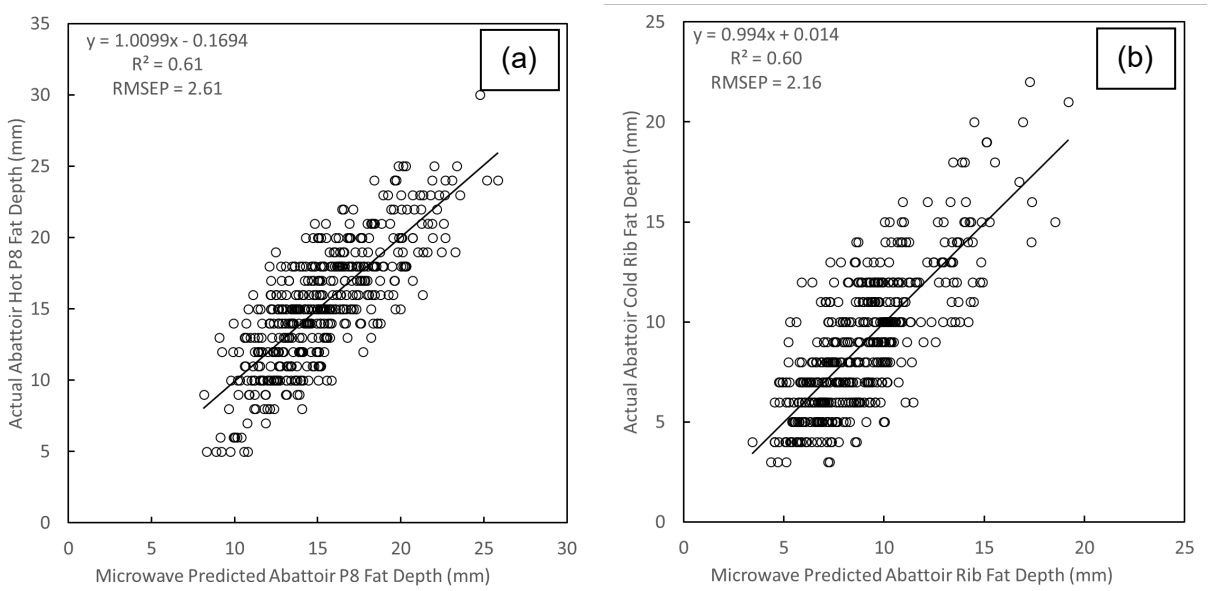

Figure 2 The association between actual and live animal microwave predicted (a) P8 site (b) Rib fat depth. The predictions are derived from the validation tests detailed in Table 6. The actual tissue depths were regressed against the predictions. Solid line represents the relationship between predicted and actual measurements.

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Table 5 Precision and accuracy estimates for leave-one-group-out cross validation models of live microwave scanning to predict (a) hot carcase P8 fat depth and (b) cold carcase rib fat depth (slaughter groups 1 – 4). Groups have been balanced for carcase fat depth (P8 or rib). Precision estimates include R^2 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 the predicted site. Ultrasound measured fat depth (mm), live weight (kg), hot standard carcase weight (kg) and carcase fat depth values reported are mean ± standard deviation (minimum, maximum) of the raw values for each of the 5 validation groups.

