Improved tenderness of alpaca carcasses using combined processing techniques

by Tamara Biffin, Dr Melanie Smith, Dr Russell Bush and Dr David Hopkins
September 2019
Improve tenderness of alpaca carcases using combined processing techniques
Publication No. 19-018
Project No. PRJ-010045

The information contained in this publication is intended for general use to assist public knowledge and discussion and to help improve the development of sustainable regions. You must not rely on any information contained in this publication without taking specialist advice relevant to your particular circumstances.

While reasonable care has been taken in preparing this publication to ensure that information is true and correct, the Commonwealth of Australia gives no assurance as to the accuracy of any information in this publication.

The Commonwealth of Australia, AgriFutures Australia, the authors or contributors expressly disclaim, to the maximum extent permitted by law, all responsibility and liability to any person, arising directly or indirectly from any act or omission, or for any consequences of any such act or omission, made in reliance on the contents of this publication, whether or not caused by any negligence on the part of the Commonwealth of Australia, AgriFutures Australia, the authors or contributors.

The Commonwealth of Australia does not necessarily endorse the views in this publication.

This publication is copyright. Apart from any use as permitted under the Copyright Act 1968, all other rights are reserved. However, wide dissemination is encouraged. Requests and inquiries concerning reproduction and rights should be addressed to AgriFutures Australia Communications Team on 02 6923 6900.

Researcher Contact Details

Tamara Biffin
Sydney School of Veterinary Science
University of Sydney
425 Werombi Road
Camden NSW 2567
02 9351 1166
tamara.biffin@sydney.edu.au

Dr Melanie Smith
Sydney School of Veterinary Science
University of Sydney
425 Werombi Road
Camden NSW 2567
02 9351 1625
melanie.smith@sydney.edu.au

Dr Russell Bush
Sydney School of Veterinary Science
University of Sydney
425 Werombi Road
Camden NSW 2567
02 9351 1785
russell.bush@sydney.edu.au

Dr David Hopkins
Centre for Red Meat and Sheep Development, NSW DPI
PO Box 129, Cowra NSW 2794
02 6349 9722
david.hopkins@dpi.nsw.gov.au

In submitting this report, the researcher has agreed to AgriFutures Australia publishing this material in its edited form.

AgriFutures Australia Contact Details

Building 007, Tooma Way
Charles Sturt University
Locked Bag 588
Wagga Wagga NSW 2650
02 6923 6900
info@agrifutures.com.au
www.agrifutures.com.au


AgriFutures Australia is the new trading name for Rural Industries Research & Development Corporation (RIRDC), a statutory authority of the Federal Government established by the Primary Industries Research and Development Act 1989.
Foreword

Rapid growth within the Australian alpaca industry has increased interest in alpaca meat as a viable alternative to traditional fibre production. The benefits arising from sustainable growth in the demand for meat include increased financial returns and genetic gains on farm with the sale of otherwise unprofitable animals. Through investment into research based guidelines for optimal management throughout the supply chain, alpaca meat eating quality and consistency can be maximized. High quality and consistent product aids in establishing rapport with consumers, the implications of which are beneficial to the industry as a whole.

Presenting the findings of three studies conducted by researchers from the University of Sydney and NSW Department of Primary Industries, this report aims to address key knowledge gaps in the areas of seasonality, transport and lairage stress and combined processing treatment effects on alpaca meat quality. Through this research, both producers and processors are provided with research based recommendations that aid in maximising alpaca meat eating quality and efficiency throughout the supply chain.

The research successfully quantified alpaca behaviour during transportation, identifying points at which stressors can be minimised, and providing a basis for future behavioural studies. Alpaca meat quality was affected by season, particularly through winter and early spring when pasture availability was limited. It was determined that a resting period of 7 days post transportation did not aid in improving meat quality, indicating that a direct to slaughter approach is optimal after transport trips of up to 4hrs in duration. Through studies looking at both split and whole alpaca carcases, it was determined that combined medium voltage electrical stimulation and tenderstretching significantly improved quality traits across multiple alpaca muscles. As a result, it is proposed that the application of these two processing treatments in combination represent best practice alpaca carcase processing procedures.

The project was funded with industry revenue from Illawarra Prime Alpacas and matched by AgriFutures Australia core funds provided by the Australian Government. This report is an addition to AgriFutures Australia’s diverse range of over 2000 research publications and it forms part of our Emerging Industries arena, which aims to support new and emerging rural industries.

Most of AgriFutures Australia’s publications are available for viewing, free downloading or purchasing online at: www.agrifutures.com.au.

Mr Michael Beer
General Manager, Business Development
AgriFutures Australia
About the Authors

Ms Tamara Biffin is a PhD candidate at the University of Sydney, with the research contained within this report forming the basis of her thesis. Tamara has a cattle production background and keen interest in the enhancement of product quality throughout the supply chain, graduating from the University of Sydney in 2015 with a Bachelor of Animal and Veterinary Bioscience (Hons 1). Throughout the course of her PhD Tamara has gained expertise in the areas of alpaca management on farm, through transport and in processing factors affecting alpaca meat quality. She has presented her research at both national and international conferences, including the International Congress of Meat Science and Technology (ICoMST).

Dr Melanie Smith is a Postdoctoral Research Associate at the University of Sydney in the area of livestock production and meat science. Melanie enjoys working at the interface between research and the meat supply chain and is passionate about engaging and educating the next generation about the red meat industry. Melanie grew up on the South Coast of NSW and completed a Bachelor of Animal and Veterinary Bioscience at the University of Sydney in 2012. Melanie has always had a passion for the livestock industry and it was during her honours in sheep productivity that she developed a keen interest in research. Melanie completed her PhD in 2017 through the University of Sydney in collaboration with the New South Wales Department of Primary Industries. Her PhD was titled factors impacting on the attributes of alpaca meat and methods to improve quality and investigated on farm, processing and consumer factors impacting alpaca meat quality from the farm through to the fork. Over the past 7 years Melanie has presented her research at both international and domestic scientific and industry conferences to present her research on alpaca meat production. In addition she has published 6 scientific journal articles, 9 conference papers and co-authored 2 scientific journal articles and 6 conference papers on alpaca meat.

Dr Russell Bush is an Associate Professor in Livestock Production at the University of Sydney. He is a whole farm value chain specialist and agricultural systems educator, having his own beef cattle and sheep operation. He has over 18 years’ experience conducting international and domestic livestock research in health, production and welfare in species such as cattle, buffalo, sheep, goats, alpacas and crocodiles. His research program includes specializing in issues impacting food security as well as the associated socioeconomic implications. This also involves supervision of honours, masters and PhD students.

Dr. David Hopkins is a meat scientist with NSW DPI and has published more than 500 scientific papers on meat and carcass studies including 20 review papers. David sits on the editorial board of two journals, is the Chief Editor, Journal of Meat Science. He has been an Adjunct Professor (University of New England, Armidale, Australia) and is currently an Adjunct Professor at Charles Sturt University, Wagga, Australia and Shandong Agricultural University, Taian, China and a Distinguished Professor and International Supervisor for IST, CAAS, China. He has authored numerous seminal papers and has written 18 book chapters. David has lead several projects in the Australian Sheep CRC and conducted numerous studies with Meat and Livestock Australia and Australian Meat Processors Corporation funding and works collaboratively with scientists internationally. He also supervises post-graduate students from Australia and overseas. He has been involved in research that has achieved commercial adoption such as VIASCAN®, new generation electrical stimulation, and SMARTSHAPE™.
Acknowledgments

The authors would like to acknowledge NSW DPI technical staff Mr. Matthew Kerr and Mrs. Kristy Bailes, along with research staff Dr. Benjamin Holman. The staff of Illawarra Prime Alpaca and the cooperating abattoir (Milton District Meats Pty Ltd) are also acknowledged for their assistance. Mr Chris Mudford (StimTech Pty Ltd, QLD, Australia) is acknowledged for supply of the immobilisation and stimulation equipment and Mr. Ben McWhirter for the design, creation and installation of camera brackets for the transportation research. In addition, Miss Courtney Nelson, Miss Bridgette Logan and Miss Sarah Legge, the University of Sydney, are acknowledged for their immeasurable assistance across studies.

The authors would like to thank Associate Professor Peter Thomson and Dr Evelyn Hall from the University of Sydney, and Dr Remy van de Ven, Dr. Damian Collins and Stephen Morris from NSW DPI for their assistance with the statistical analysis.

This project was funded by AgriFutures Australia with in-kind contributions from Illawarra Prime Alpaca.

Abbreviations

- ADF: Acid detergent fibre
- AF: Adductor femoris, hindquarter muscle
- AH: Achilles hung
- ALA: Alpha linolenic acid
- BCS: Body condition score
- CCW: Cold carcase weight
- CP: Crude protein
- DDM: Digestible dry matter
- DL: Drip loss
- DM: Dry matter
- ES: Medium Voltage Electrical stimulation
- FA: Fatty acid
- IMF: Intramuscular fat
- LL: Longissimus lumbarum (lumbar section of the loin, shortloin)
- LMM: Linear mixed model
- LT: Longissimus thoracis (thoracic or rack section of the loin)
- LTL: Longissimus thoracic et lumbarum, loin
- LW: Live weight
- ME: Metabolisable energy
- MUFA: Monounsaturated fatty acid
- NDF: Neutral detergent fibre
- NES: No Medium Voltage Electrical stimulation
- pHu: Ultimate pH
- PUFA: Polyunsaturated fatty acid
- SFA: Saturated fatty acid
- SF: Shear force
- SM: Semimembranosus, topside, hindquarter muscle
- ST: Semitendinosus, hindquarter muscle
- TL: Psoas major, tenderloin
- USFA: Unsaturated fatty acid
Publications generated from this work

Peer Reviewed Journal Articles


Conference Papers


Contents

Foreword........................................................................................................................................ iii
About the Authors .............................................................................................................................. iv
Acknowledgments ............................................................................................................................. v
Abbreviations ..................................................................................................................................... v
Publications generated from this work ............................................................................................... vi
  Peer Reviewed Journal Articles ...................................................................................................... vi
  Conference Papers ........................................................................................................................ vi
Executive Summary ........................................................................................................................... xii
Introduction ......................................................................................................................................... 1
Objectives ........................................................................................................................................... 2
Methodology ....................................................................................................................................... 3
  1. Investigation into transportation stress in Australian alpacas and methods to mediate stress related effects on meat quality ......................................................................................... 3
    1.1. Experimental design ............................................................................................................... 3
    1.2. On-farm ................................................................................................................................ 3
    1.3. Pasture analysis ..................................................................................................................... 3
    1.4. Transportation component .................................................................................................. 4
    1.5. Lairage component ................................................................................................................ 6
    1.6. Carcase Processing .............................................................................................................. 6
    1.7. Sample collection and measurements ................................................................................. 6
    1.8. Meat quality testing ............................................................................................................. 6
    1.9. Statistics .............................................................................................................................. 8
  2. The effect of combining tenderstretching and electrical stimulation on alpaca meat tenderness and eating quality ............................................................................................................. 8
    2.1. Experimental design ............................................................................................................... 8
    2.2. On-farm ................................................................................................................................ 9
    2.3. Pasture analysis ..................................................................................................................... 9
    2.4. Carcase Processing .............................................................................................................. 10
    2.5. pH decline ........................................................................................................................... 10
    2.6. Sample collection and measurements ................................................................................. 11
    2.7. Meat quality testing ............................................................................................................. 11
    2.8. Statistics .............................................................................................................................. 13
  3. Development of best practice methods for alpaca carcase processing and the assessment enzymatic infusion on alpaca loin tenderness ......................................................................... 13
    3.1. Experimental design ............................................................................................................... 13
    3.2. On-farm ................................................................................................................................ 14
    3.3. Pasture analysis ..................................................................................................................... 14
    3.4. Carcase Processing .............................................................................................................. 14
    3.5. Sample collection, infusion and measurements .................................................................... 14
    3.6. Meat quality testing ............................................................................................................. 15
    3.7. Statistics .............................................................................................................................. 16
Results ................................................................................................................................................ 17
1. Investigation into transportation stress in Australian alpacas and methods to mediate stress related effects on meat quality ................................................................. 17
   1.1. On-farm ........................................................................................................ 17
   1.2. Pasture analysis ............................................................................................ 17
   1.3. Transportation component ......................................................................... 20
   1.4. Blood cortisol analysis ............................................................................... 21
   1.5. Carcase traits .............................................................................................. 21
   1.6. Meat quality testing .................................................................................... 21

2. The effect of combining tenderstretching and electrical stimulation on alpaca meat tenderness and eating quality ............................................................... 24
   2.1. Animal live weight and body condition ...................................................... 24
   2.2. Pasture ....................................................................................................... 24
   2.3. Meat quality testing ................................................................................... 25

3. Development of best practice methods for alpaca carcase processing and the assessment enzymatic infusion on alpaca loin tenderness ........................ 33
   3.1. On-farm .................................................................................................... 33
   3.2. Pasture ...................................................................................................... 33
   3.3. Carcase data .............................................................................................. 34
   3.4. Meat quality testing .................................................................................. 34

Implications ......................................................................................................... 37

   1. Transportation behaviour ............................................................................. 37
   2. Seasonality of product quality ..................................................................... 37
   3. Processing treatments .................................................................................. 37
   4. Infusion treatment ....................................................................................... 38

Recommendations ............................................................................................... 39

References .......................................................................................................... 40
Tables

Table 1. Ethogram developed for behavioural observation of alpacas, broken down into postures, state and event behaviours. ..................................................................................................................... 5

Table 2. Pasture qualitative analysis (calculated from raw data) in the lead up to each transportation trip. Traits include; dry matter (DM), crude protein (CP), metabolisable energy (ME), acid detergent fibre (ADF), neutral detergent fibre (NDF), digestible dry matter (DDM) and (ALA). Collection periods were at 0, 3 and 6 weeks from each transportation day................................................. 19

Table 3. Predicted means (± s.e.) for weather parameters (temperature, humidity and wind speed) recorded within the stock crate across the 8 replicate trips.......................................................... 20

Table 4. Predicted means ± standard error across seasons for alpaca cold carcase weight (CCW) and evaporative loss, as well as ultimate pH (pHu), intramuscular fat (IMF), drip loss, purge, and cooking loss in the alpaca longissimus thoracis ................................................................. 24

Table 5. Average concentration (mg/100g) of common fatty acids within alpaca longissimus 24

Table 6. Pasture qualitative analysis (calculated from raw data) in the lead up to animal processing. Traits include; dry matter (DM), crude protein (CP), neutral detergent fibre (NDF), acid detergent fibre (ADF), digestible dry matter (DDM), metabolisable energy (ME), and vitamin E. Collection periods were at 0, 3 and 5 weeks from each kill (or processing) day.............................................. 25

Table 7. Predicted means ± standard errors for lightness ($L^*$), redness ($a^*$) and yellowness ($b^*$) fresh colour parameters of the alpaca longissimus lumborum each treatment level (stimulation and hang) 27

Table 8. Predicted means ± standard errors for muscle sarcomere length at each treatment level (stimulation and hang) on an individual muscle basis (m. longissimus thoracis, LT; m. adductor femoris, AF; m. semimembranosus, SM; m. semitendinosus, ST)................................................................. 28

Table 9. Predicted means ± standard errors at each treatment level (stimulation and hang) and on an individual muscle basis (m. longissimus thoracis, LT; m. longissimus lumborum, LL; m. adductor femoris, AF; m. semimembranosus, SM; m. semitendinosus, ST; m. psoas major, TL) for ultimate pH, purge, and cooking loss......................................................... 30

Table 10. Predicted means ± standard errors for average muscle particle size (µm) at each treatment level (stimulation and hang) on an individual muscle basis (m. longissimus thoracis, LT; m. adductor femoris, AF; m. semimembranosus, SM; m. semitendinosus, ST; m. psoas major, TL)................................................................. 31

Table 11. The effect of medium voltage electrical stimulation and hang method on thiobarbituric acid reactive substances (TBARS) in the alpaca longissimus thoracis and adductor femoris. 32

Table 12. Pasture qualitative analysis (calculated from raw data) in the lead up to animal processing. Traits include; dry matter (DM), crude protein (CP), neutral detergent fibre (NDF), acid detergent fibre (ADF), digestible dry matter (DDM), metabolisable energy (ME), and vitamin E. Collection periods were at 0 and 2 weeks from each kill (or processing) day.................................................. 33

Table 13. The effect of processing treatments (no electrical stimulation + Achilles hung; NES + AH and electrical stimulation + tenderstretch; ES + TS) on lightness ($L^*$), redness ($a^*$) and yellowness ($b^*$) fresh colour parameters of the alpaca longissimus lumborum................................................................. 34
Table 14. The effect of infusion treatments (no infusion, No; infusion with water, Water; and infusion with actinidain enzyme; Enzyme) on *longissimus thoracis* lightness (*L* *b*), redness (*a* *b*), yellowness (*b* *b*), and oxy/met ratio colour parameters during simulated retail display. Values are averaged across processing treatments and measurement period.

