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CONTENTS

1 INTRODUCTION

Determining carcase fatness in meat producing animals is essential to enhance productivity and profitability of lamb supply chains. Carcase trading in Australia is largely based on carcase weight, where the saleable meat yield of a carcase is affected by the amount of subcutaneous fat (Gardner et al., 2018). Fat is the most variable component of a carcase, both in its amount and distribution. Current Australian industry standards for measuring single site fat and tissue depth in lamb carcases are by subjective palpation estimates or invasive objective cut techniques and measured using callipers (Anonymous, 2005). Having the ability to estimate fat depth non-invasively with precision and accuracy is important to underpin optimised carcase boning, producer feedback and value-based trading (Scholz et al., 2015).

Non-invasive methods of measuring fat depth, which do not cause pain (in live animals) or destruction of the tissues, are preferred over invasive techniques. One non-invasive solution that may have the potential to determine carcase fatness is a Microwave System (MiS) which measures tissue depth using low power non-ionizing electromagnetic waves (Marimuthu et al., 2016). Microwave technologies provide opportunities because of the understanding that biological tissues such as skin, fat, muscle and bone feature a high contrast in dielectric properties ($\varepsilon^* = \varepsilon' - j\sigma$, ε' = permittivity and σ = conductivity), allowing for microwave frequencies to distinguish between the different layers (Marimuthu et al., 2016)). Recent work (Marimuthu et al., 2018, Marimuthu et al., 2020) has demonstrated the capacity of the prototype MiS to evaluate C-site fat depth in lamb carcases. The most recent of these studies reported precision and accuracy indicators of microwave predicted C-site fat depth across pooled and balanced datasets were RMSEP 1.53 mm, R^2 0.54, and bias of 0.03 mm (Marimuthu et al., 2020).

The objective of this study was to explore the suitability of using Microwave predicted carcase C-site fat depth as a correlated trait for the invasive standard calliper measured carcase C-site fat depth.

2 MATERIALS AND METHODS

Experimental Data Set

This study utilised 15 slaughter groups totalling 2,114 lambs from 2017 to 2019. Groups 1 to 7 utilised slaughter lambs from the Meat and Livestock Australia Genetic Resource Flock (MLA-GRF) (formerly known as the Sheep CRC Information Nucleus Flock (INF). The design of this MLA-GRF flock has been described in detail previously (Fogarty et al., 2007, van der Werf et al., 2010). MLA-GRF lambs born in 2016 to 2018 were used in this study across two research sites, (Temora, New South Wales, and Katanning, Western Australia). Lambs were the progeny of Merino or Border-Leicester X Merino dams artificially inseminated using 286 industry sires. The objective of the MLA-GRF is to produce lambs with a broad cross-section of genotypic and phenotypic traits where different sire types are chosen to represent the full range of Australian Sheep Breeding Values from the major breeds used in the Australian sheep industry (van der Werf et al., 2010). The sire types include Merino sires (Merino, Poll Merino), Maternal sires (White Dorper, Border Leicester, Booroola Leicester, Coopworth, Bond, Corriedale, Dohne Merino, East Friesian, Prime South African Meat Merino) and Terminal sires (Hampshire Down, Ile De France, Poll Dorset, Southdown, Suffolk, Texel, White Suffolk).

A small cohort of commercial Australian lambs (Bordertown cohort) were also available as part of the training data (Marimuthu et al., 2020). These animals formed part of a larger body of research, with the background methodology described in full by Payne et al. (2020). The lambs from Straun, South Australia and Greta, Victoria, were mixed sex lambs raised under similar management to the MLA-GRF flocks, grazing extensive pasture systems, weaned at 90 days, all male lambs castrated and each flock

genetically linked by sires and dams. These animals did not have sufficient pedigree and animal information to be include in a genetic evaluation or the validation data sets.

Fat depth traits

Carcase C-site Fat depth

C-site fat was recorded using digital callipers, corresponding to the depth of subcutaneous fat (mm) over the loin eye muscle at the exposed cut surface, taken approximately 45 mm from the midline corresponding to the deepest part of the longissimus muscle (Mortimer et al., 2010).