Validation Group	N in validation	Live weight (kg)	Hot standard carcase weight (kg)	Carcase Fat depth (mm)	R^2	RMSEP (mm)	Bias (mm)	Slope (mm)
(a) Prediction of carcase P8 fat depth using Microwave Scanner								
1	63	542.46 ± 65.87 (420 – 774)	297.41 ± 40.15 (219 – 435)	16.15 ± 4.16 (5 – 24)	0.67	2.38	-0.226	+0.04
2	63	561.25 ± 70.72 (412 – 816)	307.75 ± 46.30 (214 – 468)	16.19 ± 4.12 (7 – 25)	0.47	3.01	+0.550	-0.05
3	63	560.34 ± 55.50 (462 – 736)	306.36 ± 33.40 (234 – 418)	16.24 ± 4.10 (8 – 25)	0.54	2.75	-0.110	-0.04
4	63	555.75 ± 67.67 (431 – 740)	305.59 ± 42.68 (226 – 418)	16.58 ± 4.37 (9 – 30)	0.66	2.60	+0.160	-0.11
5	63	551.19 ± 65.14 (413 – 696)	303.05 ± 43.66 (222 – 412)	16.51 ± 4.16 (9 – 25)	0.50	3.03	-0.393	+0.08
	Average	**553.87 ± 65.09* (412 – 816)**	**303.87 ± 41.37* (214 – 468)**	**16.28 ± 4.20* (5 – 30)**	**0.57**	**2.75**	**0.288****	**0.06****
(b) Prediction of carcase Rib fat depth using Microwave Scanner								
1	63	553.69 ± 70.79 (413 – 816)	307.05 ± 45.42 (223 – 468)	10.32 ± 3.42 (3 – 20)	0.48	2.46	+0.003	+0.05
2	63	552.72 ± 63.56 (412 – 774)	303.15 ± 38.96 (214 – 435)	10.36 ± 3.50 (3 – 21)	0.48	2.51	+0.032	-0.06
3	63	549.02 ± 60.44 (434 – 708)	299.33 ± 40.84 (223 – 402)	10.36 ± 3.45 (4 – 21)	0.47	2.54	+0.098	+0.16
4	63	564.07 ± 68.78 (454 – 736)	310.98 ± 41.64 (240 – 418)	10.38 ± 3.44 (4 – 21)	0.55	2.35	-0.499	-0.17
5	63	563.34 ± 67.27 (431 – 740)	308.70 ± 43.92 (226 – 418)	10.39 ± 3.45 (5 – 22)	0.57	2.31	+0.398	-0.18
	Average	**556.59 ± 66.13 (412 – 816)**	**305.84 ± 42.15 (214 – 468)**	**10.36 ± 3.43 (3 – 22)**	**0.51**	**2.43**	**0.206****	**0.12****

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

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Table 6 Precision and accuracy estimates for leave-one-group-out cross validation models of live microwave scanning to predict (a) hot carcase P8 fat depth and (b) cold carcase rib fat depth (slaughter groups 1 – 5). Groups have been balanced for carcase fat depth (P8 or rib). Precision estimates include R^2 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 the predicted site. Ultrasound measured fat depth (mm), live weight (kg), hot standard carcase weight (kg) and carcase fat depth values reported are mean ± standard deviation (minimum, maximum) of the raw values for each of the 5 validation groups.

