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**Final Report
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The University of Melbourne
Prof Frank Dunshea
Royal Parade
Parkville, VIC 3010

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Executive Summary

At slaughter, muscle glycogen (which is present in muscle as an energy store) breaks down to glucose (sugar) and is converted to lactic acid. This causes an increase in the acidity of the muscle, reducing its pH. Muscle pH influences the water-holding capacity of meat, which affects yield, colour, drip loss and pork quality. For the production of high eating quality meat, it is important to manage the relationship between muscle pH and muscle temperature decline. For pork, the ideal pH range of the loin muscle at 24 hours post-slaughter is between 5.50 - 5.70.

Understanding the post-farm gate factors that influence pork pH and eating quality may provide levers that can be used to improve pork eating quality and reduce the failure rate. While it is difficult for a processor to influence production factors, if there was an understanding of the post-farmgate factors that affect pH then it would be possible for a supply chain to use these levers to ensure a high and consistent pork eating quality. Therefore, APL in partnership with the pork industry and academia conducted a national pH audit with the following objectives:

- 1) Conduct a national pH Audit as the basis of an industry-wide meat quality improvement program in pork,
- 2) Conduct data analysis and reporting of results independently (and confidentially), and
- 3) If required, provide assistance with interpreting the results for a specific plant.

Data were collected from 8 different supply chains. pH and carcass temperature were collected by trained supply chain abattoir or research staff as near as possible to 24 h post-slaughter or to ship out. Two questionnaires were used to collect information relating to transport, lairage and chiller management for lots. Individual carcass data included loin pH, loin temperature at time of pH measurement, time after slaughter of pH measurement, carcass weight and P2. Data were extracted and collated prior to analyses using the General Linear Model (GLM) in Genstat 21.

A total of 16732 pH measures across 4 years (2019, 2020, 2021 and 2022) from supply chains were analysed to determine the main off-farm factors influencing loin muscle pH at 24 hours post-slaughter or whenever carcasses were transported from the abattoir. The mean and median pH were approximately 5.70 with lower and upper quartile cut-offs of 5.59 and 5.78. Therefore, 50% of values were within a range of 0.20 pH units around 5.69 with a relatively normal distribution. These data indicate that low pH isn't a major problem for the Australian pork industry since only 17% of carcasses have a pH of 5.55 or below. Only 8% of samples were at a pH 5.50 or below.

The final statistical model accounted for 17.6 % of the variation in carcass pH. The major off-farm factor contributing to variation in carcass pH was supply chain (11.1%) followed by month, year, chiller type, time after slaughter of pH measurement, carcass temperature at pH measurement, time in lairage and carcass weight (Table 2). Supply chain captures a variety of on- and off-farm factors, including genetics, environment, nutrition and abattoir factors and so it isn't surprising that it contributes the most to variation in carcass pH, albeit it still only accounts for ca. 11%.

The introduction of quick chilling into one of the supply chains increased pH by 0.18 pH units and appears to be the most effective, albeit very expensive, means of increasing carcass pH. Carcass pH varied across the year such that pH obtained in late summer through early winter are significantly lower than in other months.

In conclusion, it appears that low pH is not an issue for most of the Australian pork industry which is pleasing given that there do not appear to be many post-farm gate factors to manipulate pH in supply chains where best practice animal welfare is in place and attention is paid to chiller management. However, it is recommended that individual processors periodically monitor pH on a subset of carcasses, particularly during the period from February to July.

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1. Background to Research

At slaughter, muscle glycogen (which is present in muscle as an energy store) breaks down to glucose (sugar) and is converted to lactic acid. This causes an increase in the acidity of the muscle, reducing its pH. Muscle pH influences the water holding capacity of meat, which affects yield, colour, drip loss and pork quality. For the production of high eating quality meat, it is important to manage the relationship between muscle pH and muscle temperature decline. For pork, the ideal pH range of the loin muscle at 24 hours post-slaughter is between 5.50 - 5.70. For example, Jose *et al.* (2013) found that over the range of ultimate pH (pHu) of 5.35 to 5.65 there was a linear increase in eating quality and a decrease in drip loss. Using a meta-analysis approach, Channon *et al.* (2018) found that pork with a pHu within the range of 5.5 to 5.70 had a pork eating quality score (PQS) of 4.3 units above that below 5.50, although this was largely influenced by the study of Jose *et al.* (2013).

