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The effect of removal technique and post-removal handling on velvet antler colour

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Abstract Asian markets for velvet antler perceive the colour of the core as a primary indicator of quality. The factors which influence colour are not known, but the market preference in Korea is for an even mid-red colour. The aim of the present study was to determine whether removal technique and post-removal handling influence velvet colour. Investigations took place at AgResearch Invermay and at Mount Hutt Station in mid Canterbury, New Zealand. The influences on velvet antler colour of sedative drug, mild stress, local anaesthetic administration, timing of tourniquet application, and restraint of the stags in a crush or workroom for velvet antler removal were investigated. The effects of antler orientation post-removal and post-removal environmental temperature on velvet antler colour were also investigated. In all studies, velvet antler was frozen and held at –20°C before being dried either by freeze drying or commercially. In all trials, a consistent pattern of both lightness and hue angle was shown from the tip of the velvet antler stick to the base; the tip was lighter and browner, the mid section was darker and redder, and the base was lighter and browner. There were no significant overall effects of drug treatment on colour, but there were significant differences among sections. Specifically, sedative drug treatments resulted in less red velvet antler than in control antlers removed using local analgesic only. Mild stress and method of local analgesic administration had no effect on any aspect of velvet antler colour. Placing the velvet at an angle of 15° (tip down) gave a darker and redder antler than the typical fully inverted position. There were no significant differences in colour whether the velvet antler was frozen immediately after removal or held at 4°C or ambient temperature for up to 6 hours prior to freezing. Overall, the use of sedative drugs produces velvet antler that is lighter and less red, and post-removal handling technique can influence colour.

Keywords deer velvet; antler; removal; handling; colour; microbiological contamination

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INTRODUCTION

Velvet antler has been removed commercially from farmed stags in Asia for hundreds of years and in New Zealand for over 20 years. In the principal world market for velvet antler, South Korea, product from New Zealand must compete with that from Russia and China. Although New Zealand holds up to 65% of the market in terms of quantity, New Zealand antler is perceived in the market place as being of lower quality than that from Russia. Although many of the reasons for the preference for Russian antler are traditional, the market perceives New Zealand antler to be less "effective" than Russian product and hence of lower value and quality.

The velvet antler research programme at Invermay has, as a general goal, the determination of possible factors which might influence quality. The Asian market uses velvet core colour as a primary indicator of quality. However, the factors that influence velvet antler colour are not known. Processors, who dry frozen antler for export,

attempt to produce an even colour throughout the core of the dried product by redistributing the blood in the antler. This is necessary because, after removal, blood (and hence red colour) tends to pool in the upper regions of the antler. Processors believe that present velvet removal and post-removal handling techniques result in less blood in the antler for redistribution than was formerly the case. This necessitates longer processing times, so that the remaining blood, which settled near the antler tip when it was inverted after removal, can be spread evenly throughout the antler.

In deciding to investigate the reasons for specific patterns of colour distribution and intensity in the antler, we were conscious that there were no published studies for reference. Therefore, we decided to investigate the possible reasons for the processors' observations that there was a low level of blood in New Zealand antler before processing. Velvet antler removal has changed on an industry basis since the first deer farms began in New Zealand. Stags are quieter and are handled in better facilities, veterinarians are more familiar with analgesia systems for deer, and overall post-harvest handling of velvet antler has improved. It is possible that improved handling and effective analgesia of stags have resulted in less blood in the antler at the time of antler removal, by lowering stress and hence blood pressure and blood flow. Likewise, post-harvest treatment of antler could influence the distribution of blood and interact, in a complex manner, with the initial quantity of blood to produce the pattern observed by the processors.

The overall aim of the studies described in this report was to investigate the effects of type of removal technique and post-removal handling on velvet antler colour. It is acknowledged that it is not clear that "antler colour" and "antler quality" are synonymous, but as this is the criterion the marketplace chooses to use, it must form the basis, at least, of a scientific evaluation.

MATERIALS AND METHODS

Investigations were carried out at two locations, AgResearch, Invermay, and Mount Hutt, mid Canterbury, during the 1993 and 1994 antler-growing seasons.

Trial 1, Invermay

The influences on antler colour of drug type, mild stress induced by holding stags in the yards prior to velveting, and of local anaesthetic administration method were investigated.

Animals and treatments

Thirty rising 3-year-old red deer stags were maintained under standard deer husbandry conditions on pasture. For the removal of velvet antler they were allocated to one of six treatments as follows, with $n = 5$ per treatment.

1. Local anaesthetic only; restraint in a crush; immediate antler removal.
2. Local anaesthetic only; restraint in a crush; held in yards for four hours before antler removal.
3. Xylazine plus local anaesthetic; immediate antler removal.
4. Xylazine plus local anaesthetic; held in yards for four hours before antler removal.
5. "Fentazin" plus local anaesthetic; immediate antler removal.
6. "Fentazin" plus local anaesthetic; held in yards for four hours before antler removal.

In addition, for each stag, one antler was randomly allocated to local anaesthetic applied in a ring block and the other to local anaesthetic applied as a modified regional nerve site block.

All drugs were administered by a veterinarian experienced in velvet antler removal.

Local anaesthetic ("Lopaine", 2% lignocaine HCl, Ethical Agents Ltd) was injected subcutaneously either as a standard ring block (10 ml per pedicle) or using a modified regional nerve block. In the latter technique, injections were given over the zygomaticotemporal and supraorbital nerves, and a third injection was given caudal to the pedicle and overlying the zygomatic process of the temporal bone over the auriculo-palpebral branch of the facial nerve. This nerve is known to innervate the antler in about 20% of red deer (Suttie et al. 1985).

Xylazine ("Rompun", 10% xylazine hydrochloride, Bayer NZ Ltd) was given at a dose rate of 1 mg kg^{-1} by intra-muscular administration such that full recumbency was induced. Yohimbine hydrochloride (0.25 mg kg^{-1}) was given after antler removal to reverse the sedative effects of xylazine.

"Fentazin" (0.4 mg ml^{-1} fentanyl citrate, 50 mg ml^{-1} xylazine hydrochloride, and 3.2 mg ml^{-1} azaperone, Parnell Laboratories Ltd) was given by intra-muscular administration at a dose rate (1 ml 70 kg^{-1}) such that full recumbency was induced. "Contran H" (1% Yohimbine and 0.01% Naloxone,

Parnell Laboratories NZ Ltd) (1 ml 40 kg^{-1}) was given after antler removal to reverse the effects of "Fentazin".

The day on which each stag cast its previous hard antler buttons was recorded. When more than a day elapsed between left and right antler casting, the date of the first antler casting was used to calculate subsequent harvest date. Antler removal took place 60 ± 1 days later. Each day during the antler-harvesting season, the stags were brought into the yards at 8 a.m. and those animals which were to have velvet removed that day were drafted. The drafted stags were then separated into two groups: those which were to have antlers removed immediately, and those which were to be held in the yards for four hours prior to antler removal. The stags to be held were given adequate space, but were neither fed nor given access to water prior to antler removal.

For both groups, antler removal took place as follows. The stags were identified with their drug treatments and administration of xylazine and "Fentazin" took place as appropriate. While these drug treatments were taking effect, stags to be treated with local anaesthetic were restrained in the crush and "Lopaine" was administered. Velvet antler removal took place 4 min after administration of local anaesthetic. A flexible rubber tourniquet was placed around both pedicles and the antlers were removed with a medium-tooth meat saw. After removal the antlers were inverted, allowed to cool, and placed in a freezer. When the stags which had been treated with xylazine or "Fentazin" were recumbent, local anaesthetic administration and antler removal was performed as described above. All recoveries from drug treatment were uneventful.

Antler processing

All antlers were frozen at –20°C until processed. When required for processing, antlers were cut into pieces (upper main beam, lower main beam, and tines) and skinned. The antler pieces were dried in a cabinet freeze-drier (Cuddon Ltd). The antlers were dried in two batches. Although both batches were intended to be dried at 20°C, due to a technical problem the drying of one batch was carried out at 30°C and one at 20°C. Antlers had been randomly allocated to each drying batch.

The dried antlers were cut into 1-cm discs, perpendicular to the vertical axis of the main beam, using a band saw fitted with an 18-mm fine blade.