Validation Group	N in validation	Live weight (kg)	Hot standard carcase weight (kg)	Carcase Fat depth (mm)	R^2	RMSEP (mm)	Bias (mm)	Slope (mm)
(b) Prediction of carcase P8 fat depth using Microwave Scanner								
1	104	527.11 ± 66.61 (391 – 732)	285.53 ± 42.63 (187 – 402)	15.39 ± 4.52 (5 – 28)	0.63	2.55	-0.151	-0.01
2	104	531.87 ± 64.60 (408 – 716)	288.48 ± 42.16 (221 – 403)	15.37 ± 4.51 (5 – 29)	0.54	2.63	-0.273	+0.07
3	103	525.48 ± 66.71 (384 – 774)	285.07 ± 42.01 (203 – 435)	15.38 ± 4.56 (5 – 30)	0.73	2.45	+0.359	-0.15
4	103	525.61 ± 63.51 (407 – 736)	284.02 ± 40.62 (222 – 418)	15.36 ± 4.64 (5 – 30)	0.59	2.72	-0.041	-0.03
5	103	534.30 ± 70.35 (408 – 816)	288.11 ± 44.59 (221 – 468)	15.49 ± 4.85 (5 – 30)	0.54	2.72	+0.069	+0.10
	Average	**528.89 ± 66.24 (384 – 816)**	**286.24 ± 42.30 (187 – 468)**	**15.39 ± 4.60 (5 – 30)**	**0.61**	**2.61**	**0.179****	**0.07****
(b) Prediction of carcase Rib fat depth using Microwave Scanner								
1	104	524.50 ± 60.19 (384 – 664)	283.79 ± 39.12 (203 – 388)	9.12 ± 3.61 (3 – 20)	0.58	2.39	+0.212	+0.05
2	104	518.73 ± 61.37 (402 – 696)	279.27 ± 38.15 (207 – 412)	9.11 ± 3.61 (3 – 20)	0.63	2.24	-0.491	-0.13
3	103	539.01 ± 69.76 (415 – 816)	291.87 ± 43.66 (221 – 468)	9.12 ± 3.67 (3 – 21)	0.48	2.05	-0.344	+0.15
4	103	531.21 ± 60.12 (408 – 708)	285.97 ± 40.53 (222 – 398)	9.11 ± 3.68 (3 – 21)	0.64	2.09	+0.422	-0.13
5	103	530.96 ± 78.29 (391 – 740)	290.30 ± 48.69 (187 – 418)	9.24 ± 3.87 (3 – 22)	0.67	2.04	+0.038	+0.06
	Average	**528.89 ± 66.24 (384 – 816)**	**286.24 ± 42.30 (187 – 468)**	**9.14 ± 3.68 (3 – 22)**	**0.60**	**2.16**	**0.301****	**0.10****

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

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3.3 Discussion (Experiment One: On Farm Microwave scanning)

The prototype microwave device demonstrated greater precision and accuracy in its ability predict beef carcase fat depth than ultrasound when used on the live animal. The result that microwave prediction in the live animal to carcase trait had a lower RMSEP than both the ultrasound predicted models (direct comparison and WEKA linear regression) provides robustness to the precision of this device. To be expected the microwave precision indicators improved with an increased data set, however as the accuracy indicators were similar and small across both data sets, this again highlights the potential of this prototype technology as live animal objective measurement technology.

This result is important for industry as ultrasound scanning is currently the gold standard of objectively determining subcutaneous P8 and rib eye fat depth in the live animal. Ultrasound is an important technology in the beef industry with fat depth, eye muscle area and intramuscular fat (IMF) of seedstock cattle informing genetic selection (Börner, Johnston, & Graser, 2013; Kemp, Herring, & Kaiser, 2002; Reverter, Johnston, Graser, Wolcott, & Upton, 2000). Ultrasound measurements of subcutaneous fatness in the live animal have demonstrated an ability to predict corresponding carcase traits (Andersen, Busk, Chadwick, Cuthbertson, Fursey, Jones, Lewin, Miles, & Owen, 1983; Brethour, 1992; Faulkner, Parrett, McKeith, & Berger, 1990; Perkins, Green, & Hamlin, 1992; Perkins, Green, Hamlin, Shepard, & Miller, 1992; Waldner, Dikeman, Schalles, Olson, Houghton, Unruh, & Corah, 1992). However ultrasound is a labour intensive technique, requiring an experienced and accredited operator and the cattle must be restrained in a crush with oil applied to the coat to obtain optimal images (Williams, 2002). Due to these constraints of ultrasound, this technology is currently only in use in Australia for young cattle seedstock genetic evaluation, it is not routinely used to measure carcase fatness to optimise turn off for slaughter. In contrast, microwave works on a point and click basis, where no specialised training is required apart from correct identification of the anatomical site to be measured. The electromagnetic waves are transmitted and reflected instantaneously thus using this device should not greatly impact the operating speed of cattle movement through a race and crush.