Microwave Predicted C-site Fat depth

Marimuthu et al. (2020) covers the measurement of fat depth using the MiS scanning equipment in greater depth. However, in summary. MiS scanning of the C-site commenced *in-situ* at 60 min post slaughter while the carcass was still hot as it entered the chiller on the rails. The VPA probe was positioned such that the centre of the probe was placed to contact the carcase at the site to be measured, C-site corresponding to a point 45 mm from spine midline over the 12th rib. The microwave probe provides for each measurement (carcase) 256 calibrated and processed frequency domain microwave signals ($|S11(f)_j|_k$ (j = 1,2, ……, 256), where k represents the individual carcase) were used to estimate C-site fat depth as per. Marimuthu et al. (2020). Two statistical methodologies for constructing prediction equations, as per. , Marimuthu et al. (2020), were explored: i) Partial Least Squares Regression (PLS) was used to derive a model consisting of 2 or 20 components and, ii) a machine learning ensemble stacking method in WEKA® 3.9.4 (The University of Waikato, Hamilton, New Zealand) – which we will term ensemble method. WEKA® is a meta-algorithm incorporating multiple machine learning techniques, combining two layers of machine learning algorithms into one predictive model to improve predictions (Elshazly et al., 2013, Ribeiro and dos Santos Coelho, 2020).

This study explored the influence of two key factors behind the reliability of the microwave prediction on the traits suitability in future genetic evaluations: i) the training dataset formalisation and its impact on the genetic variation of the microwave predicted trait, and ii) the impact of statistical methodologies when constructing the prediction equations. An in-depth description of each of the Microwave predicted c-site fat depth traits are provided in Table 1.

Table 1: Description of the microwave predicted c-site fat depth traits analysed.

TRAIT	TESTING	DESCRIPTION
HCWTPRED	Training/validation	All Bordertown data was included in the training data set only, as sire identification was not available. The remaining data from Katanning and Kirby was pooled and allocated to 1 of 5 groups, with each group balanced for Site, Year, Killgroup, Sirebreed, and finally HCWT. The 5th group was excluded, and the remaining 4 groups were pooled along with the Bordertown data to train the model, which was then validated in the 5th group. This process was repeated 5 times until predictions were validated within each of the 5 groups.
YGPRED	Training/validation	All Bordertown data was included in the training data set only, as sire identification was not available. The remaining data from Katanning and Kirby was pooled and allocated to 1 of 2 year groups, 2018 or 2019. The 2018 group was excluded, and the remaining 2019 was pooled with the Bordertown data to train the model, which was then validated in the 2018 group. This process was repeated using the 2018 data pooled with the Bordertown data to train the model, which was then validated in the 2019 group.
SBPRED	Training/validation	All Bordertown data was included in the training data set only, as sire identification was not available. The remaining data from Katanning and Kirby was pooled. All lambs from one sire breed (ie Poll Dorset) were excluded and the remainder were pooled with the Bordertown data to train the model, which was then validated in the excluded sire breed group. This process was repeated until predictions were validated within every sire breed.
ASAPRED	Training/validation	All Bordertown data was included in the training data set. The remaining data from Katanning and Kirby was pooled and used as the validation dataset. Thus the prediction model was trained in the Bordertown data, and validated in the pooled Katanning and Kirby data.
ASA2PLSPRED	Prediction equation	Based on the training / validation technique used for ***ASAPRED***. The prediction equation uses a Partial Least Squares Regression (PLS) to derive a model consisting of 2 components.
ASA20PLSPRED	Prediction equation	Based on the training / validation technique used for ***ASAPRED***. The prediction equation uses a Partial Least Squares Regression (PLS) to derive a model consisting of 20 components
ASA2ESPRED	Prediction equation	Same trait as ***ASAPRED***
ASA20ESPRED	Prediction equation	Based off the training / validation technique used for ***ASAPRED***. The prediction equation uses a machine learning ensemble stacking method consisting of 20 components

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Statistical analyses

Variance components and genetic parameters for each trait were estimated using a linear mixed model and REML methods with ASReml software (Gilmour et al., 2015). Fixed effects included type of birth, contemporary group, age of the animal, sex (male or female) and the linear and quadratic effect of the age of dam (12 levels, for 1 to 12 years of age). The quadratic function of hot carcass weight were included to adjust the carcase fat measures traits.

