Technical Report

Program and KPI: Sub-program 1.2 KPI 3.11

Report Title: Report on the accreditation of the lamb DXA device to predict fat%, lean%, and bone% in sheep carcases

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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.

Executive Summary

This report has been prepared by ALMTech on behalf of the manufacturer of the lamb DXA device (Scott Automation and Robotics) for the accreditation for predicting CT Fat%, CT Lean% and CT Bone% of sheep carcases. The accreditation trial was conducted at WAMMCO, Katanning, using lamb carcases collected over multiple kill groups from February 2022 through to July 2022 (n=338), with a further group of high weight carcases collected between August 2022 and December 2022 (n=139).

The experimental and analytical procedure used to assess the repeatability and accuracy performance of the DXA device has been described. The performance of the DXA device was compared against version 1 of the AMILSC approved guidelines for experiments to achieve accreditation of technologies for predicting fat%, lean%, and bone% in sheep carcases ("A carcase composition trait for sheep meat grading technologies" presented to AMILSC on the 17/2/2022). As total carcase composition is loosely associated with hot carcase weight, the accreditation requirements for DXA are tested within three weight categories: light (<22kg); medium (22-28kg); and heavy (>28kg). This creates nine discrete groups within which accreditation tests are applied.

We recommend accreditation of DXA for predicting composition in carcases <22kg with CT Fat % between 10.9% and 30.3%, CT Lean% between 53.2% and 65.0%, and CT Bone % between 14.9% and 25.0%. Additionally, we recommend accreditation of DXA for predicting composition in carcases between 22kg and 28kg with CT Fat % between 14.9% and 35.0%, CT Lean % between 50.9% and 66.2%, and CT Bone % between 13.3% and 18.0%. Finally, we recommend accreditation of DXA for predicting composition in carcases >28kg with CT Fat % between 22.0% and 37.1%, CT Lean % between 49% and 60.6%, and CT Bone % between 11.6% and 17.5%. This is summarised in **Table *1*** below.

Table 1. Requested accreditation ranges for lamb DXA

HCWT category	Tissue Type		
	Carcase Fat%	**Carcase Lean%**	**Carcase Bone%**
<22kg	10.9% - 30.3%	53.2% - 65%	14.9% - 25.0%
22-28kg	14.0% - 35.0%	50.9% - 66.2%	13.3% - 18.0%
>28kg	22.0% - 37.1%	49% - 60.6%	11.6% - 17.5%

The repeatability of lamb DXA has been tested on the JBS Bordertown DXA device, with the results published in a scientific journal (Connaughton, Williams et al. 2020). This experiment showed that coefficients of correlation between three repeated scans were close to 1 (0.992-0.995).

Installation and utilisation of a synthetic phantom block at each site has been used to adjust the accreditation algorithm according to start of day scan results. This process has been described.

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Based on this report, the lamb DXA device is eligible for AUS-MEAT accreditation for predicting CT Fat %, CT Lean %. and CT Bone % for all weight ranges.

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Contents

Executive Summary 2
Contents 4
1 Objective 5
2 Function and calibration of DXA 5
3 Testing DXA accuracy versus CT 8
3.1 Methodology 8
3.1.1 Experimental Design 8
3.1.2 DXA Scanning 11
3.1.3 CT Scanning 11
3.1.4 Statistical Analysis 12
3.2 Results 12
3.2.1 Low weight (<22kg) 14
3.2.2 Mid weight (22-28kg) 16
3.2.3 High weight (>28kg) 19
4 Testing DXA repeatability 22
4.1 Within device repeatability using chilled lamb carcases 23
4.1.1 Experimental design 23
4.1.2 Results 24
4.2 Within device repeatability using hot lamb carcases 24
4.2.1 Experiment design 24
4.2.2 Results 25
4.3 Between device repeatability using a synthetic phantom 25
4.3.1 Experimental design 25
4.3.2 Results 26
4.3.3 DXA calibration using the Scott phantom 26
5 Recommendations 29
6 References 30

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1 Objective

The objective was to seek accreditation of the on-line lamb DXA device against the AMILSC accreditation standards of measuring CT Fat %, CT Lean % and CT Bone %.

2 Function and calibration of DXA

The lamb DXA device designed and installed by Scott Automation and Robotics is an on-line system that produces and analyses two unique radiographs of a lamb carcase via dual detectors separated by a copper filter. An initial 'start of day' calibration scan is acquired, with one scan collected from the detectors with no tube operation (Dark), one with full tube operation but no attenuation of the X-rays (Light). These two images are used to establish a reference point for the rest of the days' images, by setting the reference maximum unattenuated value as 4095 for all lines of the high and low energy images by the equation:

Equation 1

$$Pixel = 4095 * \frac{Pixel_{Raw} - Calibration_{Dark}}{Calibration_{Light} - Calibration_{Dark}}$$

Any pixel with a value greater than 3700 is deemed to be unattenuated space and is removed from all further calculations. After the Light and Dark scans are acquired, another scan is undertaken with the attenuation of a plastic phantom block at full tube operation. This phantom is designed as a mixture of three plastics – nylon, polyethylene and acrylic and shown in **Figure *1*** - **Figure *3*** below.

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Figure 1. The Scott plastic phantom as installed at the Bordertown DXA device

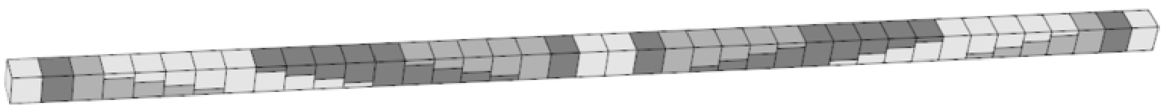

Figure 2. a 3D illustration of the different mixtures of plastics along the length of the phantom

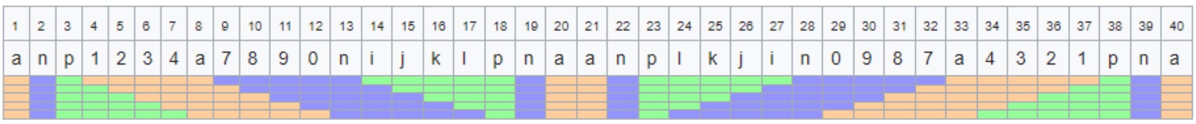

Figure 3. Illustration of the design of the plastic phantom block. This shows the mixtures of acrylic (a), nylon (n) and polyethylene (p)

The synthetic phantom block is scanned at the start of every day, and the linear attenuation (μ) of each block can be calculated by the following equation:

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Equation 2

$$\mu = -\frac{\ln\left(\frac{I}{I_0}\right)}{t}$$

Where t represents the thickness of material that the x-ray beam is passing through (adjusted for height of block and beam angle), I represents the attenuation measured for a given pixel for the low energy detector, and I_0 represents the value of an unattenuated pixel passing through air, which in this case has been calibrated to a value of 4095 in these images.

After calibration, carcase scanning can commence. For each carcase scanned, two images are acquired. These two images are converted into a single R-value image of all tissue by the equation:

Equation 3

$$Rvalue = \frac{\ln\left(\frac{I_L}{I_0}\right)}{\ln\left(\frac{I_H}{I_0}\right)}$$

Where I_L and I_H represent the attenuation measured for a given pixel for the low energy and high energy detectors, and I_0 represents the value of an unattenuated pixel passing through air, which in this case has been calibrated to a value of 4095 in these images.

A proxy for tissue thickness is calculated by the log of the low energy image values. Determination of whether a pixel contains bone or not is calculated by the following equation:

Equation 4

$$BoneValue = \ln\left(\frac{R^2}{t}\right)$$

Where R is the R-value calculated from Equation 3 above, and t is the pixel thickness estimated from the log of the low energy pixel value.

BoneValues greater than the cut-off value of 0.074 (this will vary from site to site as per the R-value and thickness adjustments that are required and is calculated based on those changes) are determined to be bone, and any remaining pixels are considered soft tissue. Total numbers of pixels containing bone and soft tissue are quantified.

Predicted weight of soft-tissue is calculated as a function of HCWT, thickness and the ratio of soft-tissue to bone pixels. The fat % of the soft tissue containing pixels is predicted by a function of thickness and R-values, which is then multiplied by the predicted proportion of soft tissue weight to HCWT to calculate the final prediction of **CT Fat %**. The calculation for **CT Lean %** is the inverse of this final step, instead taking 100% - fat % of a soft tissue pixel to calculate the lean % within the soft tissue.

CT Bone % is calculated by a function of bone pixel R-values and the ratio of bone to soft tissue pixels.

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3 Testing DXA accuracy versus CT

3.1 Methodology

3.1.1 Experimental Design

The main portion of this accreditation was conducted in 2022 at WAMMCO, Katanning. It was conducted over 5 site visits:

8th February 2022 – 100 carcases

21st March 2022 – 140 carcases

3rd May 2022 – 50 carcases

27th June 2022 – 35 carcases

26th July 2022 – 50 carcases

22nd August 2022 – 75 carcases

5th December 2022 – 64 carcases

Some carcases were subsequently excluded due to incorrect orientation through the DXA, inappropriate levels of trim, or corrupted CT image files (n=37). This protocol assumes that all carcases comply with the AUS-MEAT defined hot standard carcase weight trim, hence the exclusion of those carcases that did not comply. Furthermore, during routine commercial operation protocols must be in place to ensure that carcases are correctly oriented with the brisket facing toward the x-ray tube during scanning.

Carcases were selected to achieve a spread of weights and fat percentages that would represent the distribution seen commercially by Australian lamb abattoirs. **Table *2*** shows the number of carcases that were selected across a carcase weight by CT Fat% matrix. The areas of the table shaded in grey represent carcase phenotypes rarely found in Australian abattoirs and have therefore not been acquired.

Table 2. Carcase selection matrix consisting of a wide range of hot carcase weights and CT Fat %

	CT Fat %					
	<20%	**20-24%**	**24-28%**	**28-32%**	**32-36%**	**>36%**
<22kg	96	38	23	4		
22-28kg		51	45	24	9	
>28kg			71	50	52	11

Figure *4*, **Figure 5** and **Figure *6*** demonstrate the raw data range for CT Fat%, Lean%, and Bone% of the carcases acquired during this accreditation process, in all cases plotted against their corresponding DXA prediction.