The precision and accuracy indicators of live animal microwave fatness scanning to predict carcase traits were similar to those reported by Marimuthu *et al* (2021) using the same microwave device and VPA on the carcase to predict P8 and rib. Using microwave scanning on the hot carcase P8 to predict manual ruler measurement, Marimuthu *et al*. (2021) reported an average RMSEP of 2.86 mm, R^2 0.58, bias 0.087 mm and slope deviating 0.07 mm from 1. Microwave scanning cold carcase rib fat to predict manual ruler measurement, Marimuthu *et al*. (2021) reported an average RMSEP of 2.60 mm, R^2 of 0.55, bias 0.095 mm and slope deviating 0.03 from 1. The similarity of the live animal results in this study to the carcase predictions was unexpected as microwave signal can be detrimentally impacted by factors such as dust and debris in the coat, and varying coat and skin thickness between breeds. Furthermore the practicality of obtaining the measurement on a carcase is significantly easier as in the live animal it can be impacted by sudden movement. While only one antenna type was tested in this experiment due to farm accessibility restrictions from Covid-19, it demonstrates the capacity of a portable microwave system to estimate fatness in live cattle. Future experiments will directly comparing different antenna and probe design.

Live animal microwave scanning to predict corresponding ultrasound measurement was greater at the rib site than the P8 site, with liveweight having minimal impact on either model.

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As the dielectric properties in the coat and skin should be the same between the two sites, it is unclear why the prediction was inferior at the P8 site. As the P8 site is more difficult for the operator to access than the rib site the difference may be simply due to the design of the antenna and system. Further experiments need to test varying physical design of the unit on precision and accuracy.

The precision and accuracy indicators of live animal microwave to ultrasound rib site were similar to live animal microwave to carcase rib site prediction. However the live animal microwave to ultrasound prediction had substantially lower R^2 than live microwave to carcase P8 prediction. Whilst ultrasound is currently the only industry objective technology for determining carcase fatness, it is not without error, with studies demonstrating at extremes of fatness inaccuracies may occur (Brethour, 1992; Charagu, Crews Jr, Kemp, & Mwansa, 2000; Perkins *et al* 1997 as cited in Williams, 2002). While ultrasound remains an important industry technology it is essential that emerging technologies are validated and trained rigorously against the best available gold standard measurement. Future microwave experimental work should continue to be trained on manually measured carcase traits along with the current gold standard measurement for carcase lean meat yield, Computed Tomography.

4 Experiment Two: abattoir microwave scanning

4.1 Methods (Experiment Two: abattoir microwave scanning)

4.1.1 Experimental design and slaughter protocol

One slaughter group of commercial organic grass fed Australian beef cattle were used in one experiment, to test the calibration and validation of the commercial microwave device (C-MiS) to predict carcase traits in live cattle in the abattoir immediately prior to slaughter. One group of *Bos Taurus* (n=482) mixed sex cattle was sourced from one farm.

Cattle were consigned to slaughter at a Meat Standards Australia (MSA) accredited commercial abattoir located in Queensland. Cattle were consigned direct from farm-gate to slaughter and processed within 48 h from leaving the farm, with less than 12 h in lairage prior to slaughter, adhering to MSA protocols (MSA, 2016). Cattle were washed using an automatic sprinkler system in lairage and were very wet prior to being sent up to chute for slaughter. While cattle were standing in the chute immediately prior to the knocking box, microwave scanning was performed. Due to abattoir configuration, cattle were scanned on their right-hand side. The centre of the antenna was placed over the Musculus longissimus thoracis between the 12^{th} and 13^{th} rib. Correct anatomical placement of C-MiS over the rib eye was challenging due to movement of cattle.

Cattle were processed under standard commercial operating systems with electrical stimulation, identified via a carcase ticket and trimmed according to AUSMEAT standards (AUSMEAT, 2016). Carcases were graded according to AUSMEAT chiller assessment measurements by a single in plant commercially accredited expert grader (Anonymous, 2005). The left carcase was quartered at the $12/13^{th}$ rib, across the rib eye muscle. Subcutaneous fat depth was measured with a metal ruler over the exposed cut surface, 75% across the dorsal surface of the rib eye muscle (Anonymous, 2005).