The univariate models included either the random effects of sire (explored but not reported) or animal where the animal effects represented the additive genetic variance. Heritabilities for each trait were estimated from the univariate analyses where phenotypic variance was calculated as the sum of the additive genetic (or sire variance were relevant), and the residual variance. Whilst genetic groups which account for the flock of origin and the sheep type (Swan et al., 2015) are often fitted to larger datasets, the small number of records meant that this would not be suitable.

Phenotypic and genetic covariance for all traits and correlations between traits were estimated using bivariate analysis between all combinations of traits using ASReml. Fixed and random effects for the bivariate models were fitted using significant effects from the univariate analysis. The genetic correlations were also estimated by correlating the breeding values from the univariate analyses for the represented sires.

3 RESULTS AND DISCUSSION

Data summary

Just over 2k carcases have a microwave and calliper measured measurement of C-site fat depth. The carcases are represented across 20 contemporary groups and are the progeny of 286 sires. A summary of the records analysed is provided in Table 2. The mean digital calliper measured fat depth across the carcases was 5.1 mm with the average fat depth for the microwave predictions ranging from 3.65 to 5.07mm. This suggests that the microwave predictions are slightly under predicting the observed fat depth. It should be noted that the sub-population of carcases with microwave predictions was fatter than the larger INF/Resource flock population which has a mean calliper measured fat depth of 4.29 mm. All the microwave predicted fat depths, except MiS - ASA20PLSPred, had a lower standard deviation than was observed for the calliper measurement. The variation within the sub-population was similar although slightly lower than observed in the larger dataset.

Table 2: Summary of calliper and microwave predicted measures of lamb carcase C-site fat depth.

TRAIT	UNITS	RECORDS	SIRES	CGS	MEAN	SD
MIS - HCWTPRED	(mm)	2,114	286	20	5.07	1.58
MIS - YGPRED	(mm)	2,114	286	20	4.26	1.71
MIS - SBPRED	(mm)	2,114	286	20	5.07	1.59
MIS - ASAPRED	(mm)	2,114	286	20	4.06	1.66
MIS - ASA2PLSPRED	(mm)	2,114	286	20	3.65	1.86
MIS - ASA20PLSPRED	(mm)	2,114	286	20	4.39	3.28
MIS - ASA2ESPRED	(mm)	2,114	286	20	4.06	1.66
MIS - ASA20ESPRED	(mm)	2,114	286	20	4.13	1.80
CALIPPER C-SITE FAT	(mm)	2,114	286	20	5.10	2.38
CALIPPER C-SITE FAT	(mm)	32,411	2,259	1,371	4.29	2.43

Phenotypic Relationship between predicted and measured fat depth

The Pearson correlations between the raw microwave predictions and the calliper measured fat depth ranged from 0.66 to 0.43 with the strongest correlation observed with MIS - HCWTPRED and the weakest with MiS - ASA20PLSPred. A visual representation of the association between the microwave prediction traits and the calliper measurement is provided in Figure 1.