Trial 2, Invermay

Due to the antler processing problems experienced in Trial 1, it was decided to repeat the drug administration treatments in Trial 2. As there were no significant differences found in Trial 1 in antler colour due either to the holding period in the yards prior to velvet removal, or to local anaesthetic administration method, the timing of application of the tourniquet was instead investigated in Trial 2. As the "figure-of-eight" tourniquet system with rubber straps was used, antlers had to be treated as pairs. This meant that within-deer comparisons, such as those used for methods of local anaesthetic administration in Trial 1, could not be carried out.

Animals and treatments

Thirty rising 3-year-old red deer stags were maintained under standard deer husbandry conditions on pasture. For antler removal they were allocated to one of six treatments as follows, with $n = 5$ per treatment.

1. Local anaesthetic only; restraint in a crush; tourniquet applied immediately after local administration ("early tourniquet").
2. Local anaesthetic only; restraint in a crush; tourniquet applied immediately before antler was removed ("late tourniquet").
3. Xylazine plus local anaesthetic; tourniquet applied immediately after local administration ("early tourniquet").
4. Xylazine plus local anaesthetic; tourniquet applied immediately before antler was removed ("late tourniquet").
5. "Fentazin" plus local anaesthetic; tourniquet applied immediately after local administration ("early tourniquet").
6. "Fentazin" plus local anaesthetic; tourniquet applied immediately before antler was removed ("late tourniquet").

Procedures were the same as for Trial 1, except that the tourniquet was placed around the pedicles immediately after local administration or 4 min later, immediately prior to velvet removal. In each case, local anaesthetic was administered as a ring block.

Antler processing

The same technique and equipment were used as for Trial 1, except that the antlers were freeze dried whole and all batches were dried at 20°C.

Trial 3a, Mt Hutt Station

The influences of drug type and of crush or workroom animal handling systems on antler colour were investigated.

Animals and treatments

One hundred and eight rising 3-year-old stags were maintained under standard husbandry conditions on pasture. Antler removal took place on the most appropriate day as assessed by the producer using New Zealand Game Industry Board guidelines. Prior to antler removal stags were allocated to one of six main treatments (see below for details) with $n = 18$ per treatment. Treatments 1 and 3 were further broken down into 3 and 2 sub-treatments, respectively.

1. Local anaesthetic only, using one of the following administration methods; antlers removed in a workroom-type hydraulic restraint device as made by the farmer.
   - 1a Left hand side (LHS) ring block, right hand side (RHS) ring block, $n = 6$.
   - 1b LHS ring block, RHS modified regional block, $n = 6$.
   - 1c LHS modified regional block, RHS modified regional block, $n = 6$.
2. Low dose xylazine plus local anaesthetic; antlers removed with the stags restrained in a workroom-type hydraulic restraint device.
3. Low dose xylazine plus local anaesthetic; antlers removed with the stags restrained in a standard deer handling crush with their heads positioned as follows:
   - 3a Head-up in crush, $n = 9$.
   - 3b Head-down in crush, $n = 9$.
4. Xylazine and local anaesthetic; antlers removed with the stags recumbent or standing in the pen.
5. "Fentazin" and local anaesthetic; antlers removed with the stags recumbent or standing in a pen.
6. Carfentanil/xylazine combination and local anaesthetic; antlers removed with the stags recumbent or standing in a pen.

All drugs were administered by a veterinary surgeon experienced in velvet antler removal.

Local anaesthetic ("Local", Lignocaine hydrochloride BP 20 mg ml^{-1}, Techvet Laboratories Ltd) was injected as a modified regional block (unless otherwise stated), in the same manner as for Trial 1 at Invermay.

Xylazine ("Xylase Injection", xylazine hydrochloride 20 mg ml^{-1}, Parnell Laboratories Ltd) was given by intra-muscular injection at dose rates varying according to the temperament and weight of the stag and the prescribed treatment. The low dose xylazine treatments (20–30 mg per stag, Treatments 3a and 3b) were not sufficient to produce recumbency, but quietened the stags in the crush or the workroom. The heavier dose of xylazine (40–60 mg per stag, Treatment 4) produced recumbency in most cases.

"Fentazin", was given by intra-muscular injection at a dose rate (0.6-0.8 ml per stag) that varied depending on the temperament and the weight of the stag.

"Carfentanil" ("Thiazine 50 & Wildnil Combination", carfentanil 50 µg ml^{-1}, xylazine hydrochloride 50 mg ml^{-1}, Techvet Laboratories Ltd) was given by intra-muscular injection at a dose rate (0.5-0.75 ml per stag) that varied depending on the temperament and the weight of the stag.

Stags selected for velveting were treated according to their experimental allocation and as described above. The modified regional nerve block was the same technique as described in Trial 1.

Velvet antler removal took place approximately 4 to 6 min after administration of local anaesthetic. Immediately (seconds) before the antler was removed, a rubber tourniquet was applied around both pedicles. The antlers were removed using a medium-tooth meat saw, either in a workroom crush or in a pen, as per the allocated treatment.

Following removal the antlers were inverted, graded, and weighed. Once cool, the velvet antlers were frozen, inverted, in a large walk-in freezer.

All recoveries from drug treatment and antler removal were uneventful.

Trial 3b, Mt Hutt Station

This trial investigated the effect of post-removal antler position on antler colour.

Animals and treatments

Two hundred rising 3-year-old stags were maintained under standard husbandry conditions on pasture. When judged by the producer to be ready for harvesting using New Zealand Game Industry Board guidelines, antlers were removed under light xylazine sedation, in a crush with the animal's head down. Local anaesthesia was administered using a modified regional nerve block (see description for Trial 1), and the tourniquet was applied immediately

before antler removal. One antler was allocated at random to a control treatment. This involved immediate inversion of the antler upon removal, followed by being held for 3 h at ambient temperature in the yards and then frozen at –20°C, all with the antler in the same vertical orientation with the tip pointing downwards. The opposite antler was pre-allocated, before antler removal, to one of the following treatments:

1. Antler inclined (tip down) at approximately 15° for 3 hours after removal and frozen inverted, i.e., with the tip down (n = 40).
2. Antler inclined (tip down) at approximately 15° for 3 hours after removal, but turned after 1.5 hours so that the lower side became the upper side, and then frozen inverted (tip down) (n = 40).
3. Antler inclined (tip down) at approximately 15° for 3 hours after removal and frozen inclined at approximately 15° (tip down) (n = 40).
4. Antler inverted (tip down) for 3 hours after removal and frozen inclined at approximately 15° (tip down) (n = 40).
5. Antlers inverted (tip down) for 3 hours after removal and frozen upright (tip up) (n = 40).

Each pair of antlers was also pre-allocated, at random, to processing at one of two commercial antler processing plants (Plant A and Plant B).

Trial 4, Mt Hutt Station

This trial investigated the effects of post-removal temperature and of time to freezing on antler colour and on the extent of microbiological contamination.

Animals and treatments

One hundred and twenty rising 3-year-old stags were maintained under standard husbandry conditions on pasture. On the optimal day of antler removal, as assessed by the producer, stags were randomly allocated to one of six treatments (n = 20 per treatment) as follows:

1. Both antlers frozen immediately after removal.
2. One antler at random per stag held at ambient temperature for 30–60 minutes and then frozen. The remaining antler was placed in a refrigerator at 4°C for 30 minutes and then frozen.

3–7. As for Treatment (2), but antlers held for 60–120 min (Treatment 3), 120–180 min (Treatment 4), 180–240 min (Treatment 5), 240–300 min (Treatment 6), or 300–360 min (Treatment 7) either in a refrigerator or at ambient temperature before freezing.

All antlers were removed using the same technique as in Trial 3a, Treatment 3b.

The cut surfaces of the antlers were swabbed for microbiological examination immediately prior to their being placed in the freezer. A sterile 5-cm^2 metal template was pressed onto the exposed, cut surface of the antler base. A sterile swab stick was moistened with sterile 0.1% peptone water solution and was rubbed up, down, and across the entire exposed area in the template. The swab was turned over and the process repeated. A second, dry swab was rubbed over the sample area in the same manner. The ends of both swabs were broken off below the handling area of the sticks and were put into a sterile container containing 0.1% peptone water and glass beads, and were sent for laboratory analysis.

Total aerobic plate counts (TAC), and yeasts and moulds plate counts, were carried out according to the compendium of Methods for the Microbiological Examination of Foods, APHA, 3rd Edition, 1992 (Methods 3.513 and 16.51, respectively).

All antlers were processed at a commercial drying plant, Plant A.

