Research support for new uses and improved production of deer velvet

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ABSTRACT

Deer velvet is a novel agricultural product and has presented the New Zealand (NZ) agricultural and food sectors with a number of opportunities. When the velvet industry emerged in the early 1970s, almost the entire product was exported to the traditional oriental medicine markets, which remain the basis of the deer velvet industry in NZ. This market did not require scientific validation of efficacy as the health promoting effects of velvet were accepted. However, as this is a mature market there is interest in growing new business, notably the Western dietary supplement market. This requires new knowledge on health benefits as potential purchasers have no initial product knowledge. We have conducted research to investigate whether or not deer velvet will help promote health. We chose to look for a verifiable benefit to athletes as the athletic market would be less likely to be price sensitive than other sectors. We also investigated the application of specific velvet extracts as topical agents to promote healing of open wounds, based on their ability to promote the growth of blood vessels (angiogenesis). This paper discusses the research that set out to demonstrate velvet efficacy and quality and describes research on nutrition and breeding designed to increase velvet quality and quantity.

Keywords: deer; velvet; antlers; production; quality; wound healing; dietary supplement; health benefits.

INTRODUCTION

Deer velvet has been produced commercially in New Zealand (NZ) for about 30 years and over that time it has become a profitable sector of an expanding deer industry. The prices paid for velvet in NZ are typically high but volatile, and are largely dictated by market conditions in Korea.

In the late 1990s, driven by low commodity prices in Asia, the NZ Deer Industry decided to pursue the Western dietary supplements market to develop a new deer velvet export market. It became clear that this market could not rely on traditional knowledge of efficacy and quality but rather would require scientific evidence to persuade new prospective consumers of its benefits. The main thrust of the Velvet Antler Research NZ (VARNZ) programme since the late 1990s has been to develop this scientific knowledge. In addition, work has been done on breeding and nutrition to improve velvet quality. This paper documents some of the key research in both of these areas.

QUANTITATIVE MEASURE OF DEER VELVET QUALITY

Most natural products that are offered for retail are either standardised to a particular marker substance, which may or may not be active, or the concentration of that marker substance is stated. Consumers make choices on the basis of price and quality (amount of active/marker ingredient present) when deciding whether or not to purchase the product. Deer velvet is a highly complex mixture of substances and at present no specific activity is linked to a specific active – at least for the natural product industry. This is a threat to deer velvet sales. The consumer may choose not to buy if no comparative quality measure is given on the label, or could buy a 'defective product' with no perceptible health benefit. In either case that buyer will subsequently avoid deer velvet. If a simple quality standard could be developed, and applied to NZ deer velvet we could produce an effective label that consumers could depend on and purchase with confidence.

We believe that the active ingredients in deer velvet are likely to be organic compounds. Thus, if the organic compounds were extracted and quantitatively assessed we would have an effective measure of velvet quality. We have evidence from cell culture (Suttie & Haines, 2000) that biological activity (growth enhancement) is proportional to extract yield. A quality index has now been developed, which is termed the Velvet Activity Index™ (VAI™). This combines the yields of extractable lipids and low molecular weight proteins into a single dimensionless index which gives equal weight to the two classes of biologically active components.

Method

Samples of deer velvet powder were analysed for total lipid content by automated Sohxlet extraction. Further samples of the same velvet powders were extracted with aqueous phosphate buffer and the resulting water extracts were analysed by gel filtration chromatography. Total proteins having molecular weights less than 10 000 Daltons was determined in each sample relative to a known amount of a standard protein included in the same assay. The protein and total lipid contents of the sample were then normalised to an arbitrary value of 10 for each component in the

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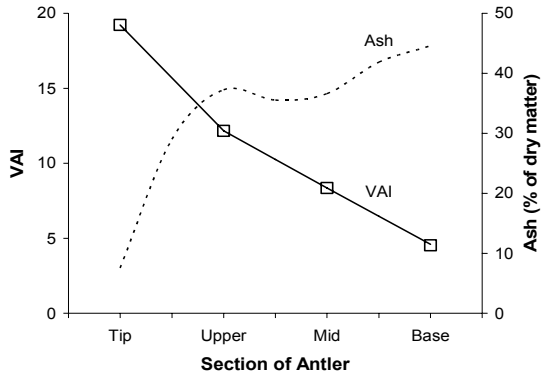
antler tip, in order to give both equal weight when combined to give the final VAI value.