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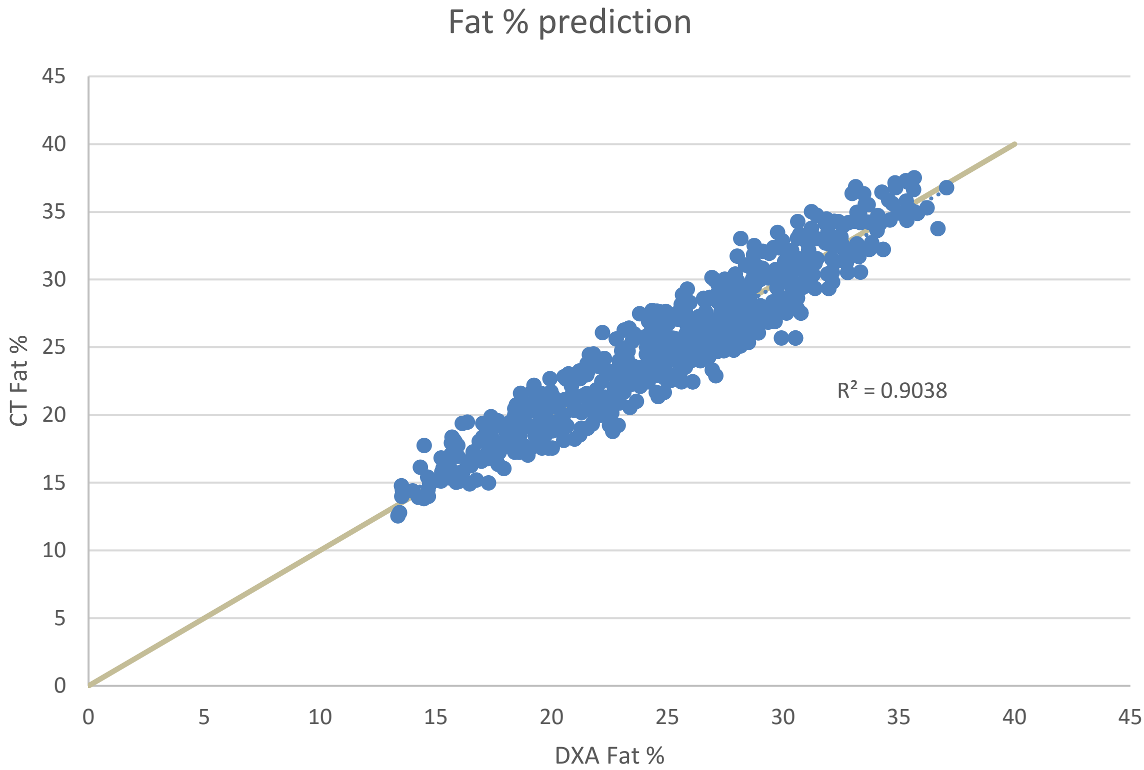

Figure 4. Comparison of CT Fat % and DXA predicted Fat %. Carcases have been pooled across all carcase weight categories.

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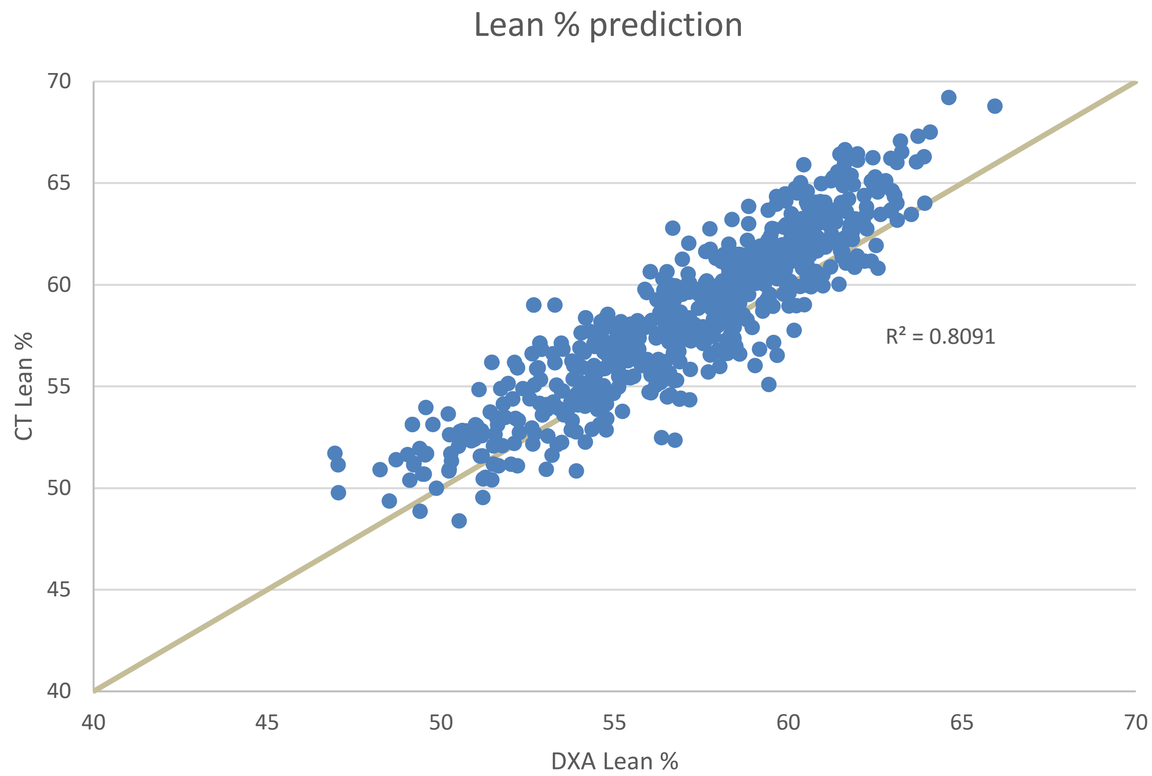

Figure 5 - Comparison of CT Lean % and DXA predicted Lean %. Carcases have been pooled across all carcase weight categories.

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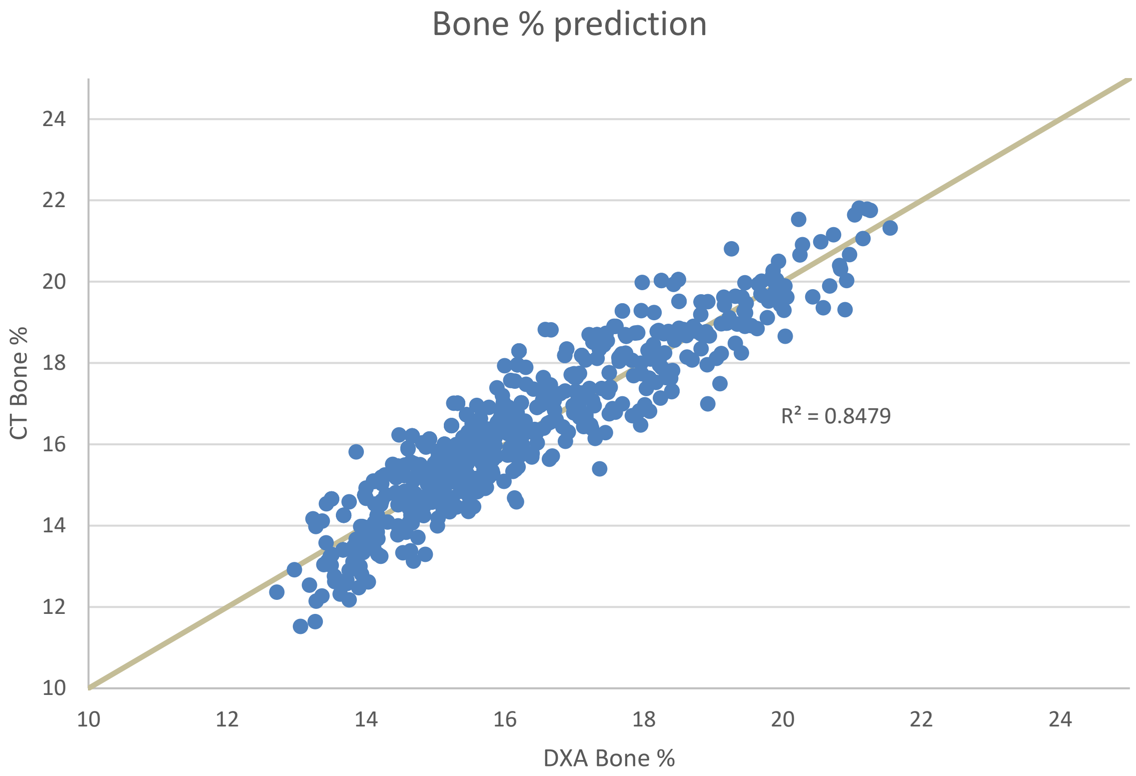

Figure 6. Comparison of CT Bone % and DXA predicted Bone %. Carcases have been pooled across all carcase weight categories.

3.1.2 DXA Scanning

The lambs were scanned as they passed beyond the weigh station and electrical stimulator at the end of the production floor, immediately prior to marshalling for chiller allocation.

The WAMMCO DXA device operates at 140kV, 10mA tube current, and will typically operate at chain speed anywhere between 7 and 9 carcases per minute. The order of carcases was noted manually as they passed the DXA terminal and were labelled as returning to Murdoch University.

The DXA images were acquired immediately after the scanning of the final lamb through the DXA research computer in the DXA systems room at WAMMCO. These files were saved and can still be accessed in their raw state at any time if required. The algorithm for accreditation was applied to these images and the results were saved.

All carcases were transported back to Murdoch University 24 hours after DXA scanning.

All raw DXA files were saved and are still available for future analysis if required.

3.1.3 CT Scanning

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.

CT scanning was conducted at The Animal Hospital Murdoch University on a Canon CT device at 120kV, 5mm slice width. The DICOM images for each lamb carcase was analysed using Image J v1.52a to determine fat, lean and bone densities, and pixel count, which was multiplied through the 5mm slice width to arrive at a final CT Fat %, CT Lean % and CT Bone % value. This is consistent with the protocol established for the sheep meat carcase composition trait, as described in the AMILSC submission "A carcase composition trait for sheep meat grading technologies" on the 17/2/2022.

All raw DICOM files were saved and available for analysis if required.

3.1.4 Statistical Analysis

Analysis of the accuracy standards were undertaken using the method described in the AMILSC submission "A carcase composition trait for sheep meat grading technologies (17/2/2022)".

The allowable error tolerances for total carcase composition are:

- Within ±3% CT Fat % for at least 67% of sample collected, and ±6% CT Fat % for at least 95% of samples collected
- Within ±3% CT Lean % for at least 67% of sample collected, and ±6% CT Lean % for at least 95% of samples collected
- Within ±1.6% CT Bone % for at least 67% of sample collected, and ±3.2% CT Bone % for at least 95% of samples collected

This was required for testing across three separate bands of lamb hot carcase weight - <22kg, 22-28kg and >28kg.

To ensure that the accuracy threshold is met across each quarter of the accreditation range, a Markov Chain Monte Carlo (MCMC) stochastic simulation was applied to the data to characterise the distribution of residuals (observed – predicted). Accuracy was then assessed within quarters of the data range that the technology is seeking accreditation for. This simulation is available using the online application:

https://accreditationapps.shinyapps.io/Sheep_Meat_IMF_Percent_V4/

This process was repeated for each tissue type (fat, lean and bone), and undertaken separately within each of the three weight bands, creating a total of 9 accreditation accuracy analyses.

3.2 Results

The results are displayed for fat%, lean% and bone% predictions within their weight categories (<22kg, 22-28kg, >28kg).

Table 3 outlines the accuracy results of the DXA devices predicting carcase composition in the nine discrete accreditation groups. The carcase composition range for fat%, lean% and bone% is listed within each weight category.