However, it is not just pHu that influences pork eating quality since the pattern of pH decline during chilling is also an important indicator of meat eating quality meat. The measurement of pH and temperature in the loin muscle from 12 to 24 hours post-slaughter can provide useful information to processors to determine whether issues with pork quality will be experienced – particularly colour, drip loss and tenderness. If pH issues are detected, corrective action and controls can be implemented to maximise a supply chain's ability to consistently achieve high-quality pork. Understanding the post-farm gate factors that influence pork pH and PQS may provide levers that can be used to improve pork eating quality and reduce fail rate. Therefore, APL in partnership with the industry conducted a national pH Audit with the following objectives.

2. Objectives of the Research Project

- 1) Conduct a national pH Audit as the basis of an industry-wide meat quality improvement program in pork.
- 2) Conduct Data analysis and reporting of results independently (and confidentially) through the University of Melbourne.
- 3) If required, assistance with interpreting the results for a specific plant through The University of Melbourne.

3. Introductory Technical Information

Production factors, including genotype, sex, production system, metabolic modifiers, stress, nutrition and season and processing factors, including stunning method, carcass chilling regime, moisture infusion, hanging method and aging, all influence pHu and eating quality (Channon *et al.* 2017a,b;2018). Many of these factors have the ability to manipulate pH, which in turn can have a positive or negative effect on meat quality. Pork with a high pHu of 5.8-6.0 results in eating quality score than pork with a low pHu (Bryhni *et al.*, 2003; Jose *et al.* 2013; Channon *et al.* 2018) while pork with a lower ultimate pH seems to be correlated with a negative sour/acidic flavour (Aaslyng *et al.*, 2007; Myers *et al.*, 2009) as well as decreased juiciness and tenderness (Lonergan *et al.*, 2007). While it is difficult for a processor to influence production factors, if there was an understanding of the post-farmgate factors that affect pHu then it would be possible for a supply chain to use these levers to ensure a high and consistent pork eating quality.

4. Research Methodology

4.1 Data collection

Data were collected from 8 different supply chains. pH and carcass temperature were collected by trained supply chain abattoir or research staff as near as possible to 24 h post-slaughter or to ship out. Two questionnaires were used to collect information relating to transport, lairage and chiller management for lots (see Supplemental data). Individual carcass data included loin pH, loin temperature at time of pH measurement, time after slaughter of pH measurement, carcass weight and P2. Data were provided to Prof Frank Dunshea at the University of Melbourne via email either directly or via APL.

4.2 Data analysis

Data were extracted from individual excel spreadsheets and collated for individual supply chains and then aggregated across supply chains. Supply chains were coded to remain anonymous. The data were subject to analyses using the General Linear Model (GLM) in Genstat 21 and the maximal model included supply chain, minutes after slaughter, the temperature at pH measurement, carcass weight, carcass P2, sex, chiller type, season, month, year, time in transport, time in lairage, total time in transport and lairage, ambient temperature and use of showers. Factors were step-wise tested to determine their influence on carcass pH.

A machine learning model was developed by testing different artificial neural network (ANN) training algorithms using an automated customized code written in Matlab® R2018b. An ANN fitting model with hidden neurons was developed using the normalized pH values as inputs and normalized values (-1-1) of the various data as targets. Only data from the first 2 years of data collection were used for this model fitting.

4.3 Dissemination of findings

Progress findings were disseminated to all supply chains on an individual and anonymous aggregated basis. Where possible tailored responses were provided to each Supply Chain. Periodic presentations have been made to the Pork Processor Referral Group (PPRG). A presentation has also been made to the European Association of Animal Production in Porto in September 2022.

5. Results

A total of 16732 pH measures across 4 years (2019, 2020, 2021 and 2022) from supply chains were analysed to determine the main off-farm factors influencing loin muscle pH at 24 hours post slaughter or whenever carcasses were transported from the abattoir. The statistics for the global data are reported in Table 1.

Table 1. Summary statistics for global pH pooled across supply chains, seasons and years (n=16732).