Results

Figure 1 shows the VAI values determined for individual sections of a single freeze dried velvet antler. Values decreased in a consistent fashion down the length of the antler, from a maximum of 19.2 at the tip to a minimum of 4.5 at the base. For comparison, the ash content of a typical freeze dried velvet antler is also presented on the same graph. Ash is the inverse of organic matter, and is currently used as the standard quality indicator for deer velvet. In contrast to the VAI results, ash showed a complex pattern down the length of the antler. Ash content was very low at the tip, rose rapidly and was fairly stable with moderately high values in the upper to mid sections, before it gradually increased to higher values at the base.

FIGURE 1: VAI™ values for sections of a freeze dried velvet antler, overlaid on a plot of the pattern of ash content for a similar antler.



VAI values of a number of commercial deer velvet products were also determined. These ranged between 8.3 and 4.1, compared with a value of 9.2 obtained for a control sample of ground powder derived from freeze-dried antlers (whole stick).

This simple index ('VAI') provides a measure of the extractable yield of lipid and protein present in deer velvet and derived products. These components were chosen to make up the VAI index since they comprise the two major biologically active fractions of deer velvet, and are both readily determined. Commercial velvet products have been found to give 'sensible' VAI values, intermediate between those of antler tip (high quality) and antler base (low quality) samples.

The VAI index has been validated (Haines *et al*., 2004), and moves are underway to make it available to the velvet industry by transfer of the assay to an accredited service laboratory. It is believed the new VAI index will provide the velvet industry with a much more robust and understandable guide to quality than the current *de facto* standard, ash.

THE EFFECTS OF ORAL DEER VELVET TREATMENT ON STRENGTH GAIN DURING TRAINING

The main objective of this experiment was to determine if deer antler products are able to enhance the strength gained by athletes undergoing self-determined training programmes.

Methods

Thirty-two males between the ages of 18 and 35 with at least 4 years of weight lifting experience were randomly assigned using a double-blind procedure into either a placebo or deer velvet powder treatment group. The placebo group received sugar capsules, and the velvet group received 1350 mg deer velvet powder, once in the morning and again immediately prior to bed-time. Random assignment was done in matched pairs (1 placebo; 1 deer velvet). Prior to and immediately following a 10-week period of supplementation, each subject participated in a series of measurements. These procedures included the measurement of maximal aerobic capacity ($\dot{V}O_2max$), maximal power output on a cycle ergometer, a determination of maximal strength (1-RM) for the bench-press and squat, a comprehensive blood chemistry profile, body composition analyses (DEXA), and a 3-day dietary recall. Of the original 32 subjects recruited for this study, 56% of the subjects properly completed all aspects of the study. Dropouts were evenly divided between each treatment group, leaving the placebo and the velvet treatment groups each with nine subjects.

Results and interpretation

For the placebo group, only the absolute 1-RM values for the bench press (Pre: 123.2 ± 24.0 kg; Post: 128.3 ± 27.5 kg, 4.1% change; $P < 0.05$) and the squat (Pre: 150.5 ± 28.2 kg; Post: 156.6 ± 30.4 kg, 4.1% change; $P < 0.05$) improved after the intervention period. When normalized for kilograms of total body weight, the placebo group did not show any significant differences for the 1-RM measurements in either the bench press or the squat exercises. In contrast, the deer velvet group showed significant improvements in the 1-RM values both in absolute terms and relative to total body weight. In absolute terms, the 1-RM for the bench press of this group increased 4.2% (Pre: 120.0 ± 23.6 kg; Post: 125.0 ± 25.7 kg; $P < 0.05$) while the squat 1-RM improved 9.9% (Pre: 159.3 ± 42.7 kg; Post: 175.0 ± 43.5kg; $P < 0.01$). When expressed relative to total body weight, 1-RM values for the bench press and squat also significantly improved ($P < 0.05$) by 4.0% and 10.1%, respectively, in the NZDAV group. One of the most interesting findings of this study was the fact that there was also a significant improvement in aerobic capacity in the velvet treatment group, despite the fact that the athletes did not undertake any aerobic training. In litres/minute, $\dot{V}O_2max$ increased significantly by 9.8% from the pre- to post-treatment period (4.30 ± 0.45 to 4.72 ± 0.60 litres/minute $P < 0.01$). When expressed

relative to total body weight in kilograms, $\dot{V}O_2max$ remained significantly elevated by 9.4% (46.5 ± 8.1 to 50.0 ± 8.9 ml/kg/minute) in the velvet group following the training-supplement intervention.

