Recent Advances in Deer Velvet Research

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Abstract

Over the last four years the Deer Velvet Research programme in New Zealand has re-aligned many of its activities to assist developing health food and dietary supplement markets. These fall into four main areas 1) product safety, 2) product efficacy, 3) velvet removal techniques and 4) product activity This paper will present data on the first three areas, and a brief discussion of the fourth.

We have carried out acute, sub-chronic, reproductive and developmental oral toxicity research and have shown that there are no product safety issues in these key areas Efficacy research has advanced considerably from cell culture and animal research to full scale human clinical trials These trials have focussed on velvet's effects on athletic performance and recovery after injury. We have shown that New Zealand deer velvet powder assists muscle strength and endurance training, but there was no significant protective effect on exercise-induced muscle damage. These results are crucial to attract and develop new overseas markets for New Zealand deer velvet Purified active ingredients from deer velvet are needed as standards and marker substances to identify velvet in mixed samples and for quality assurance parameters. This research is progressing rapidly due to recent technical innovations and targeted overseas collaborations.

The overall aim is to provide an integrated package of data to assist the marketing of New Zealand deer velvet in overseas markets.

Introduction

The Velvet Antler Research New Zealand (VARNZ) research programme was originally set up to evaluate velvet antler for the Traditional Korean market. Over the last four years the programme has gradually repositioned to predominantly provide support for the emerging North American dietary supplement market.

This market has several cornerstone product requirements. These are that a product must be non-toxic (safe), effective for the stated purpose, an active ingredient should be known and that the product is produced in an environment and welfare friendly way

The VARNZ research programme has developed objectives which address these cornerstones

The aim of this paper is to briefly summarise progress in these four areas. The summaries are brief as full publication is being developed currently in three of the four areas

1. Product Safety

Potential toxic effects of acute and sub-chronic dosage regimens of deer velvet powder have been assessed in rats, following OECD guidelines. In the acute study, rats of both sexes were exposed to a single dose of 2 g/kg body weight. There was no mortality or other signs of toxicity during 14 days observation Furthermore, no significant alteration either in relative organ weights or their histology was discernible at terminal autopsy In the 90-day sub-chronic study, deer velvet was administered in 1 g/kg daily doses by gavage to rats A control group of rats received water only There was no effect on body weight, food consumption, clinical signs, haematology or most parameters of blood chemistry including carbohydrate metabolism, liver and kidney function. No significant differences in the mean organ weights of the adrenal, kidney and brain were seen between rats treated with deer velvet and control rats However, there was a significant difference in the mean liver weights (15.1 ± 2.4 vs 18 1 ± 2 9 g) of deer velvet-treated and of control male rats, respectively. The gross necropsy and pathological examination of rats treated with deer velvet did not reveal any abnormalities in tissue

morphology Based on these results, it may be concluded that rats had no deer velvet treatment-related toxicological and histopathological abnormalities at the administered doses, despite the observed minor changes in liver weight

New Zealand deer velvet powder was tested for reproductive and developmental toxicity in Wistar rats, using the OECD guideline 421 (Reproductive/Developmental Toxicity Screening Protocol) New Zealand deer velvet was administered in 1 g/kg daily doses by gavage to treatment group rats. The control group rats received water only. Dams and litters were sacrificed on postpartum day 4 and males were sacrificed within the following week There was no apparent maternal, reproductive, or developmental toxicity No clinical signs of toxicity and no effects on body weight, food consumption, or absolute organ weights were observed No microscopic changes were observed in reproductive organs of parental animals. There were no differences in mean number of corpora lutea, implantation sites, or live pups per litter, and no gross anomalies were observed In conclusion, the dose of New Zealand deer velvet evaluated in the rats was far greater than any doses anticipated for human consumption. Thus, it is likely that no reproductive and developmental toxicological risk would occur with doses of New Zealand deer velvet commonly consumed by humans

2. Product Efficacy

Two studies have been performed with the objective of studying the effects of Deer Antler products on the health and performance of human male subjects. Both studies were approved by the Southern Regional Health Authority Ethics Committee.