Table 15. The effect of processing treatments and infusion with water and actinidin on consumer sensory evaluation of 10 day aged alpaca *longissimus lumborum*.

Figures

Figure 1. Images showing drip loss samples suspended in inflated bags for measurement via the bag method (right) and a core sample within the specialised container for EZ drip analysis (left).

Figure 2. Diagrammatic representation of treatment structure, indicating the four treatment combinations (Achilles Hung + No Electrical Stimulation, AH + No ES; Achilles Hung + Electrical Stimulation, AH + ES; Tenderstretch + No Electrical Stimulation, TS + No ES; Tenderstretch + Electrical Stimulation; TS + ES).

Figure 3. Alpaca carcase (neck removed) split ventrally down the vertebral column, with the left hand side suspended by the pelvic bone (obturator foramen) for tenderstretching (left) and the right hand side hung conventionally by the Achilles tendon (right).

Figure 4. Diagrammatic representation of muscles and sampling location, and approximate sample weights for shear force (SF), particle size (PS), ultimate pH (pHu), sarcomere, collagen and sensory samples across the (A) m. longissimus thoracis, LT; (B) m. longissimus lumborum, LL; (C) m. adductor femoris, AF; (D) m. semimembranosus, SM; (E) m. semitendinosus ST; and (F) m. psoas major, TL. Image is inclusive of aging period for each sample. LT SF block 1 and 2 were randomly allocated to aging day 5 or 10 at sample collection.

Figure 5. Images demonstrating the infusion equipment and process. Samples were infused using a Baxter Flo-Gard GSP single syringe pump (top) set to infuse at a rate of 20 mL/min through 8 individual 18 gauge needles (bottom left). The kiwifruit extract was mixed with distilled water and prepared to a concentration specified by the manufacturer, prior to being drawn into syringes (bottom right; volume determined by *longissimus thoracis et lumborum* weight).

Figure 6. Shear force values within the alpaca *longissimus thoracis* across season and lairage treatments. Significant differences between means denoted by superscripts.

Figure 7. The effect of medium voltage electrical stimulation and hang method (Achilles hung, AH; and Tenderstretch, TS) on pH decline in the alpaca *longissimus lumborum* (top) and from medium voltage electrical stimulation on pH decline in the alpaca *semitendinosus* (bottom) from 36 to 3 °C. The solid line indicates the fitted pH trend across the decline period, based on predicted pH averages. The dotted line at pH 6 demonstrates that alpaca muscle pH did not reach the predicted window expected of lamb (to reach pH 6 at 18 – 35 °C) within the measurement period.

Figure 8. Changes to lightness (*L* *b*), redness (*a* *b*), and yellowness (*b* *b*) colour parameters of the alpaca *longissimus thoracis* during simulated retail display. Measurements were taken at 0, 24, 48 and 72 h. Significant differences between means denoted by superscripts. Superscripts were generated on an individual trait basis, as indicated by the use of upper case, lower case and asterix.

Figure 9. The effect of electrical stimulation on the alpaca *longissimus thoracis* (loin), *adductor femoris, semimembranosus* (topside) and *psoas major* (tenderloin). Significant differences between means denoted by superscripts. All superscripts were generated on an individual muscle by treatment level and are not applicable to means across muscles.
Figure 10. The effect of tenderstretching on the alpaca *longissimus thoracis* (loin), *semimembranosus* (topside) and *psoas major* (tenderloin). Significant differences between means denoted by superscripts. All superscripts were generated on an individual muscle by treatment level and are not applicable to means across muscles. ................................................................. 29

Figure 11. The effect of tenderstretching within the *adductor femoris* (additional hindquarter muscle). N.B. number ‘1’ and ‘2’ equate to ‘sample 1’ and ‘sample 2’ - two separate samples collected from different locations within the same muscle (Figure 4). Significant differences between means denoted by superscripts. ........................................................................ 29

Figure 12. The effect of electrical stimulation (ES) and hang method (Achilles Hung; AH and Tenderstretch; TS) on consumer sensory evaluation of 10 day aged alpaca *longissimus lumborum*. Significant differences between means denoted by superscripts. Superscripts were generated on an individual trait by treatment level basis and are not applicable to means across traits. .................. 33
Executive Summary

What the report is about

The research aims to deliver an integrated analysis of alpaca meat production from farm gate to product sale. Building on past research findings and focusing on season, transportation and lairage stress, as well as combined processing techniques and post-boning enzyme infusion in the loin, the research answers questions relating to the factors effecting Australian alpaca meat eating quality. In addition, the report outlines the guidelines for best practice alpaca carcase processing to be adopted across Australia.

Who is the report targeted at?

This report is aimed at both producers and processors that are looking to supply the alpaca meat market. Producers are provided with information relating to the finishing of alpacas on-farm, as well as animal preparation for transportation. The report also aids those producers considering incorporating meat production into their alpaca enterprise. Processors will benefit from research recommendations relating to processing guidelines and post-boning interventions. The investigation into transportation and lairage stress will aid both producers and processors, with the outcomes being the first research based findings relating to the commercial road transportation of alpacas in Australia. Finally, the report provides an insight for both producers and processors into the consumer perceptions of alpaca meat, and the benefits of employing value-adding techniques at slaughter.

Where are the relevant industries located in Australia?

The Australian alpaca industry has a nationwide presence with producers primarily located throughout the temperate regions of Australia. The Australian herd is currently estimated to be greater than 400,000 animals (Clarke, 2016). In recent years both traditional smaller scale alpaca operations (less than 100 animals managed over small blocks of land) and the number of larger scale commercial farms (managed similarly to extensive Merino operations) have increased. As a result, the national alpaca herd is forecast to reach 1 million by 2021. Alpacas are kept primarily for their fibre, as show and stud animals, as well as guard animals and pets. A small proportion of alpacas are currently destined for meat, with approximately 50 tonne of alpaca meat supplied to restaurants annually. The major alpaca processing plants, located in New South Wales and in South Australia, as well as producers across Australia, will benefit from the information contained in this report.

Background

Rapid growth within the Australian alpaca industry has increased interest in alpaca meat as a viable alternative to traditional fibre production. This has driven research into alpaca meat eating quality as a means to deliver a consistently high quality product to market. Recent studies have focused on post slaughter components such as carcase and muscle composition, salable meat yield, suitable processing techniques and product ageing. Such research has been fundamental in establishing a framework for the development of a competitive alpaca meat market. This research also highlighted knowledge gaps in the areas of seasonality, transport and lairage stress and combined processing treatment effects on alpaca meat quality. In order to maximize quality and efficiency throughout the alpaca meat supply chain, the following research was conducted to address the knowledge gaps.
Aims/objectives

The research aims to determine guidelines for the processing of alpaca carcasses in Australia, to deliver a more consistent eating experience to the consumer and to further enhance alpaca meat quality.

The research explores four distinct phases, including:

1. The impact of transportation, lairage and season on meat quality,
2. The effects of combining carcase processing techniques (electrical stimulation and tenderstretching) on alpaca meat tenderness,
3. The effect of post boning tenderisation by means of protease infusion of alpaca meat products and
4. Whole carcase application of processing techniques and product tenderisation to develop industry standards for carcase processing.

Methods used

1. **Investigation into transportation stress in Australian alpacas and methods to mediate stress related effects on meat quality**

A total of 160 castrated male huacaya alpacas (23 ± 1 month of age) were transported to slaughter over a 12 month period. Animals were moved in two groups of 20 per season (winter, spring, summer and autumn), generating 8 replicate trials across the year (2017). Live weight, body condition score and pasture samples were collected on-farm in the lead up to slaughter and analysed to determine nutritional state throughout the year. Alpacas were transported via a stock truck at a density of 0.6 m$^2$ for 4 hours to an abattoir via an identical route for each of the 8 replicate trips. Animals were monitored for the duration of the journey using 8 GoPro cameras (GoPro Hero4 Black, GoPro, San Mateo, California, US) mounted on the corner of each pen within the truck. Footage was later analysed to validate alpaca behaviour during transportation and report variations in agonistic behaviours relating to stress.

Immediately following transport, animals were allocated to one of two treatment groups (overnight lairage pre slaughter or seven day rest period with access to feed pre slaughter). Animals were processed under commercial conditions and chilled for 24 h prior to the removal of the rack section of loins (Longissimus thoracis; LT) from the right hand side of each carcase. Meat quality analysis included fresh colour, glycogen, ultimate pH, drip loss, shear force, purge, cooking loss, intramuscular fat and fatty acid analysis.

2. **The effect of combining tenderstretching and electrical stimulation on alpaca (Vicugna pacos) meat tenderness and eating quality**

Thirty-six castrated male huacaya alpacas (23 ± 1 month of age) were processed over 2 days, two months apart. Carcases were split in half down the vertebral column prior to treatment application. Treatments were paired into 6 combinations, to balance for side, and randomly allocated to the 36 split carcases in a 2x2 factorial arrangement. Treatments included:

1. No Electrical Stimulation + Achilles Hung (control)
2. Electrical Stimulation + Achilles Hung
3. No Electrical Stimulation + Tenderstretch
4. Electrical Stimulation + Tenderstretch

Once carcases were split, the hanging position of sides allocated to the tenderstretch treatment was altered from suspension by the Achilles tendon to suspension by the pelvic bone (obturator foramen). After 24 hours chilling, carcase sides were broken down and the longissimus thoracic et lumborum (LTL), Adductor femoris (AF), Semimembranosus (SM), Semitendinosus (ST) and Psoas major (TL)
muscles were extracted for meat quality evaluation. Meat quality analysis included fresh colour, glycogen, ultimate pH, retail colour, sarcomere length, shear force, purge, cooking loss, lipid oxidation and intramuscular fat analysis. In addition a sensory evaluation was conducted following methods outlined by Smith et al. (2016).

3. Development of best practice methods for alpaca carcase processing and the assessment enzymatic infusion on alpaca loin tenderness

Thirty-six entire male huacaya alpacas (23 ± 1 month of age) were processed over two days, two weeks apart. Carcases were randomly allocated to one of two processing treatments (achilles hung + no electrical stimulation and tenderstretch + electrical stimulation) in order to investigate the effect of combined processing methods on whole alpaca carcases. Carcases were processed under normal commercial conditions and remained whole for the duration of chilling.

After 24 h, carcases were broken down with both the left and right hand side LTL (loin) of each carcase allocated to one of three infusion treatments (no infusion, infusion with water and infusion with enzyme). The allocation was constrained to ensure equal representation of carcase processing treatments across the three infusion treatments (n = 12 loins per processing × infusion treatment). In addition to loins, the SM (topside) from the right hind leg of each carcase was collected for meat quality evaluation. Meat quality analyses aligned with those performed in experiment one and two, following the same procedures.

Results/key findings

- Alpaca behaviour during transportation was successfully quantified and the results of this research provide a basis upon which future behavioural studies can be built

- Alpaca meat quality was affected by season, with reduced muscle tenderness and increased product moisture loss in seasons where on farm feed availability was limited

- A resting period of 7 days did not aid in improving meat quality, with an overall negative effect on most quality traits, suggesting a direct to slaughter approach is optimal after 4hrs transportation

- Combined medium voltage electrical stimulation and tenderstretching processing treatments significantly improved quality traits in multiple alpaca muscles when applied to both split and whole carcases

- The application of these processing treatments, in combination, is proposed as a best practice alpaca carcase processing procedure

- The infusion of alpaca loins with a proteolytic enzyme did not improve overall product quality

- There is evidence to suggest alpaca undergoes limited oxidation (both colour and lipid) during retail display, which provides a unique advantage to industry if moving toward supply of retail fresh product to consumers

Implications for relevant stakeholders

Producers now have additional information which will assist in the decision making process when preparing animals for transportation and slaughter. In addition, they are provided with a greater understanding of how on farm factors and seasonality affect product quality. Processors are provided with a clear outline of best practice carcase processing methods. Industry now has additional information relating to alpaca meat advantages comparative to other red meats and directions for future research.
Recommendations

In order of application throughout the supply chain, from paddock to consumer:

1. Product quality is reduced if animals are processed during periods of limited on farm feed availability. During those periods when pasture availability is limiting, animals should be adequately supplemented or held back from slaughter until they have had access to ample high quality feed for at least 4 weeks leading up to slaughter. This will ensure that alpacas are on a rising plane of nutrition prior to slaughter.

2. Variables that increased agonistic behaviours during transportation included time spent in crush and points at which the vehicle stopped. In addition, there was an observation of potential increased aggression with increased fleece length. Future research should investigate this potential relationship, along with stock crate flooring impacts on animal standing and sitting behaviour. Such research will aid in directing producers with their decision making processes surrounding commercial transportation.

3. There is no advantage to resting alpacas for 7 days prior to slaughter, with novel stressors arising from the location change and interaction with other species causing decreased quality of the end product. Results of this research indicate that higher product quality will be achieved when sending animals direct to slaughter (with an overnight lairage period). This is applicable for trips up to 4 h in length and it should be noted that outcomes may change if transport exceeds this time period.

4. The post slaughter processing techniques of medium voltage electrical stimulation and tenderstretching should be applied, in combination, to alpaca carcases as a standard processing procedure. There is ample evidence to indicate the overall positive effects of these techniques on multiple muscles within whole alpaca carcases.

5. If alpaca enters into the fresh retail market, research is required to determine consumer acceptability thresholds for the unique alpaca colour parameters.
Introduction

Rapid growth within the Australian alpaca industry has recently increased interest in alpaca meat as a viable alternative to traditional fibre production. This has driven research into alpaca muscle biochemistry and meat quality as a means to deliver a consistently high quality product to market. Given the initial paucity of information relating to Australian alpaca carcases, recent studies have primarily focused on post slaughter components such as carcase and muscle composition, salable meat yield, suitable processing techniques and product ageing (Smith et al., 2015; Smith et al., 2016; Smith et al., 2017a). Such research has been fundamental in establishing a framework for the development of a competitive alpaca meat market. However, the factors influencing meat quality are multifaceted, with both intrinsic and extrinsic dynamics (Cheng & Sun, 2008; McPhail at al., 2014). Such factors were identified throughout the previous alpaca meat research, with knowledge gaps identified in the areas of seasonality, transport and lairage stress and combined processing treatment effects on alpaca meat quality. In order to maximise quality and efficiency throughout the meat supply chain, further research is required to address such knowledge gaps.

Pre-slaughter activities can have a significant influence over meat quality. It is commonly understood that high stress during mustering, transport and lairage of beef cattle can lead to undesirably dark cutting and dry meat (Wariss, 1990). This occurs through the breakdown of glycogen, resulting in increased ultimate muscle pH. Resting livestock with access to feed after exposure to stressors such as transportation can allow physiological recovery and has been shown to improve meat quality traits in multiple species, including cattle and pigs, (Mcveygh, et al., 1979; Warriss et al., 1984; Salmi et al., 2012). Season (summer, autumn, winter, spring) has also been shown to have significant impacts on both beef and lamb quality due to exposure to heat and cold stress, and nutritional variability (Knee et al., 2004; McPhail et al., 2014). Through the investigation of pre-slaughter stressors and seasonality on alpacas, an appreciation of the resultant effects on meat quality within the species can be gained and thereby managed accordingly.

In addition to the management of pre-slaughter stressors, post slaughter factors are still highly important in maximising product eating quality and consistency. The leanness and inconsistent external fat distribution exhibited by alpaca carcases (Smith et al., 2017a) presents a unique challenge in the management of carcasses within processing facilities. If carcasses chill too rapidly post-slaughter, muscle fibres will shorten, resulting in a tough end product. Identification of the ideal processing procedures and hang methods that minimize carcase to carcase variation and muscle shortening may be the key in ensuring maximum eating quality and consistency. Recent research has explored the application of processing techniques such as electrical stimulation (ES) and tenderstretching (TS; carcase suspension by the pelvic bone) separately as a means to improve alpaca meat tenderness (Smith et al., 2016; Smith et al., 2017a). The research concluded that these individual techniques improved tenderness traits in different areas of the carcase, but not the entire carcase. Electrical stimulation improved loin (Longissimus thoracis et lumborum; LTL) tenderness (Smith et al., 2016) while TS had a positive effect on hind quarter muscles (Smith et al., 2017a). There is potential for an additive effect across the whole alpaca carcase when applying these techniques in combination, particularly in improving the quality and consistency of the higher value loin (through ES) and hind leg (through TS) cuts, including the topside (Semimembranosus; SM) and outside (Adductor femoris; AF).