Conclusion

The results of this study suggest that deer velvet treatment may have positive effects on strength/power in men undergoing resistance training (Broeder *et al*., 2004). The observed enhancement of aerobic capacity without specific training was noteworthy, and this unexpected result warrants further investigation.

IN VITRO ANGIOGENIC ACTIVITY AND THE ABILITY TO ENHANCE THE RATE OF WOUND CLOSURE OF A SPECIFIC DEER VELVET EXTRACT

Methods

A specific extract of deer velvet, predominantly containing proteins with molecular weights less than 10,000 Daltons, was prepared using a patented method (Haines, 2003). A total protein extract was made from 5 g of freeze dried velvet powder using 100 ml of 0.5 M phosphate buffer solution (pH 6.9). The mixture was stirred for an hour at room temperature and was then filtered through glass fibre filter paper (Whatman GF/A). The filtrate was centrifuged at 11,500 rpm for 30 minutes at 4°C. The supernatant was decanted into weighed Schott bottles and was shell frozen before being freeze-dried at 15°C.

An *in vitro* cell proliferation assay was performed by culturing human umbilical vein endothelial cells (HUVEC) in Medium 199 (GibcoBRL) supplemented with 10% foetal bovine serum (GibcoBRL), 50 U/ml penicillin, 50 mg/ml streptomycin, 2 mM L-glutamine and 1 ng/ml basic fibroblast growth factor (bFGF). Cells were trypsinised and seeded in 96-well plates at a density of 3000 cells/well/200 ml and cultured for 3 days. Following starving in 1% serum for 24 hours the cells were treated with 1% serum containing 1 ng/ml bFGF in the presence or absence of the deer velvet extracts for a further 48 hours. Two hours before incubation was terminated, 20 ml of Celltiter 96® Aqueous One Solution Reagent was added into each well. After the completion of incubation the optical densities of the wells at 490 nm (OD490) were recorded.

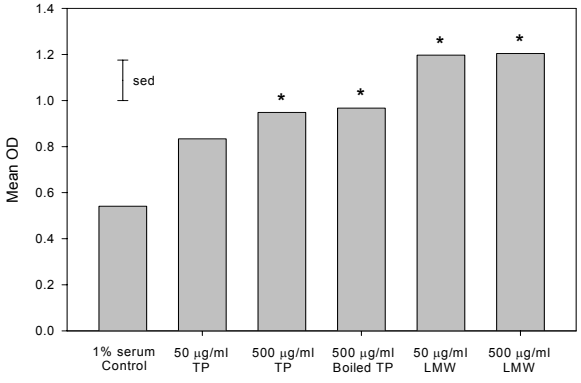
The ability of velvet extracts to promote migration of bovine aortic endothelial (BAE) cells *in vitro* was also assessed. BAE cells were allowed to grow to confluence in Dulbecco's modified Eagle medium (DMEM, GibcoBRL®) containing 10% fetal bovine serum (GibcoBRL) in 12-well plates (Nunclon™). The monolayers were then 'wounded' by scraping a disposable pipette tip across the dishes. After washing with Dulbecco's phosphate buffered saline solution plus calcium (0.1 g/L) (GIBCO™, Invitrogen Corporation), the wounded monolayers were cultured for a further 48 hours in fresh 1% serum in the presence or absence of the deer velvet extracts. The degree of movement of cells in the wounded monolayers was determined by photomicrography at the time of the initial wounding and 48 hours later. A grid with lines 1.5 cm apart and 10 cm long running parallel to a baseline was placed over the photograph. The baseline was placed on the 'wounding line' above which the cells had originally been scraped off. The number of cells intercepted by the lines 1.5, 3, 4.5, 6, 7.5 or 9 cm away from the baselines was recorded.

In vivo wounding healing experiments where conducted by making two 0.8 mm punch biopsies on the backs of rats (6 animals per group). One wound was a control wound that was treated only with carrier (saline solution) while the other wound was the treated with the specific velvet extract in saline solution. The wounds were examined and photographed at regular intervals. Wound sizes at each time point were assessed from the digitised photographs, and were expressed as percentage closure relative to their original sizes.