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Table 3. Mean error (Fit mean), standard deviation of error (Fit SD), percentage of samples within 1 times the accreditation threshold, percentage of samples within 2 times the accreditation threshold, and sample count for each quarter of the accreditation range for each tissue component, is shown within the low (<22kg), medium (22-28kg), and heavy (>28kg) weight bands.

		Low Weight (<22kg)					Mid Weight (22-28kg)					High Weight (>28kg)				
		Fit (mean)	Fit (SD)	% within 3 Fat/Lean, 1.6 Bone	% within 6 Fat/Lean, 3.2 Bone	Count	Fit (mean)	Fit (SD)	% within 3 Fat/Lean, 1.6 Bone	% within 6 Fat/Lean, 3.2 Bone	Count	Fit (mean)	Fit (SD)	% within 3 Fat/Lean, 1.6 Bone	% within 6 Fat/Lean, 3.2 Bone	Count
Range		Fat% accreditation range = 10.9% - 30.3%					Fat% accreditation range = 14.9% - 35.0%					Fat% accreditation range = 22.0% - 37.1%				
Fat	Q 1	0.25	1.45	95.71	99.99	38	-0.27	1.35	96.94	100	18	0.28	1.94	87.45	99.75	12
	Q 2	1.25	1.44	88.8	99.95	72	-0.17	1.34	97.29	100	40	0.93	1.92	84.16	99.52	21
	Q 3	1.36	1.46	86.79	99.9	23	-0.34	1.33	97.15	100	34	-0.21	1.9	88.21	99.78	24
	Q 4	0.46	1.46	94.98	99.99	21	0.37	1.37	96.6	100	14	0.31	1.92	87.7	99.77	21
		Lean% accreditation range = 53.2% - 65.0%					Lean% accreditation range = 50.9% - 66.2%					Lean % accreditation range = 49% - 60.6%				
Lean	Q 1	0.32	1.41	96.14	99.99	12	1.49	1.93	77.09	99.02	22	1.17	1.31	91.81	99.98	20
	Q 2	1.18	1.4	90.25	99.96	22	1.96	1.91	70.48	98.18	29	1.85	1.31	81.13	99.92	25
	Q 3	0.79	1.38	94.35	99.99	50	1.64	1.9	75.55	98.85	42	2.35	1.31	69.15	99.71	30
	Q 4	2.37	1.38	67.78	99.55	46	1.52	1.97	76.5	98.83	13	2.44	1.3	66.82	99.65	22
		Bone% accreditation range = 14.9% - 25.0%					Bone% accreditation range = 13.3% - 18.0%					Bone% accreditation range = 11.6% - 17.5%				
Bone	Q 1	-0.89	0.84	80.18	99.7	26	-1.13	0.76	73.36	99.62	13	-1.23	0.78	68.15	99.37	9
	Q 2	-1.36	0.83	61.14	98.69	54	-0.72	0.75	87.87	99.96	39	-0.94	0.75	81.39	99.84	31
	Q 3	-1.27	0.83	65.63	98.98	58	-0.4	0.75	94.15	99.98	33	-0.69	0.76	88.49	99.91	29
	Q 4	-1.34	0.84	62.09	98.6	16	-0.32	0.76	95.03	99.98	18	-0.4	0.78	93.24	99.97	9

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3.2.1 Low weight (<22kg)

Fat

For the low carcase weight category, the DXA estimated Fat% meets the accreditation requirements between 10.9% - 30.3% across all 4 quarters of the range (see **Table *3***, and **Figure *7***).

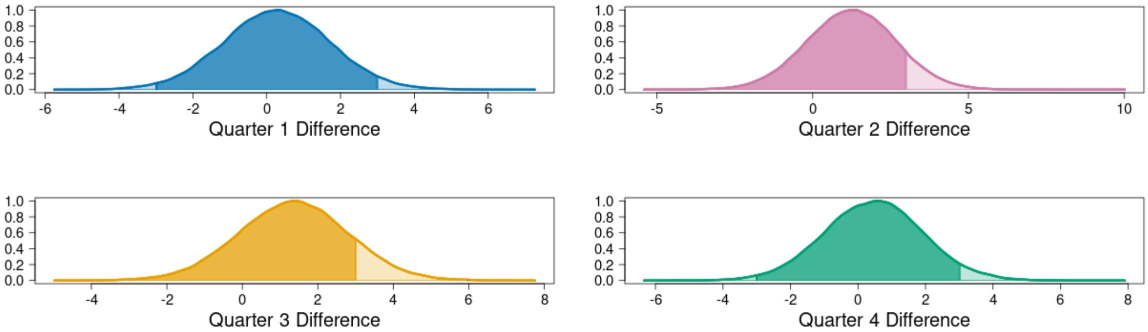

Figure 7 - Fitted Posterior Distributions of DXA device predicting CT Fat % for each quarter of the dataset. Difference is reported as the CT result minus the DXA estimate.

A scatter plot depicting the relationship between CT Fat% and DXA predicted Fat% is shown in Figure 8. CT Fat % was predicted by DXA with an RMSEP of 1.439%, and an R^2=0.928. The slope was 0.905 and had a bias of 0.91 CT Fat%.

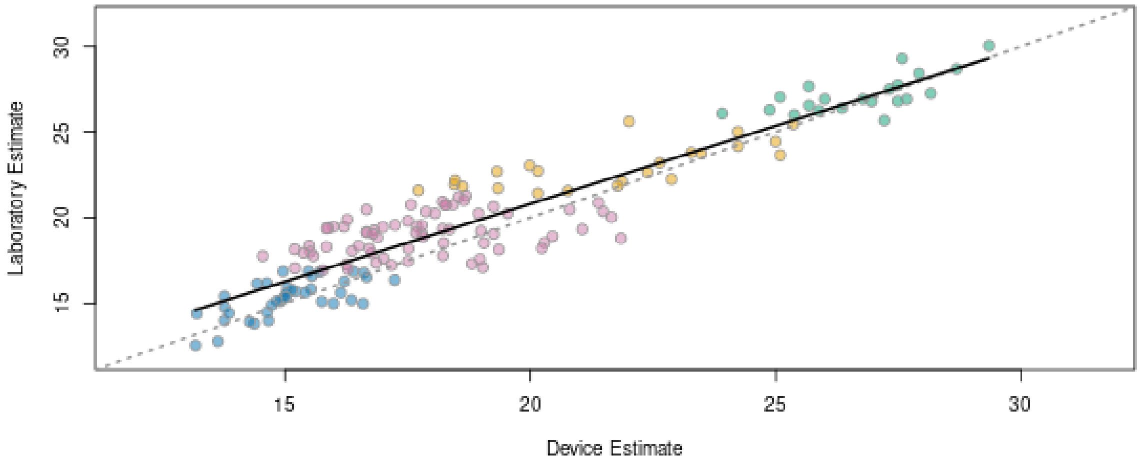

Figure 8. Relationship between CT Fat % (laboratory estimate) and DXA fat % (device estimate). The solid line represents the line of best fit, icons represent individual data points colour coded to fit the quarter to which they are assigned. The dashed line represents a 1:1 relationship.

Lean

For the low carcase weight category, the DXA estimated Lean% meets the accreditation requirements between 53.18% - 65% across all 4 quarters of the range (see Table 3, and Figure 9). The only limitation to this was that quarter 1 was not represented by a full 20

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samples, however given that the accuracy standards are met within this quarter with such confidence (ie accuracy thresholds well exceed the requirements), we propose conditional accreditation across this full range subject to meeting this requirement upon the 12 month re-validation for this trait.

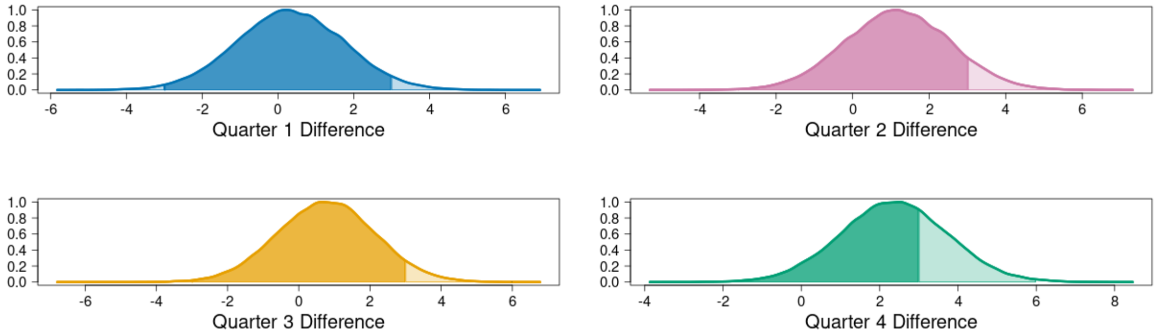

Figure 9. Fitted Posterior Distributions of DXA device predicting CT Lean % for each quarter of the dataset. Difference is reported as the CT result minus the DXA estimate.

A scatter plot depicting the relationship between CT Lean% and DXA predicted Lean% is shown in Figure 10. CT Lean % was predicted by DXA with an RMSEP of 1.52%, and an R^2=0.814. The slope of this relationship was 0.88 and had a bias of 1.7 CT Lean %.

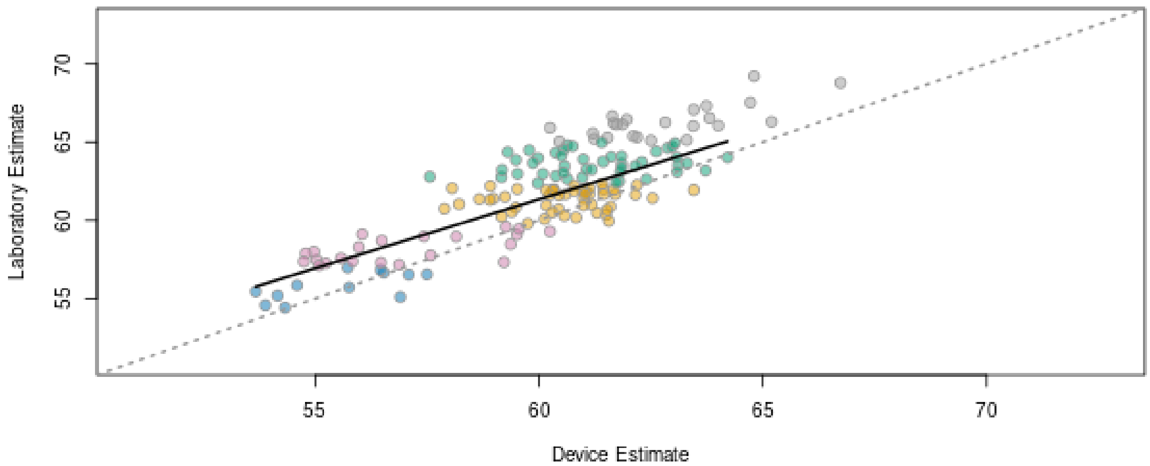

Figure 10. Relationship between CT Lean % (laboratory estimate) and DXA lean % (device estimate). The solid line represents the line of best fit, icons represent individual data points colour coded to fit the quarter to which they are assigned. The dashed line represents a 1:1 relationship. Raw data is coloured to represent the quarter to which it is assessed, with grey points representing data falling outside the accreditation range and not used by the analysis.