A. Strength Trial

The main objectives of the experiment were to

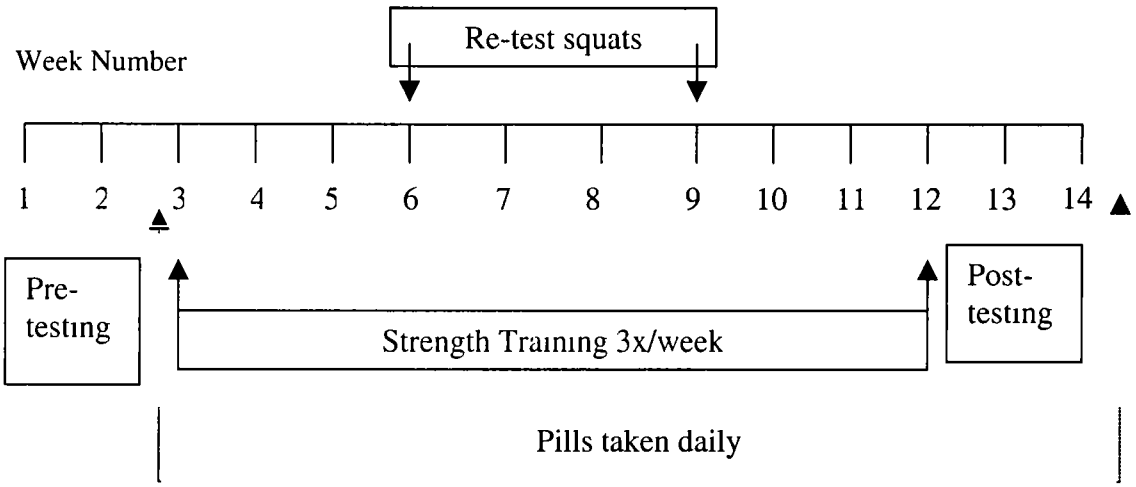
1 Determine if Deer Antler Extract (Extract) and/or Deer Velvet Powder (Powder) acted as an ergogenic aid in enhancing the muscular strength and endurance gains experienced during a 10-week resistance training study

2 Determine the effects of Extract and Powder on blood lipids and lipoproteins, immunopotentiation, blood volume and erythropoiesis, anabolic factors, and maximal aerobic power

Methodology

Fifty-one active male subjects (3 groups of 17) from 19 to 24 years old were recruited for this study Physiological testing took place pre-training, and the participants were split into 3 groups in a randomised procedure that gave a high covariate efficiency for preliminary strength measurements Participants trained for muscular strength-endurance using parallel squats (Smith machine) and isokinetic leg extension and flexion (Biodex) for 10 weeks while consuming deer velvet extract (300 mg/day), deer antler powder (1 5 g/day), or placebo in a double blind fashion (Figure 1). The capsules were identical in appearance and all testing and training was identical for the three groups of participants Physiological testing included measures of anthropometry, muscular strength and endurance, aerobic power, blood chemistry, blood volume and its major fractions, and a gonadotropin releasing hormone (GnRH) challenge test The anthropometry measurements were body mass, standing height and sum of eight skinfolds. The measures of muscular strength and endurance were isoinertial parallel squat (six-repetition maximum - 6 RM), isokinetic knee extensor strength measure (3 maximal voluntary contractions - MVC) at an angular velocity of 60 deg/s, an isokinetic endurance measure (25 MVC) of the knee extensors at an angular velocity of 120 deg/s, and a 90 second cycle ergometer sprint test The 90 second cycle test loaded the trained knee extensors as well as other contributing muscles, and required energy contribution from both aerobic and anaerobic metabolism. The aerobic power test utilised an incremental stepwise treadmill test to exhaustion in which oxygen consumption was measured directly using open-circuit spirometry. Haematological measures included blood lipids and lipoproteins, red and white blood cell differentials, testosterone, insulin-like growth factor-1 (IGF-1), and erythropoietin. Blood volume was measured using a carbon monoxide (CO) re-breathing procedure that allowed direct determination of red blood cell mass

Figure 1: *Strength Study Design*



Results & Interpretation

The main finding of this study was that isokinetic strength and muscular endurance improved to a greater extent in the group that was supplemented with Velvet Powder when compared with the Placebo group and the group supplemented with Velvet Extract However, other measures of strength, endurance, and power did not improve differentially between groups and no mechanisms could be identified that explained these significant differences

