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Seasonal variation in venison drip loss, calpain activity, colour and tenderness

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Seasonal variation in venison drip loss, calpain activity, colour and tenderness

Prepared for DEEResearch

E. Wiklund, P. Dobbie, A. Stuart & R. Littlejohn

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Inquiries or requests to:
Eva Wiklund
Email: Eva.wiklund@agresearch.co.nz
Food, Metabolism & Microbiology,
AgResearch Ltd
Private Bag 3123, Hamilton, New Zealand

Mike North
Food, Metabolism & Microbiology
Food & Textiles Group, AgResearch Limited

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1. Background

Venison is a high quality product with several attributes attractive to consumers – it is tender, has low a fat content, a favourable fat composition and high levels of minerals (Hoffman & Wiklund, 2006). However, NZ venison processors have indicated a seasonal variation in the amount of drip and that poor colour stability can be a problem, sometimes even in vacuum bags and in association with high content of drip in the bags after extended storage of chilled venison.

Deer have a seasonal growth pattern with maximum accretion of body tissue (muscle and fat) in spring and summer, and minimal accretion or even live weight loss during autumn and winter (Suttie & Webster, 1998). Assuming this seasonal variation in protein metabolism is related to proteolytic enzyme activity and possibly meat tenderness, it would likely impact on venison water-holding capacity. Venison is more tender than beef, and for some deer species ageing of the meat is not necessary at all (Barnier *et al*., 1999; Sims *et al*., 2004).This phenomenon has been explained by high activity of tenderizing enzymes in venison (Farouk *et al*., 2008; Wiklund *et al*., 1997) but has never been correlated to deer seasonality or to variation in other important meat quality attributes. Further, venison has poor colour stability, probably related to the high myoglobin content and also the high content of pro-oxidants like iron (Stevenson-Barry *et al*., 1999), but the mechanisms around the very fast browning of venison have not been fully explained.

This study has for the first time determined seasonal variation in drip loss, colour, calpain activity and tenderness in venison. In addition, using the rapidly tenderising venison as a model, has highlighted the relationship between tenderisation and formation of drip during extended storage of chilled meat.

2. Material and methods

A total of 64 young red deer (*Cervus elaphus*) stags (< 2 years old) were included in this study. The animals were slaughtered at four different times of the year (December; n=17, March; n=8, June; n=20 and September; n=19) representing ages of 12, 15, 6 and 9 months, respectively. The reason for a reduced number of samples in the March group was that 8 of the 20 samples collected were from older stags (*i.e*. 27 months) and therefore removed as non-representative. Further, 4 younger animals from the same group were excluded from the results because of high ultimate pH (DFD: Dark, Firm, Dry meat). Slaughter of the deer and meat sample collections were carried out at the two deer slaughter premises in Rotorua (Silver Fern Farms and Duncan Venison). Animals were slaughtered according to normal practices and staff assisted the AgResearch team during sampling on the slaughter line and in the boning room. Muscle samples (approximately 5 g of the left side loin, *M. longissimus*, at the rump end) for calpain analysis were collected on the slaughter line just before carcass grading. The rest of the same loin muscles were collected at 1 day post slaughter. Loins were divided in four parts and randomly allocated to storage times of 1 day, 3, 9 or 14 weeks at -1.5°C and then vacuum packaged. The loin samples were then transported to AgResearch MIRINZ, Hamilton for storage and meat quality measurements. After each of the four storage times, drip, colour and tenderness were measured.

pH measurements

Meat pH was measured with a portable pH meter (Testo® 230, Germany) with automatic temperature compensation. The pH meter was calibrated at pH 7.0 and 4.0 with buffers (Mallinckrodt Chemicals, USA) stored at room temperature (20 °C).

Drip loss, purge and cooking loss

Drip loss/water-holding capacity was measured using different methods:

1. for the fresh meat samples (1 day post slaughter) the Honikel Bag method (Honikel, 1998) was used. Samples of 50-100 g were cut off the loin samples, weighed, placed in plastic netting and then suspended in inflated plastic bags that were sealed. The sealed bags were hung in a chiller (4 °C) for 48 h before samples were removed, blotted dry and re-weighed.
2. At each storage time, drip loss was also measured using a centrifuge method (Kristensen & Purslow, 2001). From a slice of the venison loins, small samples were cut parallel to the fibre direction. The samples were approximately 1 cm long, 0.4 x 0.4 cm in cross-sectional area and weighed 0.3 – 0.5 g. Meat samples were weighed and transferred to open-ended tubes (Mobicols from MoBiTec, Göttingen). To prevent surface drying, the Mobicols were closed with a screw-on cap. The Mobicols were put in 2 ml Eppendorf tubes and centrifuged at 40 g for 1h at 4 °C. Centrifugation loss of the meat was calculated as the difference in weight before and after centrifugation.
3. Purge in the vacuum bags was measured after 3, 9 and 14 weeks of storage. Loins were removed from their packages, dabbed dry with a paper towel and then weighed. Purge loss was calculated as the difference in the weight of the loins before and after storage expressed as a percentage of the original weight of the loins.
4. Cooking loss was measured after each of the four storage times. Loin samples were weighed before cooking and after cooking the meat samples for tenderness measurements, were blotted dry and re-weighed. The cooking loss was calculated as amount of weight lost and expressed as a percentage of the original sample weight.