Bone

For the low carcase weight category, the DXA estimated Bone% meets the accreditation requirements between 14.94% - 25% across all 4 quarters of the range (see Table 3, and **Figure 11**).

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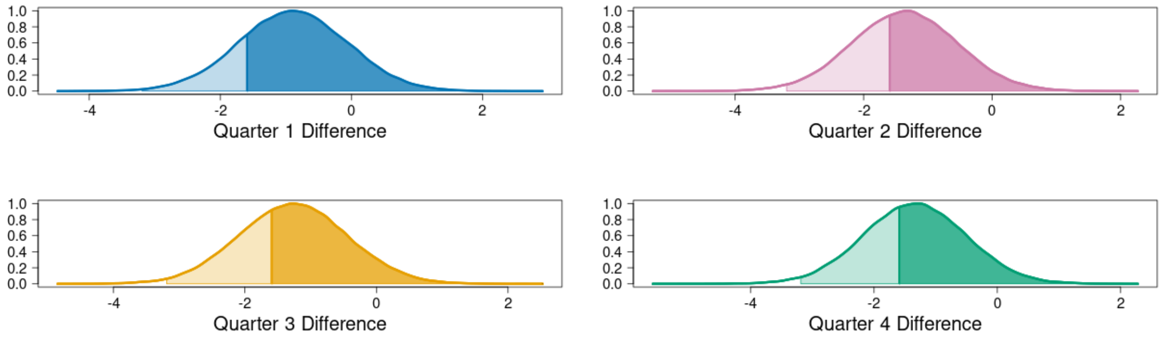

Figure 11. Fitted Posterior Distributions of DXA device predicting CT Bone % for each quarter of the dataset. Difference is reported as the CT result minus the DXA estimate.

A scatter plot depicting the relationship between CT Bone% and DXA predicted Bone% is shown in **Figure 12**. CT Bone % was predicted by DXA with an RMSEP of 0.683%, and an R^2=0.896. The slope was 0.752 and had a bias of -1.24 CT Bone %.

The only limitation to this was that quarter 4 was not represented by a full 20 samples, however given that the accuracy standards are met within this quarter with such confidence (ie accuracy thresholds well exceed the requirements), we propose conditional accreditation across this full range subject to meeting this requirement upon the 12 month re-validation for this trait.

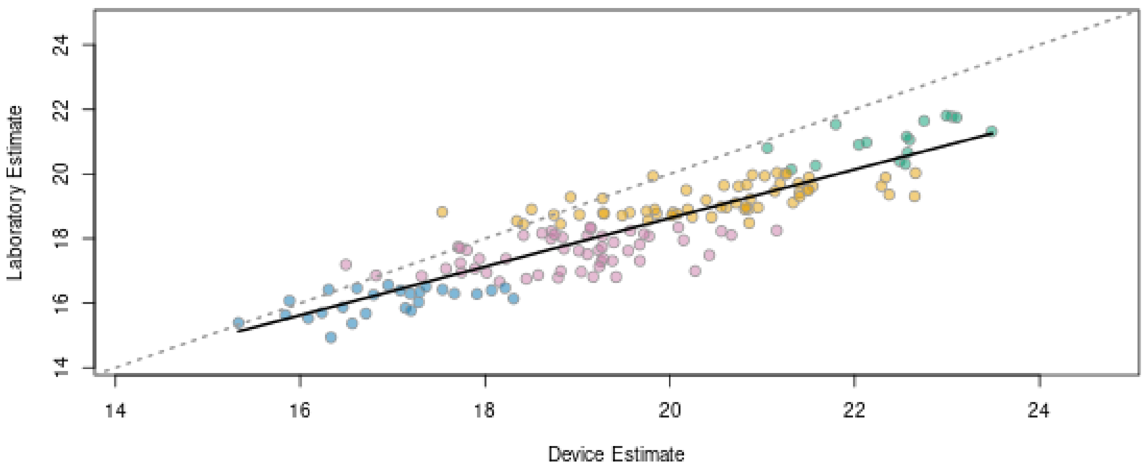

Figure 12. Relationship between CT Bone % (laboratory estimate) and DXA bone % (device estimate). The solid line represents the line of best fit, icons represent individual data points colour coded to fit the quarter to which they are assigned. The dashed line represents a 1:1 relationship.

3.2.2 Mid weight (22-28kg)

Fat

For the mid carcase weight category, the DXA estimated Fat% meets the accreditation requirements between 14.87% - 35.01% across all 4 quarters of the range (see Table 3, and **Figure 13**).

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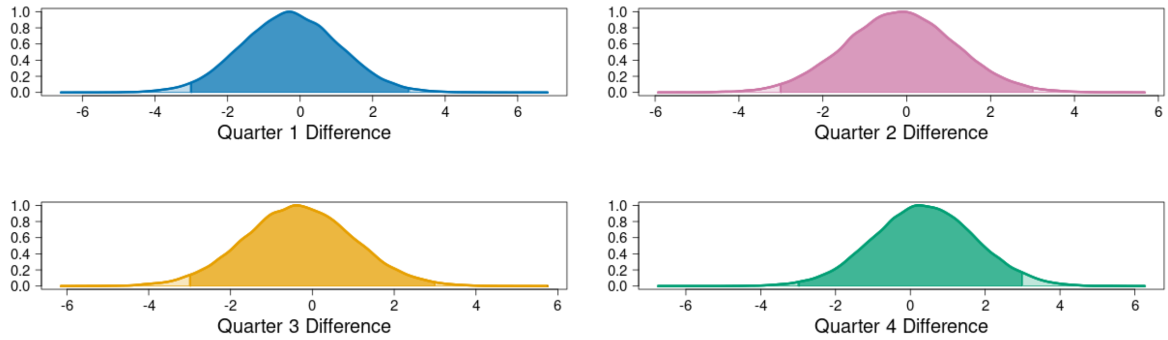

Figure 13. Fitted Posterior Distributions of DXA device predicting CT Fat % for each quarter of the dataset. Difference is reported as the CT result minus the DXA estimate.

A scatter plot depicting the relationship between CT Fat% and DXA predicted Fat% is shown in **Figure 14**. CT Fat % was predicted by DXA with an RMSEP of 1.23%, and an R^2=0.949. The slope was 0.933 and had a bias of -0.16 CT Fat %.

The only limitation to this was that quarters 1 and 4 were not represented by a full 20 samples, however given that the accuracy standards are met within these quarters with such confidence (ie accuracy thresholds well exceed the requirements), we propose conditional accreditation across this full range subject to meeting this requirement upon the 12 month re-validation for this trait.

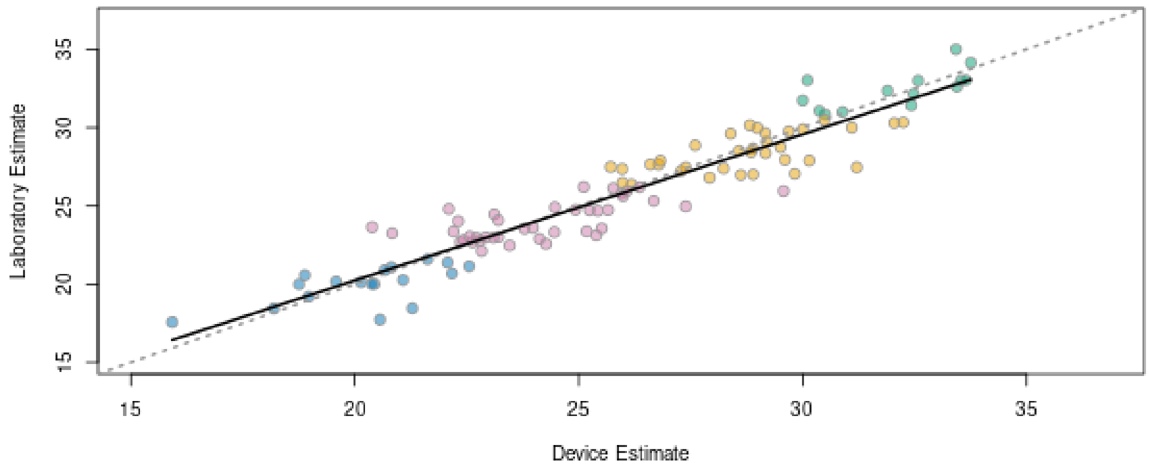

Figure 14. Relationship between CT Fat % (laboratory estimate) and DXA fat % (device estimate). The solid line represents the line of best fit, icons represent individual data points colour coded to fit the quarter to which they are assigned. The dashed line represents a 1:1 relationship.

Lean

For the mid carcase weight category, the DXA estimated Lean% meets the accreditation requirements between 50.9% - 66.21% across all 4 quarters of the range (see Table 3, and **Figure 15**).

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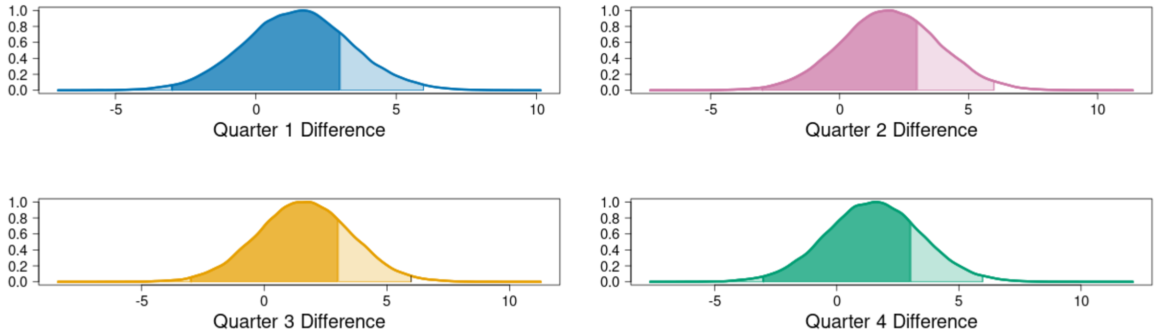

Figure 15. Fitted Posterior Distributions of DXA device predicting CT Lean % for each quarter of the dataset. Difference is reported as the CT result minus the DXA estimate.

A scatter plot depicting the relationship between CT Lean% and DXA predicted Lean% is shown in **Figure 16**. CT Lean % was predicted by DXA with an RMSEP of 1.66%, and an R^2=0.869. The slope was 0.782 and had a bias of 1.68 CT Lean %.

The only limitation to this was that quarter 4 was not represented by a full 20 samples, however given that the accuracy standards are met within this quarter with such confidence (ie accuracy thresholds well exceed the requirements), we propose conditional accreditation across this full range subject to meeting this requirement upon the 12 month re-validation for this trait.