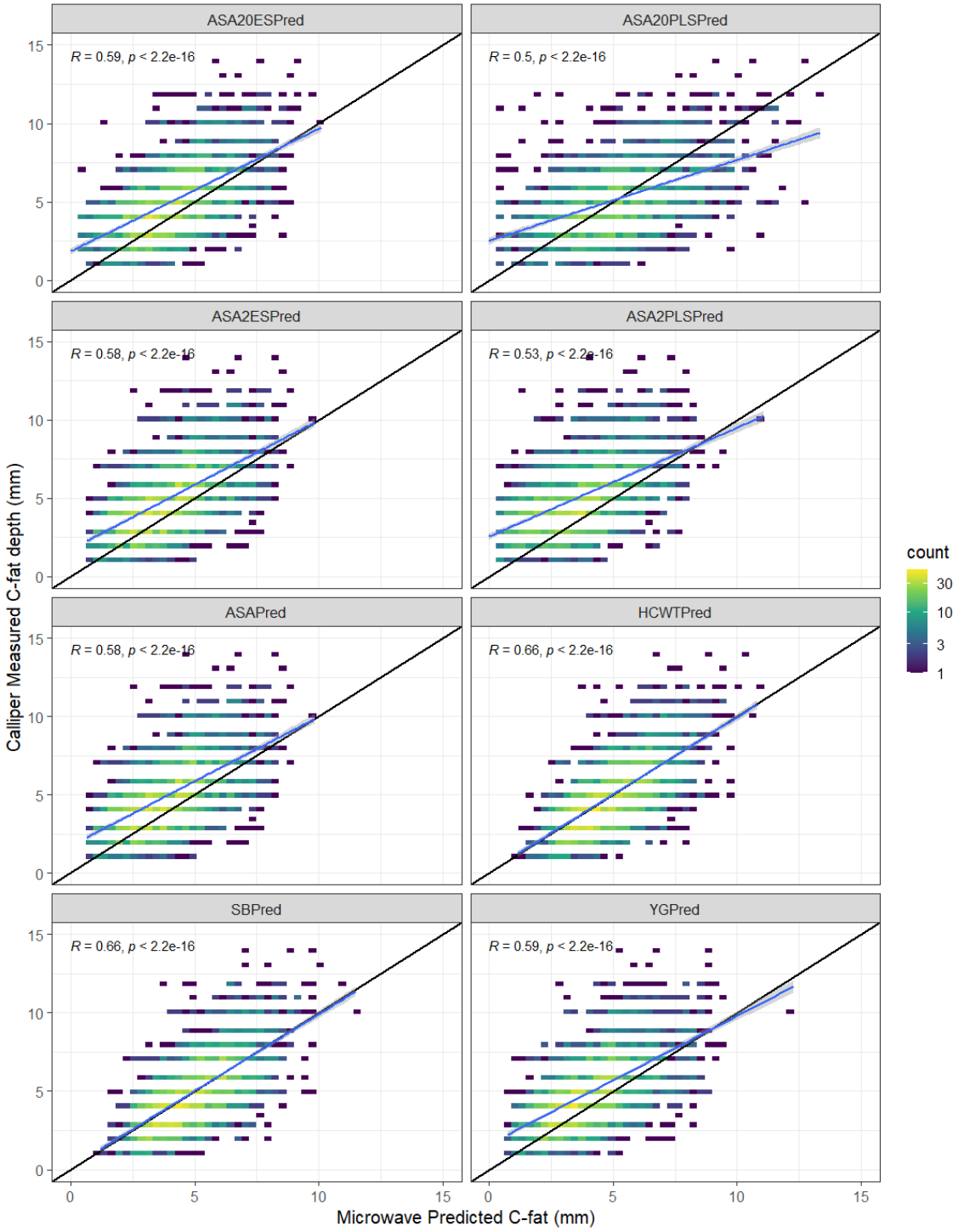

Figure 1: Observed relationships between Carcase C-site Fat depth (Y-axis) and the Microwave predicted Carcase C-site Fat depth measurements (X-axis).

Genetic Parameters

Parameter estimates from the uni-variate animal model analysis of the fat depth traits are provided in Table 3. The heritability of the calliper measured fat depth in the larger MLA-GRF dataset was 0.30 ± 0.01. This estimate compares well but are slightly higher than earlier estimates on the larger dataset (Mortimer et al., 2010, Mortimer et al., 2018) and in other populations (Fogarty et al., 2003, Safari et al., 2005). Whist the mean and variation differed between the sub-population and the larger MLA-GRF dataset the additive variance and heritability estimates were reasonably consistent.