The significant change in isokinetic muscular strength was not accompanied by similar differences in the extent of isoinertial strength adaptation (6 RM strength), peak power output on the 90 second cycle ergometer, or in the level of circulating total testosterone in the blood. Similarly, the significant change in isokinetic average power in muscular endurance was not accompanied by the expected changes in extent of the 90 second cycle ergometer test, maximal oxygen consumption, or differences in the extent of change in volume of red blood cells, total blood volume, and circulating erythropoietin A possibility exists that the velvet powder may have provided an analgesic effect, reducing the pain experienced by the athletes during the muscular endurance test, or that the powder acted as a buffer and minimised the acidosis and associated fatigue the athletes experienced during the isokinetic endurance test If that was the case however, we would have also expected to see improved performance in the 90 second endurance. Therefore, these theories can not be substantiated based on the results we have found It is quite possible that the lower starting levels of isokinetic strength and endurance in the velvet powder group contributed to the greater changes observed with training and that the powder supplement had little effect.

No significant changes were observed in the plasma immune parameters or lipid parameters that were monitored. However these variables were only of peripheral interest to the study and deficiencies in the experimental design were present Based on the results of the present study it appears that there is no treatment effect of velvet powder or extract on these parameters in the male population studied.

We believe that this is the most comprehensive experiment that has been performed on the effects of deer velvet on human performance and health As with any scientific study it is impossible to examine exhaustively all the potential effects of this product in one project The large number and variety of variables analysed in this study made coordinating an ideal study design for every variable virtually impossible Because the primary aim was to determine if Extract and/or Powder acted as an ergogenic aid to the muscular strength and endurance gains experienced during a 10-week resistance training study, the study design was optimised for this purpose The secondary aims were constructed around the design of the primary aim, and the addition of some variables once the study was already underway meant that the design was weak for these variables This study has only scratched the surface of the potential for collaborative research between the Human Performance Centre and

AgResearch - there is still much work to be done in the area of human performance and health benefits of deer velvet

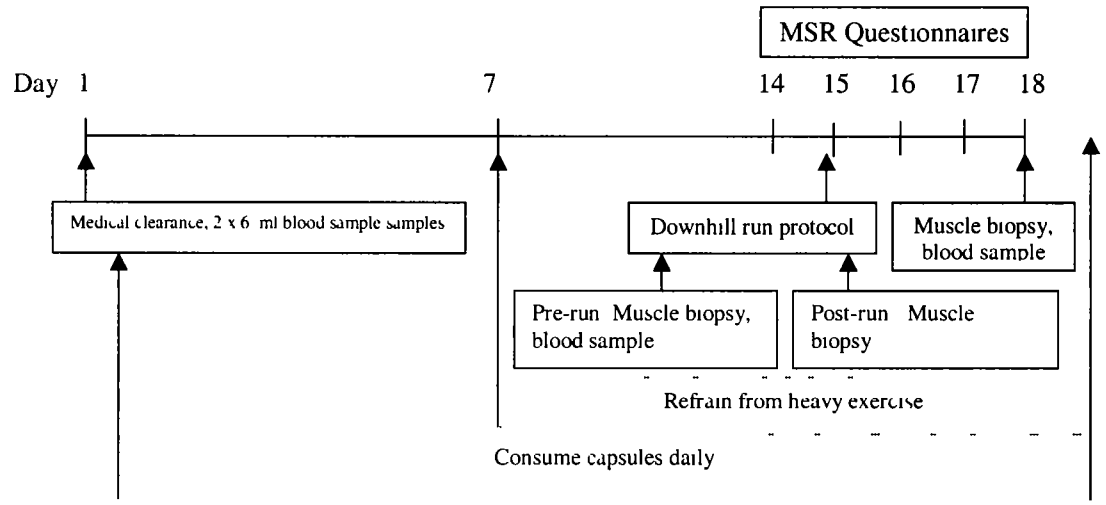
B. Skeletal Muscle Damage Trial

The main objective of this experiment was to determine if deer antler products have an enhancing role in human athletic performance by preventing ultrastructural muscle damage, and/or enhancing repair.