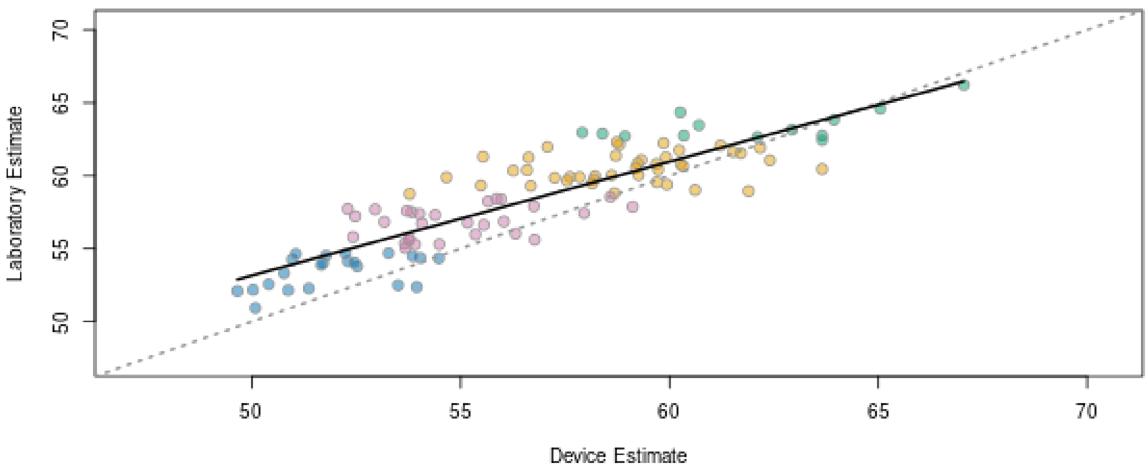

Figure 16. Relationship between CT Lean % (laboratory estimate) and DXA lean % (device estimate). The solid line represents the line of best fit, icons represent individual data points colour coded to fit the quarter to which they are assigned. The dashed line represents a 1:1 relationship.

Bone

For the mid carcase weight category, the DXA estimated Bone% meets the accreditation requirements between 13.29% - 18% across all 4 quarters of the range (see Table 3, and **Figure 17**).

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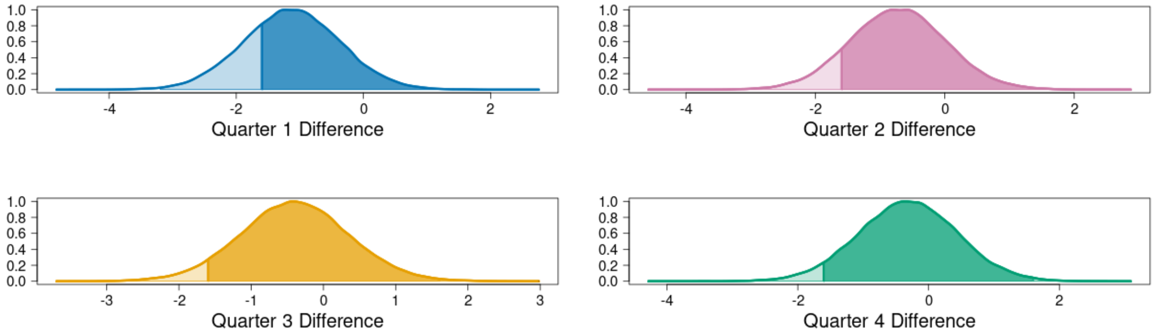

Figure 17. Fitted Posterior Distributions of DXA device predicting CT Bone % for each quarter of the dataset. Difference is reported as the CT result minus the DXA estimate.

A scatter plot depicting the relationship between CT Bone% and DXA predicted Bone% is shown in **Figure 18**. CT Bone % was predicted by DXA with an RMSEP of 0.748%, and an R^2=0.78. The slope was 0.8065 and had a bias of -0.58 CT Bone %.

The only limitation to this was that quarters 1 and 4 were not represented by a full 20 samples, however given that the accuracy standards are met within these quarters with such confidence (ie accuracy thresholds well exceed the requirements), we propose conditional accreditation across this full range subject to meeting this requirement upon the 12 month re-validation for this trait.

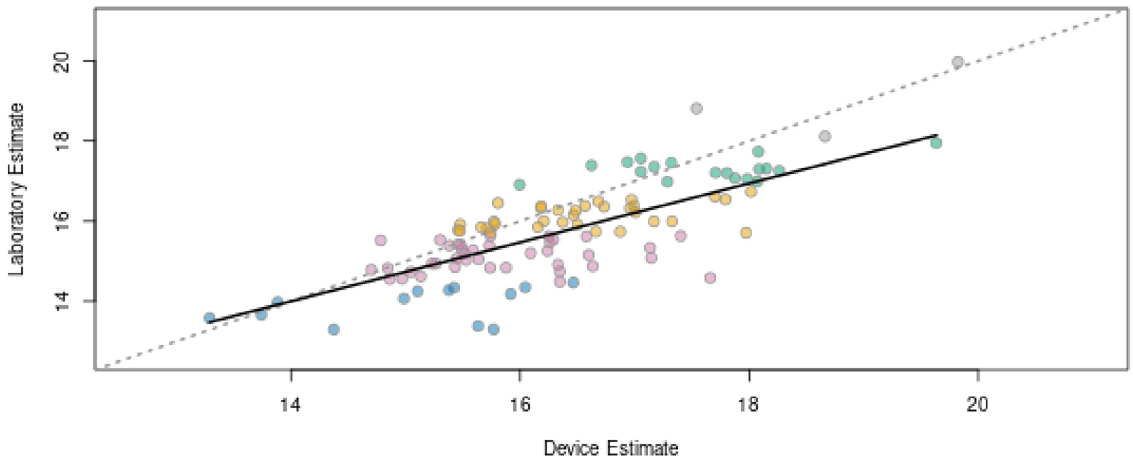

Figure 18. Relationship between CT Bone % (laboratory estimate) and DXA bone % (device estimate). The solid line represents the line of best fit, icons represent individual data points colour coded to fit the quarter to which they are assigned. The dashed line represents a 1:1 relationship.

3.2.3 High weight (>28kg)

Fat

For the high carcase weight category, the DXA estimated Fat% meets the accreditation requirements between 22.03% - 37.14% across all 4 quarters of the range (see Table 3, and **Figure 19**).

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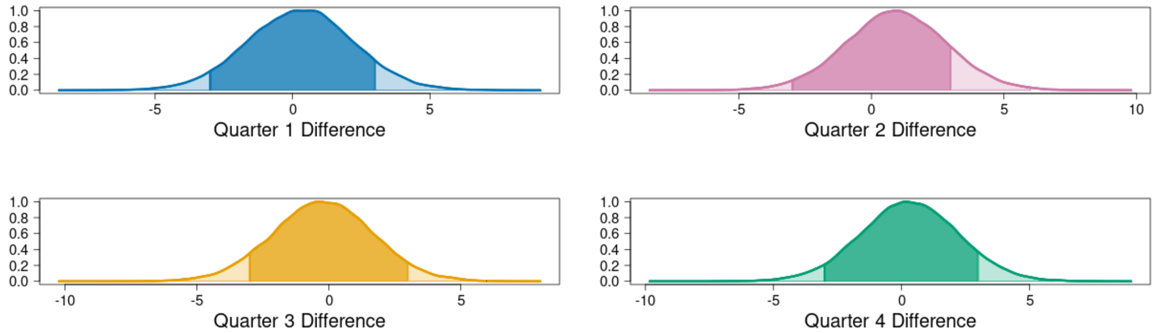

Figure 19. Fitted Posterior Distributions of DXA device predicting CT Fat % for each quarter the dataset. Difference is reported as the CT result minus the DXA estimate.

A scatter plot depicting the relationship between CT Fat% and DXA predicted Fat% is shown in **Figure 20**. CT Fat % was predicted by DXA with an RMSEP of 1.63%, and an R^2=0.903. The slope was 0.792 and had a bias of 0.31 CT Fat %.

The only limitation to this was that quarter 1 was not represented by a full 20 samples, however given that the accuracy standards are met within this quarter with such confidence (ie accuracy thresholds well exceed the requirements), we propose conditional accreditation across this full range subject to meeting this requirement upon the 12 month re-validation for this trait.

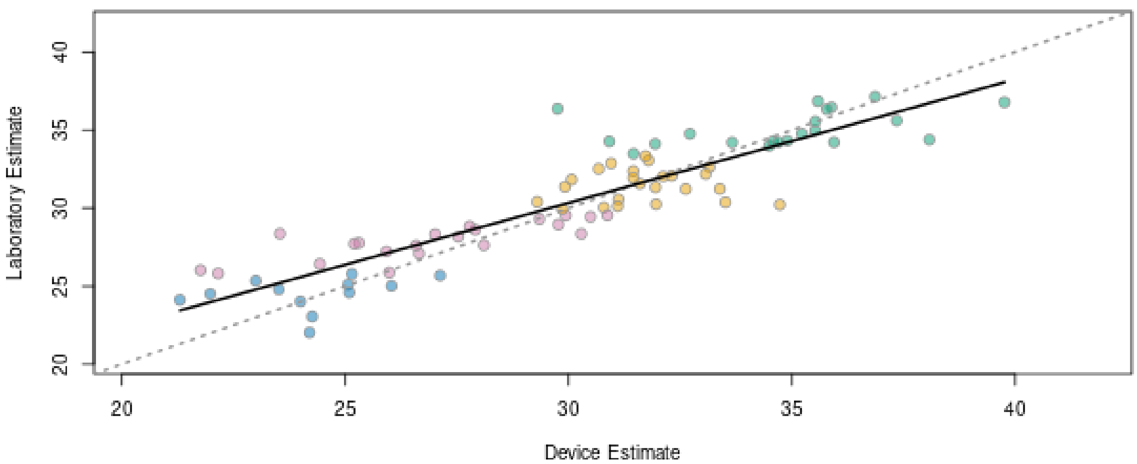

Figure 20. Relationship between CT Fat % (laboratory estimate) and DXA fat % (device estimate). The solid line represents the line of best fit, icons represent individual data points colour coded to fit the quarter to which they are assigned. The dashed line represents a 1:1 relationship.

Lean

For the high carcase weight category, the DXA estimated Lean% meets the accreditation requirements between 49% - 60.6% across all 4 quarters of the range (see Table 3, and **Figure *21***).