The phenotypic variation for the microwave traits was significantly lower than observed for the calliper-measured trait, on the same animals, ranging from 1.41 to 2.88 compared to the expected 3.24 ± 0.03. This reduced variation was observed in both the residual and additive variance although proportionally more in the residual. This has resulted in higher heritability estimates. The heritability of the microwave traits ranged from 0.41 to 0.55 (se. range from 0.05 to 0.07). The high heritability for the microwave prediction should be approached with caution. It is possible that the predictions are capturing an underlying structure of the data which has inflated the predictions and underestimate the non-genetic variation existing in the data. It is hypothesised that as the data set grows and the heritability will reduce to be in line with the calliper-measured trait.

Table 3: Variance and heritability estimates for carcase C-site fat depth and the microwave predicted carcase C-site fat depth using an animal model

TRAIT	PHENOTYPIC VARIANCE		RESIDUAL VARIANCE		ADDITIVE VARIANCE		HERITABILITY	
MICROWAVE DATASET								
MIS - HCWTPRED	1.41	(0.05)	0.69	(0.09)	0.73	(0.11)	0.51	(0.07)
MIS - YGPRED	1.52	(0.05)	0.75	(0.10)	0.78	(0.12)	0.51	(0.07)
MIS - SBPRED	1.45	(0.05)	0.71	(0.09)	0.74	(0.11)	0.51	(0.06)
MIS - ASAPRED	1.45	(0.05)	0.70	(0.09)	0.75	(0.11)	0.52	(0.07)
MIS - ASAPRED 2PLS	1.73	(0.06)	0.83	(0.11)	0.90	(0.13)	0.52	(0.07)
MIS - ASAPRED 20PLS	2.88	(0.10)	1.71	(0.18)	1.17	(0.21)	0.41	(0.05)
MIS - ASAPRED 2ES	1.45	(0.05)	0.70	(0.09)	0.75	(0.11)	0.52	(0.07)
MIS - ASAPRED 20ES	1.45	(0.05)	0.66	(0.10)	0.79	(0.12)	0.55	(0.07)
CARCASE C-SITE FAT	3.24	(0.03)	2.24	(0.05)	1.00	(0.06)	0.31	(0.01)
LARGER DATASET								
CARCASE C-SITE FAT	3.58	(0.04)	2.50	(0.06)	1.08	(0.07)	0.30	(0.01)

Sire Breeding Value Correlations

The large difference in variance estimates and heritability would indicate that the microwave predicted traits may not be the same trait as the calliper measurement. Due to the relatively small number of records available the strength of the genetic relationship was first explored by comparing the sire's breeding values from each of the respective uni-variate analyses (Figure 2).

The Pearson correlation between the breeding values for the microwave prediction and the calliper measured fat depth traits ranged from 0.53 – 0.71 (Table 4). Reducing the numbers of sires to only compare the breeding values for the more accurate sires (more progeny) improved the strength of the correlations (Table 4). However, the number of high accuracy sires within the sampled population is low, only 55 sires (19%) have 12 or more progeny. The correlations between the microwave

predictions and the calliper fat depth are relatively consistent across the prediction models indicating that genetically the prediction models are ranking the sires equally.