Mean =	5.69
Median =	5.68
Minimum =	4.46
Maximum =	7.13
Lower quartile =	5.59
Upper quartile =	5.78
Skewness =	0.70
Kurtosis =	4.88

The mean and median pH were approximately 5.70 (5.69 and 5.68, respectively) with lower and upper quartile cut-offs of 5.59 and 5.78. Therefore, 50% of values were within a range of 0.20 pH units around 5.69 with a relatively normal distribution as indicated by the skewness of 0.70 and kurtosis of 4.88 (moderate skew to the right and moderate tails). The distribution of global pH values is provided in Figure 1. These data indicate that low pH isn't a major problem for the Australian pork industry since only 17% of carcasses have a pH of 5.55 or below. Only 8% of samples were at a pH 5.50 or below. Nevertheless, it would be advantageous if the bottom tail of pH measurements could be increased by 0.05 units as this would reduce drip loss and increase PQS across the industry. High pH doesn't appear to be a major issue although it may be for the individual supply chain.

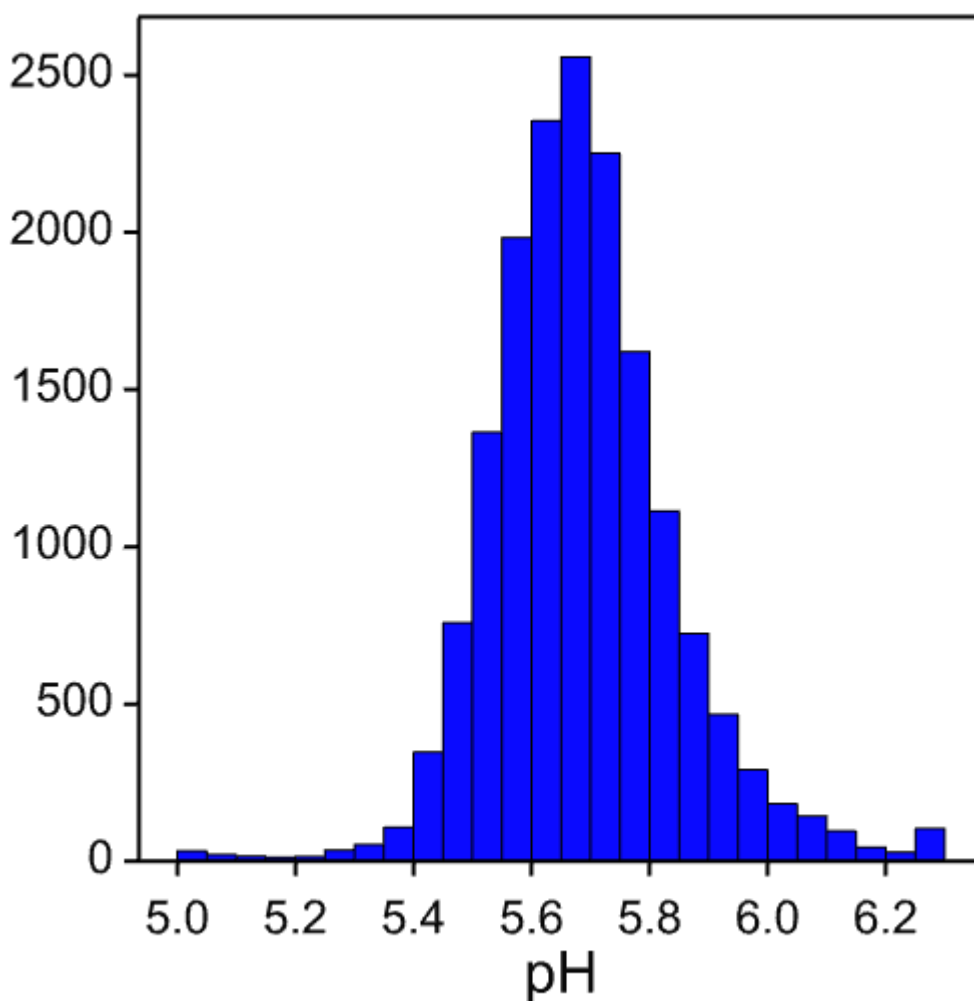


Figure 1. Distribution of carcass pH pooled across supply chains, seasons and years

The data were subject to analyses using GLM and the maximal model included supply chain, minutes after slaughter, temperature at pH measurement, carcass weight, carcass P2, sex, chiller type, season, month, year, time in transport, time in lairage, total time in transport and lairage, ambient temperature

and use of showers. Factors were step-wise tested to determine their influence on carcass pH. The final contribution from the various factors accounted for are reported in Table 2.