As the alpaca loin is one of the highest yielding muscles of the alpaca carcase (6.1 % of cold carcase weight; Smith et al., 2015) and a highly valued ($/kg) cut, there are both marketing and versatility gains to be made by the industry if loin tenderness could be further improved. Currently, the alpaca loin is approximately 25 % less tender than the alpaca topside (82.4 N vs 48.6 N shear force, respectively; Smith et al., 2016) when no processing treatments are applied. The research to date indicates that a 16 % improvement in loin tenderness can be achieved when applying processing procedures, such as electrical stimulation, prior to chilling (Smith et al., 2016). It is evident that there is further room for improvement in alpaca loin tenderness. The effects of value adding through
product enhancement after the point of carcase breakdown have not yet been reported for alpaca. Actinidain, extracted from kiwifruit, is a naturally occurring enzyme that has been shown to increase protein and collagen solubility within beef and lamb and thereby improve muscle tenderness (Lewis & Luh, 1988; Han et al., 2006; Toohey et al. 2011). While other plant based enzymes have a tendency to over-tenderise product, actinidain use has resulted in fewer reports of an undesirable final texture in meat (Bekhit et al., 2017). This is particularly important with alpaca as there is the potential to align loin tenderness with that of the hindquarter when infusing post-boning, without significantly altering the unique properties of this emerging red meat. Consumer sensory panels have been utilized across many red meats in the past (Thompson et al., 2005a; Perry et al., 2001; Smith et al., 2016) to gain insights into consumer acceptability and variation in product quality, whether positive or negative. Throughout the following research, consumer analysis was undertaken with the aim of reporting processing and infusion effects on alpaca meat eating quality.

Tenderness is one of the main drivers of consumer satisfaction (Thompson et al., 2005b). Therefore, there are significant marketing and financial gains to be made by the Australian alpaca meat industry as it expands if improvements to product tenderness and eating quality can be achieved throughout the supply chain. The following report aims to build on the fundamental research conducted in recent years, in order to deliver an integrated analysis of alpaca meat production from paddock to consumption. Specifically, the report will help answer questions relating to alpaca meat eating quality as a result of season, transport and lairage stress, combined processing techniques and post-boning enzyme infusion in the loin.

Objectives

The main objectives of this study are:

1. To determine the impact of seasonality, transportation and lairage on alpaca meat quality
2. To determine the effects of combining two processing techniques (electrical stimulation and tenderstretching) on alpaca meat quality traits
3. To determine the effect of post boning tenderisation by means of protease infusion of alpaca meat
4. To establish best practice guidelines for carcase processing, including the application of tenderstretching and electrical stimulation to whole carcasses and post boning tenderization by means of protease infusion
Methodology

1. Investigation into transportation stress in Australian alpacas and methods to mediate stress related effects on meat quality

1.1. Experimental design

A total of 160 animals (23 ± 1 month of age) were transported to slaughter over a 12 month period (January 2017 – December 2017). Animals were moved in two groups of 20 per season (winter, spring, summer and autumn), generating 8 replicate trials across the year. Animals were randomly allocated to a location within a standard stock crate (7.2 x 2.5 m, L x W), either a front or rear pen (2.4 x 2.5 m, L x W; n = 10 animals per pen), and monitored for the duration of the trip. Immediately following transport, animals were allocated to one of two treatment groups:

1. Overnight lairage pre slaughter (n = 10 per transport group)
2. Seven day rest period with access to feed pre slaughter (n = 10 per transport group).

In order to account for potential variation in the exposure to transport stressors as a result of the two separate within truck locations, animals were allocated to treatments in a way that ensures equal numbers from both the front and rear pens were assigned to each lairage treatment (n = 5 animals per pen directed to one of the two treatment groups).

1.2. On-farm

Animal live weight (LW) and body condition score (BCS; 1 – 5 scale with 0.25 unit increments; Keinprecht et al., 2016) was recorded at one day prior to each transportation day. Radio frequency identification (RFID) tags were used to identify individuals and LW and BCS measurements recorded using Gallagher™ Weigh Scales and Livestock Manager TSi 2 Data Recorder technology (Gallagher Group Limited, Epping VIC, Australia).

Pasture samples were collected at 0, 3 and 6 weeks prior to each transportation day for qualitative (DM, CP, ME, NDF and ADF) and fatty acid analysis. Twenty representative 30 cm x 30 cm (900cm²) quadrants were sampled across the paddocks in which the animals were grazed in the lead up to transportation. Quadrants were cut from ground level, pooled and a fresh pasture weight recorded at the point of collection. Each quadrant was assessed for pasture composition by recording the percentage of legume, desirable grass, mature/dry grass, weeds, litter and bare ground as a means to capture changes in composition across the year (quadrat method for estimation of pasture composition, available at http://mbfp.mla.com.au/Pasture-growth/Tool-27-Field-based-pasture-measurements). On several occasions, pasture within some quadrants was deemed too short and sparse for animal grazing. This was noted and taken into account when analysing pasture composition data and was utilised as an indicator of pasture availability. Pasture samples were then frozen at – 20 °C until subsequent analysis.

1.3. Pasture analysis

Prior to analysis, pooled samples from each collection week were freeze dried and ground to a 1 mm particle size. Sample analysis was performed in duplicate for analytical dry matter, neutral detergent fibre (NDF), acid detergent fibre (ADF) and crude protein (CP) following the official methods of analysis protocols (AOAC, 2005). The NDF and ADF analysis was performed as described by Van Soest (1963), modified for an automated Ankom 200 Fibre Analyser and F57 filter bags (A2000 ANKOM Technologies, New York, USA). Sample nitrogen (N) content was quantified by the Kjeldahl method (method 984.13; AOAC, 1990) using a Leco-428 Analyser (Michigan, USA) and the CP content then calculated using the conversion factor CP = N x 6.25 (McDonald, Edwards,
Greenhalgh, & Morgan, 2002). Pasture metabolisable energy (ME) was determined from the fibre and nitrogen content of pasture using methods previously described by NSW Agriculture (1983) and Oddy et al., (1983). Calculations were as follows:

$$\text{DDM} \% = 83.58 - 0.824 \times (\text{ADF} \%) + 2.62 \times (\text{N} \%)$$

$$\text{ME} (\text{MJ/kgDM}) = 0.17 \times (\text{DDM} \%) - 2$$

For pasture fatty acid analysis, 20 mg of pasture sample was mixed with 2 ml methanol:toluene (reagent 4:1 containing \(\mu\)g/mL C19:0 internal standard) and methylated by adding 0.2 mL acetyl chloride drop-wise while continually vortexing the sample. Samples were incubated at 100 ℃ for 60 minutes. Once cooled, 5 mL of 6 % potassium carbonate was added to halt the reaction. Tubes were centrifuged at 3660 rpm for 10 min and the upper layer (approximately 0.1 mL) containing the fatty acid methyl esters (FAME) transferred to a 0.4 mL insert within a glass vial for analysis using gas chromatography.

1.4. Transportation component

1.4.1. Loading and transportation

The 20 animals for each transportation week (\(n = 8\)) were selected at random from a larger herd one day prior their respective trips. The group was held on pasture with ad lib access to water in the lead up to transport. The following day, alpacas were loaded by an experienced stock handler and transporter as per normal on farm procedure and in accordance with the Standards and Guidelines for the Land Transport of Livestock (Edition one, 2012; USyd ethics approval prj 2016/1061). Animals were transported at a density of 0.6 m\(^2\) for 4 hours to the abattoir via an identical route for each of the 8 replicate trips. Variation in road surface type and any adverse events were recorded manually throughout the duration of each trip.

1.4.2. Environmental variables

Temperature (ºC), humidity (%) and wind speed (km/hr) within the stock crate were recorded using a portable weather station (WS5019 HOLMAN iWeather Weather Forecaster, Osborne Park, WA, Australia) connected wirelessly to a tablet held within the truck cab. These variables were logged every 10 minutes and averaged within trips for analysis.

1.4.3. Behavioural observation

Animals were monitored for the duration of the journey using 8 GoPro cameras (GoPro Hero4 Black, GoPro, San Mateo, California, US) set to record at 1080 pixels, 24 frames per second and a wide field of view. Individual cameras were mounted on the corner of each pen, with the lens positioned on a diagonal to ensure complete coverage of the pen. An additional two cameras were fitted to the rear pen facing outwards from the truck to monitor the loading of the alpacas and any adverse incidents occurring behind the truck during transit. A VIRB Action Camera (Garmin Ltd. Olathe, Kansas, US) with inbuilt GPS and speedometer, recording at 1080 pixels, 30 frames per seconds in a wide field of view was mounted on the truck dashboard to monitor any events that may influence behaviour during the trip. Animal safety and camera function were monitored throughout the trip by streaming footage via a Bluetooth connection in real time to a tablet held in the cab of the truck.

The camera footage underwent detailed behavioural analysis at the conclusion of all transportation weeks. The observation periods commenced at 0 hrs of the journey, once all alpacas were loaded onto the truck. Each alpaca was sampled for 5 minutes with observations repeated every 20 minutes. A pause was made in the journey as the truck stopped to rest the driver after 2 hrs of driving and observations continued once the truck resumed the journey. During each observation, the posture (sitting or standing; Table 1) and main behaviour (exploring, alert, aggressive etc.; Table 1) of each
alpaca was recorded every minute for the 5-minute period. Point behaviours including; interactions between alpacas, slips and falls were additionally recorded throughout each journey.

Footage from the 8 transportation days was observed by a single observer across multiple sittings. Each recording sheet noted the trip number, observation number, pen, alpaca ID and road surface for that period. Road surfaces were visually determined utilising the VIRB Action Camera (Garmin Ltd. Olathe, Kansas, US) and classified as, gravel, surfaced, and further divided into suburbs (speed of 60km/hr or less), highway (80km/hr or more), bends, declines and road work zones (consisting of stop start traffic and unpredictable trajectory). A behavioural ethogram was generated with the assistance of an animal behaviour scientist to assist with behavioural assessment (Table 1).

Table 1. Ethogram developed for behavioural observation of alpacas, broken down into postures, state and event behaviours.

<table>
<thead>
<tr>
<th>Behaviour</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Postures</td>
<td></td>
</tr>
<tr>
<td>Standing, head up</td>
<td>Animal is in an upright position with all 4 limbs touching the ground. Animal’s head is raised above the shoulder.</td>
</tr>
<tr>
<td>Standing, head down</td>
<td>Animal is in an upright position with all 4 limbs touching the ground. Animal’s head is resting below the shoulder.</td>
</tr>
<tr>
<td>Cush (sitting)</td>
<td>Animal’s limbs are folded beneath them, with thorax and abdomen not visible.</td>
</tr>
<tr>
<td>Prostrate</td>
<td>Lying flat, in a stretched-out position on either left or right side. Head resting on ground.</td>
</tr>
<tr>
<td>State Behaviours1</td>
<td></td>
</tr>
<tr>
<td>Exploring</td>
<td>Ears upright or smelling or touching nose to objects or observing and actively investigating surroundings.</td>
</tr>
<tr>
<td>Alert</td>
<td>May be standing or lying, animal has head up with ears forward (can be moving/flicking ears) and actively observing surroundings. Not actively moving around or displaying inquisitive behaviours (i.e. not exploring).</td>
</tr>
<tr>
<td>Aggression</td>
<td>Can be categorised as severe (containing 3 or more of the below examples OR greater than 3 bouts of any one aggressive behaviour within one minute) or mild (containing 1 -2 of the below examples). Aggressive behaviours: kicking, spitting, ears down, tail up or biting.</td>
</tr>
<tr>
<td>Submissive</td>
<td>Ears down, exhibiting signs of discomfort through ears back and avoiding interaction with dominant animals by physical distance of the whole body when standing or head and neck when sitting.</td>
</tr>
<tr>
<td>Unsettled</td>
<td>Flaring nostrils, unnatural stance, indecisive between standing/lying, shaking.</td>
</tr>
<tr>
<td>Point Behaviours2</td>
<td></td>
</tr>
<tr>
<td>Neutral Interaction</td>
<td>Purposeful/intentional contact between two animals (e.g. communication or aiding in balance, such as the disturbance of one animal’s behaviour by another such as sitting on another/being sat on).</td>
</tr>
<tr>
<td>Negative Interaction</td>
<td>Purposeful/intentional contact between two animals, such as agonistic behaviours inclusive of biting, lunging, spitting and threatening.</td>
</tr>
<tr>
<td>Chewing</td>
<td>An alpaca is ruminating/ preparing bolus for spiting.</td>
</tr>
<tr>
<td>Spitting</td>
<td>The expulsion of contents from the mouth.</td>
</tr>
<tr>
<td>Fall</td>
<td>Animal loses footing and falls completely to knees or ground. Part of animal’s body must hit the ground.</td>
</tr>
<tr>
<td>Slip/stumble/Unsteady on feet</td>
<td>Animal loses footing but regains balance. Animal may be struggling to keep steady but does not fall to knees or onto the ground.</td>
</tr>
<tr>
<td>Shaking</td>
<td>An alpaca is unable to maintain normal stance causing the shaking of two or more limbs.</td>
</tr>
<tr>
<td>Urinating/defecating</td>
<td>Animal is urinating or defecating.</td>
</tr>
<tr>
<td>Scratching/grooming</td>
<td>Animal is scratching foreleg/forequarter with hind-leg or using teeth to scratch and/or groom fleece.</td>
</tr>
</tbody>
</table>

1State behaviours are measurable by duration and quantified by time.
2Point behaviours are without a measurable duration and quantified by frequency.
1.5. Lairage component

Upon arrival at the abattoir, 5 animals from each truck pen were selected at random and placed into lairage (lairage; n = 10). The remainder of the animals were directed toward a holding paddock where they were held for 7 days prior to slaughter (rested; n = 10) with ad lib access to feed (pasture and/or hay) and water until being placed into lairage the afternoon prior to processing. This was repeated for each of the 8 replicate groups, generating 16 kill weeks across the year.

1.6. Carcass Processing

The alpacas were slaughtered and dressed out under commercial conditions within a camelid certified abattoir on the south coast of New South Wales. A conventional captive bolt was used to render animals insensible, followed by exsanguination through severing of the jugular veins and carotid arteries. At this point blood was collected into pre-labelled lithium heparin vacutainers and placed on ice prior to being centrifuged (15 min at 3500 rpm) to obtain blood serum for the determination of cortisol concentration. Immediately following exsanguination, animals were immobilized to prevent excess kicking during carcass dressing procedures. Prior to entering chillers, a glycogen core sample was collected from the right hand side of each carcass in the caudal region of the *longissimus lumborum* (LL, shortloin section of the loin). Core samples were snap frozen in liquid nitrogen and stored at – 80 ºC prior to analysis.

1.7. Sample collection and measurements

After 24 hours chilling, carcass sides were weighed to obtain cold carcass weight and broken down for sample collection. The loin was removed from the right hand side of each carcass, prepared into the *longissimus thoracic* (LT; rack section of the loin) and vacuum packaged for transport after a fresh colour measure was performed. Samples were transported at an average temperature and humidity of 6.0 ºC and 85.6 %, respectively, for 3 hours to a laboratory for sample preparation. Moving cranially along the LT from the 12th/13th rib, toward the 4th rib, approximately 90 g was removed for ageing and weighed, followed by three approximately 60 g samples for drip loss analysis. The remaining muscle was diced, placed into 50 ml tubes and frozen at – 20 ºC for intramuscular fat and fatty acid analysis. Drip loss samples were prepared as outlined below. After 10 days ageing (3.0 ºC and 83.3 % humidity), the 90 g blocks were weighed to obtain post ageing weights and prepared into one 65 g block for shear force (SF) analysis and one 5 g sample for ultimate pH (pHu) assessment. Shear force and pHu samples were frozen at – 20 ºC until subsequent analysis.