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4.1.2 Description of microwave hardware and signal analysis

Please refer to section 3.1.2.

4.1.3 Statistical analysis

All equations were performed in WEKA® 3.9.4 (The University of Waikato, Hamilton, New Zealand). Please refer to section 3.1.3 for description of prediction equations.

The C-MiS predictions were pooled and divided into 5 groups stratified for ribfat depth. A k-fold cross validation (k=5) methodology was used to test the prediction, where 80% of the data (4 groups) were used for training, and validated on the remaining 20% (5^{th} group). This was repeated so every group was validated against, totalling 5 validation groups. Only validation results are reported, no training data is included.

The precision of C-MiS to predict ribfat depth is expressed as root mean square error of the prediction (RMSEP) and R-square (R^2). Accuracy of the model is described by bias and slope. The difference between the predicted and expected values at the mean of the dataset is the bias. The slope is the deviation of the slope of the relationship from 1. The absolute values |x| for bias and slope were used to calculate the average across the 5 validation groups.

4.2 Results (Experiment Two: abattoir microwave scanning)

Descriptive statistics are provided in *Table 7*, demonstrating the range in manually measured rib fat depth.

Table 7 Descriptive statistics including animal number, and mean ± standard deviation, minimum and maximum for liveweight and carcase ribfat depth

Kill Date	n	Live weight (kg)	Ribfat (mm)
01/06/2022	482	664.18±53.08 (301.00 – 833.00)	6.16±2.31 (3.00 – 15.00)

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The precision of C-MiS on the live animal rib fat site to predict corresponding carcase trait had an average RMSEP of 2.214 mm, with the R^2 on average explaining only 2% of the variation (*Table 8*). The range in values across the 5 validation tests for RMSEP was 0.514 mm, and R^2 varied by 0.07 units. The maximum bias was 0.257 mm and at most the slope deviated 1.064 mm from 1.

Table 8 Precision and accuracy estimates for k-fold (k=5) models of C-MiS scanning live cattle at the rib fat site to predict carcase rib fat. 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 the predicted site.

Validation Group	N in validation	R^2	RMSEP (mm)	Bias (mm)	Slope (mm)
1	97	0.07	1.858	0.160	-0.282
2	97	0.00	2.372	0.021	1.064
3	96	0.01	2.280	-0.257	0.583
4	96	0.00	2.278	0.001	0.997
5	96	0.00	2.278	0.046	0.860
	Average	0.02	2.214	0.019	0.757

4.3 Discussion (Experiment Two: abattoir microwave scanning)

Scanning cattle in the abattoir immediately before the knocking box, the C-MiS device had poor ability to predict beef rib fat depth, with the average R^2 explaining only 2% of the variation. We postulate multiple reasons why the precision was poor scanning cattle immediately before the knocking box. Firstly the cattle were very wet from multiple washing episodes prior to the measurement being taken. Water heavily attenuates microwave signals (Vijay, Jain, & Sharma, 2015), making the estimation of traits almost impossible. Secondly, there was limited clear access to the cattle when taking measurements due to abattoir configuration. Measurements were taken on the animal's right hand side as the left was inaccessible, and correct antenna placement was difficult to achieve due to movement of cattle within the race. Furthermore dehiding and carcase trimming may have artificially altered subcutenous fat depth. Dehiding of cattle using a downward hide puller can cause tearing of fat over the loin and hindquarter regions (Thompson, 2009). Additional disruption to the fat can occur if powered flaying knives or curved hand knives are used during the dehiding process or if any fat trimming occurs prior to carcase measurements being obtained. Figure 3 demonstrated various trimming which has occurred over the hot carcase rib fat site prior to any measurements being obtained. This experiment demonstrates that microwave scanning needs to be performed prior to washing, in facilities with good access for antenna placement and carcases need to be monitored for excess disruption of subcutaneous fat from dehiding or trimming.