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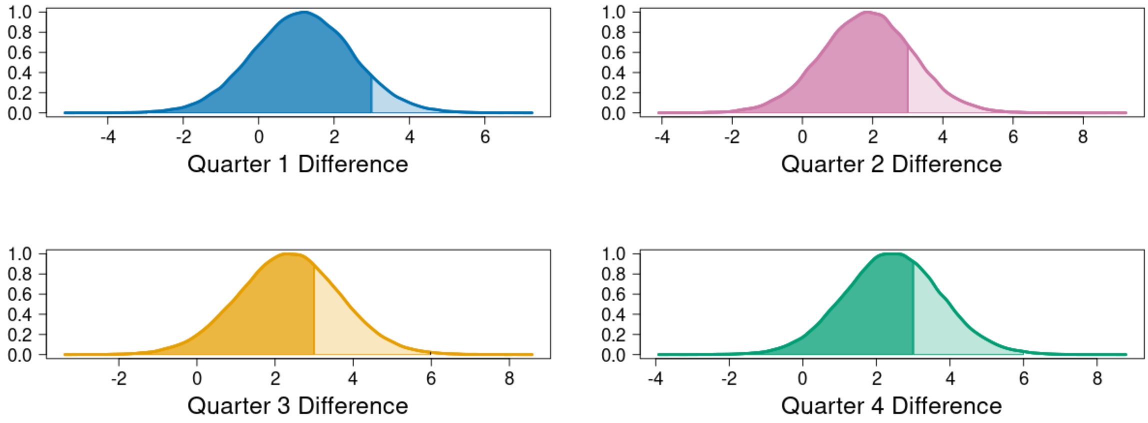

Figure 21. Fitted Posterior Distributions of DXA device predicting CT Lean % for each quarter of the dataset. Difference is reported as the CT result minus the DXA estimate.

A scatter plot depicting the relationship between CT Lean% and DXA predicted Lean% is shown in **Figure *22***. CT Lean % was predicted by DXA with an RMSEP of 1.33%, and an R^2=0.81. The slope was 0.939 and had a bias of 2.08 CT Lean %.

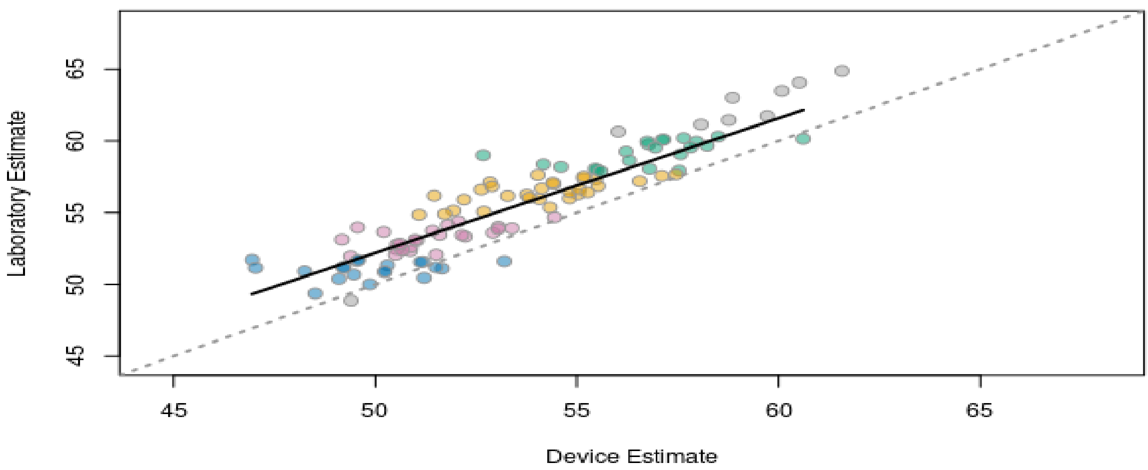

Figure 22. Relationship between CT Lean % (laboratory estimate) and DXA lean % (device estimate). The solid line represents the line of best fit, icons represent individual data points colour coded to fit the quarter to which they are assigned. The dashed line represents a 1:1 relationship.

Bone

For the high carcase weight category, the DXA estimated Bone% meets the accreditation requirements between 11.63% - 17.52% across all 4 quarters of the range (see Table 3, and **Figure 23**).

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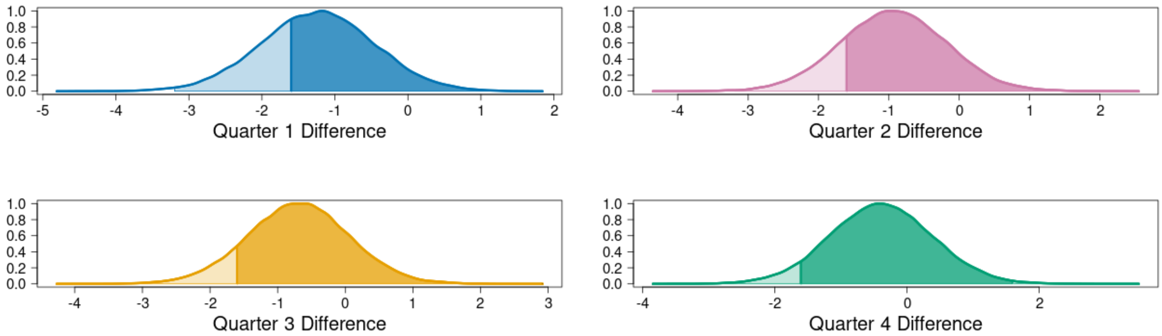

Figure 23. Fitted Posterior Distributions of DXA device predicting CT Bone % for each quarter of the dataset. Difference is reported as the CT result minus the DXA estimate.

A scatter plot depicting the relationship between CT Bone% and DXA predicted Bone% is shown in **Figure 24**. CT Bone % was predicted by DXA with an RMSEP of 0.704%, and an R^2=0.74. The slope was 0.741 and had a bias of -0.81 CT Bone %.

The only limitation to this was that quarters 1 and 4 were not represented by a full 20 samples, however given that the accuracy standards are met within these quarters with such confidence (ie accuracy thresholds well exceed the requirements), we propose conditional accreditation across this full range subject to meeting this requirement upon the 12 month re-validation for this trait.

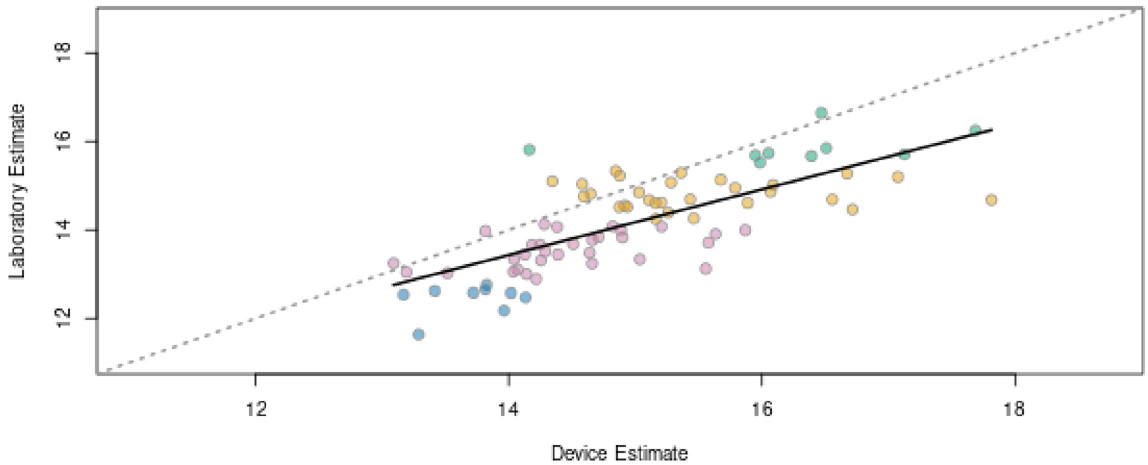

Figure 24. Relationship between CT Bone % (laboratory estimate) and DXA bone % (device estimate). The solid line represents the line of best fit, icons represent individual data points colour coded to fit the quarter to which they are assigned. The dashed line represents a 1:1 relationship.

4 Testing DXA repeatability

As DXA is a fixed install device along an abattoir chain, there are logistical difficulties in testing the repeatability of this device, both within a single device and especially across multiple devices.

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Nevertheless, studies have been conducted in the past to assess repeatability within two separate devices, and one study assessing the repeatability across multiple devices. The experiments were conducted by Murdoch University, an institution independent of Scott Automation and Robotics. These experiments were logistically difficult, and due to high biosecurity concerns and travel limitations within the last few years, repeating such experiments with AUS-MEAT support has been almost impossible.

One constraint in undertaking accreditation of DXA is that the installation requires integration into lamb slaughter chains and involves significant site works. On this basis it is not possible to install multiple systems at one site to conduct repeatability tests between multiple devices. Furthermore, this “hot” scanning system is located at the end of the WAMMCO kill chain, and at this site it is not feasible to undertake repeat scanning of the same hot carcases prior to chiller entry. Therefore, in accordance with the submitted report accepted by AMILSC titled “Accreditation of destructive or fixed-installation technologies predicting beef rib-eye traits”, we will use evidence from the repeat scans of an identically manufactured plastic phantom which is installed at each facility with a DXA device. These were undertaken at the same time as the acquisition of the accreditation data presented in this report.

To further support the demonstration of repeatability, we have conducted other experiments where “chilled” carcases were repeat scanned at two separate JBS abattoirs where DXA was installed, and then the carcases were transported between these sites to be re-scanned. We acknowledge that these are imperfect tests as they are undertaken using chilled carcases which were therefore no longer a “hot standard carcase”. Furthermore, for the between site comparison the carcases are also likely to have slightly changed composition through dehydration. None-the-less this evidence is presented to provide evidence of repeatability within this submission.

4.1 Within device repeatability using chilled lamb carcases

4.1.1 Experimental design

This experiment was conducted in 2017, and is described in detail within a published international journal article in Meat Science (Connaughton, Williams et al. 2020).

Six groups of ten chilled lamb carcases were DXA scanned three times in quick succession at JBS Bordertown. DXA estimates of CT Fat %, CT Lean % and CT Bone % were calculated, and the coefficient of correlation between the three scans was calculated for CT Fat % as an example in SAS 9.1. The predicted mean deviation of the CT Fat % predictions from each of the six groups was calculated for each of the three scans to assess repeatability between scans. Furthermore, the predictions of CT Fat %, CT Lean % and CT Bone % for each repeated scan was compared to the first scan of each carcase, and the differences were assessed to determine if they fell within the minimum accuracy requirements for a device.

This experiment was repeated at Bordertown the following year, with five repeated scans taking place rather than three on a total of 40 carcases. The difference of predicted CT Fat %, CT Lean % and CT Bone % from the first scan of each carcase was calculated and compared to the minimum accuracy requirement s of this device.

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4.1.2 Results

There was very high repeatability between the three repeat scans across all six groups. The coefficients of correlation are displayed in **Table 4**.

Table 4. Coefficients of correlation between the Fat % predictions of each rep across all six groups, with simple within the top right portion, and partial correlation within the bottom left portion

		Scan		
		1	2	3
	1	1	0.993	0.994
Scan	2	0.992	1	0.995
	3	0.995	0.994	1

The mean differences across the three scans of the 10 carcases in each of the 6 groups are displayed in **Table 5**. This result demonstrates that the repeatability of this device in the worst case demonstrated a mean deviation from the group mean of 0.53 CT Fat %, well within the allowable threshold for CT Fat % accuracy of less than 3 CT Fat%. Of the 180 carcase scans, 100% of the scans were within ±3 CT Fat %. This same assessment was made for repeatability of the CT Lean% and CT Bone% predictions, and as expected 100% were within ±3 CT Lean %, and 100% were within ±1.6 CT Bone %.