1.8. Meat quality testing

1.8.1. Fresh colour

Twenty-four hours post slaughter, a fresh surface was cut from LT samples and allowed to bloom at ambient temperature (6 – 7 ºC) for 40 minutes, after which time the meat colour was measured using a Minolta Chroma (model CR400, Osaka, Japan). The chromameter was set to the L*, a*, b* system and illuminate D65, observer angle of 2º, aperture size of 5.0 mm and a closed cone. The chromameter was calibrated using a standardised white tile prior to measurement, and three measurements were taken across the face of the LT for each sample.

1.8.2. Glycogen and ultimate pH

For the determination of glycogen content, 1 g of frozen LL sample was incubated in 10 mL of Milli-Q water for 5 minutes at 100 ºC, homogenised (two bursts of 15 s at 22000 rpm) and then centrifuged (Model CPR, Beckman Instruments, CA, USA) for 15 min at 3500 rpm (De Brito et al., 2016). The supernatant from each sample was plated in duplicate and compared to glycogen standards using the colorimetric protocol detailed by the commercial glycogen assay kit (Sigma-Aldrich, MO, USA). Absorbance was measured at 570 nm using a micro-plate reader (FLUOstar OPTIMA, BMG,
Labtechnologies, Victoria, Australia) in order to calculate total glycogen concentration of each sample.

Ultimate pH analysis was performed using methods outlined by De Brito et al. (2016). Briefly, 1 g of frozen aged sample was homogenised in an iodoacetate buffer and suspended in a water bath at 22 °C. Ultimate pH was determined as the average of duplicate measures using a temperature and pH meter (Model smartCHEMC-CP, TPS Ltd., Queensland, AUS) calibrated at 22 °C in pH 4.01 and 6.86 buffers.

1.8.3. Drip loss

Drip loss, an evaluation of the meat water holding capacity, was measured via two methods. Two methods were utilised in order to compare and validate the use of the more streamlined ‘EZ’ method in the evaluation of drip loss in alpaca meat, compared to the standardised ‘Bag’ method. From the three 60 g LT blocks, two were weighed to 3 decimal places (A & D Company limited, FZ-500i 520 g/0.001 g, SA, Australia) and suspended inside an inflated bag (Figure 1) according to the standardised bag method protocol (Honikel, 1987). For analysis via the EZ method, the third 60 g block was trimmed to 2.5 cm perpendicular to the muscle grain. From this, two core samples were cut parallel to the muscle fibre using a purpose built cylindrical (2.5 cm diameter) muscle corer. These samples were weighed to 3 decimal places and placed within specialised EZ drip loss containers (Figure 1) according to methods outlined by Rasmussen & Andersson (1996).

All samples were placed within the same chiller for 48 h at an average temperature of 3.3 °C and average humidity of 83 %. After 48 h, the residual drip was removed from samples with paper towel and samples were individually weighed to 3 decimal places to obtain post-drip weights.

Figure 1. Images showing drip loss samples suspended in inflated bags for measurement via the bag method (right) and a core sample within the specialised container for EZ drip analysis (left)

1.8.4. Shear force

Frozen samples were prepared and cooked for shear force analysis as described by Hopkins & Thompson (2001). Shear force blocks were randomly allocated to one of 8 cook batches. The allocation was constrained in such way that ensured equal representation from each kill week and each season across the batches. Shear force was measured as the average of 6 peak force recordings across each muscle block using a Lloyd (Model LRX, Lloyd instruments, Hampshire UK) fitted with a Warner Bratzler shear v blade.
1.8.5. Purge and cooking loss

Purge was calculated from the pre and post age (10 d) weight of the 90 g aged LT sample, and expressed as a percentage of the pre-age weight. Cooking loss was calculated as a percentage of the pre-cook weight on all shear force blocks, using the pre-cook frozen weight and post cook weight as outlined by Hopkins & Thompson (2001).

1.8.6. Intramuscular fat (IMF) and muscle fatty acid (FA)

Prior to analysis, samples were freeze dried and ground using a FOSS KnifetechTM1095 mill and stored (−20°C) until analysis. The IMF and FA analysis was conducted using methods described by Hopkins et al. (2014). In brief the IMF analysis consisted of a 3 g freeze dried and ground sample bring extracted with 85 ml hexane for 80 min, in a FOSS Soxtec 2050 machine, before being removed and dried (30 min and 105°C) and weighed. For fatty acid analysis, 10 mg of freeze dried and ground sample was methylated and evaluated as outlined above for pasture samples.

1.9. Statistics

Statistical analysis was carried out in consultation with a biometrician. All traits for experiment one were analysed using linear mixed models (LMM) regressions and fitted in the statistical program Genstat 18th edition (VSN International Ltd., Hertfordshire, UK).

2. The effect of combining tenderstretching and electrical stimulation on alpaca meat tenderness and eating quality

2.1. Experimental design

Thirty-six castrated male huacaya alpacas were randomly selected from a commercial property on the southern tablelands of New South Wales. Animals were grazed on late summer and autumn pastures in the lead up to slaughter and processed over 2 days, two months apart (9/03/2016 and 4/05/2016; n=18 animals per processing).

Carcases were split in half down the vertebral column prior to treatment application. Treatments were paired into 6 combinations, to balance for side, and randomly allocated to the 36 split carcases in a 2×2 factorial arrangement (n=18 carcase halves per treatment; Figure 2).

Treatments included:

1. No Electrical Stimulation + Achilles Hung (control); No ES + AH
2. Electrical Stimulation + Achilles Hung; ES + AH
3. Electrical Stimulation + Tenderstretch No ES + TS
4. Electrical Stimulation + Tenderstretch; ES + TS
Figure 2. Diagrammatic representation of treatment structure, indicating the four treatment combinations (Achilles Hung + No Electrical Stimulation, AH + No ES; Achilles Hung + Electrical Stimulation, AH + ES; Tenderstretch + No Electrical Stimulation, TS + No ES; Tenderstretch + Electrical Stimulation; TS + ES)

2.2. On-farm

Animal LW and BCS were recorded at 0, 5 and 8 weeks prior to each processing day, as per standard husbandry procedure. Radio frequency identification (RFID) tags were used to identify individuals and measurements recorded using Gallagher™ Weigh Scales and a Livestock Manager TSi 2 Data Recorder (Gallagher Group Limited, Epping VIC, Australia).

Pasture samples were also collected at 0, 5 and 8 weeks prior to each processing day for qualitative assessment (DM, CP, ME, NDF and ADF) and the analysis of vitamin E content. Six representative 30 cm x 30 cm (900 cm²) quadrates were sampled across the 55 acre paddock in which the animals were grazed in the lead up to slaughter. Quadrates were cut from ground level and a fresh pasture weight recorded at the point of collection, as outlined for experiment one. An additional grab sample was collected from each sample point and pooled to generate a representative vitamin E sample per collection week.

2.3. Pasture analysis

Samples from each quadrate within each collection week were pooled and dried at 100 °C for 24 h in preparation for qualitative analysis. Prior to analysis, all samples were ground through a 1 mm screen. Sample analysis was performed in duplicate as outlined in experiment one.

Prior to vitamin E analysis, pooled grab samples from each collection week were freeze dried and ground to a 1 mm particle size. Pasture vitamin E content was determined using high performance liquid chromatography (HPLC) measuring α-tocopherol content according to methods described by McMurray & Blanchflower (1979).
2.4. Carcase Processing

The alpacas were slaughtered and dressed out under commercial conditions. A conventional captive bolt was used to render animals insensible, followed by exsanguination through severing of the jugular veins and carotid arteries. Immediately following exsanguination, animals were immobilized (2000 mA peak current at 500 µs pulse interval and 1000 µs pulse width for 10 s) to prevent excess kicking during carcase dressing procedures.

Prior to treatment application, the neck was removed at the junction of the 5th and 6th cervical vertebrae and carcases split ventrally in half (Figure 3) using a cattle brisket saw. Post dressing and splitting of carcasses, the hanging position of carcase sides allocated to the tenderstretch treatment was altered from suspension by the Achilles tendon for dressing purposes to suspension by the pelvic bone (obturator foramen). This resulted in carcase suspension in the typical tenderstretch position for the duration of chilling, as the carcase passed through rigour mortis (Figure 3).

Prior to entering chillers, a glycogen core sample was collected from the right hand side of each carcase and medium voltage electrical stimulation applied to all carcase sides allocated to the electrical stimulation treatment. Electrical stimulation was applied using a portable electrical stimulation unit (StimTech Pty Ltd., Brisbane, AUS) set to the medium voltage parameters of ~ 300 V with a 600 mA peak at 68 ms pulse interval and 1000 µs pulse width for 40 s.

Figure 3. Alpaca carcase (neck removed) split ventrally down the vertebral column, with the left hand side suspended by the pelvic bone (obturator foramen) for tenderstretching (left) and the right hand side hung conventionally by the Achilles tendon (right)

2.5. pH decline

Once carcases entered the chillers, pH and temperature were measured in the caudal section of the LL and m. semitendinosus (ST) of all carcase halves. Measurements were taken at half hourly intervals from approximately 35 ºC to 18 ºC and then again at 6 h and 24 h post slaughter using hand held pH meters with temperature compensation (WB-80, winTPS Pty. Ltd., Brisbane, AUS), a polypropylene spear-type gel electrode (Ionode IJ 44) and cylindrical stainless steel probe attachment. The meters were calibrated intermittently at ambient temperature using pH buffers 4.01 and 6.86 (TPS Pty Ltd., Brisbane, AUS).
2.6. Sample collection and measurements

After 24 hours chilling, carcase sides were weighed to obtain cold carcase weight (left + right side weights) and broken down for sample collection. The LTL was removed and prepared into the longissimus thoracic (LT) and longissimus lumborum (LL) sections (Figure 4). Samples for meat quality analysis were prepared from the LT, Adductor femoris (AF), Semimembranosus (SM), Semitendinosus (ST) and Psoas major (TL) muscles. The LL from all carcase halves was prepared into 15 cm long blocks for the purpose of aging and sensory analysis. All other muscles were sub-sectioned for analyses as follows.

Figure 4. Diagrammatic representation of muscles and sampling location, and approximate sample weights for shear force (SF), particle size (PS), ultimate pH (pHu), sarcomere, collagen and sensory samples across the (A) m. longissimus thoracis, LT; (B) m. longissimus lumborum, LL; (C) m. adductor femoris, AF; (D) m. semimembranosus, SM; (E) m. semitendinosus ST; and (F) m. psoas major, TL. Image is inclusive of aging period for each sample. 1LT SF block 1 and 2 were randomly allocated to aging day 5 or 10 at sample collection.

2.7. Meat quality testing

2.7.1. Glycogen and ultimate pH

Glycogen samples were stored and analysed as outlined for experiment one.

Ultimate pH (pHu) was analysed using 10 day aged (3.1 °C and 75.7 % humidity) LT and AF samples from all carcase sides. Samples were stored and analysed as described above.

2.7.2. Fresh colour

At the point of collection, a fresh surface was cut from the LL sensory sample and allowed to bloom at ambient temperature (6 – 7 °C) for 40 minutes. After this time the meat colour was measured using a Minolta Chroma (model CR400, Osaka, Japan), as outlined in experiment one.
2.7.3. Retail colour

For retail colour stability analysis, a fresh surface was cut from 3cm thick 5 day aged LT, overwrapped with 15 µm PVC film and placed under simulated retail display for 40 minutes bloom time. After this time, the initial (0 h) measure was taken using a Hunter Lab Mini Scan XE plus, followed by measurements at 24, 48 and 72 h. The colourometer was set to the L*, a*, b* system and illuminate D65.

2.7.4. Sarcomere length and shear force

At 24 h post slaughter, a 5 g sample was prepared from the 5 muscles (LT, AF, SM, ST and TL) of all carcase sides and stored at –20 ºC until subsequent sarcomere length measurement. Samples for analysis of sarcomere length in the AF were taken from the caudal end of the muscle. Sarcomere length was measured using laser diffraction (Bouton et al., 1973), where a thin slice (< 1 mm) was cut from the frozen muscle sample and mounted on a glass microscope slide. Sarcomere length was calculated as the average of five replicate measures read across the slide using a laser diffraction machine.

Approximately 80 g was sub-sectioned from each muscle (LT, AF, SM, ST and TL of all carcase sides; Figure 4), vacuum packaged and chilled for 10 days at an average of 3.1 ºC and 75.7 %. A second 80 g sample was taken from the LT and aged for 5 days (LT 5 d; 3.2 ºC and 75.7 % humidity) for a within muscle aging comparison (Figure 4A). Likewise, a second 80 g sample was sub-sectioned from the AF (AF 2; aged for 10 days at an average of 3.1 ºC and 75.7 % humidity) for an intramuscular comparison of shear force across the muscle (Figure 4C). At the conclusion of the aging period, muscle samples were prepared into a 65 g shear force block and frozen at –20 ºC until subsequent analysis. Samples were prepared and cooked for shear force analysis as outlined in experiment one. Shear force blocks from the two kill days were randomly allocated to cook batches, on an individual muscle basis.

2.7.5. Purge and cooking loss

Purge was recorded for both the LL and AF. This was calculated from the pre and post age (10 d) weight of the sensory block for the LL and both (AF 1 and AF 2) shear force blocks for the AF, and expressed as a percentage of the pre-age weight. Cooking loss was calculated as a percentage of the pre-cook weight on all shear force blocks, using the pre-cook frozen weight and post cook weight as outlined by Hopkins & Thompson (2001).

2.7.6. Collagen and particle size

Samples for particle size determination were prepared from each muscle sample and each aging period (AF 1, AF 2, LT 5 d, LT 10 d, SM, ST, TL; Figure 4). Analysis of particle size was performed as described by Karumendu et al. (2009), where approximately 1 g of frozen muscle sample was homogenized in 15 mL of ice cold buffer prior to measurement with a laser diffraction particle size analyser (Beckman Coulter, Model LS 13 320, Miami, USA). Samples were tested in duplicate.

For the analysis of total and soluble collagen, samples were prepared from the right hand side of each carcase (Figure 4). Approximately 50 g was collected from the LT and AF at 24 h post slaughter, diced and placed in 50 mL tubes before being frozen at –20 ºC. Samples were freeze dried and ground prior to being analysed using methods described by Starkey, Geesink, Oddy, & Hopkins (2015).

2.7.7. Lipid oxidation

Lipid oxidation was determined via the TBARS assay previously described (Smith et al., 2016), using 1 g of frozen LT subsampled from pre and post retail colour display samples. Sample concentrations (mg) of malondialdehyde were determined using a spectrophotometer reading absorbance at 540 nm.
2.7.8. Sensory evaluation

Sensory samples collected from the LL were aged for 10 days (3.1 °C; 75.7 % humidity) prior to all sensory blocks (excluding those from two animals that were condemned at the abattoir; *n* condemned blocks = 4) being sub-sectioned to generate 5 steaks per carcass side (*n* steaks = 340). Sensory analysis was conducted according to methods described by Smith et al. (2016). Briefly, 680 grill samples were served to 86 untrained panellists across 2 sessions and 3 nights (6 sessions in total). There were 14 to 16 participants in each session and sessions were made up of 9 tasting rounds.

The allocation of grill samples to panellists within sessions was multi-tiered, with the inclusion of the initial treatment to carcass side randomisation. Within the allocation, both sides for each carcass were randomised to a single tasting session. Samples within the same treatment group were distributed as uniformly as possible across the 6 sessions. The two halves (grill samples) for each steak were allocated to two panellists within one tasting round of a session. Additionally, pairs of samples arising from the same carcass, with sides exposed to different treatments, were tested by the same panellist in sequential tasting rounds to maximise the efficiency of treatment comparison.

On the day of testing, samples for each session were thawed, prepared and cooked as outlined by Smith et al. (2016). Prior to tastings, panellists were asked for demographic information regarding age, income, gender, cultural background, frequency of red meat consumption, the number of adults and children in the household and their preferred degree of doneness. These were later converted into ordinal categories for inclusion as covariates within the statistical analysis. Panellists were also asked to evaluate grill samples for 5 subjective traits including tenderness, juiciness, flavour, overall liking (on a 0 – 100 scale) and satisfaction (4 categories; unsatisfactory, good every day, better than every day or premium product).

2.8. Statistics

Animal, carcase and laboratory based meat quality traits were analysed using linear mixed models (LMM) regressions within Genstat 18th edition (VSN International Ltd., Hertfordshire, UK). Consumer sensory analysis and pH decline data were modelled within the ASREML package (Butler, 2009) under R (R Core Team, 2013), in consultation with a biometrician.