Colour measurement

All antlers from Trials 1 and 2, and antlers randomly allocated within treatments from Trials 3a (n = 18) and 4 (n = 20), were assessed for colour using a Lab Scan 6000 scanning reflectance visible spectrometer (Hunter Associates Inc., USA). These antlers were cut into 1-cm discs, perpendicular to the vertical axis of the main beam using a band saw fitted with an 18-mm fine blade. All antlers from Trials 3a, 3b, and 4 were analysed using a Chroma Meter CR-200 (Minolta, Japan). Colour measurements were taken immediately after removal, after thawing but before processing, mid way through the drying process after the lower tines had been cut off above the brow tine or bez tine (if present), and at the conclusion of processing at the base (both plants) and the cut surface of the mid beam (Plant A only).

The Lab Scan 6000 scanning reflectance visible spectrometer had 0° illumination, adjustable beam diameter, and 45° viewing geometry with the specular component excluded. The instrument was calibrated with a white standard supplied by the manufacturers and conforming to the National

Bureau of Standards perfect white. An IBM XT microcomputer performed all colour calculations from the digitised spectral data. Each antler disc was placed over a 10-mm-diameter open circular port with a 6-mm-diameter illuminated spot (area) in the horizontal upper surface of the sensor module. Ten reflectance spectra were obtained over the wavelength range 400–700 nm for different, but overlapping, regions approximately 10 mm apart. The spectra were averaged, and their CIELAB (Commission International d' Eclairage) co-ordinates (L*, a*, and b* values) were recorded for the CIE standard source D65 and the CIE 10° standard observer. These values correspond to the visually perceived colour attributes of "lightness" (L*) on a scale between 0 (white) and 100 (black), and of saturation and hue according to their red versus green (a*) and yellow versus blue (b*) attributes. From a* and b*, the hue angle was calculated. This angle measures colour in relation to the red colour axis, which is defined as having a hue angle of 0°. Antlers having low hue angles are thus redder than those with greater hue angles (which appear browner).

The Chroma Meter CR-200 is a compact, portable tristimulus colour analyser for measuring reflective colours from surfaces. It has an 8-mm-diameter measuring area and uses diffuse illumination and a 0° viewing angle (specular component included). The meter was calibrated using a white standard supplied by the manufacturer. Three reflectance spectra were obtained in a single place for each antler cut surface. From the spectra, mean L*, a*, and b* co-ordinates were calculated.

The Lab Scan 6000 is generally accepted to be more accurate but is not portable, so it was used for in-depth studies of antlers from the Invermay Trials 1 and 2, and selected antlers from Trials 3 and 4. Because the Chroma Meter CR-200 is portable, albeit slightly less accurate, it was used to measure large numbers of antlers on the farm and at the processing plants. Correlations between data analysed using both measurement systems indicate a very high level of agreement and reliability.

Biometric analysis

The colour measurements were analysed by analysis of variance (ANOVA). Data from the lower cut surfaces of each section are presented, and the antlers measured using the Lab Scan 6000 were analysed according to anatomically defined sections (Fig. 1). ANOVA provided the standard error of the difference (SED) values and the standard error of the ratio (SER) values. Regression analyses were used to examine the relationships between time to freezing, and microbiological measurements in Trial 4.

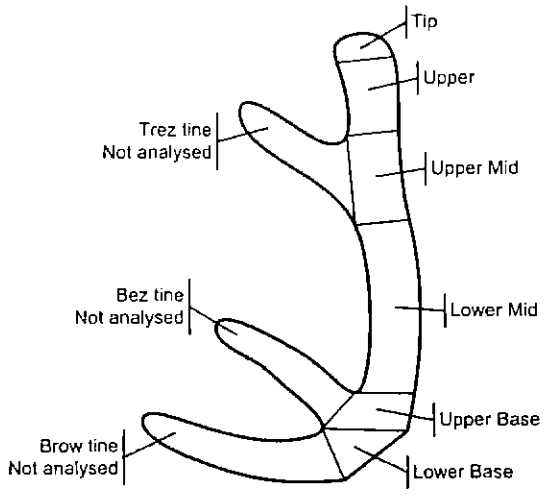

Fig. 1 Sections of antler studied. Preliminary studies indicated high levels of variability within the tines, and consequently they were excluded from further analysis.

RESULTS

Trial 1, Invermay

Antlers from all drug treatment groups displayed similar patterns of L* and hue down their main beams from the tip to the base (Fig. 2). The upper and upper mid sections were generally darker and redder than either the tips or lower sections. ANOVA revealed no significant overall effects of drug treatment on L* or hue, but there was an individual significant difference in hue for the upper sections. This comparison revealed that local-only antlers were redder in this region than those of the xylazine-treated animals. In general, the xylazine antlers tended to be less red (but not significantly so), than those of the other treatments. There were no significant differences in L* or hue due to holding treatment or due to analgesic administration technique in stags treated with local anaesthesia only (Fig. 3, 4). In contrast, the temperature at which the antlers were dried had major effects on L* and hue (Fig. 5). Antlers dried at 20°C were overall significantly redder and darker than those dried at

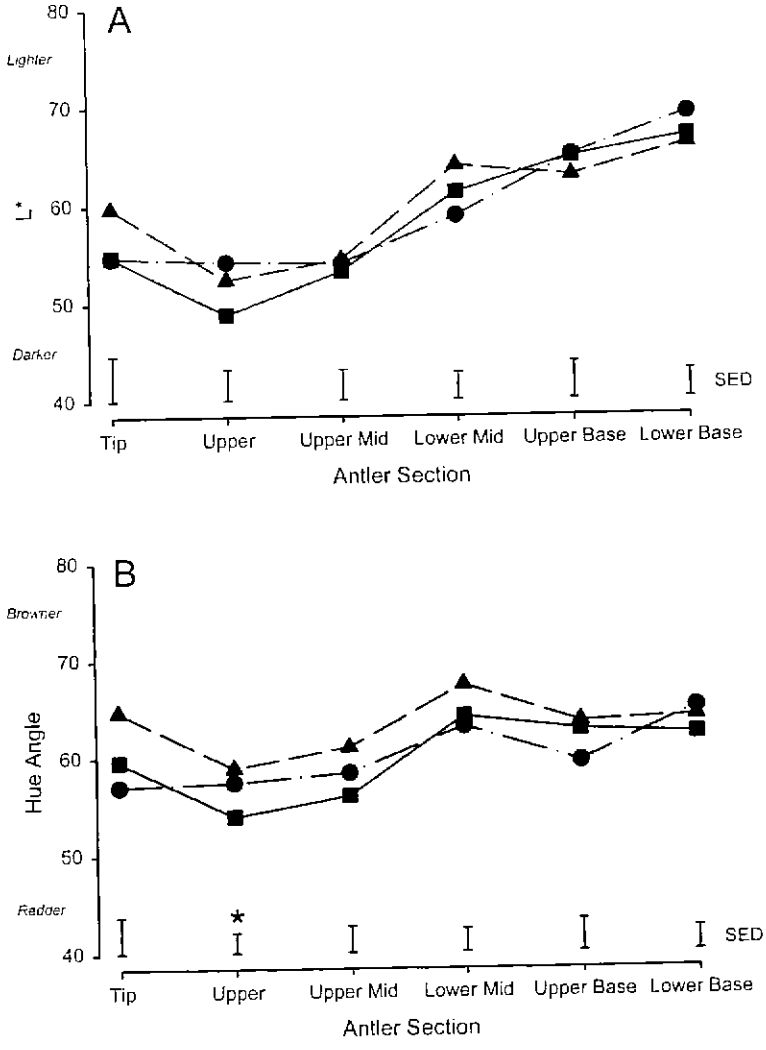

Fig. 2 The effect of drug treatment on **A**, L* (lightness) and **B**, hue angle values of antler sections in Trial 1. SED is the standard error of the difference. ■, local only; ▲, Xylazine; ●, Fentazin. *, $P < 0.05$.

30°C. Moreover, the patterns of colour within the antler varied with drying temperature. The antlers processed at 30°C were darkest at the tip and were progressively lighter toward the base. In contrast, the antlers dried at 20°C were light at the tip, darker in the upper and upper mid sections, and lighter toward the base.

Trial 2, Invermay

There were no significant effects of the drug treatments alone, or of the timing of the application of the tourniquet in the local only antlers, on L* or hue (Fig. 6). In contrast, there were highly significant interactions between xylazine/"Fentazin" treatment and the timing of the tourniquet application. However, detailed examination of this interaction revealed a further interaction with ambient temperature on the day of removal. Antler colour in relation to tourniquet application requires clarification in a further study. The distribution of L* for the xylazine/"early tourniquet" group differed from all other groups, in that minimum L* (i.e., maximum darkness) was detected in the lower-mid rather than the upper-mid section.