Methodology

Thirty active male subjects (3 groups of 10) between the age of 16 and 26 years (mean age 21.3 ± 2 9 years) were recruited from the University of Otago for this randomised double blind study. Participants were eligible for the study if they were active and healthy, unaccustomed to downhill running, and had no pre-existing musculoskeletal disorders. Following medical clearance, participants were randomised to receive either Extract (300 mg/day), Powder (1 5 g/day) or placebo, as in the Strength Trial 14 days later (Figure 2) participants were made to perform a downhill run to induce damage to the quadriceps muscle group.

Figure 2: *Skeletal Muscle Damage Study Design*



Participants arrived at the laboratory in pairs to perform the downhill run protocol. Body mass (kg) and standing height (cm) were measured with the subjects wearing light clothing and no shoes The pre-run muscle biopsy was taken and venous blood samples drawn before starting the treadmill protocol. Subjects ran on a motorised treadmill (Quinton Q65, Series 90, Seattle) for 35 minutes discontinuously on a 12% downhill grade. Heart rate was monitored using a telemetric heart rate monitor (Polar Sport Tester, PE 4000, Kemplele, Finland). Following a five minute warm up at a comfortable self-selected pace, the treadmill decline was set at 12%, and the treadmill velocity adjusted to elicit a heart rate of 70% of age-predicted maximum for each subject

Muscle biopsies were harvested from the lateral quadriceps, immediately prior to, immediately following, and four days following the downhill run protocol. Muscle soreness rating (MSR) questionnaires were completed by subjects immediately after, and on each of the four days following the downhill run. MSR was scored on a scale of 1 (Normal) to 10 (Very Sore).

Results & Interpretation

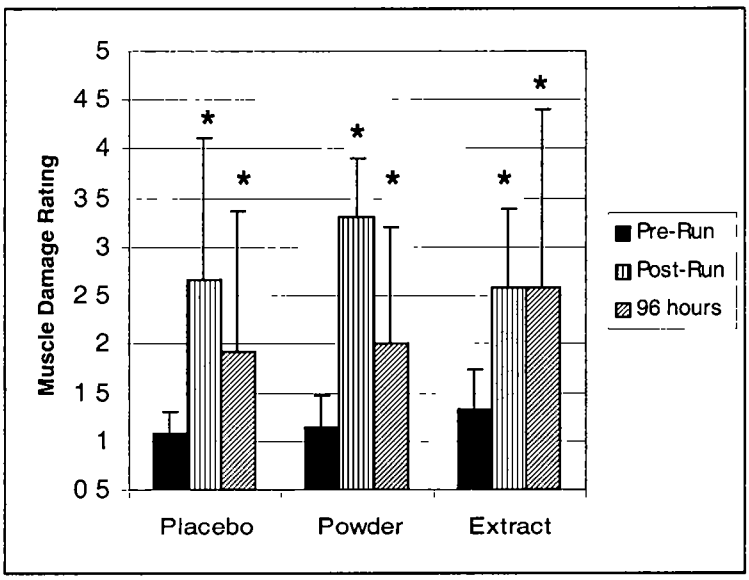
The main findings of this study were

1 Serum creatine kinase was significantly lower 96 hours post exercise in the group supplemented with velvet powder

2 There was no direct evidence that supplementation with deer antler products enhanced muscle repair at an ultrastructural level

The downhill treadmill run produced ultrastructural muscle damage that, on a scale of 1 (representing normal muscle) to 5 (indicating widespread sarcomere destruction), was ranked as "minor" to "moderate". However, there were no significance differences between treatment groups (Figure 3).

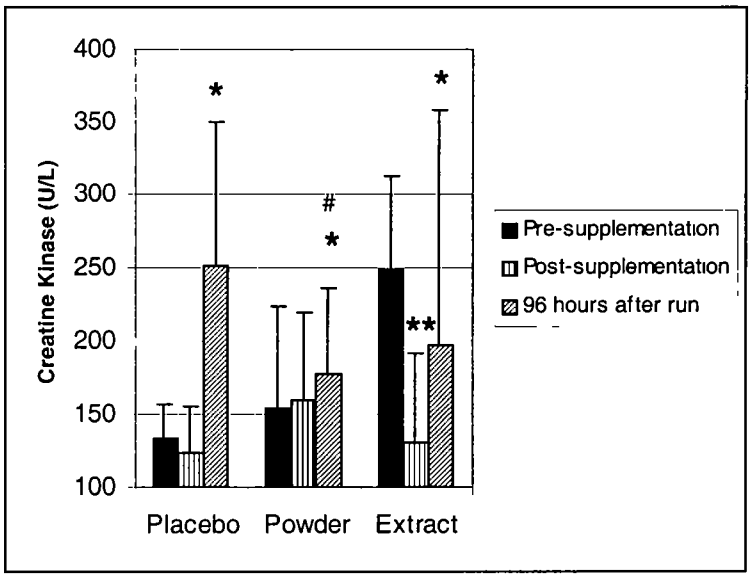
Figure 3: *Ultrastructural damage pre-run, immediately post-run, and 96 hours following the downhill run for the Placebo, Powder, and Extract groups*