3. Development of best practice methods for alpaca carcase processing and the assessment enzymatic infusion on alpaca loin tenderness

3.1. Experimental design

Thirty-six entire male huacaya alpacas (23 ± 1 month of age) were selected at random from a commercial property on the southern tablelands of New South Wales. Animals were grazed on spring pastures in the lead up to slaughter and processed over two days, two weeks apart (26/09/2018 and 10/10/2018; *n* = 18 animals per processing). Carcasses were randomly allocated to one of two processing treatments (achilles hung + no electrical stimulation and tenderstretch + electrical stimulation) in order to investigate the effect of combined processing methods on whole alpaca carcases. After 24 h, carcases were broken down with both the left and right hand side loins of each carcass allocated to one of three infusion treatments (no infusion, infusion with water and infusion with enzyme). The allocation was constrained to ensure equal representation of carcase processing treatments across the three infusion treatments (*n* = 12 loins per processing x infusion treatment).
3.2. On-farm

Animal LW and BCS were recorded at 0 and 2 weeks prior to each processing day as outlined above. Pasture samples were also collected at 0 and 2 weeks prior to each processing day for qualitative analysis (DM, CP, ME, NDF and ADF). Ten representative 30 cm x 30 cm (900cm²) quadrates were sampled across the paddocks in which the animals were grazed in the lead up to slaughter, pooled, weighed and dried as outlined in experiment 2.

3.3. Pasture analysis

Samples from each quadrant within each collection week were pooled and dried at 100 °C for 24 h in preparation for qualitative analysis. Prior to analysis, all samples were ground through a 1 mm screen. Sample analysis was performed in duplicate as outlined in experiment one.

3.4. Carcase Processing

The alpacas were slaughtered and dressed out as outlined in experiment one, with carcases remaining whole and necks retained to align with current commercial practice. Animals were immobilized using the portable immobilization unit described in experiment two (2000 mA peak current at 500 µs pulse interval and 1000 µs pulse width for 10 s).

Post dressing of carcases, the hanging position of those allocated to the tenderstretch + electrical stimulation treatment was altered from suspension by the Achilles tendon to suspension by the pelvic bone (obturator foramen). Prior to entering chillers, a glycogen core sample was collected from the right hand side of each carcase. Electrical stimulation was applied using the same portable medium voltage electrical stimulation unit outlined in experiment two, set to the same parameters (~ 300 V, 600 mA peak at 68 ms pulse interval and 1000 µs pulse width for 40 s.).

3.5. Sample collection, infusion and measurements

At 24 h post slaughter, carcases were weight to obtain cold carcase weight. Carcases were broken down, with the LTL (left and right hand side) and SM (right side only) retained for sampling. A 3 g sample was removed at the point of collection and frozen at –20 °C for subsequent sarcomere length analysis. Longissimus samples were weighed and prepared for fresh colour analysis by cutting at the 12th/13th rib marking prior to vacuum packaging. Semimembranosus samples were weighed, packaged and transported along with the LTL for 3 h (at 5.3 °C and 86.6 % humidity) to laboratory facilities.

Longissimus samples were patted dry, reweighed and infused using a Baxter Flo-Gard GSP single syringe pump set to infuse at a rate of 20 mL/min through 8 individual 18 gauge needles (Figure 5). The kiwifruit extract (Figure 5) was prepared according to the manufacturer’s guidelines (OT1005X, Earlee Products Pty Ltd., Brisbane, Australia) and reconstituted using distilled water.
Images demonstrating the infusion equipment and process. Samples were infused using a Baxter Flo-Gard GSP single syringe pump (top) set to infuse at a rate of 20 mL/min through 8 individual 18 gauge needles (bottom left). The kiwifruit extract was mixed with distilled water and prepared to a concentration specified by the manufacturer, prior to being drawn into syringes (bottom right; volume determined by *longissimus thoracis et lumborum* weight).

3.6. Meat quality testing

3.6.1. Glycogen and ultimate pH

Glycogen and ultimate pH were analysed as outlined in experiment one.

3.6.2. Fresh colour

*Longissimus* samples were prepared into the LT and LL by cutting at the 12th/13th rib and a fresh colour reading was taken across the surface of the LL after 40 minutes bloom time, as outlined in experiment one.

3.6.3. Retail colour

A fresh surface was cut from 5 day aged LT, overwrapped with 15 μm PVC film and placed under simulated retail display for 40 minutes bloom time. Measurements were at 0, 24, 48 and 72 h, as outlined in experiment two.
3.6.4. Sarcomere length and shear force

Sarcomere length was measured as outlined in experiment two.

After 10 days ageing (3.1 ºC and 75.3 % humidity) SF blocks were prepared from the LT and SM as outlined above. A second SF sample was collected from each loin for a within muscle aging comparison, and aged to 5 days. Shear force samples were stored at – 20 ºC until subsequent analysis, and the analysis performed as outlined above.

3.6.5. Cooking loss

Cooking loss was calculated as outlined in experiment one.

3.6.6. Particle size analysis

Samples for particle size determination were prepared from LT and SM from each ageing period (i.e. 5 and 10 days for LT and 10 days for SM). Analysis of particle size was performed as described in experiment two.

3.6.7. Sensory evaluation

Sensory samples collected from the LL were aged for 10 days (3.1 ºC; 75.3 % humidity) and then sub-sectioned to generate 5 steaks per loin (n steaks = 360). Sensory analysis was conducted according to methods described by Smith et al. (2016). Briefly, 720 grill samples were served to 96 untrained panellists across 2 sessions and 3 nights (6 sessions in total). There were 16 participants in each session and sessions were made up of 9 tasting rounds.

The allocation of grill samples to panellists within sessions was multi-tiered, balancing for the initial processing and infusion treatment allocation to sessions and ensuring that each panellist tasted each treatment at least once. The allocation was constrained to ensure all samples from each carcase were tasted within the same session, but that repeat tests on a treatment were not offered to the same individual in sequential tasting rounds. The two halves (grill samples) for each steak were allocated to two panellists within one tasting round of a session.

The remainder of the sensory evaluation was conducted as outlined in experiment two.

3.7. Statistics

Animal, carcase, fresh colour and sarcomere traits were analysed using linear mixed models (LMM) regressions within Genstat 18th edition (VSN International Ltd., Hertfordshire, UK). All other meat quality traits were modelled within the ASREML package (Butler, 2009) under R (R Core Team, 2013), in consultation with a biometrician.
Results

1. Investigation into transportation stress in Australian alpacas and methods to mediate stress related effects on meat quality

1.1. On-farm

Animal live weight averaged 61.6 ± 0.07 kg across the year, ranging from an average of 57.8 ± 1.59 kg (spring) to 64.06 ± 1.59 kg (autumn). With the exception of transport group 6 (54.0 ± 2.25 kg), animal live weight did not vary across trip groups. Body condition score also did not vary across trips or season, with animals retaining an average BCS of 2.75 ± 0.03 across the year (2017).

1.2. Pasture analysis

Pasture availability (indicated here by percentage of grass of desirable grazing length) and quality varied across the year. Pasture growth followed a traditional annual trend where summer pastures were of higher fibre content and lower digestible dry matter (DDM; though ample availability). This was followed by pasture availability dropping off toward the end of autumn and into winter, increasing again through spring (Table 2). Spring pastures exhibited the highest CP (%), ME (MJ/kg DM) and DDM (%). This also aligned with an increase in alpha linolenic acid (ALA), where spring pastures exhibited an average of 11.6 mg ALA/100 g tissue, compared to 1.3, 5.0 and 7.0 mg/100 g for summer, autumn and winter, respectively. Increases in ALA aligned to decreases in pasture DM. This is to be expected as ALA is found at higher concentrations within green leafy plant material (Ponnampalam et al., 2014). Alpha linolenic acid is a noteworthy polyunsaturated fatty acid within pasture as it is a precursor to many health claimable long chain fatty acids (omega-3, EPA, DHA) within meat (Ponnampalam et al., 2014).
Table 2. Pasture qualitative analysis (calculated from raw data) in the lead up to each transportation trip. Traits include; dry matter (DM), crude protein (CP), metabolisable energy (ME), acid detergent fibre (ADF), neutral detergent fibre (NDF), digestible dry matter (DDM) and (ALA). Collection periods were at 0, 3 and 6 weeks from each transportation day.

<table>
<thead>
<tr>
<th>Trip number</th>
<th>Season</th>
<th>Week from kill</th>
<th>Paddock number</th>
<th>DM (%)</th>
<th>Grass of desirable grazing length (%)</th>
<th>CP (%)</th>
<th>ME (MJ/kg DM)</th>
<th>ADF (%)</th>
<th>NDF (%)</th>
<th>DDM (%)</th>
<th>ALA (mg/100 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Summer</td>
<td>6</td>
<td>3</td>
<td>1</td>
<td>68</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>1.8</td>
</tr>
<tr>
<td>2 Summer</td>
<td>6</td>
<td>3</td>
<td>1</td>
<td>68</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>0.6</td>
</tr>
<tr>
<td>3 Autumn</td>
<td>6</td>
<td>3</td>
<td>1</td>
<td>88</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>2.8</td>
</tr>
<tr>
<td>4 Autumn</td>
<td>6</td>
<td>3</td>
<td>2</td>
<td>83</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>1.0</td>
</tr>
<tr>
<td>5 Winter</td>
<td>6</td>
<td>3</td>
<td>2</td>
<td>48</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>0.9</td>
</tr>
<tr>
<td>6 Winter</td>
<td>6</td>
<td>3</td>
<td>2</td>
<td>42</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>6.9</td>
</tr>
<tr>
<td>7 Spring</td>
<td>6</td>
<td>3</td>
<td>2</td>
<td>31</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>6.9</td>
</tr>
<tr>
<td>8 Spring</td>
<td>6</td>
<td>3</td>
<td>3</td>
<td>33</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>11.9</td>
</tr>
</tbody>
</table>

*The 6 week sampling period for animals within transport group one was missed*
1.3. Transportation component

1.3.1. Environmental variables

Temperature, humidity and windspeed data collected from within the stock crate during transit varied across the trips (Table 3). Despite this variation, environmental variables were shown to have no effect on behavioural outcomes, indicating suitability for transportation across all seasons. Season had no effect on the behaviour of alpacas across the eight trips ($P = 0.92$). However, it is important to note the temperate climate experienced throughout the trial (minimum average temperature 12.4 ºC and maximum average 27.7 ºC). Further research is required to determine if environmental extremes during transport increase the incidence of behaviours indicative of heat or cold stress.

Table 3. Predicted means (± s.e.) for weather parameters (temperature, humidity and wind speed) recorded within the stock crate across the 8 replicate trips.

<table>
<thead>
<tr>
<th>Trip Number</th>
<th>Season</th>
<th>Temperature (ºC)</th>
<th>Humidity (%)</th>
<th>Windspeed (km/hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Summer</td>
<td>27.7 ± 0.7a</td>
<td>52.3 ± 2.5ad</td>
<td>16.8 ± 1.5a</td>
</tr>
<tr>
<td>2</td>
<td>Summer</td>
<td>21.7 ± 0.7b</td>
<td>52.6 ± 2.4ad</td>
<td>12.8 ± 1.5b</td>
</tr>
<tr>
<td>3</td>
<td>Autumn</td>
<td>19.9 ± 0.7c</td>
<td>84.6 ± 2.4b</td>
<td>17.9 ± 1.6ac</td>
</tr>
<tr>
<td>4</td>
<td>Autumn</td>
<td>16.5 ± 0.7d</td>
<td>69.6 ± 2.5c</td>
<td>15.0 ± 1.6ab</td>
</tr>
<tr>
<td>5</td>
<td>Winter</td>
<td>12.4 ± 0.7c</td>
<td>76.6 ± 2.5c</td>
<td>16.3 ± 3.7ab</td>
</tr>
<tr>
<td>6</td>
<td>Winter</td>
<td>14.2 ± 0.7c</td>
<td>51.5 ± 2.5a</td>
<td>13.4 ± 2.95ab</td>
</tr>
<tr>
<td>7</td>
<td>Spring</td>
<td>22.1 ± 0.7b</td>
<td>33.0 ± 2.7c</td>
<td>14.3 ± 1.6bc</td>
</tr>
<tr>
<td>8</td>
<td>Spring</td>
<td>18.1 ± 0.7d</td>
<td>58.7 ± 2.5d</td>
<td>12.5 ± 1.6bc</td>
</tr>
</tbody>
</table>

Significant differences between means denoted by superscripts. All superscripts were generated on a parameter basis and are not applicable to means in different columns.

1.3.2. Behavioural observation

1.3.2.1. Postural state

Alpacas sat (cush) for 51 % of the observed time when averaged across the 8 trips. This is lower than the period of time estimated by industry (Animal Health Australia, personal communication, August 3rd 2016) and increases the risks of slips and falls during transit. It is unclear from this study alone whether this presents an aversion to the standard cattle flooring utilised within the stock crate for the current study, or if this represents normal proportion of postural behaviour for alpacas in transport. In order to determine if flooring type impacts postural behaviour, the use of grooved rubber matting within the stock crate could be investigated.

The location of alpacas within the truck (front and rear pen) had no effect ($P = 0.140$) on postural state. This suggests that irrespective of within truck location, animals respond similarly to transportation and therefore no alterations to current commercial loading practices are proposed.

1.3.2.2. State behaviours

Road surface had a significant effect ($P < 0.05$) on the proportion of time spent sitting, as well as on levels of aggressive, submissive, alert and unsettled behaviours. In addition, postural state (sitting or standing) affected ($P < 0.001$) the incidence of other state behaviours. For example, aggression doubled when alpacas were sitting rather than standing (14.92 ± 1.3 % and 7.30 ± 0.6 % of the observed time, respectively). This is likely a result of increased stability and proximity to other alpacas while sitting. Aggressive and submissive behaviours while sitting also increased ($P < 0.001$) at roadworks and around bends, when compared to other road surfaces. Aggressive, submissive, alert and unsettled behaviours were observed less during decent.
The level of aggressive behaviour observed between trips differed significantly ($P = 0.013$). The level of aggression in trip eight was the highest of all trips with 24 (± 6.7) % of observations resulting in aggression as the main behaviour. Averaged across postural states, trips two and six resulted in the lowest incidence of aggression at just 5 (± 1.6) % of the total observed time. It should be noted that fleece length was not standardised for this study. Alpacas transported in trip one were fresh off shears, while alpacas transported in trip eight were in full fleece. When analysing the incidence of aggression while sitting across trips, trip one exhibited the lowest proportion of aggressive episodes and trip eight, the highest. This could indicate a relationship between fleece length and aggressive episodes during transport due to increased proximity between alpacas when sitting in full fleece. Fleece length should be considered in any future research that focuses on alpacas during transportation.

Within other red meat species, research has shown that the grouping animals of similar age, size and sex for transport will minimise the incidence of aggressive episodes (Cockram, 1997; Grigor, 1998). In addition, the grouping of livestock prior to transport and the avoidance of mixing groups of animals within a fortnight of transport allows for acclimatisation and the formation of herd structures (Warriss, 1990; Grandin, 1997). This results in fewer aggressive and submissive episodes during transport. While this was not investigated in the current study, it is likely that the same trend would be observed for alpacas and should be taken into account in future studies.

1.3.2.3. Point behaviours

The frequency at which point behaviours were observed varied across the trips. Trip two had the highest number of slips ($n = 122$) which aligned with a lower level of aggression and time spent sitting (just 18 % of time spent sitting across the 4 h journey). Neutral interactions varied across all trips with the highest level being observed in trip one. Trip one also had the highest incidence of negative interactions. Trip six and eight had the highest count of spitting behaviour.

1.4. Blood cortisol analysis

Blood plasma cortisol levels did not vary across season or resting treatments ($P = 0.61$ and 0.11 respectively). Plasma cortisol, averaged across season and lairage treatments, was 1.05 mg/dL at the point of slaughter.

1.5. Carcase traits

Cold carcase weights were lowest in spring and highest in autumn (Table 4). There was a significant relationship between cold carcase weights and evaporative loss. Evaporative loss, also known as chiller loss or shrinkage, refers to weight lost from a carcase as moisture during carcase chilling over 24 hours. Evaporative loss was calculated as the difference between hot carcase weight and cold carcase weight, expressed as a percentage of the initial hot carcase weight. There was no variation in carcase evaporative loss across season or between lairage treatments through 2017. Evaporative loss percentages ranged from 3 – 4% of the initial hot carcase weight across the year which is toward the higher end of that observed for beef and lamb (1 – 3%). However, this is to be expected given the lean nature of the product compared to beef and lamb. Through optimising animal body condition on farm, desirable carcase fat cover and weights can be achieved in plant and in turn, can help to minimise evaporative loss from carcasses.