Trial 3a, Mt Hutt Station

There were no significant differences due to drug treatment found in L* or hue values in the subset of antlers analysed using the Lab Scan 6000 (Fig.

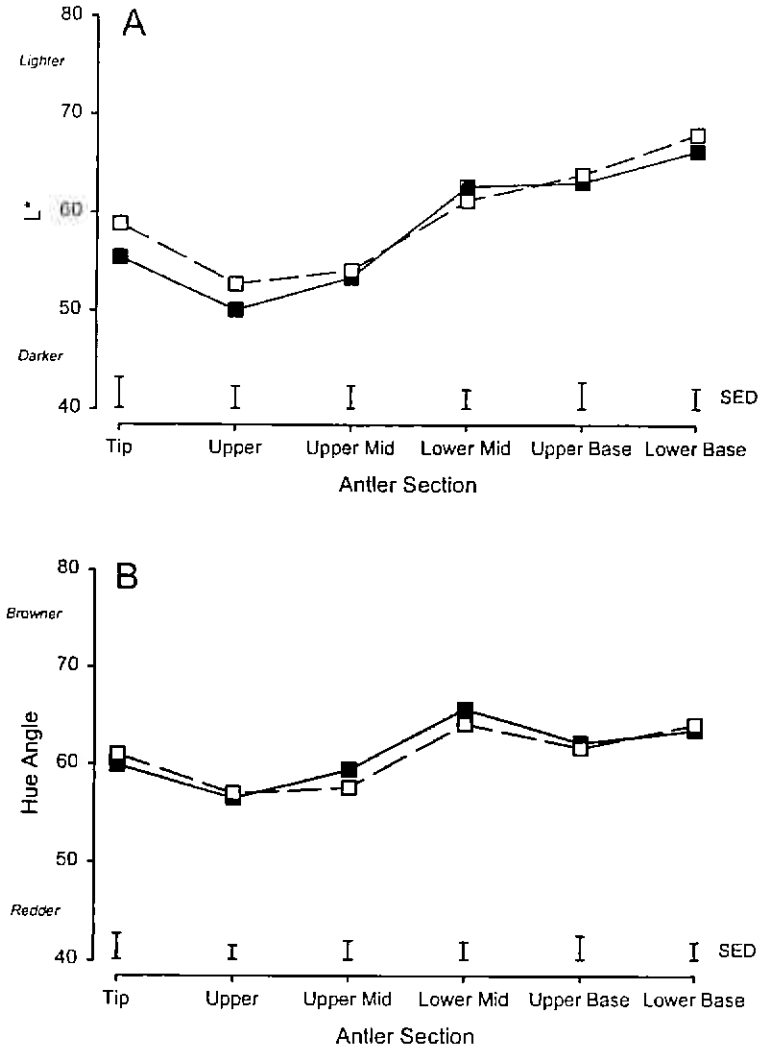

Fig. 3 The effect of holding treatment on **A**, L* (lightness) and **B**, hue angle values of antler sections in Trial 1. SED is the standard error of the difference. ■, holding; □, immediate.

7). Overall, these antlers became darker and redder from the base toward the tip. Antlers which were removed from stags in a workroom (with local only or with low dose xylazine), and those from stags that were sedated with "Carfentanil", tended to be darker and redder in the upper-mid sections than antlers from the other treatment groups.

In the full set of antlers analysed using the Chroma Meter CR-200, significant differences in L* and hue due to antler removal treatment were revealed (Tables 1 and 2). Immediately after removal, the bases of antlers from stags treated with xylazine at a sedation level, or with "Fentazin", were lighter and less red than those from stags treated with local anaesthetic only. This trend continued during processing. The xylazine, "Fentazin", and "Carfentanil" treatments each significantly increased lightness and reduced redness in fully processed antlers, both in the mid beam and at the base. There were strong trends that the low dose of xylazine also increased lightness and reduced redness compared with local anaesthetic only. Among the local-only treatments there were, overall, no significant differences in lightness or red colour due to mode of administration. Head position of animals in the crush after xylazine treatment did not significantly influence either measure of antler colour in stags lightly sedated with xylazine. Overall, the most important finding was that use of sedative drugs during

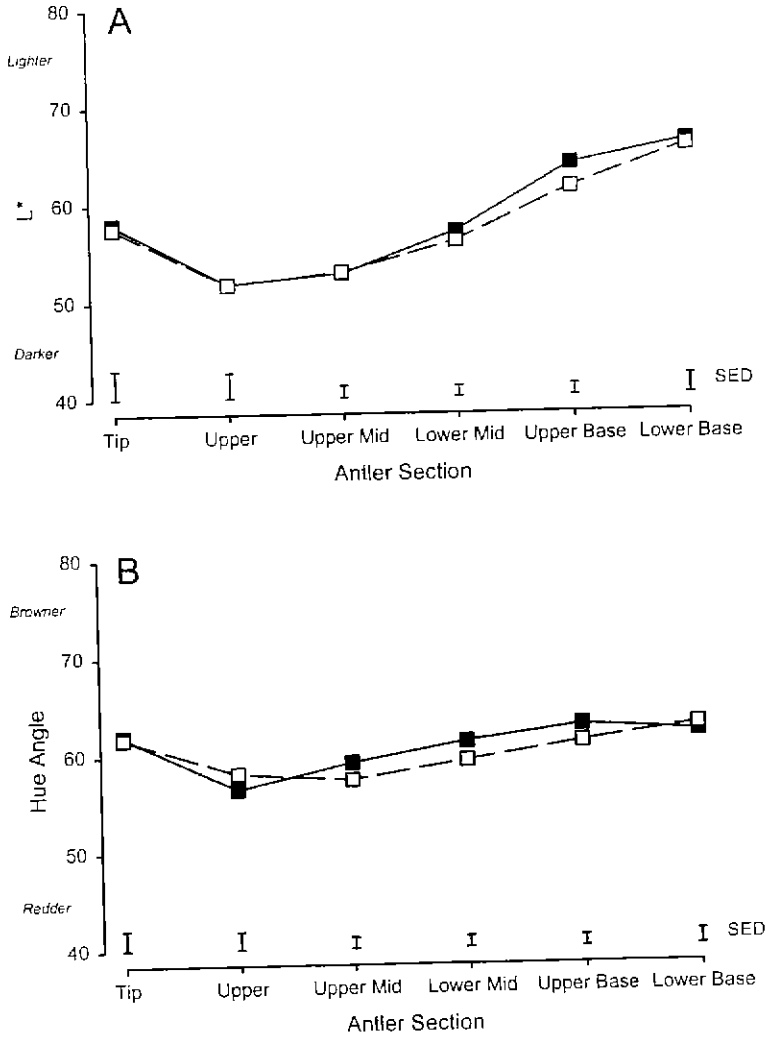

Fig. 4 The effect of local analgesic administration technique on **A**, L* (lightness) and **B**, hue angle values of antler sections in Trial 1. SED is the standard error of the difference. ■, nerve block; □, ring block.

removal treatment resulted in increased lightness and reduced redness of antlers.

Trial 3b, Mt Hutt Station

At all stages of post-removal handling, including drying at both processing plant, it was clear that one treatment consistently resulted in antlers that were darker and more red, i.e., antler inclined at an angle of 15° at all times after removal and during freezing (Tables 3–6). However, all treatments in which the antler was held at an angle of 15° before freezing produced antler with slightly more preferred colour than the standard post-removal antler inversion technique, which was used as the control treatment. The adverse effects of the standard inversion technique on antler colour were partially reversed by freezing the antler upright, i.e., in the anatomical position.

Although no statistical comparisons were carried out, the agreement in the data between the two processing plants (Tables 3–6) is worthy of note.

Trial 4, Mt Hutt Station

Antlers from this trial that were analysed using the Lab Scan 6000 had overall patterns of L* and hue, in relation to regions of the main beam, that were more similar to those of Invermay antlers freeze dried at 20°C than to the antlers of the previous Mt Hutt Station experiment (Trial 3).