Tenderness

Loins were cooked in bags submerged in boiling water until the internal temperature of the sample reached 75°C. A thermocouple was inserted in each sample to measure the temperature at the centre of the sample during cooking. After cooking the samples were immediately cooled on ice. Ten 1 cm x 1 cm cross-section slices (bites) were prepared from the cooked sample with the muscle fibres running longitudinally along the slice. Each sample was then sheared with the long axis of the fibres running perpendicular to the blade, using a MIRINZ tenderometer. The results were expressed as shear force (kgF).

Calpains

The relative activity of extracted muscle calpain was measured after separation using DEAE Sephacel ion exchange chromatography (Wheeler & Koohmaraie, 1991) with slight modifications (Sainz *et al*., 1992). The activity of the calpain fractions was

assessed by means of the enzymatic digestion of a fluorometric dye (Calbiochem Calpain-1 Substrate, Fluorogenic Cat. No. 208748). In brief the soluble muscle extract was filtered then loaded onto a 10x100 mm DEAE Sephacel ion exchange column and equilibrated with Buffer A (40mM Tris pH7.5) Using a step-wise salt gradient calpastatin and its bound calpain was eluted with buffer A + 100 mM NaCl, μ-calpain with buffer A + 200 mM NaCl and m-calpain with Buffer A+300 mM NaCl. 2 ml fractions were collected and these were analysed for calpain activity as follows. 20 μl of each fraction was mixed with 120 μl of Buffer A, 5 μl of 100 mM $CaCl_2$ and 3.33 μg of fluorogenic substrate.

The activity was measured as the rate of appearance of fluorescence due to digestion of the fluorescent substrate when sample was excited at 490nm and fluorescent emission measured at 520 nm. The area under the curve of fractions collected for each calpain type were bulked and adjusted for sample weight to yield the relative activity values reported.

Colour display life

Triplicate colour measurements were made on each freshly cut 2.5 cm thick steak 2 h after opening the vacuum bag and then daily using a Minolta Chroma meter (CR-300, Osaka, Japan) as found appropriate for venison (Wiklund *et al*., 2006).

Statistical analysis

Each variable at each observation time was analysed by analysis of variance, with month as the treatment term. Display life (days of acceptable colour) for each sample was calculated as the time taken to reach a mean a^* value of 12, using linear interpolation between consecutive samples, as has been used previously for venison (Stevenson-Barry *et al*., 1989; Wiklund *et al*., 2001). For some samples the mean a* value was greater than 12 for each observation, so the algorithm of Taylor (1973) for pre-processing censored data for analysis of variance was used. For most analyses (except where otherwise stated) the main effect of month was highly significant ($P<0.001$).

3. Results

Carcass measurements and pH

The animals slaughtered in December (Group 1) and March (Group 2) had significantly higher carcass weights and GR scores than the deer slaughtered in July (Group 3) and September (Group 4) (Table 1).

The measured pH values in the loin samples also varied between the different groups of animals. Meat from deer in Group 1 had the lowest pH values at all storage times (Table 1). It was also clearly demonstrated that the pH in chilled loin samples from all four groups increased (with an average of 0.16 (SE 0.013) pH units) over the storage period (Figure 1).