Table 2. Cumulative contribution of various off-farm factors to carcass pH.

Variable	% variation accounted for
Supply chain	11.1
+ Month	13.8
+ Year	16.0
+ Chiller type	16.8
+ Minutes after slaughter of pH measurement	17.1
+ Carcass temperature at pH measurement	17.3
+ Time in lairage	17.4
+ Carcass weight	17.6

Table 3. Parameter estimates for the best model to predict carcass pH as indicated in Table 2¹.

Parameter	estimate	s.e.	t(16310)	t pr.
Constant	5.5106	0.0204	269.60	<.001
Supply B	0.1032	0.0130	7.94	<.001
Supply C	0.1403	0.0137	10.25	<.001
Supply D	0.0735	0.0127	5.77	<.001
Supply E	0.1179	0.0140	8.42	<.001
Supply F	0.0709	0.0153	4.62	<.001
Supply G	0.3613	0.0147	24.65	<.001
Supply H	0.3179	0.0189	16.79	<.001
Month FEB	-0.05893	0.00660	-8.93	<.001
Month MAR	-0.07981	0.00626	-12.75	<.001
Month APR	-0.03702	0.00827	-4.48	<.001
Month MAY	-0.09302	0.00601	-15.47	<.001
Month JUN	-0.03951	0.00628	-6.29	<.001
Month JUL	-0.06857	0.00564	-12.15	<.001
Month AUG	0.00297	0.00720	0.41	0.680
Month SEP	0.0637	0.0129	4.95	<.001
Month OCT	-0.0792	0.0129	-6.16	<.001
Month NOV	0.09819	0.00885	11.10	<.001
Month DEC	0.07867	0.00806	9.77	<.001
Year 2020	0.13065	0.00870	15.02	<.001
Year 2021	0.10458	0.00862	12.14	<.001
Year 2022	0.0354	0.0125	2.84	0.005
Chiller Quick	0.1845	0.0127	14.52	<.001
Time (minutes)	-0.00004824	0.00000582	-8.29	<.001
Temperature	-0.01146	0.00137	-8.36	<.001
Lairage (minutes)	0.00000744	0.00000303	2.46	0.014
Carcass_Weight	0.000731	0.000131	5.59	<.001

¹Parameters for factors are differences compared with the reference level:

Factor	Reference level
Supply	A
Month	JAN
Year	2019
Chiller	Conventional

The final model accounted for 17.6 % of the variation in carcass pH. The major off-farm factor contributing to variation in carcass pH was supply chain (11.1%) followed by month, year, chiller type, time after slaughter of pH measurement, carcass temperature at pH measurement, time in lairage and carcass weight (Table 2). Supply chain captures a variety of on- and off-farm factors including genetics,

environment, nutrition and abattoir factors and so it isn't surprising that it contributes the most to variation in carcass pH, albeit it still only accounts for ca. 11%.

Supply chains B-G had significantly different pH to Supply chain A while supply chain H was not different from Supply chain A (Table 3). The introduction of quick chilling into one of the supply chains increased pH by 0.18 pH units and appears to be the most effective, albeit very expensive, means of increasing carcass pH. Carcass pH varied across the year although it should be borne in mind that samples haven't been obtained from all supply chains in every month of the year and this may impact on estimates. Nevertheless, it appears that pH obtained in late summer, autumn and into early winter are significantly lower than in other months (Figure 2). October appeared to be somewhat anomalous although there were only 150 measures obtained from only one supply chain in one year (2021). Despite the monthly effects, there were no significant effects of including either ambient temperature at loading or slaughter. However, this was possibly because not all supply chains were able to include these variables in their data collection.