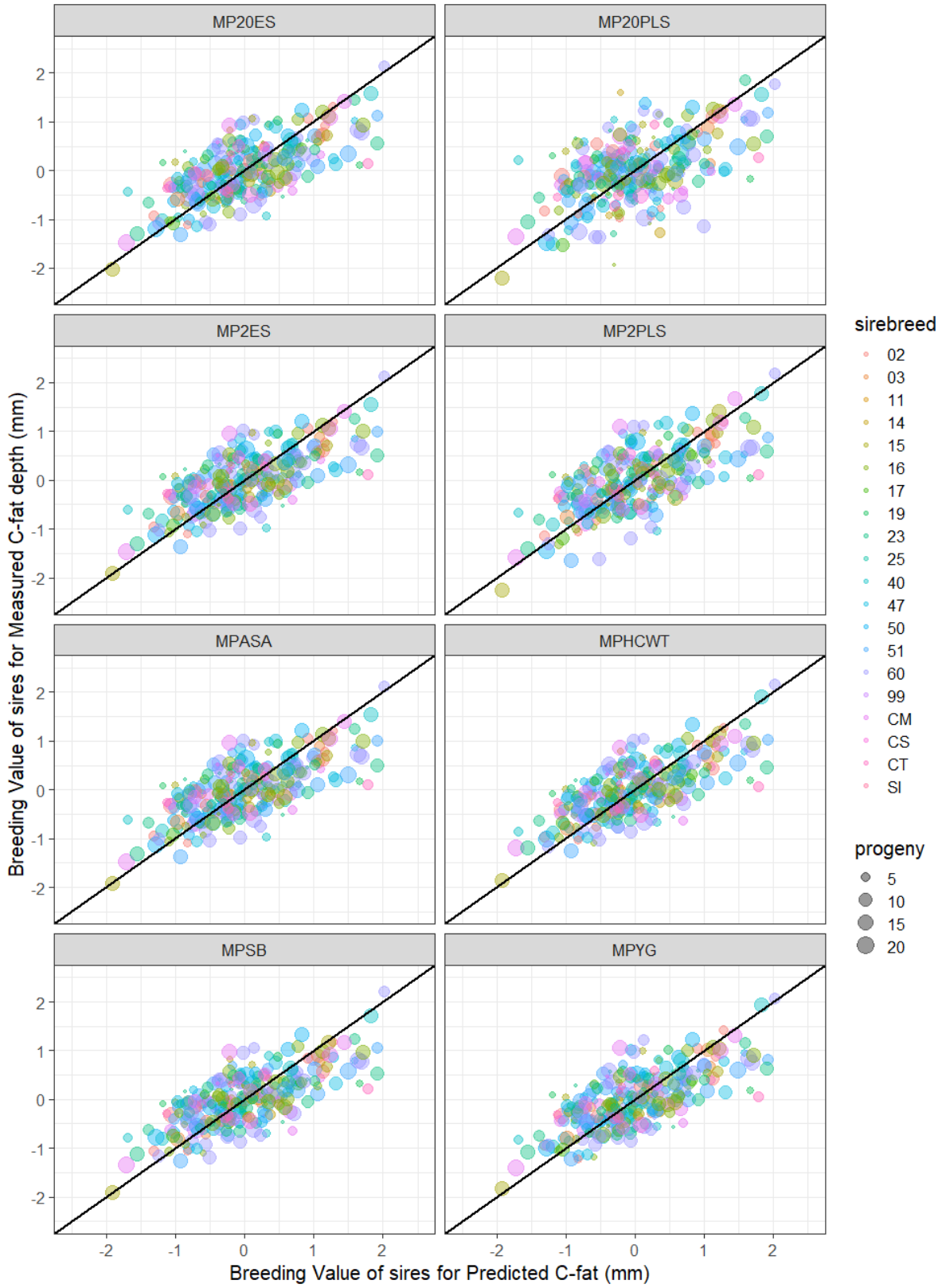

Figure 2: Relationship between sire breeding values, coloured by sirebreed and scaled by number of progeny, from the univariate models for Carcase C-site Fat depth (Y-axis) and the microwave predicted carcase C-site Fat depth traits (X-axis)

Table 4: Correlations between sire breeding values from the univariate animal models for Carcase C-site Fat depth and the Microwave predicted Carcase C-site Fat depth restricted by the number of progeny.

	ALL	>= 3 PROGENY	>= 5 PROGENY	>= 10 PROGENY	>= 12 PROGENY
SIRES	286	237	200	92	55
MIS - HCWTPRED	0.71	0.74	0.75	0.78	0.83
MIS - YGPRED	0.70	0.73	0.75	0.78	0.82
MIS - SBPRED	0.71	0.73	0.75	0.78	0.83
MIS - ASAPRED	0.69	0.71	0.73	0.77	0.82
MIS - ASAPRED 2PLS	0.67	0.69	0.71	0.76	0.81
MIS - ASAPRED 20PLS	0.53	0.58	0.62	0.70	0.79
MIS - ASAPRED 2ES	0.69	0.71	0.73	0.77	0.82
MIS - ASAPRED 20ES	0.67	0.70	0.73	0.78	0.83

Genetic Correlations

Phenotypic and genetic correlations between the c-site at depth traits are presented in Table 5. Whilst the small size of the dataset has limiting the ability to truly, predict the relationships between traits, the strength of correlations were promising. The phenotypic correlation between the microwave predictions ranged from 0.65 to 0.97 with the weakest correlations observed when the traits differed in the prediction model not the training population. The phenotypic correlations between the microwave and calliper measured trait ranged from 0.37 to 0.52.