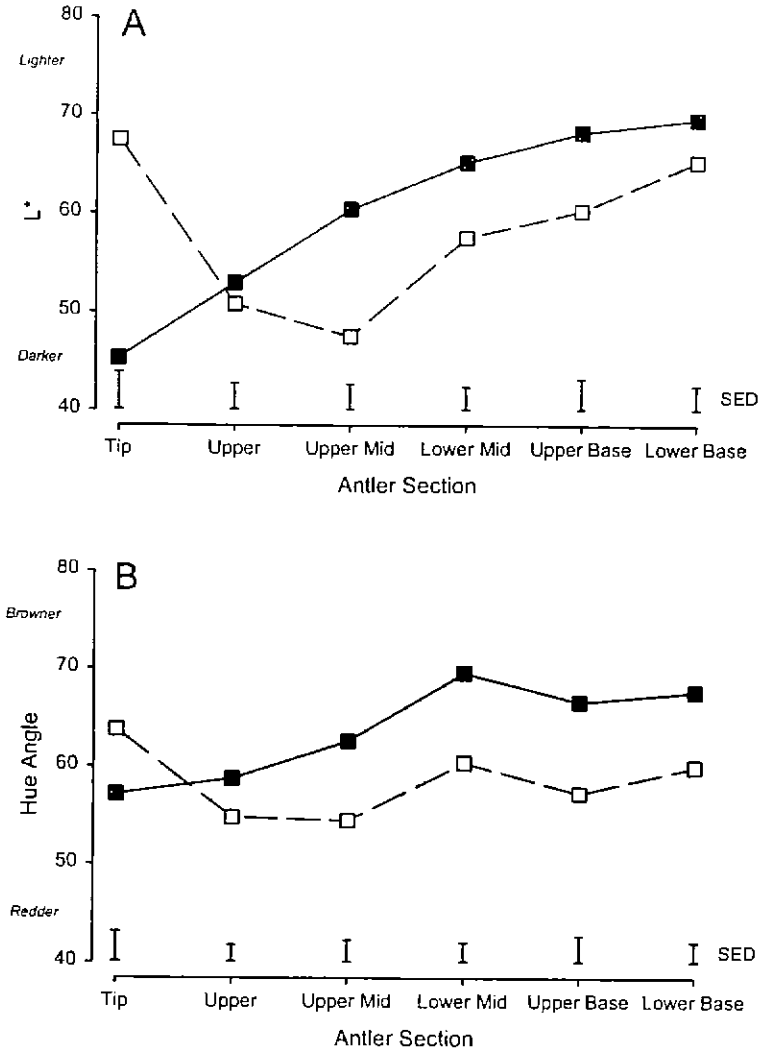

Fig. 5 The effect of drying temperature on **A**, L* (lightness) and **B**, hue angle values of antler sections in Trial 1. SED is the standard error of the difference. ■, 30°C; □, 20°C.

That is, antlers were darkest and reddest in the mid region of the main beam, while the tips and base regions were similar in lightness and brown character, instead of having the darkest and most red colour nearest the tip as in the previous Mt Hutt Station antlers. There was no significant main effect of varying the time between antler removal and freezing on L* or on hue (Fig. 8). However, at 30–60 min from removal to freezing, the upper mid section was significantly lighter and less red. There were no significant differences in L* or hue between antlers held at 4°C or at ambient temperature following removal and prior to freezing (Fig. 9).

The bases of antlers analysed using the Chroma Meter CR-200 after processing at Plant A did not show significant differences in either L* or hue due to time from removal to freezing (Table 7). No consistent patterns due to differences in time to freezing or in pre-freezing temperature emerged in the microbiological data (Table 8). There were no advantages, on microbiological grounds, in chilling the antlers to 4°C in a refrigerator compared with holding at ambient temperature prior to freezing. The yeasts and moulds data must be viewed carefully, since the actual contamination rate was very low with less than 20% of each treatment group having detectable yeasts.

DISCUSSION

The overall strategy used to achieve the aims of this study was to carry out more the intensive experimental work at Invermay, and the less intensive work at Mt Hutt Station, under conditions representative of normal deer farms. The Invermay antler was processed by freeze drying, while the Mt Hutt Station antler was commercially processed by either Plant A or B. In this discussion, the

Table 1 L* (lightness) in relation to treatment in antlers from Trial 3a, Mt Hutt Station 1993. Lower numbers correspond to increased darkness in colour. Local anaesthetic was given to all stags as a modified regional block unless otherwise stated. Colour measurements were made using the Chroma Meter CR-200. ND, no data; SED, standard error of the difference. **, $P < 0.01$; ***, $P < 0.001$.

	Time and position of colour measurement				
Antler removal treatment	Immediately after removal antler base	Pre-processing (post-thaw) antler base	Mid-processing antler mid beam	Processed antler mid beam	Processed antler base
1a Local only, ring block both sides	26.1	24.1	32.4	34.8	38.3
1b Local only, ring block one side, regional one side	25.4	23.9	28.7	32.8	37.7
1c Local only, regional block both sides	25.7	25.4	30.6	31.9	37.9
2 Low dose xylazine, workroom	27.5	27.0	33.7	36.1	40.0
3a Low dose xylazine, crush, head up position	ND	ND	ND	40.5	41.6
3b Low dose xylazine, crush, head down position	ND	ND	ND	43.3	41.7
4 Xylazine	28.4	28.9	35.5	43.4	43.6
5 "Fentazin"	29.8	29.9	34.7	39.6	41.9
6 "Carfentanil"	27.2	28.1	34.7	37.2	41.6
SED	0.71***	1.41***	1.88**	2.30***	1.79**

Table 2 Hue angle (redness) in relation to treatment in antlers from Trial 3a, Mt Hutt Station 1993. Lower numbers correspond to greater redness. Local anaesthetic was given to all stags as a modified regional block where not specifically stated. Colour measurements were made using the Chroma Meter CR-200. ND, no data; SED, standard error of the difference. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

	Time and position of colour measurement				
Antler removal treatment	Immediately after removal antler base	Pre-processing (post-thaw) antler base	Mid-processing antler mid beam	Processed antler mid beam	Processed antler base
1a Local only, ring block both sides	21.2	21.1	41.5	46.1	48.3
1b Local only, ring block one side, regional one side	20.3	21.4	37.0	41.4	45.4
1c Local only, regional block both sides	20.3	21.6	41.6	42.6	45.9
2 Low dose xylazine, workroom	21.0	22.3	44.6	46.0	48.5
3a Low dose xylazine, crush, head up position	ND	ND	ND	53.4	50.2
3b Low dose xylazine, crush, head down	ND	ND	ND	54.9	49.4
4 Xylazine	21.9	23.2	45.6	53.8	50.7
5 "Fentazin"	21.8	23.6	44.8	51.3	50.0
6 "Carfentanil"	20.9	22.2	45.0	49.5	49.6
SED	0.56*	0.69***	2.33**	2.12***	1.76