*Significant difference from the baseline damage score for group

The serological markers of muscle damage, creatine kinase (CK), lactate dehydrogenase (LDH) and aspartate amino transferase (AST) were measured at times corresponding to pre-supplementation, post-supplementation and 96 hours post-exercise. As expected, serum CK levels rose in all three groups post-exercise (Figure 4) However, the increase was significantly lower for the Powder group as compared to the controls receiving placebo. The increase in CK in the Extract group was intermediate, and was not significantly different to the controls. Consistent with this, LDH and AST levels both rose slightly more in the control group than the two velvet-treated groups, but none of the differences were significant

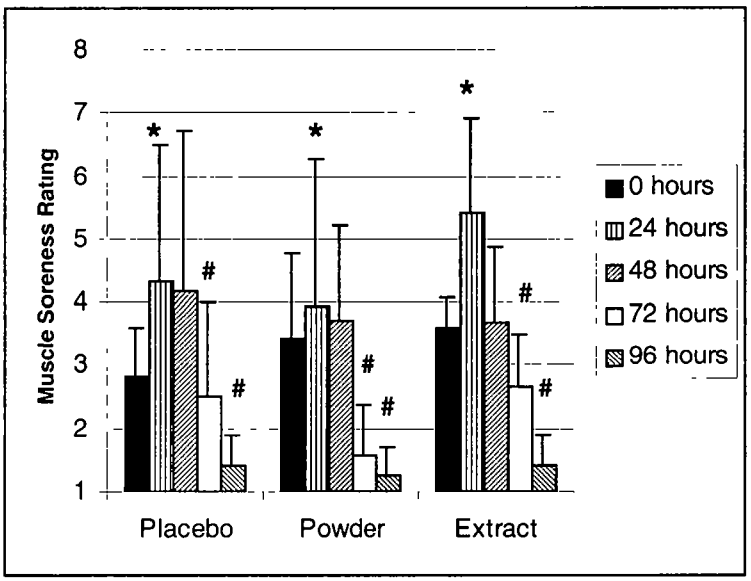
Figure 4: Creatine Kinase (CK) levels (U/L) at pre-supplementation, post-supplementation, and 96 hours following the downhill run for the Placebo, Powder, and Extract groups.



*Significantly different from post-supplementation scores for the group
**Significantly different from placebo group at baseline
#Significant difference in CK rise vs placebo group at 96 hours post-run

MSR values peaked 24 hours post-exercise in all groups (Figure 5), and then gradually declined to normal levels For the Powder group however, the decrease in MSR between 48 and 72 hours following exercise was greater than for the other two treatments As a consequence, muscle soreness in this group returned to normal levels 24 hours earlier than the Extract and the control groups.

Figure 5: Muscle Soreness Rating (MSR) immediately after (0 hours), and 24, 48, 72, and 96 hours after the downhill run for the Placebo, Powder, and Extract groups



*Significantly higher than immediately after the run (0 hours) for group
#Significantly lower than immediately after the run (0 hours) for group

However, given the lack of demonstrable difference due to treatment in the degree of ultrastructural damage to muscle tissue, the results of this study do not provide unequivocal evidence of a protective or restorative effect of velvet antler products on human skeletal muscle exposed to acute eccentric

loading It is possible that the dose rates of Powder and Extract used were not sufficiently high to produce a demonstrable effect on the parameters measured. The positive CK result and trend in MSR are encouraging, and certainly indicate that the use of deer velvet antler for prevention or amelioration of muscle damage warrants further investigation.