Table 1. Carcass and meat quality characteristics for red deer stags in the study

Trait	Group 1 (Dec)	Group 2 (March)	Group 3 (July)	Group 4 (Sept)	SED
Carcass					
weight, kg	65.2[a]	60.7[a]	45.1[b]	47.6[b]	2.58
GR[1]	6.4[a]	6.1[a]	1.9[b]	2.1[b]	1.18
pH in loin					
1 d	5.46[a]	5.64[c]	5.54[b]	5.62[c]	0.028
3 w	5.50[a]	5.74[c]	5.59[b]	5.63[b]	0.026
9 w	5.56[a]	5.64[ab]	5.67[b]	5.70[b]	0.031
14 w	5.60[a]	5.70[b]	5.74[b]	5.81[c]	0.029
Drip, %					
1 d	3.32[a]	0.97[c]	3.21[a]	2.28[b]	0.314
3 w	3.76[a]	2.46[b]	2.69[b]	2.74[b]	0.403
9 w	5.07[ab]	4.20[bc]	3.80[c]	5.87[a]	0.465
14 w	4.93[b]	5.07[ab]	4.18[b]	6.18[a]	0.462
Centrifuge drip, %					
1 d	10.62[a]	3.78[bc]	1.93[c]	4.19[b]	0.697
3 w	5.47[b]	7.95[a]	1.15[d]	2.41[c]	0.462
9 w	3.94[a]	1.73[b]	0.67[c]	0.98[c]	0.246
14 w	4.60[a]	0.47[b]	0.99[b]	0.96[b]	0.449
Cooking loss, %					
1 d	26.2[ab]	23.7[bc]	22.7[c]	27.4[a]	0.99
3 w	29.0[b]	27.2[b]	23.2[c]	32.3[a]	1.10
9 w	26.8[b]	24.0[bc]	20.9[c]	30.5[a]	1.37
14 w	28.2[b]	29.7[b]	28.3[b]	23.4[a]	1.09
Shear force, kgF					
1 d	6.18[b]	11.28[a]	9.44[a]	9.60[a]	0.761
3 w	4.06[b]	5.18[a]	3.94[b]	4.90[a]	0.266
9 w	3.29[b]	4.22[a]	4.13[a]	4.44[a]	0.251
14 w	3.34[b]	4.31[a]	3.87[ab]	4.21[a]	0.233
Calpastatin-bound					
Calpain[2]	149.0[b]	187.0[ab]	238.0[a]	163.0[b]	20.2
µ-calpain[2]	324.0[b]	344.0[b]	446.0[a]	442.0[a]	25.5
m-calpain[2]	329.0[c]	420.0[bc]	519.0[a]	469.0[ab]	30.8
Colour display life, hours					
1 d	113.1[c]	170.0[b]	175.5[b]	217.7[a]	16.32
3 w	81.2[b]	76.5[b]	83.2[b]	138.9[a]	11.85
9 w	58.4[b]	41.8[c]	48.4[c]	69.4[a]	4.34
14 w	45.9[ab]	29.5[c]	42.4[bc]	54.7[a]	5.49

Means in the same row with different letters are significantly different ($p \leq 0.05$).

[1] Tissue depth over the 12th rib, 160 mm from the mid line of the back (site of measurement adjusted from lamb carcasses to deer carcasses).

[2] Relative activity of calpastatin-bound calpain, µ-calpain and m-calpain.

Drip loss, purge and cooking loss

Drip loss measured using the Honikel bag method at 1 day post slaughter was highest in loin samples from Groups 1 and 3 compared with the other groups (Table 1). Purge loss in the vacuum bags was measured after 3, 9 and 14 weeks. After 3 weeks of storage Group 1 loin samples had the highest amount of purge (Table 1). After 9 and 14 weeks of storage the amount of purge in all loin samples had increased and approached a

similar level for all groups (Table 1). A clear trend of an increasing amount of drip/purge over the storage period was shown in loin samples averaging 2.5 % (SE 0.17) over the four groups (Figure 2).

Centrifuge drip in small samples (Figure 10) measured after drip/purge had been determined in the loin samples was highest in meat from Group 1 at all storage times except after 3 weeks of storage when Group 2 samples had the highest amount of centrifuge drip (Table 1). Over the storage period the amount of centrifuge drip tended to decrease in the loin samples from all four groups (Figure 3).

Cooking loss was highest in the loin samples from Group 4 up to 9 weeks post slaughter (Table 1). After 14 weeks of storage cooking loss values were similar for loin samples from all groups except Group 4 for which the amount had decreased markedly (Figure 4).

Tenderness

The loin samples from deer in Group 1 were very tender already at 1 day post slaughter and consistently had the lowest shear force values throughout the storage period (Table 1). However, the meat samples from the other groups also tenderized significantly during the first 3 weeks of storage (Table 1). After 3 weeks there were only small improvements in tenderness for loin samples from all four groups, and the final shear force values measured at 14 weeks post slaughter (ranging from 3.3 – 4.3 kgF) all represented very tender meat (Figure 5).

Calpains

The relative activities of the calpastatin-bound calpain, μ-calpain and m-calpain all displayed a seasonal pattern of lower values in December (Group 1), an increase at the onset of the rutting season (Group 2, March) with maximum values measured in July (Group 3), and a subsequent slight decline in September (Group 4) (Table 1, Figure 6).