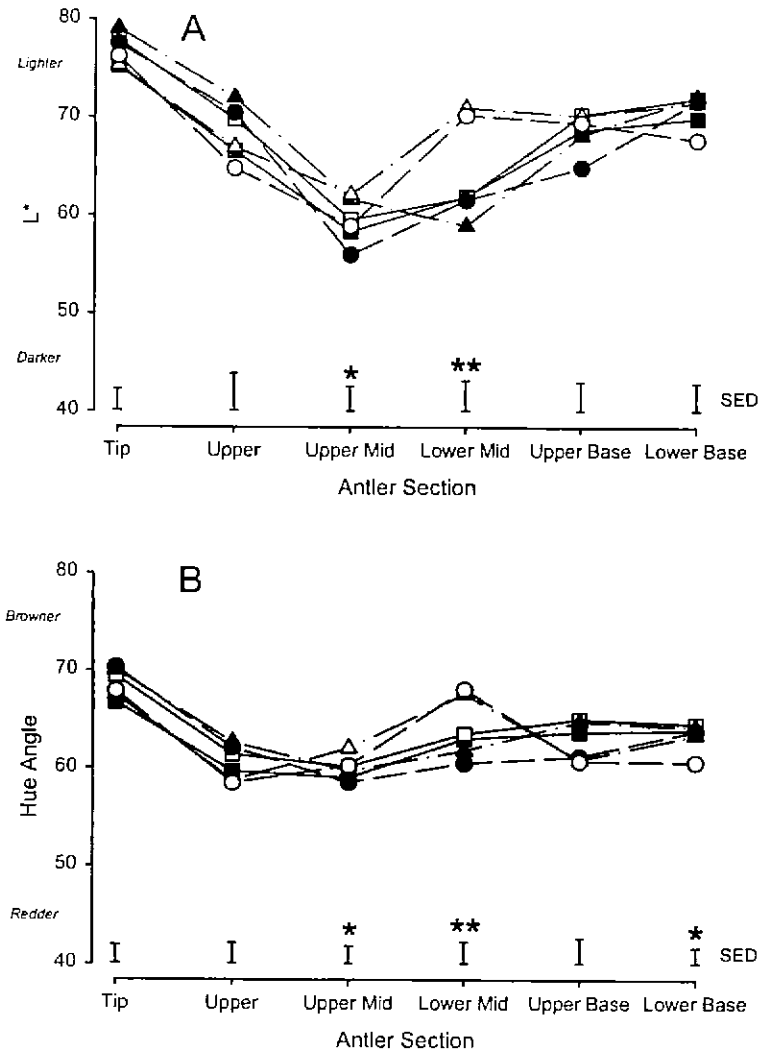

Fig. 6 The effect of drug treatment and timing of tourniquet application on **A**, L* (lightness) and **B**, hue angle values of antler sections in Trial 2. SED is the standard error of the difference. ■, local only, early tourniquet; □, local only, late tourniquet; ▲, Xylazine, early tourniquet; △, Xylazine, late tourniquet; ●, Fentazin, early tourniquet; ○, Fentazin, late tourniquet. *, $P < 0.05$; **, $P < 0.01$.

intention is to consider data from all sources in an attempt to pull together relevant information on factors which could influence antler colour, to facilitate recommendations, and to indicate directions for further work. Factors are discussed in a time sequence from pre- to post-antler removal.

Holding stags in familiar yards for 4 h prior to antler removal had no effect on antler colour. It was assumed that the stags would have become mildly stressed due to the extended nature of the yarding, but behavioural observations at the time do not bear this out. In any event, antler removal from less domesticated stags in former times was much more stressful (as judged by human observers) than in the present study. Consequently, although it can be concluded that holding stags for 4 h in our situation did not affect antler colour, the situation with respect to the effect of a higher level of stress on antler colour is not known. It would be unethical to induce stress simply for the purpose of increasing antler colour, and the risk of damage to antlers would probably obviate any improvements achieved in this way.

Taking together the trend from Trial 1 and the data from the large sample of antlers scanned in Trial 3, it is clear that the use of sedative drugs in doses sufficiently high to cause recumbency during velvet removal, reduces antler colour. This was

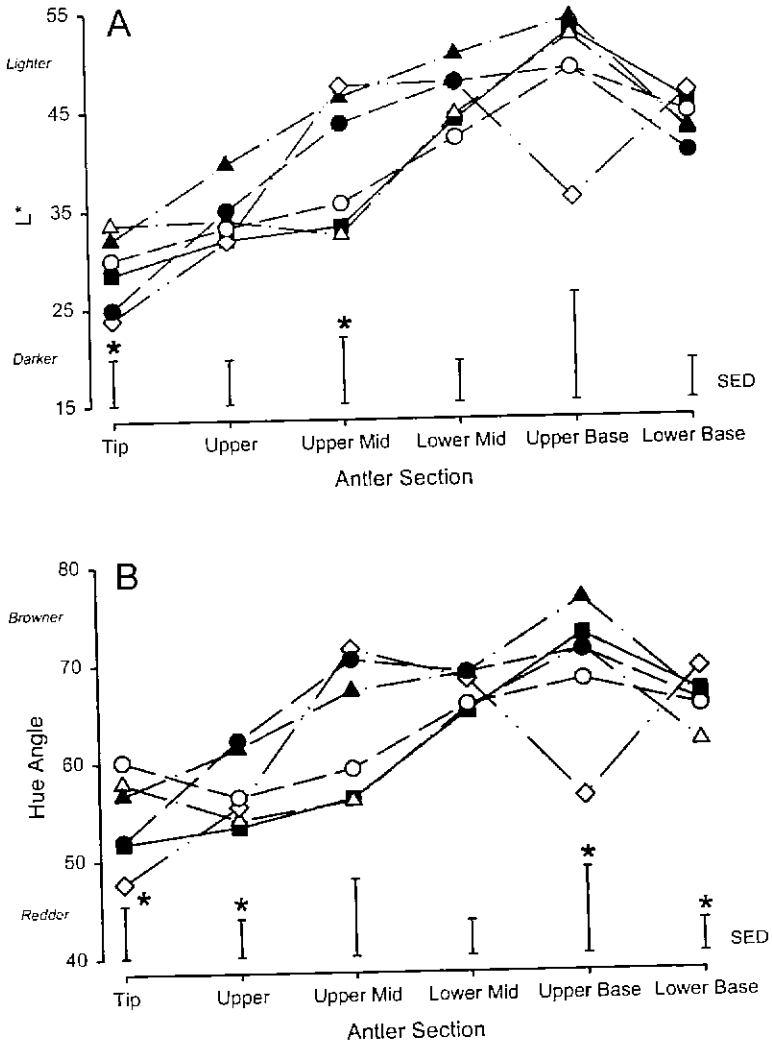

Fig. 7 The effect of drug treatment and restraint method on **A**, L* (lightness) and **B**, hue angle values of antler sections in Trial 3a. SED is the standard error of the difference. ■, local only, workroom; ▲, low dose xylazine, workroom; △, low dose xylazine, crush; ◇, xylazine, standing or sitting; ●, Fentazin plus xylazine; ○, Carfentanil plus xylazine. *, $P < 0.05$.

especially evident with xylazine. There is also a strong trend in Trial 3 that even low doses of xylazine alter antler colour and its distribution, compared with stags whose antlers are removed under local anaesthetic alone. It is highly likely that the lighter, less red colour is due to the fact that there is less blood in the antler at the time of removal. This is probably because of the blood pressure-lowering effect of xylazine.

One possible reason why the Invermay studies revealed trends rather than significant differences in antler colour pattern due to drugs is that fewer antlers were studied. It is probably best to use the detailed Invermay experiments to investigate the within-antler differences due to drug, and the Mt Hutt Station experiments to consider overall effects. Considered in this light, drugs influence not only absolute colour but also its pattern and distribution. Whether drugs *per se* influence processability of antler or simply reduce the amount of blood (and thus colour) at the time of removal is unknown. A second possible reason is that the Invermay and all Lab Scan 6000 data are based on the mean of 10 separate random measurements of each antler cross-section, while the Mt Hutt Station data obtained with the Chroma Meter CR-200 were only scanned at one position in each cross-section, in the centre. As the centre apparently has a more intense colour than the edges (rim), i.e., the colour pattern varies over an antler cross-section, then variability in the

Mt Hutt Chroma Meter data might be less. This would possibly result in a higher number of real significant differences being detected.

It is germane to point out that a single measure of colour at the base, such as would be perceived by a purchaser of processed velvet, does not necessarily reflect the colour, or its distribution, within the antler. In Fig. 6, for example, although the colour due to combined drug/tourniquet treatments is poor in the lower-mid section, this is

Table 3 L* (lightness) in relation to post-removal holding conditions in antlers from Trial 3b, Mt Hutt Station 1993. Antler was processed at Processing Plant A. Colour measurements were made using the Chroma Meter CR-200. ND, no data; SED, standard error of the difference. *, $P < 0.05$; ***, $P < 0.001$.

Antler removal treatment	Time and position of colour measurement				
	Immediately after removal antler base	Pre-processing (post-thaw) antler base	Mid-processing antler mid beam	Processed antler mid beam	Processed antler base
1 Antler inclined at 15° for 3 hours, then frozen inverted	27.8	27.5	32.2	35.6	40.8
2 Antler inclined at 15° for 3 hours, but turned after 1.5 hours so that the lower side became the upper side and then frozen inverted	27.5	28.2	31.9	34.4	40.6
3 Antler inclined at 15° for 3 hours, and frozen inclined at 15°	27.2	29.2	28.4	28.5	38.1
4 Antler inverted for 3 hours, and frozen inclined at 15°	30.1	29.8	32.8	35.3	42.3
5 Antler inverted for 3 hours, and frozen upright	27.3	ND	28.7	30.8	41.8
Control – Antler inverted for 3 hours, and frozen inverted	28.0	27.7	35.1	38.0	42.2
SED	0.84*	1.01	1.07***	1.51***	1.49

Table 4 Hue (redness) in relation to post-removal holding conditions in antlers from Trial 3b, Mt Hutt Station 1993. Antler was processed at Processing Plant A. Colour measurements were made using the Chroma Meter CR-200. ND, no data; SED, standard error of the difference. ***, $P < 0.001$.