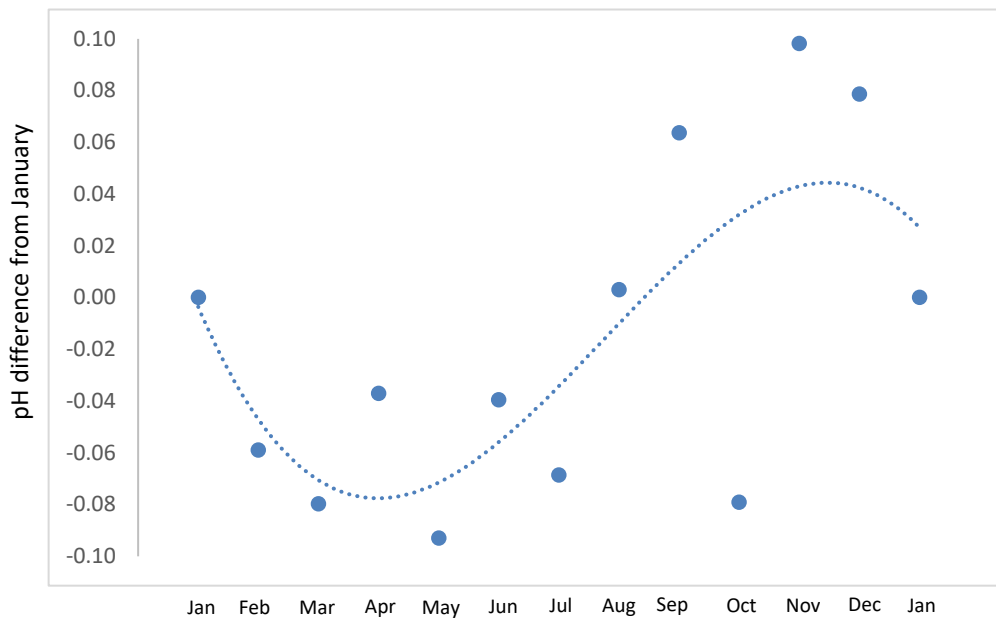


Figure 2. Monthly variation in loin pH pooled across supply chains, seasons and years

Carcass pH declined significantly with increasing time after slaughter by about 0.00005 units per minute beyond approximately 12 hours (Figure 3). Carcass pH declined by about 0.011 units for every degree increase at the time of determination of carcass pH (Table 3). Carcass pH was substantially higher in 2020 and 2021 and slightly higher in 2022 suggesting some improvement in pH over the study although the difference for 2022 is relatively small. There was a very small effect of lairage time on pH of (0.000007 units/min) and no significant effect of transport time (data not shown). Loin pH increased 0.0007 units/kg carcass weight but was not significantly influenced ($p=0.10$) by P2. Including sex into the model didn't improve the model fit although there were very differences between the different sexes (5.753, 5.758 and 5.747 for females, Improvac males and entire males, respectively, $p=0.017$).