Colour display life

The colour display life was superior in loin samples from deer in Group 4 at all storage times (Table 1). At 1 day post slaughter these samples had a display life of 9 days compared with 7 days for samples from Groups 2 and 3 and only 5 days for the loin samples from Group 1 (Figure 7). After 3 weeks of chilled storage the colour display life of the samples from Group 4 had decreased to 6 days which was still significantly better than approximately 3 days for loin samples from the other three groups (Figure 7). At 9 weeks post slaughter the colour display life for Group 4 samples and samples from the three other groups had further decreased to 3 days and 2 days, respectively (Figure 7). After 14 weeks of chilled storage all samples had a colour display life of 1-2 days (Figure 7). The decline in Minolta a* values at all storage times are illustrated in Figure 8.

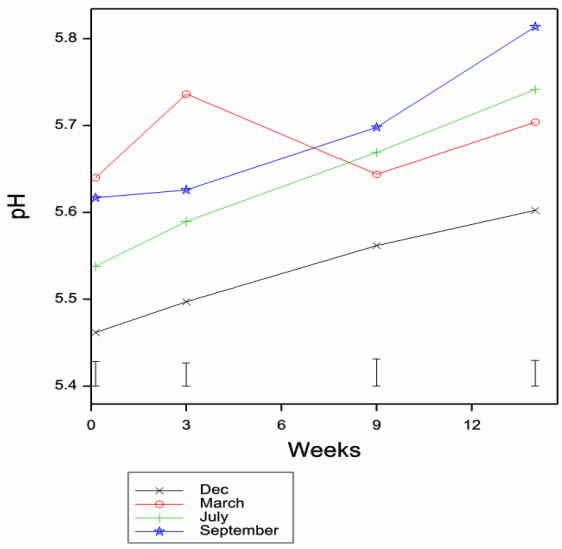

Figure 1.
Mean meat pH values in loin (*M. longissimus*) samples at four different storage times (1 day, 3 weeks, 9 weeks and 14 weeks at -1.5°C) from red deer stags included in the study, with error bars indicating standard error of difference (SED).

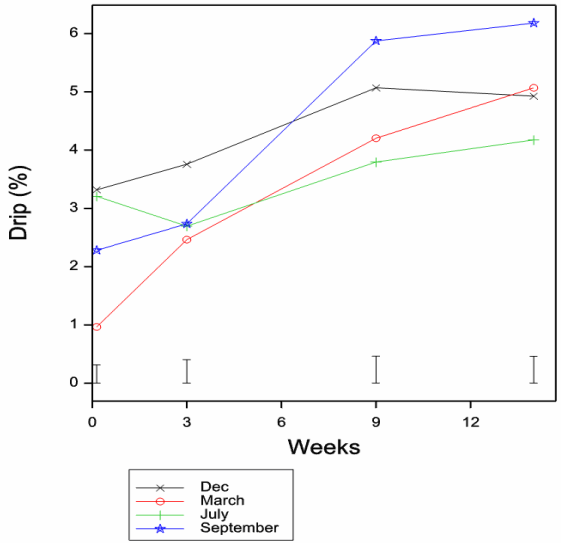

Figure 2.
Mean drip loss (1 day post slaughter) and purge values (%) in loin (*M. longissimus*) samples at three different storage times (3 weeks, 9 weeks and 14 weeks at -1.5°C) from red deer stags included in the study, with error bars indicating standard error of difference (SED).

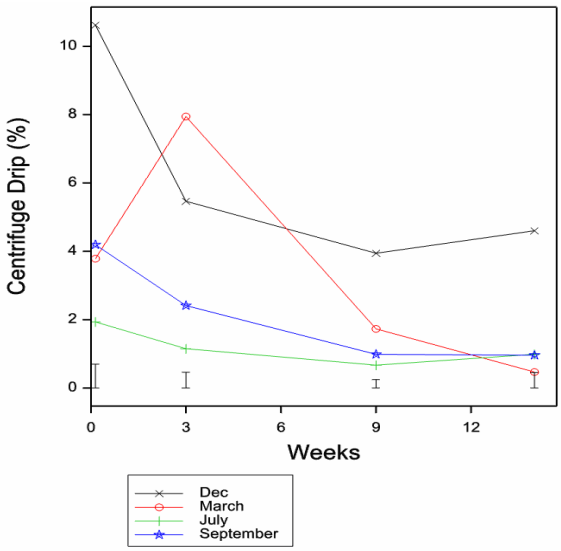

Figure 3.
Mean centrifuge drip loss values (%) in loin (*M. longissimus*) samples at four different storage times (1 day, 3 weeks, 9 weeks and 14 weeks at -1.5 °C) from red deer stags included in the study, with error bars indicating standard error of difference (SED).