Antler removal treatment	Time and position of colour measurement				
	Immediately after removal antler base	Pre-processing (post-thaw) antler base	Mid-processing antler mid beam	Processed antler mid beam	Processed antler base
1 Antler inclined at 15° for 3 hours, then frozen inverted	20.6	21.7	42.3	44.2	46.7
2 Antler inclined at 15° for 3 hours, but turned after 1.5 hours so that the lower side became the upper side and then frozen inverted	21.4	22.9	41.5	43.3	46.8
3 Antler inclined at 15° for 3 hours, and frozen inclined at 15°	20.8	23.0	38.0	37.9	43.8
4 Antler inverted for 3 hours, and frozen inclined at 15°	21.1	23.1	42.7	44.1	48.8
5 Antler inverted for 3 hours, and frozen upright	21.8	ND	37.8	38.9	43.7
Control – Antler inverted for 3 hours, and frozen inverted	21.6	22.4	45.8	49.0	50.3
SED	0.48	0.70	1.32***	1.19***	1.25***

Table 5 L* (lightness) in relation to post-removal holding conditions in antlers from Trial 3b, Mt Hutt Station 1993. Antler was processed at Processing Plant B. Colour measurements were made using the Chroma Meter CR-200. ND, no data; SED, standard error of the difference. *, $P < 0.05$; ***, $P < 0.001$.

Antler removal treatment	Time and position of colour measurement			
	Immediately after removal antler base	Pre-processing (post-thaw) antler base	Processed antler mid beam	Processed antler base
1 Antler inclined at 15° for 3 hours then froze inverted	27.1	28.6	32.9	39.1
2 Antler inclined at 15° for 3 hours, but turned after 1.5 hours so that the lower side became the upper side and then frozen inverted	28.0	27.7	32.9	35.9
3 Antler inclined at 15° for 3 hours, and frozen inclined at 15°	27.4	28.6	32.4	35.6
4 Antler inverted for 3 hours, and frozen inclined at 15°	27.1	28.6	37.5	40.8
5 Antler inverted for 3 hours, and frozen upright	27.5	ND	33.3	37.4
Control – Antler inverted for 3 hours, and frozen inverted	27.6	29.3	37.6	41.0
SED	0.46	0.83	1.47***	2.52*

Table 6 Hue (redness) in relation to post-removal holding conditions in antlers from Trial 3b, Mt Hutt Station 1993. Antler was processed at Processing Plant B. Colour measurements were made using the Chroma Meter CR-200. ND, no data; SED, standard error of the difference. Level of significance of differences between means: ***, $P < 0.001$.

Antler removal treatment	Time and position of colour measurement			
	Immediately after removal antler base	Pre-processing (post-thaw) antler base	Processed antler mid beam	Processed antler base
1 Antler inclined at 15° for 3 hours, then frozen inverted	19.9	25.1	43.5	46.1
2 Antler inclined at 15° for 3 hours, but turned after 1.5 hours so that the lower side became the upper side and then frozen inverted	20.0	24.3	45.3	46.3
3 Antler inclined at 15° for 3 hours, and frozen inclined at 15°	19.9	24.1	42.6	42.5
4 Antler inverted for 3 hours, and frozen inclined at 15°	21.0	24.6	46.0	48.5
5 Antler inverted for 3 hours, and frozen upright	21.4	ND	43.1	46.7
Control – Antler inverted for 3 hours, and frozen inverted	21.3	24.9	49.1	50.1
SED	0.30***	0.60	1.52***	2.52***

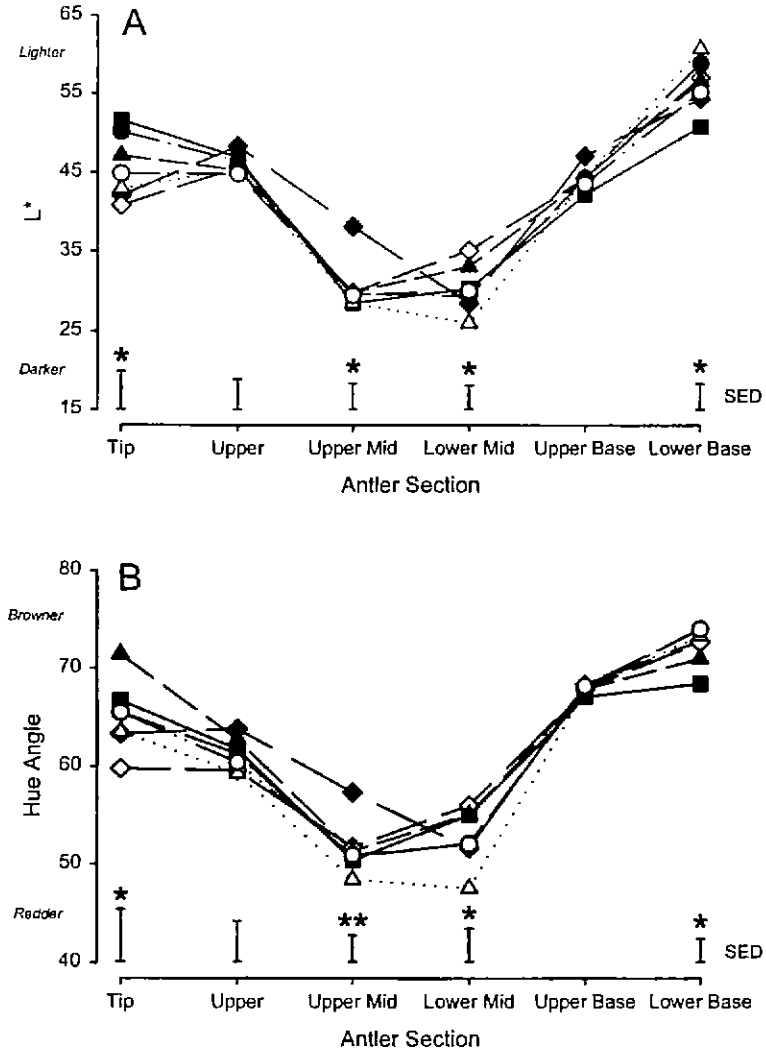

Fig. 8 The effect of time from removal to freezing on **A**, L* (lightness) and **B**, hue angle values of antler sections in Trial 4. SED is the standard error of the difference. No SED is available for the upper base section as sample sizes were low. This was because few antlers had bez tines. ■, immediate; ◆, 30–60 min; ◇, 60–120 min; ▲, 120–180 min; △, 180–240 min; ●, 240–300 min; ○, 300–360 min. *, $P < 0.05$; **, $P < 0.01$.

Table 7 L* and hue in relation to treatment in antlers from Trial 4, Mt Hutt Station 1994. Colour measurements were made using the Chroma Meter CR-200, at the base of each antler only, after processing in Plant A. SED is the standard error of the difference. There were no significant differences due to holding the antlers in the refrigerator or at ambient temperature.

Time to freezing	L*	Hue
Immediate	47.0	56.5
30–60 min	47.5	56.9
60–120 min	49.3	57.3
120–180 min	46.7	55.0
180–240 min	48.7	58.1
240–300 min	47.8	56.6
300–360 min	46.9	57.8
SED	1.5	1.2

not reflected in significant differences at the base. Problems such as this are likely to become more apparent when antler classified in South Korea as New Zealand "Nog yong", that is, the name for velvet antler in Korean, is sliced.

There is a trend observed in Trial 3a that stags treated with low dose xylazine followed by antler removal in a workroom had darker, redder antlers than those of stags treated with low dose xylazine followed by removal in a crush. This comparison needs further study.