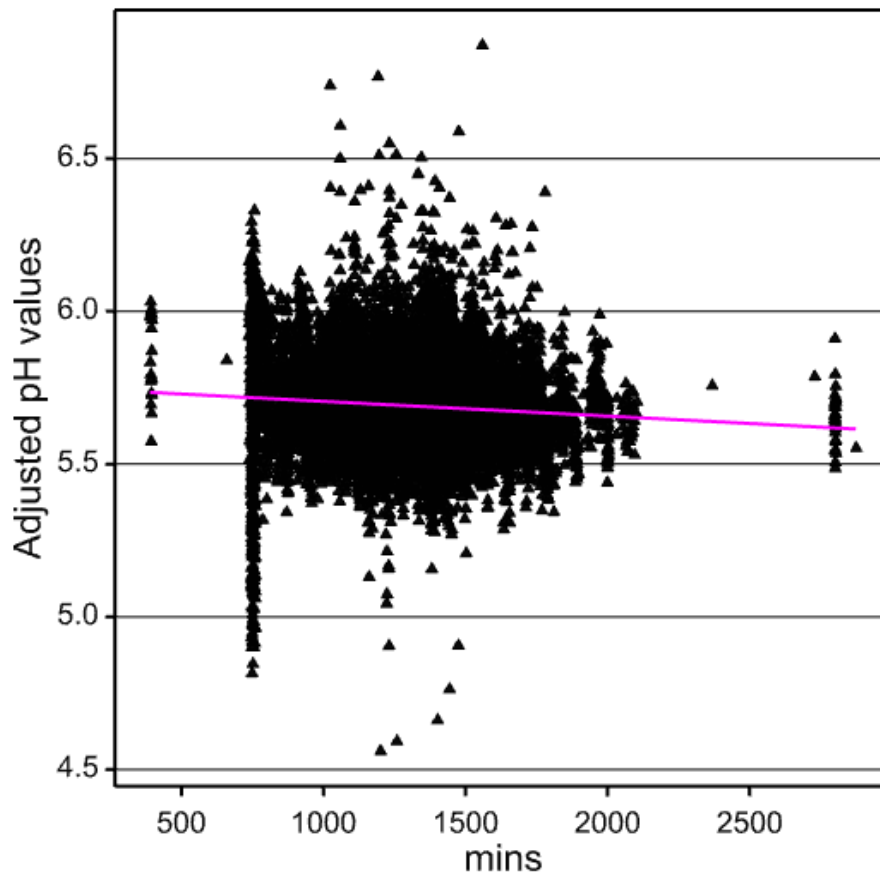


Figure 3. Effect of time post slaughter on loin pH pooled across supply chains, seasons and years

Given the relatively low estimates of variance accounted for by off farm factors to predict carcass pH, it was decided to attempt to use some machine-learning and artificial intelligence techniques to attempt to model carcass pH. Parameters chosen were some of those that could possibly be manipulated such as transport and lairage time as well as ambient temperature. However, these inputs weren't very well related to carcass pH and so there was a very poor fit between model estimates (Figure 4). This is unfortunate as we have used this approach to model other biological systems (Fuentes *et al.* 2020). However, it is consistent with our other statistical analyses indicating the little effect of these factors.

6. Discussion

The major pleasing finding from the present audit was that the Australian pork industry doesn't have a large issue with low pH since only 17 and 8% of pork samples were below 5.55 and 5.50, respectively. Indeed, 50% of the pH values were within a range of 0.20 pH units around 5.69 with a relatively normal distribution suggesting relatively consistent eating quality, at least as influenced by pH. Channon *et al.* (2018) suggested a pH threshold of 5.50 for pork eating quality with pork with a pH of 5.50 to 5.70 having a PQS of 4.3 points greater than that below 5.50. Therefore, on this basis only 8% of Australian pork samples would fall below this threshold. Jose *et al.* (2013) also found that drip loss increased

linearly with increasing pH upto a pH of 5.65. In the present audit, 50% of the samples were above 5.69 indicating that drip loss is likely not to be an issue for most of the Australian pork industry.

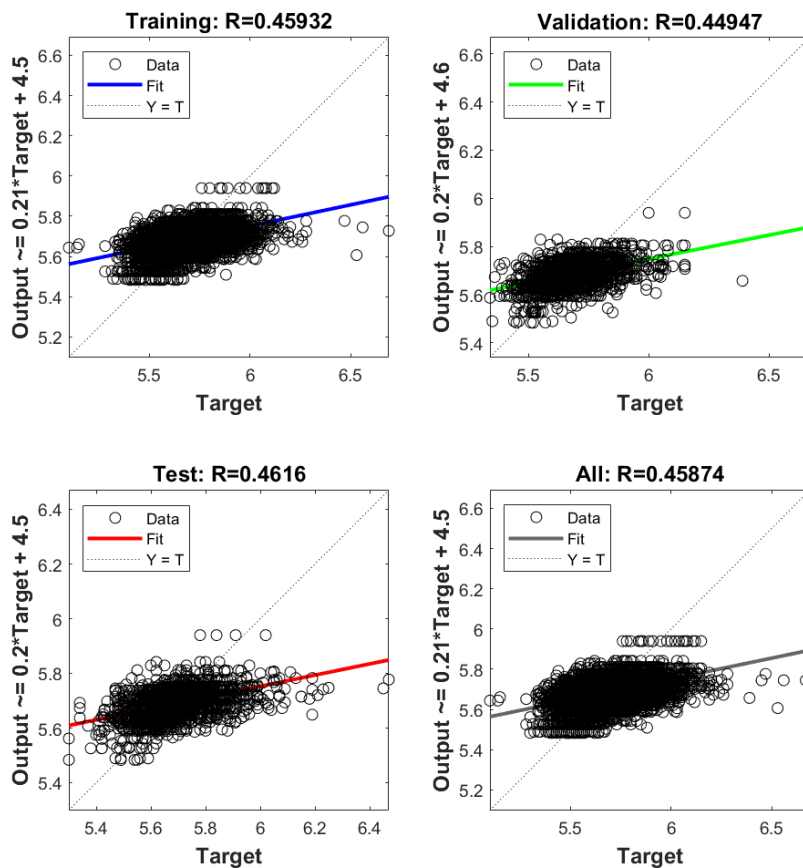


Figure 4. Regression graph of Training, Validation, Test and All data of model used to estimate carcass pH.