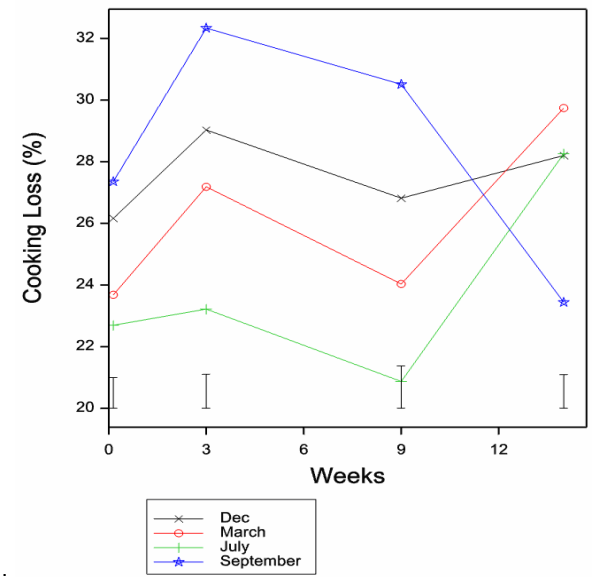

Figure 4.
Mean cooking loss values (%) in loin (*M. longissimus*) samples at four different storage times (1 day, 3 weeks, 9 weeks and 14 weeks at -1.5 °C) from red deer stags included in the study, with error bars indicating standard error of difference (SED)

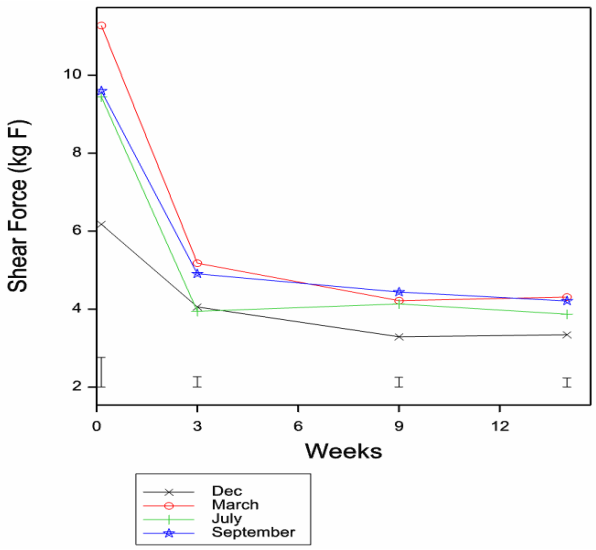

Figure 5.
Mean shear force values (kgF) in loin (*M. longissimus*) samples at four different storage times (1 day, 3 weeks, 9 weeks and 14 weeks at -1.5 °C) from red deer stags included in the study, with error bars indicating standard error of difference (SED).

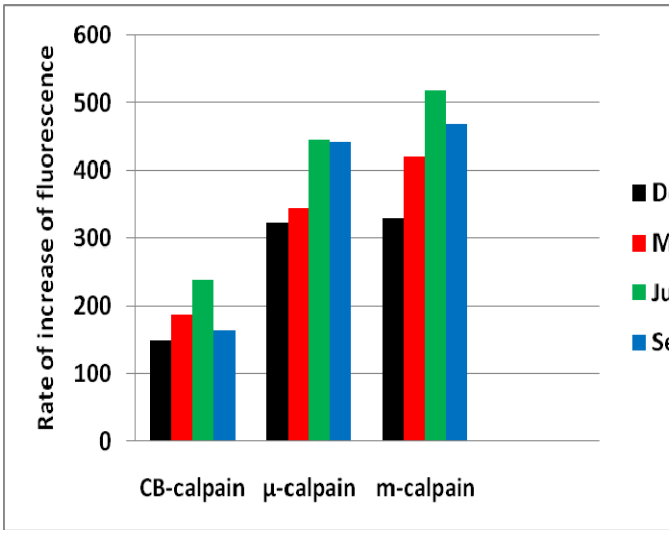

Figure 6.
Relative activity of calpastatin-bound (CB) calpain, µ-calpain and m-calpain in loin (*M. longissimus*) samples (collected 30 min post slaughter) from red deer stags included in the study