1.6. Meat quality testing

1.6.1. Fresh colour

Meat colour significantly influences consumer perceptions of freshness and quality (Faustman & Cassens, 1990). Alpaca LT fresh colour did not change across season or as a result of resting, with the exception of decreased redness in spring. Fresh colour parameters averaged 39.4 (± 1.13), 15.7 (± 0.71), 7.7 (± 0.33) for $L^*$ (lightness), $a^*$ (redness) and $b^*$ (yellowness) respectively. Research in lamb has indicated that $L^* > 44$ and $a^* > 14.1$ will result in consumer acceptability of the product (Khliji et
al., 2010). While a* scores within this study exceed the lamb threshold, L* values do not. Further research is required in order to determine exact consumer acceptability thresholds for alpaca meat fresh colour.

### 1.6.2. Glycogen and ultimate pH

Muscle glycogen content provides a snapshot of energy reserves contained within muscle at the point of sampling. Reduced glycogen results in a high pH as muscle enters rigour mortis, and can lead to a dark frim dry end product.

Within the current study, glycogen was lower ($P = 0.008$) in muscles of animals killed in autumn and winter (Table 4). These seasonality trends align with the pasture quality discussed in section 1.2 above. Pasture availability was far greater in both summer and spring. While pasture ME, CP and DDM was down comparative to the other seasons, the ample feed availability would have assisted in increasing muscle glycogen content. Glycogen levels varied significantly between direct and rested animals, with rested animals exhibiting muscle glycogen content of 6.6 mmol/kg lower, on average, than animals sent direct. This could have resulted from increased stressors within the novel lairage environment, as well as the change in nutrition from the southern tablelands to south coast across the year in which the research was conducted.

Average glycogen values were much lower in the current study, at 45.7 (± 2.8) mmol/kg, than that reported in the past for alpacas managed under similar Australian conditions (73.8 mmol/kg; Smith et al., 2017a). The contrast in glycogen content reported between these alpaca studies may be a result of differences in transportation distance and conditions, or nutritional quality in the lead up to slaughter. Alpacas were transported for a greater distance in the current study than the past and were also sourced from an alternate property. Muscle glycogen levels were also significantly lower than that reported for lamb (at 79.93-119.90 mmol/kg). A lower muscle glycogen is to be expected comparative to other red meat species given the glycolytic nature of alpaca muscle. Regardless of lower glycogen values, muscle pH was reduced well below 5.8 in the current study, suggesting that a glycogen concentration of 45.7 (± 2.8) mmol/kg is sufficient to meet a desired pHu in alpaca.

Average pHu across seasons is presented below (Table 4). There was no effect of season or resting on pHu and values align to that found in past studies (Cristofanelli et al., 2004; Salva et al., 2009; Smith et al. 2017a).

### 1.6.3. Drip loss

Water holding capacity refers to the ability of meat to retain moisture under the pressures of gravity, freezing, heating or cooking. The retention of water within meat can influence meat eating quality, with fluid loss resulting also in economic loss where meat is sold on a per kg basis. Drip loss refers to the percentage of fluid lost from meat over a set period of time under gravitational force. Within this study, two methods for the evaluation of drip loss in alpaca meat were investigated. There was no difference ($P = 0.49$) between the accuracy of these two methods (bag and EZ method) to predict drip loss in the alpaca meat. The bag method resulted in an estimation of 3.39 ± 0.24 % drip and EZ method 3.16 ± 0.22 %. Both season (Table 4) and resting had a significant effect ($P = 0.03$) on drip loss percentage when averaged across measurement methods. Muscle from rested animals exhibited higher drip than muscle from animals sent direct to slaughter (3.8 ± 0.18 and 3.2 ± 0.18, respectively).

### 1.6.4. Shear force

Muscle tenderness can be measured objectively through shear force evaluation, where the lower the value (Newtons; N), the more desirable the muscle tenderness.

Within the current study, summer and autumn muscle exhibited more desirable tenderness than winter and spring product (Figure 6). This somewhat contradicts glycogen findings. However, these SF values do reflect the harsh winter and early spring conditions experienced on farm. It is evident that if
on farm feed availability is limiting, animals should be held off from slaughter until sufficient supplementary feeding can be implemented or there has been adequate pasture availability for greater than 4 weeks (as indicated within the current study).

With the exception of autumn, muscle from animals sent direct to slaughter was of more desirable tenderness than product from rested animals (Figure 6). These results align with the glycogen data discussed above and further indicates the negative effect of resting on meat quality.

![Figure 6](image)

**Figure 6.** Shear force values within the alpaca *longissimus thoracis* across season and lairage treatments. Significant differences between means denoted by superscripts.

1.6.5. Purge and cooking loss

Purge is an indication of the moisture loss during aging (presented as a percentage of the fresh muscle, or un-aged, weight). Moisture lost as purge within the current study followed the same trend found for drip loss above, with summer purge percentage being greater than in other seasons (Table 4). Product from animals sent direct to slaughter lost significantly less ($P = 0.02$) moisture than animals that were rested. There are many potential explanations for this and it is likely that events occurring early in post-mortem chilling (resulting from greater energy reserves, lower stress etc.) are playing a significant role in this outcome.

There was no seasonal effect ($P = 0.22$) on cooking loss percentage (Table 4). Product from rested animals tended to have higher cooking loss than product from animals sent direct (21.4 ± 0.36 % and 20.3 ± 0.36 % respectively).

When moisture is lost from the product, which can occur at numerous points including carcase chilling, ageing and cooking, valuable proteins can be lost from the meat. The resultant product will be less juicy than product that has lost less moisture.

1.6.6. Intramuscular fat (IMF) and muscle fatty acid (FA)

*Longissimus lumborum* IMF did not change ($P = 0.11$; Table 4) across the year for animals sent direct to slaughter. Intramuscular fat averaged 1.3 (± 0.08) % across seasons, which higher than values previously reported for Australian alpacas (0.58 - 0.66 %; Smith et al., 2017a; Smith et al., 2017b). Alpaca IMF remains well below IMF % reported for other red meat species, with grass fed beef reported to contain 5 % IMF (Scollan et al., 2006) and lamb 4 – 5 % (Hopkins et al., 2006).
The concentration of both saturated (SFA) and unsaturated fatty acids (USFA) within the current study (Table 5) were greater than that previously reported for alpaca (Smith et al., 2017b). This could be a reflection of the increased IMF values observed within the current study, or a result of differing production systems. Animals were grazed on pastures located on the southern tablelands of NSW during the current study, while past studies looked at alpacas grazed on the south coast of NSW. Of notable importance is the low omega6:omega3 ratio and concentration of health claimable long chain fatty acids (omega-3, DHA, EPA). An omega6:omega3 ratio of < 4.0 has been linked to reduced incidence of cardiovascular disease and obesity within western society (Simopoulos, 2002).

Table 5. Average concentration (mg/100g) of common fatty acids within alpaca longissimus thoracis

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Concentration (mg/100 g meat)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C20:5n-3 (EPA)</td>
<td>23.48</td>
</tr>
<tr>
<td>C22:5n-3 (DPA)</td>
<td>16.14</td>
</tr>
<tr>
<td>C22:6n-3 (DHA)</td>
<td>1.20</td>
</tr>
<tr>
<td>EPA + DHA</td>
<td>24.68</td>
</tr>
<tr>
<td>EPA + DHA + DPA</td>
<td>40.82</td>
</tr>
<tr>
<td>Omega-6</td>
<td>98.33</td>
</tr>
<tr>
<td>Omega-3</td>
<td>44.80</td>
</tr>
<tr>
<td>Σ PUFA</td>
<td>24.51</td>
</tr>
<tr>
<td>Σ MUFA</td>
<td>458.03</td>
</tr>
<tr>
<td>Σ USFA</td>
<td>482.54</td>
</tr>
<tr>
<td>Omega 6:Omega 3</td>
<td>2.10</td>
</tr>
<tr>
<td>PUFA:SFA</td>
<td>0.05</td>
</tr>
</tbody>
</table>

2. The effect of combining tenderstrectching and electrical stimulation on alpaca meat tenderness and eating quality

2.1. Animal live weight and body condition

Animals were an average 53.8 (± 6.0) kg and body condition score 3. Animals gained an average of 2.6 kg in the 2 weeks leading up to slaughter (0.19 kg/day). Body condition did not change across the collection period.

2.2. Pasture

Pasture quality improved over the 5 week collection period, with CP and ME increasing and fibre content (NDF and ADF) decreasing (Table 6). This aligns with the animal weight gains and maintenance of body condition throughout the collection period. Vitamin E content of pasture peaked after the observed peak in pasture ME values (Table 6).
Pasture qualitative analysis (calculated from raw data) in the lead up to animal processing. Traits include; dry matter (DM), crude protein (CP), neutral detergent fibre (NDF), acid detergent fibre (ADF), digestible dry matter (DDM), metabolisable energy (ME), and vitamin E. Collection periods were at 0, 3 and 5 weeks from each kill (or processing) day.

<table>
<thead>
<tr>
<th>Collection Period</th>
<th>DM %</th>
<th>CP %</th>
<th>NDF %</th>
<th>ADF %</th>
<th>DDM %</th>
<th>ME (MJ/kg DM)</th>
<th>Vitamin E (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kill 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 wk</td>
<td>78</td>
<td>9</td>
<td>67</td>
<td>40</td>
<td>54</td>
<td>7.2</td>
<td>57.2</td>
</tr>
<tr>
<td>3 wk</td>
<td>87</td>
<td>10</td>
<td>68</td>
<td>42</td>
<td>54</td>
<td>7.1</td>
<td>96.3</td>
</tr>
<tr>
<td>0 wk</td>
<td>33</td>
<td>17</td>
<td>60</td>
<td>37</td>
<td>61</td>
<td>8.3</td>
<td>19.1</td>
</tr>
<tr>
<td>Kill 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 wk</td>
<td>44</td>
<td>21</td>
<td>54</td>
<td>32</td>
<td>66</td>
<td>9.2</td>
<td>22.2</td>
</tr>
<tr>
<td>3 wk</td>
<td>64</td>
<td>18</td>
<td>52</td>
<td>39</td>
<td>60</td>
<td>8.1</td>
<td>129.5</td>
</tr>
<tr>
<td>0 wk</td>
<td>63</td>
<td>14</td>
<td>61</td>
<td>33</td>
<td>62</td>
<td>8.5</td>
<td>178.5</td>
</tr>
</tbody>
</table>

**2.3. Meat quality testing**

**2.3.1. pH decline, glycogen and ultimate pH**

The pH decline measurement in muscle aims to capture the decrease in muscle pH caused by glycolysis and lactic acid production as muscles enter into rigour mortis. It is a useful tool for determining the effectiveness of electrical stimulation and has been utilised as a predictor of shear force in lamb (Thompson et al., 2005b).

In the current study, ES resulted in a more rapid pH decline within the ST and LL when compared to non-ES carcases (Figure 7), the magnitude of which was in line with that reported for alpaca in the past (pH difference at 36 ºC of 0.14 between ES and non-ES). This rapid decline supports findings that ES in the alpaca carcase will influence pH decline equivalently to other red meat species (Smith et al., 2016). However, both LL and ST pH did not fall to 6 until muscle temperature had stabilised at approximately 5 ºC on the day of slaughter. This is in contrast to lamb, where optimal tenderness is achieved when carcase pH reaches 6 across the temperature range 18 ºC to 35 ºC (Thompson et al., 2005b). It is evident from both the current study, and past studies in alpaca where pH decline has been reported, that alpaca pH decline will differ to that of other red meat species. It is also evident that there is no adverse effect of this on alpaca muscle shear force or eating quality. As a result, pH at 24 h may act as a better predictor for alpaca muscle shear force than measurement of pH over the decline period in future research. Despite this, measurement of the pH decline within the muscle still aids with testing the operation of processing equipment and in assessing the effectiveness of stimulation.
Figure 7. The effect of medium voltage electrical stimulation and hang method (Achilles hung, AH; and Tenderstretch, TS) on pH decline in the alpaca longissimus lumborum (top) and from medium voltage electrical stimulation on pH decline in the alpaca semitendinosus (bottom) from 36 to 3 °C. The solid line indicates the fitted pH trend across the decline period, based on predicted pH averages. The dotted line at pH 6 demonstrates that alpaca muscle pH did not reach the predicted window expected of lamb (to reach pH 6 at 18 – 35 °C) within the measurement period.

Ultimate pH ranged from 5.5 (± 0.06) in the LT to 5.7 (± 0.04) in the ST, which aligns to experiment one findings. Glycogen values averaged 45.5 (± 2.8) mmol/kg and were in line with values reported for experiment one. However, pH once again fell to the desired level, despite glycogen being lower than values observed for alpaca in the past (73.8 mmol/kg; Smith et al., 2017). This supports the notion that a glycogen concentration around 45 mmol/kg is not limiting to pH decline within the alpaca muscle.

2.3.2. Fresh colour

Electrical stimulation increased LL fresh colour lightness, redness, and yellowness (Table 7), which supports previous findings for Australian alpaca meat (Smith et al., 2016). Hang method increased (P = 0.018) b* fresh colour, with an additional trend (P = 0.063) toward increased a* values. As outlined in experiment one, research in lamb has shown that L* > 44 and a* > 14.1 will result in consumer acceptability of the lamb product. Fresh colour within this study did not exceed this threshold, supporting experiment one findings. It is evident that alpaca exhibits unique colour parameters and further research is required into consumer acceptability of these parameters, particularly if the industry moves toward a fresh meat market.
Table 7. Predicted means ± standard errors for lightness (L*), redness (a*) and yellowness (b*) fresh colour parameters of the alpaca longissimus lumborum each treatment level (stimulation and hang)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Stimulation</th>
<th>Hang</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>L*</td>
<td>39.79 ± 0.33a</td>
<td>36.54 ± 0.33b</td>
</tr>
<tr>
<td>a*</td>
<td>13.15 ± 0.42a</td>
<td>11.81 ± 0.42b</td>
</tr>
<tr>
<td>b*</td>
<td>0.66 ± 0.15a</td>
<td>-0.70 ± 0.15b</td>
</tr>
</tbody>
</table>

Significant differences between means denoted by superscripts. Superscripts were generated on an individual colour parameter level and are not applicable to means in different rows.

2.3.3. Retail colour

Product colour changed (P < 0.001) across the retail display period, with significant increases in L*, a* and b* values from 0 – 24 hr, followed by a gradual decline thereafter (Figure 8). Stimulation altered retail colour, with both L* and b* being greater for stimulated (38.96 ± 1.15 and 15.96 ± 0.35 for L* and b* respectively) than non-stimulated (37.87 ± 1.15 and 15.40 ± 0.35 L* and b* respectively) product. This confirms that alpaca responds to retail display across time similarly to other red meat species, while retaining unique colour parameters.

Oxy/met ratio values across the retail display period averaged 4.5, 4.8, 4.4 and 4.1 (± 0.10) at 0, 24, 48 and 72 h respectively. This oxy/met ratio is a reflection of meat surface brownness and is linked to the oxidation of red oxymyoglobin to brown metmyoglobin (Mancini & Hunt, 2005). A lower ratio value reflects increased surface brownness. The levels within this study are well above thresholds reported for decreased consumer acceptability in lamb (> 3.3; Khliji et al., 2010). These results demonstrate the potential retail display advantages of alpaca and indicate limited oxidation across the retail display period.

Figure 8. Changes to lightness (L*), redness (a*) and yellowness (b*) colour parameters of the alpaca longissimus thoracis during simulated retail display. Measurements were taken at 0, 24, 48 and 72 h. Significant differences between means denoted by superscripts. Superscripts were generated on an individual trait basis, as indicated by the use of upper case, lower case and asterix.
2.3.4. Sarcomere length and shear force

Electrical stimulation increased LT sarcomere length, while tenderstretching lengthened AF and SM sarcomeres (Table 8). The combination of processing techniques had a highly significant effect on sarcomere length within the TL, with AH + ES muscle exhibiting the greatest length out of any treatment group (2.67 ± 0.07 µm). Muscle from TS + ES carcases was statistically similar to muscle exposed to the control (AH + no ES). This suggests that the application of ES to TS carcases may aid in overcoming any potential shortening within the TL when TS is applied to alpaca carcases.