There were no significant differences in antler colour due to local analgesia administration technique. This is reasonable, as antler blood vessels lack control systems which depend on adrenergic

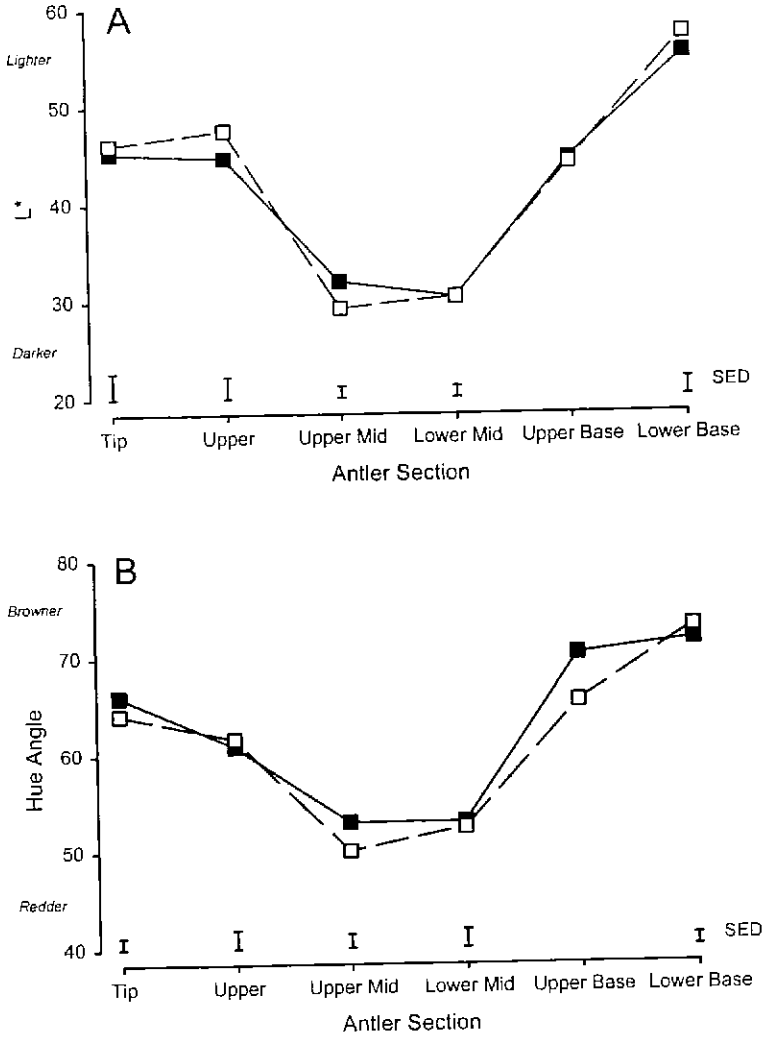

Fig. 9 The effect of pre-freezing conditions on **A**, L* (lightness) and **B**, hue angle values of antler sections in Trial 4. SED is the standard error of the difference. ■, ambient; □, fridge.

Table 8 Total aerobic and yeast and mould counts (cfu cm^{-2}) at the bases of antlers from Trial 4, Mt Hutt Station 1994. Antlers were held either in a refrigerator or at ambient temperature in the yards for up to 360 min after removal. Data presented are geometric means, and SER is the standard error of the ratio. Geometric means are significantly different when the ratio of one to another is greater than the square of the SER. ND, no data.

	Total aerobic count		Yeast and mould count	
Time to freezing	Ambient	Refrigerator	Ambient	Refrigerator
Immediate	883	ND	104	ND
30–60 min	759	1901	100	280
60–120 min	1180	317	120	97
120–180 min	358	1217	100	100
180–240 min	1476	1247	133	100
240–300 min	1588	1226	233	141
300–360 min	2243	675	102	100
SER	2.24		1.31	

or cholinergic agents (Rayner & Ewen 1981). In any case, local analgesia, properly administered to achieve insensitivity, would not be expected to differ in its effect on blood flow as a result of administration technique.

Time from removal to freezing (in the range 0–6 h) had no effect on antler colour, with a minor exception evident in the upper section at 0.5–1 h. This is the time when the blood remaining in the antler after removal would have clotted. The best advice might be to immediately freeze the antler, or to wait for at least one hour to elapse to permit the clot to disperse.

No significant effects on colour were detected after velvet was cooled in a refrigerator before freezing. This could be because the antler cools after removal anyway, and rapid cooling to 4°C does not influence the distribution of blood (and hence colour) any more than the natural rate of cooling. There is certainly no support for recommending this technique to deer farmers and veterinarians.

Analysis of total aerobic and yeast and mould counts revealed no increase in contamination resulting from increasing the time between removal and freezing. Likewise, there were no advantages evident from either microbiological measurement in chilling the antlers in a refrigerator before freezing. While this result is probably accurate in its own right, and no statistically significant difference emerged, it is still likely to be good practice to freeze antlers a maximum of 3–4 h after removal.

Conventionally, antlers are immediately inverted with tips downwards after removal, and frozen inverted, so as to retain the blood. Trial 3b revealed that this treatment results in antler having the poorest colour. This is reasonable because, when the antler is inverted, blood is likely to pool at the tip/upper section potentially making redistribution during the drying process more difficult. In comparison, inclination at 15° prior to freezing, and also during freezing, significantly enhanced antler colour. Inclining the antler at this shallow angle probably helps retain the blood while preventing undue pooling in the upper sections. Freezing the antler in an upright position (i.e., tip upwards), after inversion for 3 hours, was also a useful technique, and produced antler with colour that was almost as good as antler held at 15°. The effects of immediate freezing at 15° on antler colour should be examined.

Discussion of the results relating to the effects of processing on antler colour must be treated carefully, because none of these trials set out specifically to examine these effects. Inferences can be made from the result of drying the antler in Trial 1 at two temperatures, and from broad comparisons between the antler freeze dried at Invermay and those processed traditionally by Plants A and B.

From the Invermay trial it is clear that the absolute colour, and its pattern, differed substantially in antlers freeze dried at the higher temperature (30°C) compared with velvet freeze dried at 20°C. The higher temperature resulted, overall, in lighter, less red velvet, but with a tendency for darker, more red tips compared with bases. In contrast, in the velvet from Trials 1, 2, and 4 that was dried at the lower temperature (20°C), a different, but consistent pattern emerged. Antler was lighter and less red at the tip, darkest and most red in the mid sections, and lighter and less red at the base. It is tempting to speculate that processing temperature was largely responsible for the different patterns. Intuitively, a higher temperature might denature the proteins in the blood and hinder its even redistribution in the antler. Additionally, higher temperature might accelerate oxidation of haemoglobin and hence cause the browning effect observed in Trial 1.

Broadly comparing data from Trials 2 and 4, it is clear that freeze-dried antlers (Trial 2) are consistently lighter than those processed commercially (Trial 4). In addition, the mid sections, which are typically the reddest portions of the antler, are more red in the commercially processed than in the freeze-dried antler. Tips and bases, on the other hand, are of similar hue in antlers processed by either technique. Important caveats must be emphasised; the trials were not designed to make these between-process comparisons, and sources of antler and removal methods differ. Nevertheless, the consistency of the comparisons points strongly to an important effect of processing temperature and technique on antler colour. Both Plants A and B consistently produced antlers which were darker and more red than those freeze dried at Invermay. It is interesting to note that although the plants use different techniques, the colour patterns and absolute colours measured were very similar. Freeze drying, therefore, seems to produce less market-preferred colour than either commercial system. The effects of temperature in the commercial processes are unknown at this time.

Taking particularly Trials 1 (20°C processing), 2, and 4 together, and examining the data from the individual sections of the antler, there is an overall pattern of colour distribution within the antler. Mid

sections are darker and redder than either tips or bases. This is probably the most vascular part of the live antler, with the tips being composed of cartilage and calcifying cartilage with fine blood vessels, and the bases being most heavily calcified. These anatomical differences probably set practical limitations on the extent of antler colour redistribution achievable during processing.

The discussion of the data separately considered the effects of pre- and post-removal conditions on antler colour. This is probably simplistic, although two principal points have emerged; there are conditions which influence the absolute amount of colour initially in the antler, and conditions which influence its ability to be re-distributed during final processing. Clearly the processing itself is a third factor, but this is outside the control of producers. Prior to antler removal, the drug used and, potentially, the timing of the application of the tourniquet are very important. Another possibly relevant factor, not considered in the current study, is the stage of antler growth at time of removal (i.e., days from casting). After removal, the angle at which the antler is held seems to be very important, both before and during freezing.

Currently, farmers are paid by the weight of antler produced, within the constraints of a grading system, hence there is no financial incentive to produce antler of superior colour. When slicing regulations alter in Korea, however, it may become necessary to reward producers of antler which has superior colour. Farmers will then need to pay careful consideration to the factors highlighted by this study as being important determinants of final antler colour.

NOTE ADDED IN PROOF: Since this work was completed consumers are tending towards products free of any possible residues, hense the use of sedation drugs is discouraged.

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