The genetic correlations across all the fat depth traits ranged from 0.93 to 1.00 (se. range, 0.00 to 0.07) suggesting that they are all genetically the same trait. This aligns with the correlations observed between sire breeding values (Table 5) as the estimates of correlations and co-variances will be influenced proportionally more by the sire lines with the greatest number of progeny. It should be noted that previous research in this population has shown slightly higher genetic correlations between traits due to the sampling of diverse sire lines (Walkom and Brown, 2017). This in conjunction with the smaller than desired number of records means that the very strong correlations may not be a true reflection of the traits suitability.

Table 5: Phenotypic (above diagonal) and genetic correlations (below diagonal) between Carcase C-site Fat depth and the Microwave predicted Carcase C-site Fat depth using an animal model.

	MIS - HCWTPRED		MIS - YGPRED		MIS - SBPRED		MIS - ASAPRED		MIS - ASAPRED 2PLS		MIS - ASAPRED 20PLS		MIS - ASAPRED 2ES		MIS - ASAPRED 20ES		CARCASE C-SITE FAT	
MIS - HCWTPRED			0.96	(0.00)	0.98	(0.00)	0.95	(0.00)	0.93	(0.00)	0.67	(0.01)	0.95	(0.00)	0.93	(0.00)	0.51	(0.02)
MIS - YGPRED	0.99	(0.00)			0.96	(0.00)	0.96	(0.00)	0.94	(0.00)	0.67	(0.01)	0.96	(0.00)	0.94	(0.00)	0.50	(0.02)
MIS - SBPRED	0.99	(0.00)	0.99	(0.00)			0.94	(0.00)	0.93	(0.00)	0.65	(0.01)	0.94	(0.00)	0.93	(0.00)	0.52	(0.02)
MIS - ASAPRED	0.99	(0.00)	0.99	(0.00)	0.99	(0.00)			0.98	(0.00)	0.68	(0.01)		na.	0.97	(0.00)	0.51	(0.02)
MIS - ASAPRED 2PLS	0.98	(0.01)	0.97	(0.01)	0.98	(0.01)	0.99	(0.00)			0.67	(0.01)	0.98	(0.00)	0.95	(0.00)	0.50	(0.02)
MIS - ASAPRED 20PLS	0.95	(0.03)	0.95	(0.03)	0.93	(0.04)	0.94	(0.03)	0.93	(0.03)			0.68	(0.01)	0.83	(0.01)	0.37	(0.02)
MIS - ASAPRED 2ES	0.99	(0.00)	0.99	(0.00)	0.99	(0.00)		na.	0.99	(0.00)	0.94	(0.03)			0.97	(0.00)	0.51	(0.02)
MIS - ASAPRED 20ES	1.00	(0.01)	1.00	(0.00)	0.99	(0.01)	1.00	(0.00)	0.99	(0.00)	0.96	(0.02)	1.00	(0.00)			0.50	(0.02)
CARCASE C-SITE FAT	0.99	(0.06)	1.00	(0.07)	1.00	(0.04)	1.00	(0.04)	0.97	(0.05)	0.95	(0.07)	1.00	(0.04)	1.00	(0.05)		

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4 CONSIDERATIONS AND RECOMMENDATIONS

The preliminary analysis of microwave predicted c-site fat depth on lamb carcases indicates that the technology shows promise as an alternative measure to the invasive calliper measure. However, the results from the genetic evaluation show that the variation expressed within the microwave predicted traits is significantly less than observed in the calliper measured trait. High genetic correlations between the microwave and calliper traits show promise but should be treated with caution due to the unique data structure of the MLA-GRF and the low number of records available for the microwave traits. It is recommended that the technology is explored further and continues to be recorded on MLA-GRF and industry animals, with the analysis reported here, re-addressed when noticeably more records become available.

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