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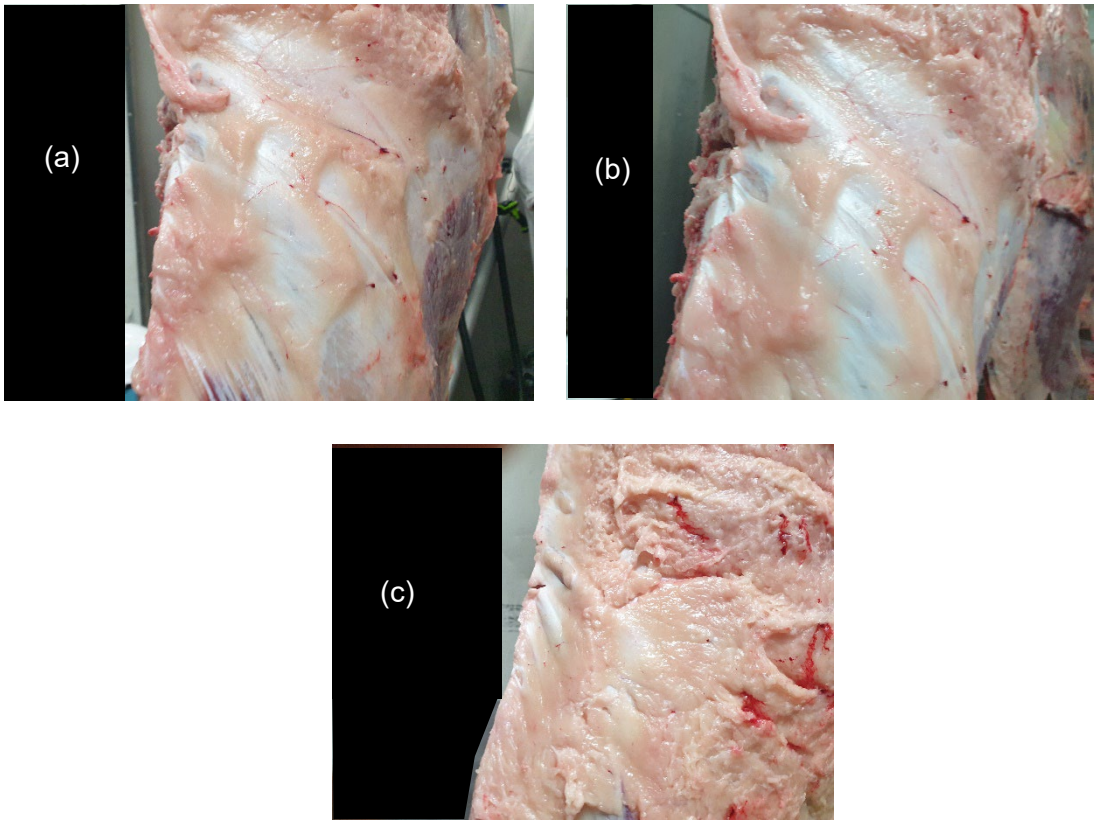

Figure 3 Demonstration of hot carcase rib fat site trimming that has occurred prior to measurements being obtained.

5 Conclusions

We conclude that the microwave system shows good potential for measuring fat depth in live cattle to predict corresponding carcase measurements when cattle are dry and restrained in good facilities with clear access. Microwave scanning has poor predictive ability when cattle are wet or there is large amounts of movement. Further validation and training of across a more diverse phenotypic and genotypic range of cattle is required along with the comparison of different microwave antennas and probes.

This project is supported by funding from the Australian Government Department of Agriculture, Fisheries and Forestry as part of its Rural R&D for Profit programme in partnership with Research & Development Corporations, Commercial Companies, State Departments & Universities.

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This project is supported by funding from the Australian Government Department of Agriculture, Fisheries and Forestry as part of its Rural R&D for Profit programme in partnership with Research & Development Corporations, Commercial Companies, State Departments & Universities.

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