3) Velvet Removal Techniques

Compression Analgesia

The requirement for a simple, ethically sound technique for analgesia during spiker velvet removal is great as large numbers of animals are involved and because velvet must be removed prior to transport and slaughter

Previous studies at the AgResearch Animal Behaviour and Welfare Research Centre (ABWRC) have demonstrated that rubber rings (bands) applied to the pedicle of the antlers of spikers (yearling red deer stags) reliably induce analgesia within 60 min of application.

(a) It was decided to assess the duration of analgesia following velveting and band removal. It was considered that analgesia that persisted for some time would be positive for welfare while immediate cessation or extended duration of analgesia may be disadvantageous for welfare.

(b) In addition an investigation of the effects of different durations of ring application (as would conceivably occur under practical conditions) on the welfare of the animals was undertaken.

Methods

A total of 38 red deer spikers (15 at the Ruakura Deer Unit and 23 at a commercial farm) were used. Animals were removed from pasture immediately before the experimental work was undertaken and randomly allocated to treatments

Two different types of analgesia treatment were used prior to velveting the spikers. In one ('local'), 15ml of lignocaine hydrochloride was administered as a ring block around each pedicle at least 5 min prior to velvet removal In the second, a doubled-over rubber band was applied to the pedicle (2cm above the base of the skull) using an Elastrator applicator In different treatment groups the rings were applied for either 1, 2 or 4 hours prior to velveting (these groups were denoted as 1-h, 2-h, and 4-h band) The bands were removed immediately after velveting (by nicking the band with a scapel) The total number of animals in each of the four treatments were 10, 9, 13 and 6 for local, 1-h, 2-h and 4-h band, respectively) Antler removal was undertaken using loppers

The effectiveness of the analgesia treatments before and after velveting was determined from measures of the level of electrical stimulation (applied to the velvet) that elicited a withdrawal response (sharp movement of the head). The baseline ('threshold') values were assessed immediately prior to the application of analgesia treatments The degree of analgesia was measured immediately before velveting, and 15 min, 180 min, 1 day, 2 or 3 days, 6 or 7 days, and 15 days post-velveting. The animals on the commercial farm were not tested on Day 15. The degree of analgesia during velvet removal involved scoring the behavioural responses of the deer during cutting on a 10 point scale (Matthews et al 1992) from zero (no response) to 9 (jump upward in the crush)

In addition, the pedicles were examined by an experienced veterinarian at each analgesic test for signs of inflammation, discharges or any other abnormalities

Analyses

The responses of the animals to each analgesia test on each spike (baseline, immediately pre-velveting, and 15 min, 180 min, 1 day, 2 or 3 days, 6 or 7 days, and 15 days post-velveting) were grouped into three categories

- 'none' (no response to noxious stimulus)
- 'partial' (a higher level of electrical stimulation than that used in 'Baseline' was required to elicit the response seen at the 'threshold')

- 'full' (a similar level of electrical stimulation to that required to elicit the threshold response during Baseline was required)

There were no significant differences between the responses at 15 min and 180 min so the data for 180 min only have been presented Similarly, there were no differences in responses between animals tested on Days 2 or 3, or between those tested on Days 6 or 7, so these data were combined (i.e Days 2 and 3 combined, Days 6 and 7 combined) and are denoted as Day 2 and Day 6, respectively.

Appropriate non-parametric statistics i.e. Kruskal and Wallis one way analysis of variance were used to analyse the significance of the data at a 'p' value of 0.05

Results

Pre-velveting

There were no significant differences between treatments with all animals reacting to the electrical stimulus on both spikes during baseline testing (before application of analgesic treatments). All animals in all treatments did not respond to the electrical stimulus (at threshold and supra-threshold levels) immediately prior to velveting (no difference between treatments, indicating that analgesia had been achieved under all treatments (Figure 6)

Post-velveting

The proportions of spikes in which full, partial, or no analgesia was observed at various intervals post-velveting are shown in Figure 6.