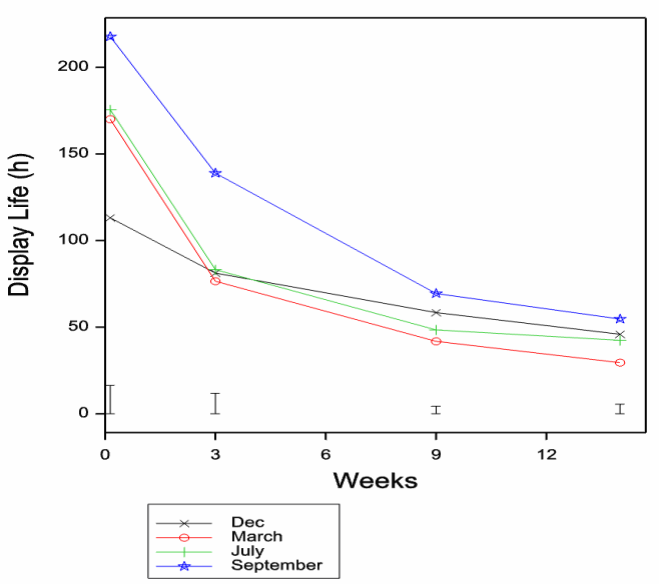

Figure 7.
Mean display life (hours of Minolta a* value ≥ 12) in loin (*M. longissimus*) samples at four different storage times (1 day, 3 weeks, 9 weeks and 14 weeks at -1.5 °C) from red deer stags included in the study, with error bars indicating standard error of difference (SED).

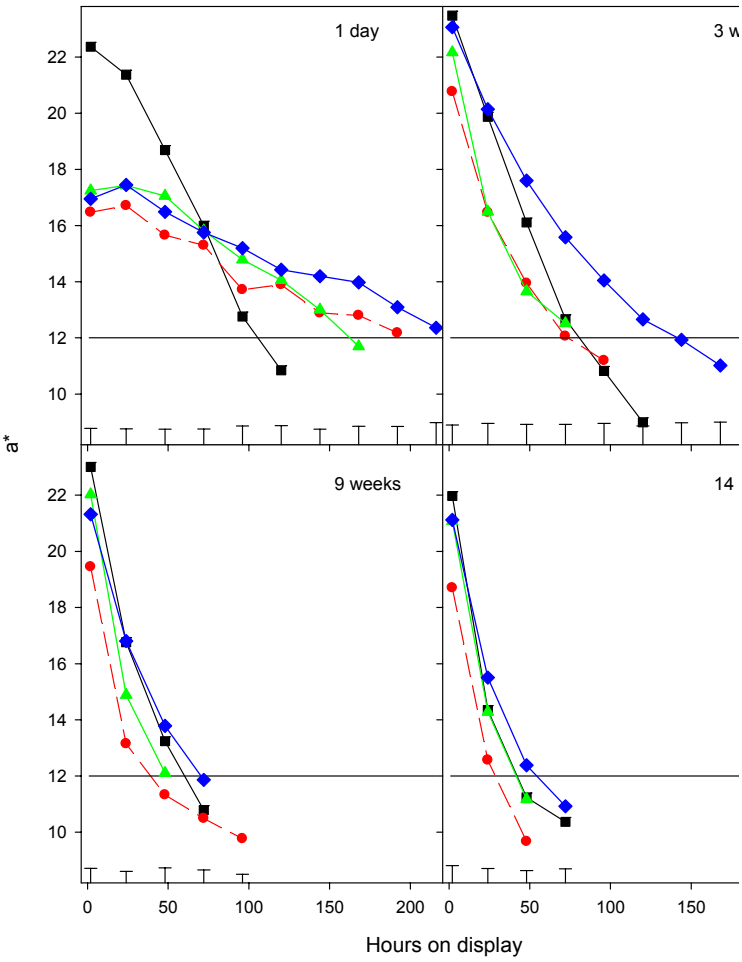

Figure 8.
Mean Minolta a* values in loin (*M. longissimus*) samples at four different storage times (1 day, 3 weeks, 9 weeks and 14 weeks at -1.5 °C) from red deer stags from four slaughter groups (■ Dec, ● March, ▲ July and ♦ Sept) included in the study, with error bars indicating standard error of difference (SED).