Approximately, 88% of the data were from 4 supply chains (A,B,C,D) and all of these supply chains had pH values that were normally distributed. Although there were quite clear differences in pH between some of the supply chains, there were very few other off-farm factors that influenced pH. The next most important parameter after the supply chain was the month with pH being lower during late summer to early winter. Interestingly, there was no effect of ambient temperature at loading or prior to slaughter on pH which indicates that it isn't temperature *per se* that contributes to low pH during these months. It is possible that the low pH may be due to carry-over effects from summer but this is conjecture. However, carryover effects on carcass composition are not unknown (Liu et al. 2020). Although there was only a very small effect of lairage and transport time on pH overall, in some individual supply chains the combination of short lairage time and short transport time resulted in slightly lower pH.

Loin pH were higher in 2020 and 2021 compared to 2019 which is possibly because supply chains were paying greater consideration to pH, maybe through attention to chiller management. Rapid chilling was one factor that did increase muscle pH and presumably, slow chilling could have the opposite effect. While the decrease in muscle pH in 2022 compared to 2020 and 2021 may be due to the seasonal nature of the sampling from a single supply chain, supply chains are encouraged to be vigilant to maintain chilling rates and minimise freezer overcrowding.

Despite the lack of effect of post-farmgate factors on pork muscle pH the opportunity arising is that it appears that there may be on-farm factors such as genetics, nutrition, production system (conventional versus deep litter) and time off feed that may be impacting carcass pH. Some of these may have been captured in the Supply Chain effects but others may not have been captured here. An opportunity exists to further explore these factors. Also, filling the seasonal gaps in carcass pH data may provide more insight into the off-farm factors impacting carcass pH.

7. Implications & Recommendations

It appears that low pH is not an issue for most of the Australian pork industry which is pleasing given that there do not appear to be many post-farm gate factors to manipulate pH in supply chains where best practice animal welfare is in place and attention is paid to chiller management. However, it is recommended that individual processors periodically monitor pH on a subset of carcasses, particularly during the period from February to July.

Further work may focus on some of the on-farm factors that may influence pork pH.

8. Intellectual Property

No intellectual property arising.

9. Technical Summary

One technical aspect that was evaluated by Megan Trezona in a related project on some of these samples was the use of continuous pH monitors. These pH meters showed very good agreement with spot measurement and may be useful in routine monitoring.

10. Literature cited

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II. Publications Arising

Dunshea, F.R., Lealiifano, A., Trezona, M., Gole, V., and Hewitt, R.J.E. (2022) National audit of pork pH in Australia. In "Proceedings of 73rd meeting of the European Federation of Animal Science", Porto, Portugal, p 390.

12. Supplemental Tables.

Supplemental Table 1. Abattoir questionnaire 1.

Abattoir Name:

1. Ramp Facilities

- (a) Type
- (b) Width (m)
- (c) Flooring
- (d) Sides
- (e) Slope

2. Yard design (including access to drinkers)

3. Race

- (a) Design
- (b) Automation of pig movement

4. Stunning system

- (a) Type
- (b) Average number of pigs per gondola
- (c) CO₂ concentration
- (d) Stunning time
- (e) Stun to stick interval for first pig in each stunning group
- (f) Stun to stick interval for last pigs in each stunning group

5. Bleeding time

6. Scald

- (a) Type
- (b) Water temperature
- (c) Scalding time

7. Evisceration

- (a) Time from stun to first stroke of knife
 - (b) Carcasses eviscerated during breaks/stoppages
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Supplemental Table 2. Abattoir questionnaire 2

Vendor/ Tattoo	Transport date	Number of pigs in consignment	Transport distance	Time of arrival at abattoir	Transport time	Resting time in lairage	Time of slaughter	Ambient temperature
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Lairage Stocking rate	Lairage Techniques used by stockperson to move pigs	Showering facilities used: Yes/No	Time stunning to chilling	Chiller air speed	Carcass temperature rate of fall	No. of carcasses in chiller
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