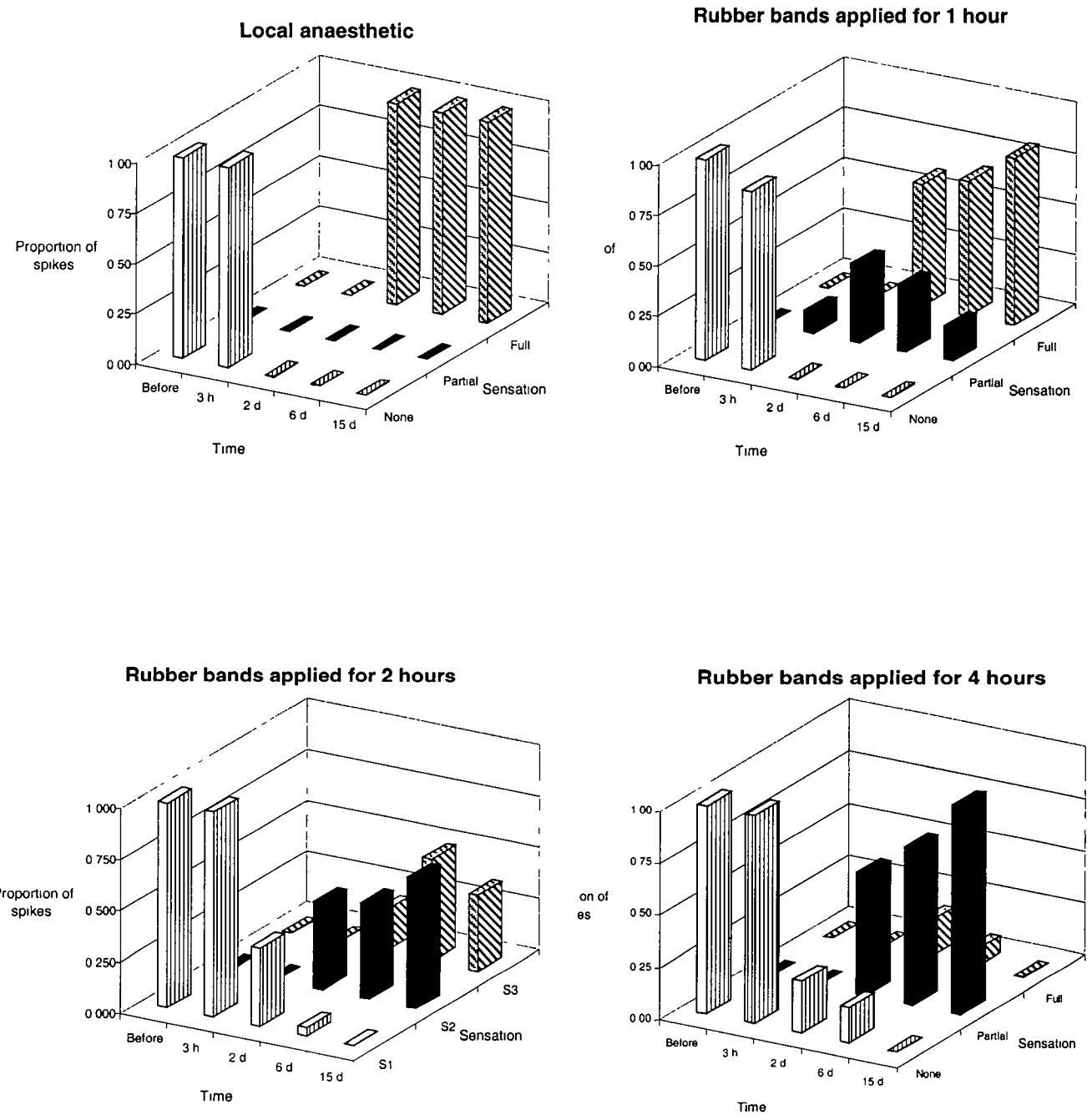
All animals in all treatments did not respond to the electrical stimulus (no difference between treatments), 30 min after velvet removal. Similarly, there were no significant differences between treatments 3 hours post-velveting, with all but one animal being totally analgesic (this animal was in the 1 hours treatment and had partial sensation)

Between 3 and 15 days post-velveting all treatments were characterised by an increasing degree of responsiveness to the electrical stimulus, but the pattern of change differed between treatments (Figure 6) At Day 3 (and subsequently), all animals in the local anaesthetic and 1-h band treatments responded (partially or fully) to the electrical stimulation These responses were similar to baseline levels in 100% of spikes given 'local', and in 61% of spikes given 1-h bands. In addition, 63% of 2-h band spikes and 75% of 4-h band spikes had recovered some sensation by Day 2 The differences between band treatments were not significant.