Table 5. Predicted mean deviation (± SE) of DXA predicted Fat% from the raw group mean for 3 repeat scans (rep 1-3) of 6 groups of 10 carcasses (A-F). For each of these groups (A-F), the StDev of the raw mean Fat % is also shown

		Predicted mean deviation of Fat % from the raw group mean (±SE)			
		Scan			
		1	2	3	Raw mean Fat % (±StDev)
Group	A	-0.16 (±0.14)	0.04 (±0.14)	0.12 (±0.14)	31.8 (±5.79)
	B	0.47 (±0.325) [a]	0.2 (±0.325) [a]	-0.48 (±0.325) [b]	23.0 (±2.49)
	C	-0.02 (±0.086)	-0.03 (±0.086)	0.05 (±0.086)	27.1 (±5.56)
	D	0.50 (±0.125) [a]	-0.25 (±0.125) [b]	-0.25 (±0.125) [b]	23.7 (±3.93)
	E	0.53 (±0.121) [a]	-0.23 (±0.121) [b]	-0.31 (±0.121) [b]	24.6 (±3.93)
	F	0.45 (±0.104) [a]	-0.21 (±0.104) [b]	-0.24 (±0.104) [b]	24.8 (±5.22)

The experiment with the five repeated scans produced similar results, with 96% of scans within ±3 CT Fat % and 97.3% of scans within ±6 CT Fat %, 96.7% of scans within ±3 CT Lean % and 98% within ±6 CT Lean %, and 96.7% within ±1.6 CT Bone % and 98% within ±3.2 CT Bone %. This easily meets the allowable error tolerances stated with the accreditation standards.

4.2 Within device repeatability using hot lamb carcases

4.2.1 Experiment design

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.

This experiment was conducted in 2019, and was performed at Gundagai Meat Processors, Gundagai, NSW.

30 carcases were selected to maximise the range of weight and fat scores, and each carcase was scanned 5 times in quick succession. DXA predictions were recorded and the correlation between each of the 5 scans was conducted across all carcases. The differences from the mean of each of the 5 scans was calculated, and the residual from the mean was also calculated to assess whether the repeated scans fall within the threshold limits.

4.2.2 Results

There was very high repeatability across the five runs, with the coefficients of correlation results displayed in **Table 6**.

Table 6. Coefficients of correlation between the Fat % predictions of each scan

		Scan				
		1	**2**	**3**	**4**	**5**
	1	1.00	0.93	0.95	0.92	0.94
	2	-	1.00	0.91	0.97	0.98
Scan	**3**	-	-	1.00	0.92	0.93
	4	-	-	-	1.00	0.97
	5	-	-	-	-	1.00

Of the total of 150 scans, 81.3% were within the error tolerance threshold of ±3 CT Fat %, and 100% were within ±6 CT Fat %. 96% of scans were within ±3 CT Lean %, with 100% within ±6 CT Lean %. Finally, 99.3% were within ±1.6 CT Bone % and 100% within ±3.2 CT Bone %.

4.3 Between device repeatability using a synthetic phantom

To further support the between device repeatability, we have undertaken tests in accordance with the submitted report accepted by AMILSC titled "Accreditation of destructive or fixed-installation technologies predicting beef rib-eye traits". This is fulfilled by the presence of an identically manufactured plastic phantom at each facility with a DXA device, which is scanned daily.

4.3.1 Experimental design

In 2017, an experiment was conducted to assess the cross-site calibration of two DXA devices – one in Bordertown, SA, and the other in Brooklyn, VIC. 60 carcases were selected in total, with 10 selected at each site over three consecutive days. The carcases were selected to maximise the range for weight and fat score.

On day 1, 10 carcases were selected and DXA scanned at each site. A synthetic phantom block (50mm depth,300mm high, 200mm wide) constructed of nylon and HDPE was also DXA scanned. The carcases were then transported to the other site overnight to be repeat DXA scanned the following day. This process was repeated two additional times, creating a total of

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6 groups each with DXA scans from both sites. After completion of DXA scanning the carcases were transported to Werribee, VIC, for CT scanning. Thus, in total all 60 carcases were scanned twice by DXA (once at each site), and then scanned by CT.

4.3.2 Results

The scanning of the phantom block at the start of each scanning session showed very consistent R values and thickness determination within each site (**Table 7**). These values differed by a small but consistent amount between the two sites (**Table 7**).

Table 7. DXA estimated R value and thickness (mm) of a nylon-6 phantom at Bordertown and Brooklyn

	Bordertown		Brooklyn	
Day	R value	Thickness	R value	Thickness
1	1.2188	59.49	1.2539	65.26
2	1.219	59.16	1.2516	63.04
3	1.2218	59.61	1.2549	62.78
4	1.2184	58.26	1.252	63.01

This difference in R values and thickness underpins the adjustment applied to DXA values, so that the values at Brooklyn equated to that at Bordertown. By doing so, the predictions of CT Fat % and CT Lean % between sites are comparable, as seen in **Table 8**. This represents the basis for the calibration of DXA between sites.

Table 8. Comparison of CT Fat % and CT Lean % predictions by DXA at the Bordertown and Brooklyn sites across the four days of experimentation after adjustment with the phantom block

	CT Fat %		CT Lean %	
Scan Day	Bordertown	Brooklyn	Bordertown	Brooklyn
1	0 ± 0.376	0.66 ± 0.375	0 ± 0.285	-0.46 ± 0.284
2	0.51 ± 0.266	0.15 ± 0.265	-0.36 ± 0.201	-0.107 ± 0.201
3	0.15 ± 0.266	0.29 ± 0.265	-0.11 ± 0.201	-0.20 ± 0.201
4	0.20 ± 0.376	0 ± 0.375	-0.14 ± 0.285	0 ± 0.284

These results demonstrate that after adjustment for the synthetic phantom, the repeatability between the two sites is well within the margin of error tolerance, with 100% of scans within ±3 CT Fat %, 100% of scans within ±3 CT Lean %, and 100% of scans within ±1.6 CT Bone %.

4.3.3 DXA calibration using the Scott phantom

Calibration of DXA systems to ensure consistent results across site requires the scanning of a synthetic phantom manufactured by Scott Automation and Robotics. This phantom is in-built within all sheep DXA installations and is designed as a mixture of three plastics – nylon, polyethylene and acrylic and is shown in **Figure *1***, **Figure *2***, and **Figure *3*** in section 2 above.

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The synthetic phantom block is scanned at the start of each day's production, and the linear attenuation (μ) of each block is calculated by the following equation:

$$\mu = -\frac{\ln\left(\frac{I}{I_0}\right)}{t}$$

Where t represents the thickness of material that the x-ray beam is passing through (adjusted for height of block and beam angle), I represents the attenuation measured for a given pixel for the low energy detector, and I_0 represents the value of an unattenuated pixel passing through air, which in this case has been calibrated to a value of 4095 in these images.

Differences between sites can be characterised by the differences in the linear attenuation coefficients of the plastics within the Scott phantom, as shown in **Figure 25** for the low energy attenuation and **Figure 26** for the high energy attenuation, and shown by **Figure 27** which demonstrates the estimated R values for these plastics prior to applying the calibration adjustment.

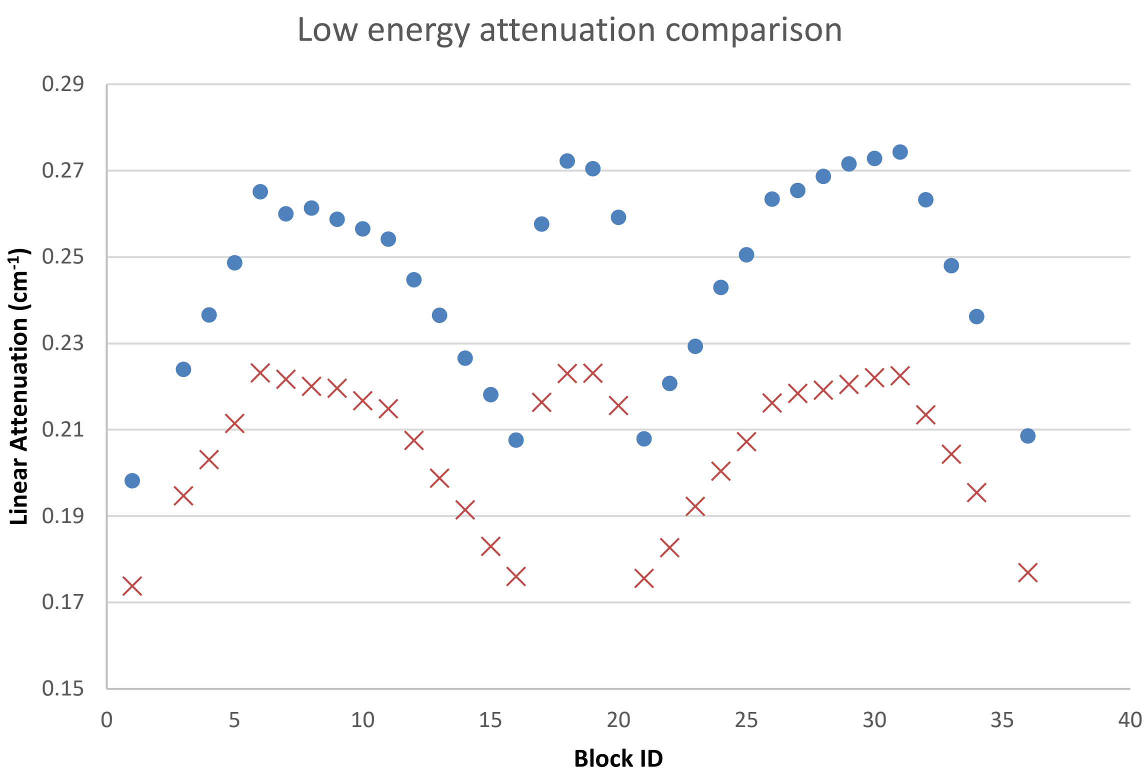

Figure 25. The linear attenuation of the synthetic phantoms blocks in the low energy images from WAMMCO (X) and GMP (O).