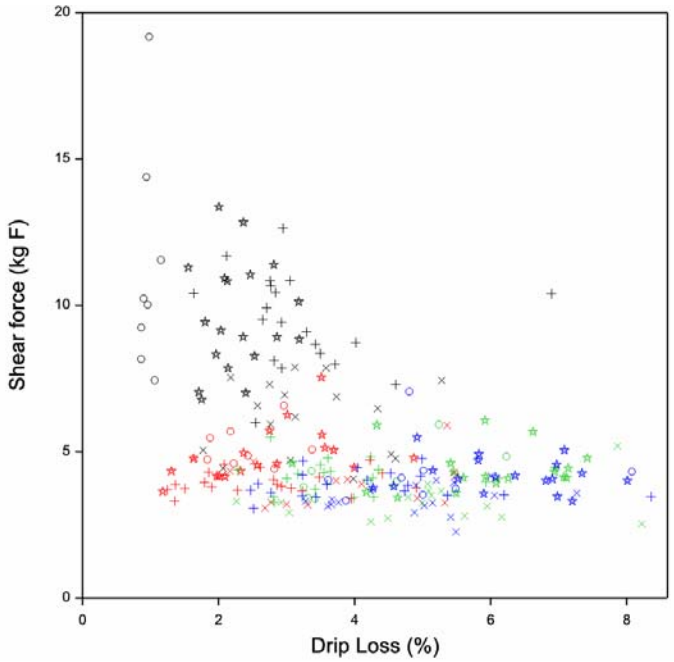

Figure 9.
Scatter plot of all measured shear force (kgF) and drip/purge loss (%) values for all groups and storage times in the study. Colour represents storage time: **black markings** = 1 day; **red markings** = 3 w; **green markings** = 9 w and **blue markings** = 14 w. Symbol represents animal group: X = Group 1; O = Group 2; + = Group 3 and ✡ = Group 4.

Figure 10.
Meat sample in the Mobicol tubes inside an Eppendorf tube and drip (on the bottom of the tube) after centrifugation.

4. Discussion

The design of a study like this inevitably means that animal groups are confounded with animal age. The deer in Groups 1 and 2 were 12 and 15 months of age, respectively and animals in Groups 3 and 4 were younger deer of 6 and 9 months of age. For the oldest animals in the study (Group 2) slaughter time (March) collided with onset of puberty and there were possibly effects of the first rutting season for these young deer. Physiological changes in the animals triggered by increasing testosterone levels at this time have a significant impact on the social interaction between animals, especially when they are kept in confined spaces, *e.g.* during transport and lairage before slaughter. The carcasses from Group 2 classified as high pH (DFD) were all from small animals, which suggests that these deer consumed most of their muscle energy stores due to stress from being "bullied" by bigger more dominant stags. Such behaviours are likely to have been accentuated by increased testosterone levels.

The nutritional status and physical condition of deer has been demonstrated to have a considerable effect on muscle glycogen content and meat ultimate pH values (Wiklund *et al*., 1996). The meat pH values of the deer in the present study (mean values ranging from 5.46 – 5.64 at 1 day post slaughter) indicated that the animals from all four groups were in good physical condition. However, deer from Group 1 had the lowest pH values and also the highest carcass weights and GR scores, suggesting these deer were in optimal condition for slaughter. The phenomenon of increased meat pH values during long term chilled storage observed in the venison samples in this study have previously been reported (Wiklund *et al*., 2001).

Purge in vacuum bags during long-term chilled storage has also been reported previously for venison, showing both lower (Wiklund *et al*., 2001) and similar (Wiklund *et al*., 2006) levels of purge loss compared with the present study. The increasing amount of purge loss over the storage period observed in this study agrees well with the earlier mentioned venison studies. Drip loss measured with the Honikel bag method or as purge in vacuum bags represents water dripping out from the meat structure with minimal force applied to the meat sample. The centrifugation method on the other hand applies force to release water from the meat sample. The reason why centrifuge drip levels decreased over the storage period when the purge loss increased in the present study was probably related to the fact that after water had already been released as purge there was less water left to be released in the centrifuge test. Previously the relationship between ageing and drip formation in pork samples have been reported (Kristensen & Purslow, 2001) but the ageing time in that study was only 10 days. We are not aware of any centrifuge drip data published for venison or any other type of meat over a period of chilled storage similar to that of the present study.

Shear force values similar to those found in the present venison loin samples have been reported previously (Wiklund *et al*., 2001; Farouk *et al*., 2007). Comparative studies have clearly shown that venison is very tender compared with beef as soon as 1-3 days post slaughter (Barnier *et al*., 1999; Farouk *et al*., 2007) as was also demonstrated at 1 day post slaughter in loin samples from deer in Group 1 in the present study. After 3 weeks of chilled storage, loin samples from the deer in Group 2 were significantly tougher than all other samples. Thus, the physiological changes in connection with puberty and rut in these young stags could be affecting meat tenderness and possibly

the role of the tenderising enzyme calpain. However, when the meat would reach the market in Europe or the US (around 6-7 weeks post slaughter), the difference in tenderness between samples from the different groups would be negligible.