Table 8. Predicted means ± standard errors for muscle sarcomere length at each treatment level (stimulation and hang) on an individual muscle basis (m. longissimus thoracis, LT; m. adductor femoris, AF; m. semimembranosus, SM; m. semitendinosus, ST)

<table>
<thead>
<tr>
<th>Muscle</th>
<th>Stimulation</th>
<th>Hang</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes</td>
<td>No</td>
<td>AH</td>
<td>TS</td>
</tr>
<tr>
<td>LT</td>
<td>1.83 ± 0.029a</td>
<td>1.74 ± 0.029b</td>
<td>1.78 ± 0.029a</td>
<td>1.79 ± 0.030a</td>
</tr>
<tr>
<td>AF</td>
<td>1.90 ± 0.046a</td>
<td>1.83 ± 0.045a</td>
<td>1.75 ± 0.045b</td>
<td>1.98 ± 0.046a</td>
</tr>
<tr>
<td>SM</td>
<td>2.10 ± 0.044a</td>
<td>1.96 ± 0.045b</td>
<td>1.82 ± 0.044b</td>
<td>2.24 ± 0.046a</td>
</tr>
<tr>
<td>ST</td>
<td>2.24 ± 0.054a</td>
<td>2.16 ± 0.054a</td>
<td>2.18 ± 0.054a</td>
<td>2.21 ± 0.055a</td>
</tr>
</tbody>
</table>

Significant differences between means denoted by superscripts. All superscripts were generated on an individual muscle by treatment level and are not applicable to means across muscles.

Trends observed for sarcomere length followed through to muscle shear force outcomes. Stimulation significantly improved ($P < 0.05$) tenderness of the LT and TL (by 22.9 and 6.4 N respectively; Figure 9), while it had no effect ($P > 0.05$) on hindquarter muscles. The more desirable tenderness of the hindquarter muscles in comparison to the loin should be noted. Results indicate that through the application of ES, LT shear force can be reduced (i.e. tenderness improved) to near that of hindquarter muscles.

Figure 9. The effect of electrical stimulation on the alpaca *longissimus thoracis* (loin), *adductor femoris*, *semimembranosus* (topside) and *psoas major* (tenderloin). Significant differences between means denoted by superscripts. All superscripts were generated on an individual muscle by treatment level and are not applicable to means across muscles.
Tenderstretching improved tenderness in the hindquarter (Figure 10; excluding the ST), while there was a trend ($P = 0.08$) toward improved tenderness of the LT. It is important to note that tenderstretching did not affect TL shear force, which supports the findings outlined above regarding sarcomere length and is positive given the commonly reported negative effects of TS on TL shear force in beef and lamb. In addition, a significant hang by shear force block location interaction was observed. The result was such that shear force values for block 1 were more in line with values for block 2 (being of more desirable tenderness) when carcases were TS, thereby decreasing within muscle variation (Figure 11). These findings indicate that TS has the potential to not only improve the tenderness of the alpaca hindquarter, but also the consistency. When combined, ES and TS significantly improve product tenderness when considering multiple muscles. Through application to a whole carcase, TS benefits may be increased with additional neck and shoulder weight.

Figure 10. The effect of tenderstretching on the alpaca *longissimus thoracis* (loin), *semimembranosus* (topside) and *psoas major* (tenderloin). Significant differences between means denoted by superscripts. All superscripts were generated on an individual muscle by treatment level and are not applicable to means across muscles.

Figure 11. The effect of tenderstretching within the *adductor femoris* (additional hindquarter muscle). N.B. number ‘1’ and ‘2’ equate to ‘sample 1’ and ‘sample 2’ - two separate samples collected from different locations within the same muscle (Figure 4). Significant differences between means denoted by superscripts.
2.3.5. Purge and cooking loss

Electrical stimulation reduced purge within 10 d aged LL (Table 9). Purge loss within the AF ranged from 7.8 (± 0.51) % to 9.3 (± 0.50) % for TS and AH caudal muscle (sample 2). It is evident that both alpaca LL and hindquarter muscles exude higher purge than other red meat species during aging, with electrical stimulation partially assisting in purge reduction.

Electrical stimulation reduced cooking loss in the LL, while hang had no effect. Tenderstretching reduced SM and AF cooking loss by 1.1 and 0.8 %, respectively. Cooking loss in the TL was increased due to TS and reduced as a result of ES application. These findings demonstrate once again that through combining ES with TS in alpacas, the potentially negative effects of TS on TL quality can be minimised.

Table 9. Predicted means ± standard errors at each treatment level (stimulation and hang) and on an individual muscle basis (m. longissimus thoracis, LT; m. longissimus lumborum, LL; m. adductor femoris, AF; m. semimembranosus, SM; m. semitendinosus, ST; m. psoas major, TL) for ultimate pH, purge, and cooking loss.

<table>
<thead>
<tr>
<th>Muscle and Trait</th>
<th>Stimulation</th>
<th>Hang</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes</td>
<td>No</td>
<td>AH</td>
<td>TS</td>
</tr>
<tr>
<td>LT Ultimate pH</td>
<td>5.54 ± 0.06</td>
<td>5.51 ± 0.06</td>
<td>5.52 ± 0.06</td>
<td>5.53 ± 0.06</td>
</tr>
<tr>
<td>LL Purge (%)</td>
<td>8.35 ± 0.69</td>
<td>10.68 ± 0.68</td>
<td>9.91 ± 0.68</td>
<td>9.12 ± 0.69</td>
</tr>
<tr>
<td></td>
<td>21.40 ± 0.39</td>
<td>23.11 ± 0.39</td>
<td>22.11 ± 0.39</td>
<td>22.40 ± 0.40</td>
</tr>
<tr>
<td>AF Ultimate pH</td>
<td>5.58 ± 0.03</td>
<td>5.59 ± 0.03</td>
<td>5.58 ± 0.03</td>
<td>5.59 ± 0.03</td>
</tr>
<tr>
<td>Purge (%)</td>
<td>8.70 ± 0.46</td>
<td>8.75 ± 0.46</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>22.63 ± 0.99</td>
<td>23.17 ± 0.99</td>
<td>23.29 ± 0.99b</td>
<td>22.50 ± 0.99a</td>
</tr>
<tr>
<td>SM Ultimate pH</td>
<td>5.60 ± 0.04</td>
<td>5.60 ± 0.04</td>
<td>5.58 ± 0.04</td>
<td>5.62 ± 0.04</td>
</tr>
<tr>
<td>Cooking loss (%)</td>
<td>20.34 ± 0.48a</td>
<td>21.03 ± 0.48a</td>
<td>21.25 ± 0.48b</td>
<td>20.12 ± 0.49a</td>
</tr>
<tr>
<td>ST Ultimate pH</td>
<td>5.74 ± 0.08</td>
<td>5.75 ± 0.07</td>
<td>5.74 ± 0.07</td>
<td>5.75 ± 0.07</td>
</tr>
<tr>
<td>Cooking loss (%)</td>
<td>19.22 ± 0.53a</td>
<td>19.17 ± 0.53a</td>
<td>19.45 ± 0.53a</td>
<td>18.93 ± 0.53a</td>
</tr>
<tr>
<td>TL Ultimate pH</td>
<td>5.62 ± 0.04</td>
<td>5.60 ± 0.04</td>
<td>5.62 ± 0.04</td>
<td>5.61 ± 0.04</td>
</tr>
<tr>
<td>Cooking loss (%)</td>
<td>19.50 ± 0.44a</td>
<td>21.57 ± 0.44a</td>
<td>19.81 ± 0.43a</td>
<td>21.26 ± 0.44b</td>
</tr>
</tbody>
</table>

Significant differences between means denoted by superscripts. All superscripts were generated on an individual muscle by treatment level and are not applicable to means in different rows

1 Inclusive of the ageing cofactor
2 Values averaged across AF block location

2.3.6. Particle size

Average particle size for muscles across treatment levels are presented in the below table (Table 10). The TL presented the lowest PS of all muscles, which aligns with its low shear force value and desirable tenderness. While there was some muscle to muscle PS variation, muscles were generally of similar PS with the largest variation resulting from processing treatments (Table 10). Electrical stimulation significantly improved loin PS, aligning it with TL PS (Table 10). There was a significant hang effect on SM particle size, improving it by 22.1 µm. Trends in PS reduction align with the improvements in overall muscle tenderness, further supporting the positive impacts of TS + ES on carcase quality when considering multiple muscles.
Table 10. Predicted means ± standard errors for average muscle particle size (µm) at each treatment level (stimulation and hang) on an individual muscle basis (m. longissimus thoracis, LT; m. adductor femoris, AF; m. semimembranosus, SM; m. semitendinosus, ST; m. psoas major, TL)

<table>
<thead>
<tr>
<th>Muscle</th>
<th>Stimulation</th>
<th>Hang</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ES</td>
<td>No ES</td>
</tr>
<tr>
<td>LT¹</td>
<td>123.3 ± 2.22²</td>
<td>148.3 ± 2.67³</td>
</tr>
<tr>
<td>AF²</td>
<td>145.6 ± 2.62³</td>
<td>146.9 ± 2.64³</td>
</tr>
<tr>
<td>SM</td>
<td>141.3 ± 2.54³</td>
<td>152.4 ± 2.74³</td>
</tr>
<tr>
<td>ST</td>
<td>162.9 ± 2.93³</td>
<td>153.1 ± 2.76³</td>
</tr>
<tr>
<td>TL</td>
<td>122.7 ± 2.21³</td>
<td>127.1 ± 2.29³</td>
</tr>
</tbody>
</table>

Significant differences between means denoted by superscripts. Upper-case superscripts denote significance between muscles and stimulation level. Lower-case superscripts denote significance between muscles and hang level. All superscripts were generated on a stimulation by muscle and hang by muscle basis.

1Values averaged for aging period
²Values averaged for the two test locations within AF

2.3.7. Muscle vitamin E

Average vitamin E concentration within the LT and AF was 5.4 ± 0.62 and 6.1 ± 0.62 mg/kg meat. Within beef and lamb, 2 mg vitamin E/kg muscle has been reported as the threshold above which there is a detectable positive impact on protein and lipid oxidation rates (Ponnampalam et al., 2014). However, Vitamin E content had no significant impact on colour stability or lipid oxidation traits within the current study. This is likely due to the limited overall oxidation observed in the current study (well below 3 mg MDA/kg meat, outlined in section 2.3.8.) and excessive vitamin E levels found within alpaca muscle. This provides alpaca meat with an advantage if a retail market develops, given consumers heavily discount oxidised red meat.

2.3.8. Lipid oxidation

TBARS values within the current study (Table 11) align with results from past alpaca research (Smith et al., 2017b) and indicate that alpaca undergoes little oxidation throughout retail display. Oxidation levels remain well below the reported 3 mg MDA/kg meat reported as detectable by consumers in lamb (Ponnampalam et al., 2012). This aligns with the retail stability findings above, where there was little change to colour over time and high oxy/met ratios across the display period. The limited oxidation of alpaca meat during retail display is advantageous should the alpaca meat market shift toward sales of fresh product direct to consumers.

Electrically stimulated LT was significantly (P = 0.036 and 0.026) more oxidized (higher TBARS values) than non-ES product at the point of both pre-retail and post-retail display (Table 11). Hang method had no effect on LT oxidation. The same trend was observed for LL samples collected at the point of sensory analysis, with ES product being 0.15 (± 0.13) mg MDA/kg meat higher (P < 0.001) than non-ES product (Table 11). Oxidation levels for pre-retail display AF samples were 0.26 (± 0.07) mg MDA/kg greater (P = 0.007) for AH product than TS product, while ES had no effect. However, by the conclusion of the 72 h retail display period, there was no longer a difference between hang methods. Conversely, ES product was 0.22 (± 0.12) mg MDA/kg higher (P = 0.030) than non-ES product at the point of post-retail sampling, despite having no difference on muscle oxidation initially.
Table 11. The effect of medium voltage electrical stimulation and hang method on thiobarbituric acid reactive substances (TBARS) in the alpaca *longissimus thoracis* and *adductor femoris*.

<table>
<thead>
<tr>
<th>Period and muscle</th>
<th>Stimulation</th>
<th>Hang</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>TS</td>
<td>AH</td>
</tr>
<tr>
<td>Pre-display</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LT</td>
<td>1.00 ± 0.09b</td>
<td>0.81 ± 0.09a</td>
</tr>
<tr>
<td>AF</td>
<td>0.89 ± 0.08a</td>
<td>0.87 ± 0.08a</td>
</tr>
<tr>
<td>Post-display</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LT</td>
<td>1.25 ± 0.07b</td>
<td>1.05 ± 0.07a</td>
</tr>
<tr>
<td>AF</td>
<td>1.32 ± 0.12b</td>
<td>1.10 ± 0.12a</td>
</tr>
<tr>
<td>Pre-sensory</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LL</td>
<td>0.84 ± 0.13b</td>
<td>0.69 ± 0.13a</td>
</tr>
</tbody>
</table>

Significant differences between means denoted by superscripts. All superscripts were generated on an individual muscle by treatment level and are not applicable to means in different rows.

2.3.9. Sensory evaluation

Consumers rated ES product 14.9 units higher for tenderness, 8.6 units higher for juiciness, 7.5 units higher for flavour and 10.4 units higher for overall liking than non-ES product (Figure 12). Rounding of consumer rating scores to the nearest whole number to reflect the statements indicated by consumers, it can be noted that ES product was rated ‘better than every day’ when compared to the non-ES product at ‘good every day’. These results indicate that consumers can not only detect ES product, but that they have a significantly more enjoyable eating experience when consuming the ES product.

Hang had no effect on consumer responses, which is not surprising given that LT shear force was also unaffected by hang method. To date, all research that has reported the effects of hang method on LTL tenderness in alpaca has utilized split carcasses, where the neck has been removed. It is possible that split carcasses, without the weight generated by the neck, lack the weight required to place tension on muscle fibres of the alpaca LL. Therefore, there is potential for an improved response to TS treated product when applied to whole carcasses in future.

Despite ES increasing product oxidation, this was not detected by consumers. When analysed within the sensory model, TBARS values did not affect sensory responses (*P* = 0.142, 0.948, 0.169, 0.294, 0.145 for tenderness, juiciness, flavour, overall liking and overall rating, respectively). This is likely due to overall TBARS values for the current study being very low, in particular being well below the minimum threshold reported to negatively affect lamb eating quality (Ponnampalam et al., 2012). Once again this outlines a potential retail advantage of alpaca meat. In addition, it reinforces the suitability of ES application to alpaca carcasses as there are little to no negative observable impacts.

This is the first study investigating the response of consumers to TS alpaca LL. Given that there is no adverse effect of TS on consumer responses, and that ES product rates higher for all parameters, it can be concluded that consumers rate TS + ES alpaca product higher than AH + non-ES product for all desirable eating quality traits.
Figure 12. The effect of electrical stimulation (ES) and hang method (Achilles Hung; AH and Tenderstretch; TS) on consumer sensory evaluation of 10 day aged alpaca longissimus lumborum. Significant differences between means denoted by superscripts. Superscripts were generated on an individual trait by treatment level basis and are not applicable to means across traits.

3. Development of best practice methods for alpaca carcase processing and the assessment enzymatic infusion on alpaca loin tenderness

3.1. On-farm

Animals were an average of 23 (± 0.2) months of age, weighing 52.6 (± 1.1) kg and averaging a BCS of 2.75 (± 0.03) at the point of slaughter. Alpacas gained an average of 4.8 (± 0.3) kg and a BCS of 0.25 (± 0.05; from 2.5 to 2.75) in the lead up to slaughter.

3.2. Pasture

Pasture quality varied most considerably (ME and CP increased by 2 units along with a reduction in fibre content and increased digestible dry matter) between the first and second collection weeks i.e. in the two weeks leading up to kill one. Quality remained constant across the following fortnight in the lead up to the second kill week (Table 12).

Table 12. Pasture qualitative analysis (calculated from raw data) in the lead up to animal processing. Traits include; dry matter (DM), crude protein (CP), neutral detergent fibre (NDF), acid detergent fibre (ADF), digestible dry matter (DDM), metabolisable energy (ME), and vitamin E. Collection periods were at 0 and 2 weeks from each kill (or processing) day.