By Day 6, three only out of a total of 41 (7%) spikes treated with bands had not recovered any sensation, two of these were in the 4-h treatment and one was in 2-h treatment. None of the 4-h treatment had full sensation at Day 15, although several animals were recorded with full sensation at Days 2 and 6 This apparent anomaly is due to the absence of data at Day 15 for some of the animals (see Methods for explanation)

There were no untoward or unusual clinical signs that would indicate that the animals were suffering in any of the analgesic treatments.

Figure 6: *The degree of sensation in spikes at various intervals before and 3h, Days 2, 6 and 15 after velvet removal under each analgesia treatment local anaesthetic, 1-h band, 2-h band; 4-h band*



Discussion

Both 'local' and the compression technique resulted in apparently effective analgesia for at least three hours with all spikes in this study, although the duration of the analgesia varied between treatments. Full sensation was recovered between the first and third day post velveting when local anaesthetic was used Previous work in our laboratory (Matthews et al 1992) would indicate that the sensation most likely returned within 24h of velveting

Amongst the compression treatments, the 1-h band method resulted in the most rapid return of sensation The longer the bands were left in place pre-velveting the slower the rate of recovery of sensation All animals in the 1-h treatment had recovered some sensation by Day 2 and most had fully recovered by Day 15. Two-thirds or more of the animals in the 2-h and 4-h treatments had partially or fully recovered by Day 2.

The majority of 2-h (96%) and 4-h (85%) animals had recovered some or all sensation by Day 6, and all were able to respond to noxious stimuli by Day 15. The rate of return to full sensation appeared to be somewhat faster in the 2-h than the 4-h band treatment

The relatively rapid return in sensation to the pedicle in the band treated spikers would suggest that the animals did not sustain severe or prolonged tissue or nerve damage from the procedure. This is supported by the absence of any untoward sequelae (e.g. inflammation, infection) in those animals in the week following velveting.

Compression analgesic using rubber rings has been approved for the removal of spiker antler in New Zealand and is welfare friendly The extension of the technique for branched antler removal is underway

4) Product Activity

The evaluation of velvet antler active ingredients is somewhat behind the others as it has received least research investment. We need to develop an effective quality standard, which can, at best, be used as a marker, which indicates the presence of velvet in an 'unknown' product This task is difficult because the antler is only an organ of an animal – it probably does not express specific substances which are unique to it and not found elsewhere in the deer. Hence no simple test is likely to be adequate We are, however, interested currently in a number of proteins which seem to be expressed in much larger amounts in velvet compared with the rest of the deer body It is possible that measurement of a combination of these may provide a test that will distinguish velvet from other tissues with an acceptable level of confidence

We are collaborating with a Chinese group who have developed a number of Traditional Chinese Medicines In addition we are investigating 'profiling' or forming a picture of velvet composition using a number of simple tests as a means to describe velvet

Future Directions

The most needed pieces of market support information are more clinical research, a technique for standardising product and more welfare research to develop drug free removal techniques

VARNZ clinical research is currently focussed on enhancement of human physical performance Some trials will be performed in the US to maximise uptake of the results by the market. Clinical research by other groups is also expanding in the US and Canada, predominantly in the areas of osteoporosis and bone and joint health At this stage, all clinical research is generic to the point where stage of development at harvest, breed, processing and part of the antler are not considered This may change with greater knowledge Positive work published in refereed journals, whatever the source, can only benefit the export of New Zealand velvet.

Natural products sold as functional foods or dietary supplements typically have a marker ingredient, which may or may not be the active ingredient, on which products are standardised for comparison. At this time there is no such agreed standards for velvet antler Developing such a standard is a necessary step The decision is based on a number of issues including cost of testing and the reliability of the test At this stage we have a number of different tests and these await marketing decisions

Welfare considerations demand full analgesia during velveting, but chemical techniques of analgesia are not the market preference due to the risk of residues More work is needed to develop better systems

All of the above issues are being addressed in the VARNZ programme in 1999/2000.

Reference

Matthews, L R , Ingram, J R , Cook, C J, Bremner, K J , Kirton, P 1992 Induction and Assessment of Velvet Analgesia Ed P R. Wilson. *Proceedings of a Deer Course for Veterinarians*, Deer Branch NZVA 9: 69-76