The drip loss and shear force results recorded at 1 day post slaughter in the present study, demonstrated that the very tender samples from deer in Group 1 also had the highest drip loss values. In addition, for loin samples from all groups, tenderness improved over the storage period to reach very low shear force values after 14 weeks of storage and was accompanied by a significant increase in purge loss (Figs. 2, 5 and 9). This suggests that there is a strong relationship between tender meat and drip loss.

The calpain activities measured in the present study followed a similar trend to that previously reported for red deer neck muscle (*M. splenius)* (Dobbie *et al*., 1997). As an effect of the onset of the rutting season, the rate of protein turnover increases particularly in male deer due to muscle "build up". Immediately after the rut the bulky muscles returns to normal size which also requires a high protein turnover. Therefore it is important that the calpain activities are high at these time points. As earlier discussed, animal group (slaughter month) and age are confounded in the present study. Thus, the oldest animals included that also likely would be influenced by onset of the rutting season is Group 2, where an increase in calpain activity was observed. For the following two groups 3 and 4, the deer were too young to be influenced by physiological changes in relation to the rut but as young fast growing animals they would be expected to have a high protein turnover and calpain activities. This assumption agrees with the present results. The relationship between high calpain activity and high growth rate in young animals has been demonstrated for cattle (Dobbie, unpublished results).

The colour display life of the loin samples in the present study was found to be better by far than had earlier been reported for long term chilled red deer venison (Wiklund *et al*., 2001; Wiklund *et al*., 2006). However, data for fallow deer (*Dama dama*) venison (loin samples) demonstrated a colour display life similar to that of loin samples from Group 4 in the present study (Wiklund *et al*., 2005). In reindeer (*Rangifer tarandus tarandus*) loin samples colour display life was similar to that of Groups 1, 2 and 3 in the present study (Wiklund, unpublished results). The browning of meat, which determines the colour display life, is due to the reaction between oxygen and myoglobin (the colour pigment in meat) where red myoglobin is oxidized to brownish metmyoglobin. The speed of this oxidation process is dependent on several factors like antioxidant content, oxygen consumption and reducing enzyme activity in the meat (Faustman & Cassens, 1990). Wiklund *et al* (2006) demonstrated a significantly better colour display life of venison from grazing animals compared to deer fed a pelleted feed, and suggested that this difference was related to the higher content of natural antioxidants (vitamin E) in the pasture compared to the pelleted feed mixture. It is suggested that the better colour display life of the loin samples from Group 4 could be related to higher antioxidant content of the superior quality pasture growing in the spring.

5. Conclusions

- Animals slaughtered in December (Group 1) had the lowest pH values and also the highest carcass weights and GR scores, indicating these deer were in optimal condition for slaughter.
- A clear trend of an increasing amount of drip/purge over the storage period was shown in the loin samples averaging 2.5 % over the four groups.
- The loin samples from deer in Group 1 were very tender already at 1 day post slaughter and consistently had the lowest shear force values throughout the storage period. The final shear force values measured in samples from the four groups at 14 weeks post slaughter (ranging from 3.3 – 4.3 kgF) all represented very tender meat.
- The relative activities of the calpastatin bound calpain, μ-calpain and m-calpain all displayed a seasonal pattern. However, there were no clear indications that this enzyme system alone was responsible for the variation in meat tenderness.
- In loin samples from all groups, tenderness improved over the storage period to reach very low shear force values after 14 weeks of storage and was accompanied by a significant increase in purge loss. This suggests that there is a strong relationship between tender meat and drip loss.
- The significantly better colour display life of the loin samples from deer slaughtered in September (Group 4) was possibly related to a higher antioxidant content of the superior quality pasture growing in the spring.

6. Technology transfer

The project has to date produced the following outputs:

Presentations

- Wiklund. E. 2008. Venison quality research update. Venison Processor's Technical Committee, 8 April, Wellington.
- Wiklund. E. 2008. Venison quality research update. Venison Processor's Technical Committee, 30 July, Wellington.
- Wiklund, E. 2008. Seasonal variation in venison quality. DEEResearch Board Meeting, 13 August, Ruakura, Hamilton.
- Wiklund, E. 2008. Seasonal variation in venison drip and tenderness. MIRINZ Meat Industry Workshop, 21 October, Ruakura, Hamilton.
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Scientific publications

The results from the project are currently being prepared for publication in the international journal Meat Science.

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