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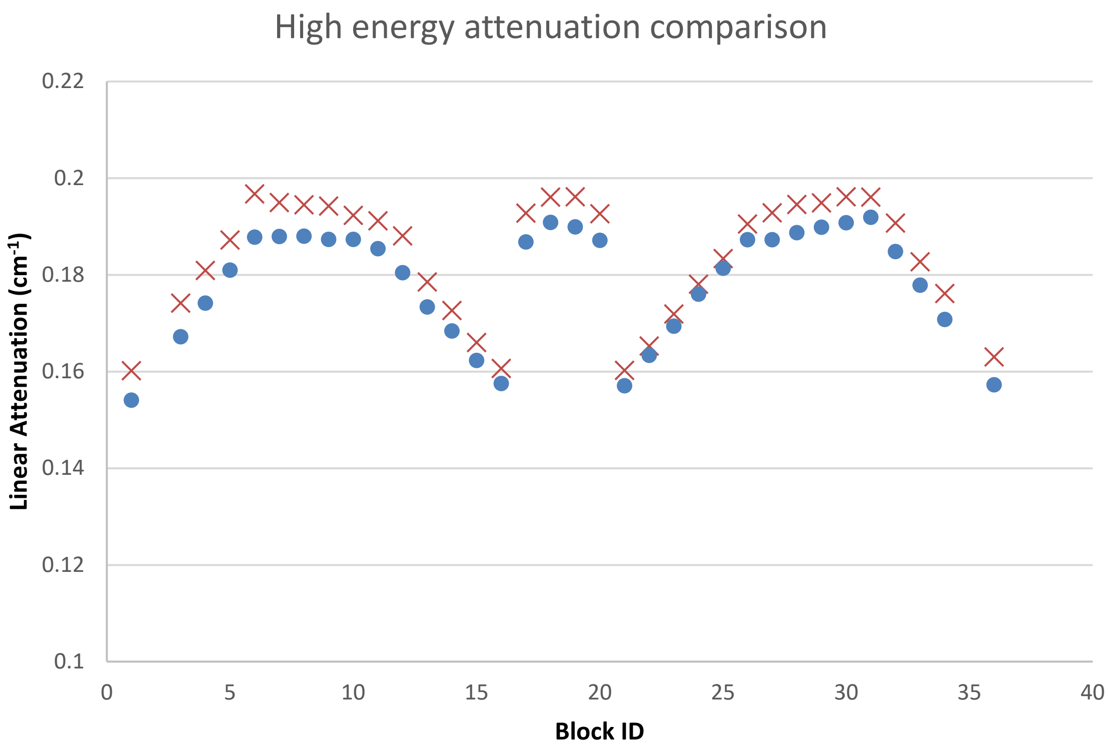

Figure 26. The linear attenuation of the synthetic phantoms blocks in the high energy images from WAMMCO (X) and GMP (O).

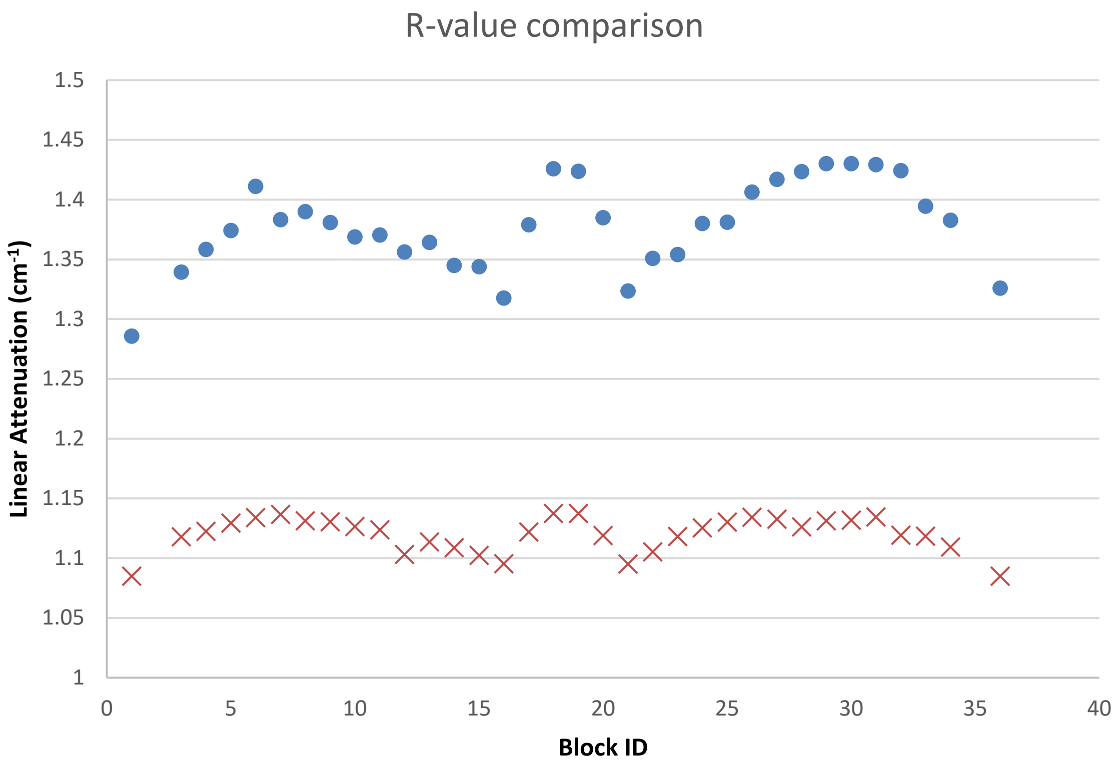

Figure 27. Comparison of R-values of each block of the synthetic phantom at WAMMCO (X) and GMP (O).

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These differences in estimated linear attenuation coefficients between devices are expected, and demonstrate why DXA scanners require calibration using synthetic phantoms (Anonymous 2011). Using the R-values and thickness estimates derived from the synthetic phantom, the differences between the sites can be estimated, and adjustments to the coefficients in the algorithm (soft tissue weight calculation, fat % in soft tissue pixel, and bone %) can be applied, proportional to the values derived from the plastic phantom.

In this manner, the results for the sister site at GMP can be adjusted to align with the WAMMCO device. The effect of this can be seen in **Figure 28** below. The red icons are from scans of 15 carcases taken at GMP prior to applying calibration, with DXA Fat% predictions shown on the X axis and CT Fat% predictions shown on the Y axis. This demonstrates the capacity of DXA calibration using synthetic phantoms to maintain accuracy across sites.

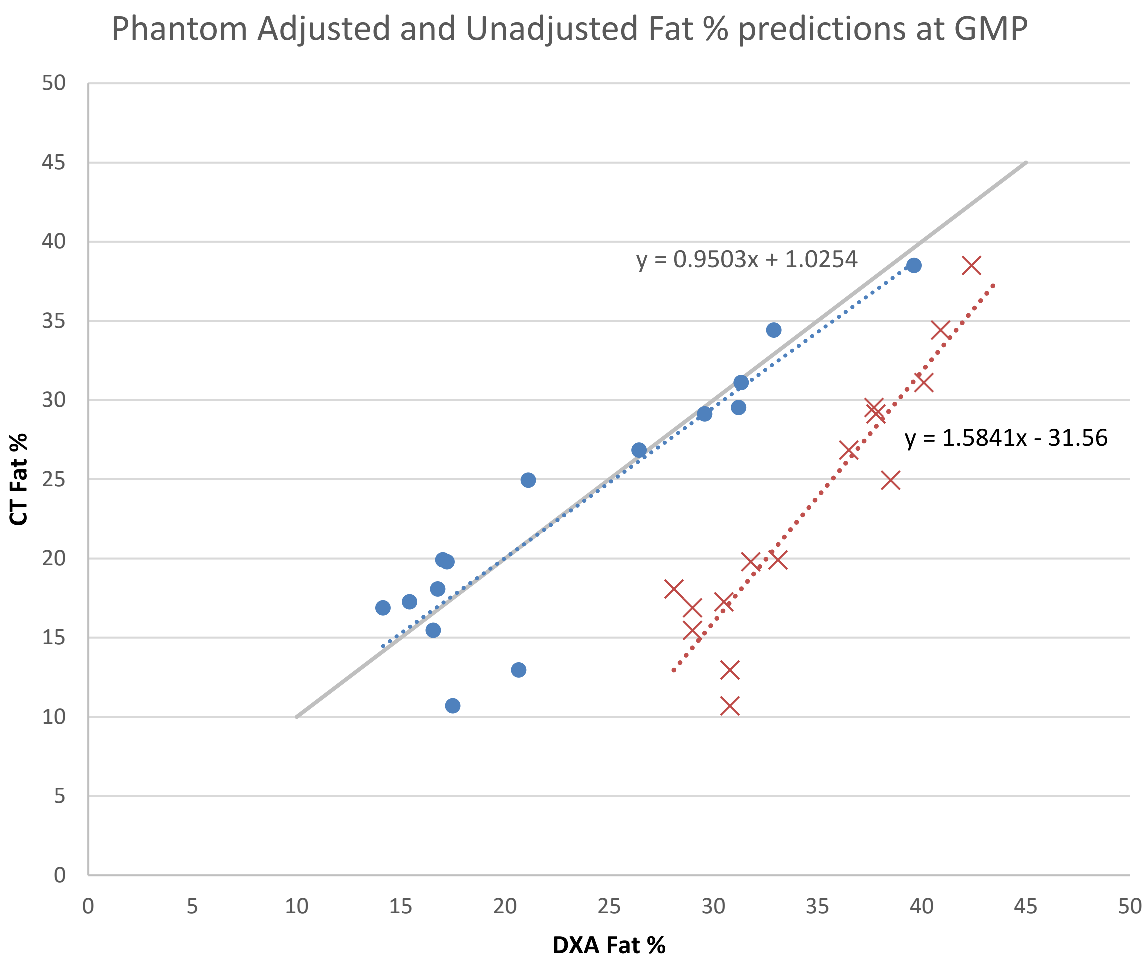

Figure 28. CT Fat% versus DXA predicted Fat% of carcases scanned at GMP prior to calibration adjustment (X) and after calibration adjustment (O) of DXA values. Solid grey line represents the 1:1 line of perfect prediction. The calibration adjusted values (X) align well with the line of perfect prediction.

5 Recommendations

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.

The lamb DXA achieves the AMILSC approved minimum requirements for predicting carcase composition within the light (<22kg), medium (22-28kg), and heavy (>28kg) weight categories. Within each weight category the accreditation range for prediction of CT Fat %, CT Lean % and CT Bone % is listed in **Table *9***.

Table 9. Accreditation ranges for lamb DXA

		Tissue Type		
		Fat	**Lean**	**Bone**
HCWT	**<22kg**	10.9% - 30.3%	53.2% - 65%	14.9% - 25.0%
	22-28kg	14.0% - 35.0%	50.9% - 66.2%	13.3% - 18.0%
	>28kg	22.0% - 37.1%	49% - 60.6%	11.6% - 17.5%

For both between device repeatability and within device repeatability, accreditation requirements were met through demonstration using previous experimental data collected using both hot and cold DXA devices.

Therefore, based on this report, we recommend that the lamb DXA device is accredited for predicting CT Fat %, CT Lean % and CT Bone % in the ranges outlined in **Table 9**.

6 References

Anonymous (2011). Dual Energy X Ray Absorptiometry for Bone Mineral Density and Body Composition Assessment. Vienna, INTERNATIONAL ATOMIC ENERGY AGENCY.

Connaughton, S. L., A. Williams, F. Anderson, K. R. Kelman, J. Peterse and G. E. Gardner (2020). "Dual energy X-ray absorptiometry predicts lamb carcass composition at abattoir chain speed with high repeatability across varying processing factors." Meat Science: 108413.

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