<table>
<thead>
<tr>
<th>Collection Period</th>
<th>DM %</th>
<th>CP %</th>
<th>ME (MJ/kg DM)</th>
<th>NDF %</th>
<th>ADF %</th>
<th>DDM %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kill 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 wk</td>
<td>40</td>
<td>22</td>
<td>7.8</td>
<td>58</td>
<td>42</td>
<td>58</td>
</tr>
<tr>
<td>0 wk</td>
<td>22</td>
<td>24</td>
<td>10.0</td>
<td>41</td>
<td>28</td>
<td>71</td>
</tr>
<tr>
<td>Kill 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 wk</td>
<td>22</td>
<td>24</td>
<td>10.0</td>
<td>41</td>
<td>28</td>
<td>71</td>
</tr>
</tbody>
</table>
3.3. Carcase data

Cold carcase weight averaged 32.4 (± 0.8) kg across the two kill days. Dressing percentage averaged 65 (± 1.2) % and carcase evaporative loss averaged 3.5 (± 0.1) %.

3.4. Meat quality testing

3.4.1. Glycogen and ultimate pH

Muscle glycogen content averaged 153.0 (± 5.82) mmol/kg, which is significantly higher than that reported for both experiment one and two. Ultimate pH values averaged 5.5 (± 0.05) in the LL and 5.6 (± 0.05) in the SM, regardless of processing or infusion treatments. This is again in line with the earlier experiments.

3.4.2. Fresh colour

As observed in experiment two, the ES + TS treatment significantly (P < 0.001) increased muscle L*, a* and b* values (Table 13). These results emphasise the positive effects of combined processing treatment application on the alpaca LL, particularly in improving lightness and redness scores toward thresholds acceptable in lamb. However, there is still a need to develop consumer acceptability thresholds specific to alpaca given its unique colour parameters if moving toward a fresh market.

Table 13. The effect of processing treatments (no electrical stimulation + Achilles hung; NES + AH and electrical stimulation + tenderstretch; ES + TS) on lightness (L*), redness (a*) and yellowness (b*) fresh colour parameters of the alpaca longissimus lumborum.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>NES + AH</th>
<th>ES + TS</th>
</tr>
</thead>
<tbody>
<tr>
<td>L*</td>
<td>35.8 ± 0.96b</td>
<td>39.2 ± 0.96a</td>
</tr>
<tr>
<td>a*</td>
<td>10.8 ± 0.55b</td>
<td>12.0 ± 0.55a</td>
</tr>
<tr>
<td>b*</td>
<td>8.4 ± 0.54b</td>
<td>9.5 ± 0.54a</td>
</tr>
</tbody>
</table>

Significant differences between means denoted by superscripts. Superscripts were generated on an individual colour parameter level and are not applicable to means in different rows.

3.4.3. Retail colour

Product colour change over the retail period followed the same trend as observed in experiment two with a significant increase (P < 0.001) across parameters from 0 to 24 hours, followed by a minor decline over the next 36 hours. Oxy/met ratio values across the retail display period averaged 4.2, 4.0, 3.8 and 3.5 (± 0.07) at 0, 24, 48 and 72 h respectively. As observed in experiment two, oxy/met values remained above thresholds reported for lamb (> 3.3; Khliji et al., 2010) across the display period, reinforcing the potential retail display advantages of alpaca when compared to other red meat species.

Processing treatments did not alter L*, a*, b* or ratio values (P = 0.32, 0.79, 0.66, 0.72 respectively), contrasting the findings of experiment two. Overall, colour parameters within the current study are higher than that observed in experiment two. This may explain the lack of processing treatment effect, with the current parameters exceeding that of ES product within experiment two.

Infusion treatments had a significant effect (P < 0.001) on LT L*, a* and ratio values (Table 14). In general, the kiwi enzyme increased lightness and decreased redness, as did water. This can be linked to increased moisture on the surface of the meat altering surface reflectance of light. Infusion with both water and enzyme resulted in increased surface brownness.
Table 14. The effect of infusion treatments (no infusion, No; infusion with water, Water; and infusion with actinidain enzyme; Enzyme) on longissimus thoracis lightness (L*), redness (a*), yellowness (b*) and oxy/met ratio colour parameters during simulated retail display. Values are averaged across processing treatments and measurement period.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Infusion treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No</td>
</tr>
<tr>
<td>L*</td>
<td>41.0 ± 0.52(^c)</td>
</tr>
<tr>
<td>a*</td>
<td>17.5 ± 0.30(^a)</td>
</tr>
<tr>
<td>b*</td>
<td>16.0 ± 0.43(^a)</td>
</tr>
<tr>
<td>Ratio</td>
<td>3.6 ± 0.09(^b)</td>
</tr>
</tbody>
</table>

Significant differences between means denoted by superscripts. Superscripts were generated on an individual colour parameter level and are not applicable to means in different rows.

3.4.4. Sarcomere length and shear force

There was a significant (P < 0.001) processing treatment by muscle interaction, where processing treatments increased sarcomere length within both muscles, but within the SM to a greater extent than the LT. Processing treatments increased sarcomere length from 1.69 ± 0.04 to 1.82 ± 0.04 µm, while SM sarcomere lengths increased from 1.79 ± 0.06 to 2.23 ± 0.06 µm. This is to be expected given the dual effects exerted on muscle by the combination of ES with TS. Results align to those in experiment two and clearly demonstrate the positive effects of combined processing methods.

Processing improved (P < 0.001) LT shear force by 18.5 (± 2.8) N and SM shear force by 25.6 (± 3.7) N. Average SF values for non-treated LT and SM were 85.8 (± 2.8) N and 70.7 (± 3.6) N, respectively. The processing treatment effect within the LT is in line with that observed in experiment two, while effects within the SM are much greater. These results, supported by positive outcomes within the additional muscles studied in experiment 2, clearly demonstrate that advantages of applying electrical stimulation in combination with tenderstretching to the alpaca carcase. Maximum gains can be made in product quality and consistency when considering the whole carcase basis.

The kiwi enzyme significantly (P < 0.001) improved LT shear force while infusion with water did not vary from the control. This suggests that the improvement in muscle tenderness due to enzyme infusion is a result of enzymatic action as opposed to mechanical force. Longissimus thoracis shear force values were 80.2 (± 2.5), 81.0 (± 2.5) and 64.0 (± 2.5) for no (control), water and enzyme infusion treatments respectively.

3.4.5. Cooking loss

Cooking loss was lower by 1.7 % for ES + TS product than NES + AH (within the LT only), aligning with results from experiment two. Averaged across processing treatments, LT cooking loss was 27.9 (± 0.3) % and SM 21.9 (± 0.4) %. Processing treatments did not change cooking loss within the SM. Cooking loss was higher by 2.3 % in infused LT (water and enzyme) than non-infused. These results again highlight the advantages of applying combined processing techniques.

3.4.6. Particle size

There was a significant difference (P = 0.001) between SM and LT PS (176.9 ± 7.34 and 151.7 ± 4.62 µm respectively). These values are higher than that reported in experiment two for the equivalent muscles. Processing and infusion treatments did not affect muscle particle size. While this does not align with that found in experiment two, it should be noted that muscle shear force was positively impacted by ES and suggests a lack of proteolytic effects.
3.4.7. Sensory evaluation

Processing treatments had a positive effect on tenderness ($P = 0.002$) and flavour ($P = 0.03$) ratings. Despite improving lab based shear force, enzyme infusion had no effect ($P = 0.10$) on consumer tenderness scores. Likewise, infusion did not affect flavour. For juiciness, processing treatments tended ($P = 0.06$) toward higher scores for TS + ES treated product, while infusion treatments resulted in lower ($P = 0.04$) consumer scores than the control (no infusion; Table 15). Both processing and infusion treatments influenced the overall liking outcomes ($P = 0.01$ and $0.05$, respectively), within processing treatments improving scores and infusion reducing scores (Table 15). Overall rating was only affected by processing treatments ($P = 0.01$). It is evident that the most significant improvement in sensory scores resulted from the application of TS and ES. Infusion treatments had little to no effect ($P = 0.10, 0.04, 0.10, 0.05$ and $0.19$ across the five quality traits) on overall eating quality.

Table 15. The effect of processing treatments and infusion with water and actinidin on consumer sensory evaluation of 10 day aged alpaca *longissimus lumborum*.

<table>
<thead>
<tr>
<th></th>
<th>Achilles + no stim</th>
<th>Tenderstretch + stim</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Tenderness</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>$58.7 \pm 5.39^{bc}$</td>
<td>$69.5 \pm 5.41^{a}$</td>
</tr>
<tr>
<td>Water</td>
<td>$51.4 \pm 5.40^{d}$</td>
<td>$68.0 \pm 5.39^{ab}$</td>
</tr>
<tr>
<td>Enzyme</td>
<td>$56.3 \pm 5.37^{cd}$</td>
<td>$70.1 \pm 5.36^{a}$</td>
</tr>
<tr>
<td><strong>Juiciness</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>$56.3 \pm 5.01^{ab}$</td>
<td>$62.6 \pm 5.02^{a}$</td>
</tr>
<tr>
<td>Water</td>
<td>$52.7 \pm 5.02^{b}$</td>
<td>$58.2 \pm 5.01^{ab}$</td>
</tr>
<tr>
<td>Enzyme</td>
<td>$52.0 \pm 4.99^{b}$</td>
<td>$57.4 \pm 4.98^{ab}$</td>
</tr>
<tr>
<td><strong>Flavour</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>$57.2 \pm 4.76^{b}$</td>
<td>$65.0 \pm 4.77^{a}$</td>
</tr>
<tr>
<td>Water</td>
<td>$55.9 \pm 4.77^{b}$</td>
<td>$60.8 \pm 4.77^{ab}$</td>
</tr>
<tr>
<td>Enzyme</td>
<td>$55.2 \pm 4.71^{b}$</td>
<td>$59.5 \pm 4.73^{b}$</td>
</tr>
<tr>
<td><strong>Overall liking</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>$56.1 \pm 5.01^{bc}$</td>
<td>$66.3 \pm 5.01^{a}$</td>
</tr>
<tr>
<td>Water</td>
<td>$52.6 \pm 5.02^{c}$</td>
<td>$60.4 \pm 5.00^{b}$</td>
</tr>
<tr>
<td>Enzyme</td>
<td>$53.5 \pm 4.99^{bc}$</td>
<td>$60.3 \pm 4.98^{b}$</td>
</tr>
<tr>
<td><strong>Overall rating</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>$2.0 \pm 0.16^{bc}$</td>
<td>$2.4 \pm 0.16^{a}$</td>
</tr>
<tr>
<td>Water</td>
<td>$2.0 \pm 0.16^{c}$</td>
<td>$2.2 \pm 0.16^{ab}$</td>
</tr>
<tr>
<td>Enzyme</td>
<td>$2.0 \pm 0.16^{bc}$</td>
<td>$2.3 \pm 0.16^{ab}$</td>
</tr>
</tbody>
</table>

Significant differences between means denoted by superscripts. Superscripts were generated on an individual trait by treatment level basis and are not applicable to means across traits.

In regards to the demographic outcomes, the age group 18 – 25 rated tenderness 14.4 units lower ($P = 0.03$) on average than the age groups 36 – 45, 46 – 55 and 56 – 65. There was no difference in tenderness rating between the other age groups. Consumers stating their income as $50K – 75K rated tenderness 12.3 units lower ($P = 0.05$) on average than the two lower income brackets. The highest income bracket (> $70K) did not differ significantly from the other income groups. There was a trend toward age and income effects on juiciness rating ($P = 0.06$ and $0.07$, respectively), with the 18 – 25 age group and $50K – 75K income group again ranking steaks lower for this trait. There were no demographic effects on flavour, overall liking or overall rating outcomes.
Implications

1. Transportation behaviour

The research into alpaca behaviour transportation in Australia is the first of its kind. The results of this study provide a fundamental basis for future research into alpaca behaviour, particularly when considering stress and animal welfare under various conditions. It is evident that alpaca behaviour during transportation does not vary across seasons. However, the weather conditions were mild across the year (2017) in which the research was conducted and further research may need to be conducted in order to determine stress responses in alpacas under heat and cold extremes.

Alpacas went to cuss for 51% of the time during transportation which is a noteworthy outcome. In order to determine if this represents normal postural behaviour for alpacas during transport, studies looking at a comparison between flooring type within stock crates is required.

Aggressive episodes during transportation increased when animals went to cuss. Likewise, there was a trend toward increased aggression as the year progressed, which may reflect a fleece length effect on aggressive episodes.

2. Seasonality of product quality

Product quality (primarily tenderness) can be linked to on farm feed availability. Throughout the year in which the study was conducted, feed quality and availability followed a traditional annual trend for the NSW southern tablelands, with evidence to indicate reduced product quality when feed was limited. This was reflected in reduced product tenderness and increased moisture loss when animals were processed in harsher seasons (winter and early spring).

There was a negative effect of resting alpacas pre-slaughter, with reduced muscle glycogen content and tenderness. Moisture loss in the form of drip and purge was greater for rested animals, resulting in a greater loss of valuable meat proteins when compared to product of animals sent direct to slaughter. This has potential impacts on meat nutritional quality, juiciness and price with meat being sold on a $/kg basis.

Muscle glycogen content varied across season and was quite low compared to other red meat species. However, muscle pH_u was unaffected by season and was reduced to desirable values irrespective of muscle glycogen concentration. With the support of experiment two findings, it can be concluded that low muscle glycogen content (of ~ 45 mmol/kg) is not limiting to alpaca pH_u. This has positive implications for product shelf life and quality.

3. Processing treatments

Combined processing technologies improved product fresh colour, with electrical stimulation improving lightness scores when applied to both split and whole carcases. Electrical stimulation and tenderstretching also significantly improved muscle tenderness, exerting a positive effect across multiple muscles (loin and hindquarter) when applied in combination to both split and whole carcases. Finally, combined processing techniques also aid in reducing product moisture loss (purge and cooking loss), providing strong evidence for the application of these techniques in combination to be standard in alpaca carcase processing.

The research suggests alpaca meat has significant retail display advantages when compared to other red meats. Both colour and lipid oxidation is minimal across a 72 h display period and can be attributed to high vitamin E (acting as an antioxidant) content within alpaca muscle.
4. Infusion treatment

Infusion treatments improved laboratory based shear force values (decreased shear force). However, this improvement was comparable to that achievable with the application of combined processing techniques. This indicates that there is no additive advantage of infusing with a proteolytic enzyme. Furthermore, laboratory based improvements to tenderness were not detected at the consumer level with trends toward decreased overall liking when applying the actinadin enzyme to the alpaca loin.
Recommendations

The results of this research can be summarised to provide the following recommendations, outlined in order from paddock to the consumer. Importantly, it should be noted that there is now sufficient evidence to suggest combined processing techniques be applied to alpaca carcases as a standard processing procedure.

1. Product quality is reduced if animals are processed during periods of limited on farm feed availability. During those periods when pasture availability is limiting, animals should be adequately supplemented or held back from slaughter until they have had access to ample high quality feed for at least 4 weeks leading up to slaughter. This will ensure that alpacas are on a rising plane of nutrition prior to slaughter.

2. Variables that increased agonistic behaviours during transportation included time spent in crush and points at which the vehicle stopped. In addition, there was an observation of potential increased aggression with increased fleece length. Future research should investigate this potential relationship, along with stock crate flooring impacts on animal standing and sitting behaviour. Such research will aid in directing producers with their decision making processes surrounding commercial transportation.

3. There is no advantage to resting alpacas for 7 days prior to slaughter, with novel stressors arising from the location change and interaction with other species causing decreased quality of the end product. Results of this research indicate that higher product quality will be achieved when sending animals direct to slaughter (with an overnight lairage period). This is applicable for trips up to 4 h in length and it should be noted that outcomes may change if transport exceeds this time period.

4. The post slaughter processing techniques of medium voltage electrical stimulation and tenderstretching should be applied, in combination, to alpaca carcases as a standard processing procedure. There is ample evidence to indicate the overall positive effects of these techniques on multiple muscles within whole alpaca carcases.

5. If alpaca enters into the fresh retail market, research is required to determine consumer acceptability thresholds for the unique alpaca colour parameters.
References


Improved tenderness of alpaca carcases using combined processing techniques

by Tamara Biffin, Dr Melanie Smith, Dr Russell Bush and Dr David Hopkins

September 2019

AgriFutures Australia Publication No. 19-018
AgriFutures Australia Project No. PRJ-010045
ISBN: 978-1-76053-041-9