Results and interpretation

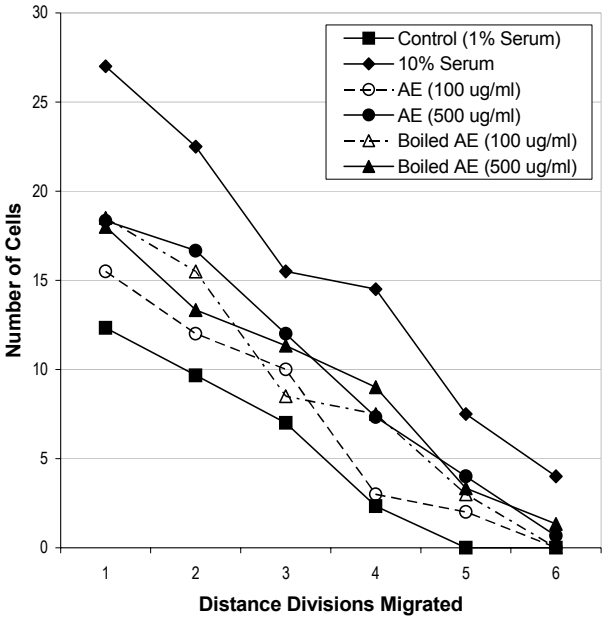
The effects of a total protein extract of deer velvet and the specific LMW velvet extract on the proliferation of HUVEC are presented in Figure 2. Both extracts enhanced the proliferation of HUVEC cells, with the LMW velvet extract having a more marked effect. Interestingly, boiling of the total protein extract had no effect on its proliferative activity. Figure 3 shows the effect of the LMW velvet extract on the migration of BAE cells. A greater number of cells treated with velvet extract were found to have migrated at each measured position out from the 'wounding' line, compared with the control treatment lacking velvet. In addition, the velvet treated cells migrated much further overall than the controls. These results show that both doses of the LMW velvet extract were effective in stimulating the migration of the endothelial cells, and that boiling the extract did not cause loss of its activity.

FIGURE 2: HUVEC proliferation in response to 1% serum (Control), a total protein extract from antler (TP) before and after boiling for 3 minutes, and the specific low molecular weight velvet extract (LMW). All treatments, with the exception of TP at 50 µg/ml, caused significantly more proliferation than the control treatment, as indicated by *. sed = standard error of the difference.



The specific LMW extract appears to be heat stable, is mitogenic for endothelial cells and enhances endothelial cell migration, which encouraged us to attempt *in vivo* experiments on wounds. Because it is heat stable, the LMW velvet extract has considerable flexibility for incorporation into a variety of clinically useful applications.

FIGURE 3: Cell migration assay. The migration of BAE cells in response to 1% serum (Control) or the specific LMW velvet extract before (AE) and after boiling for 3 min (Boiled AE). Velvet extracts were used at concentrations of 100 µg/ml and 500 µg/ml, and also contained 1% serum.

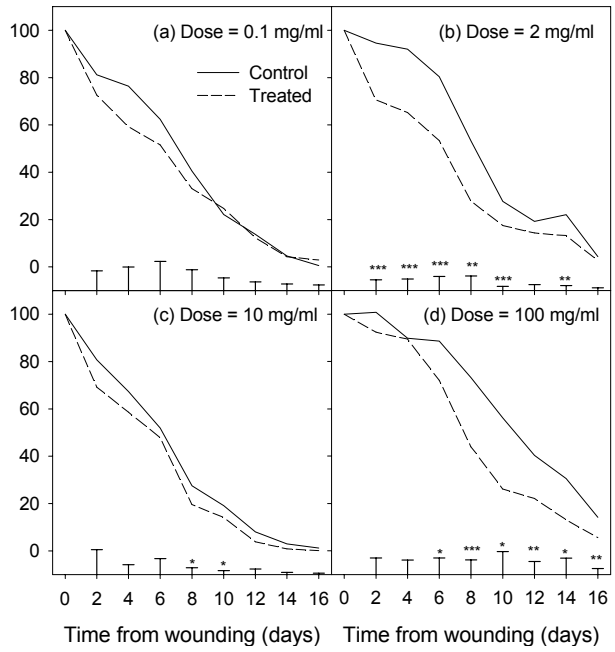


The results of various doses of LMW extract in the *in vivo* wound healing model are shown in Figure 4. At dose rates 2 mg/ml, 10 mg/ml and 100 mg/ml, significant improvement in degree of wound closure was observed at the time points analysed. No significant response was found with 0.1 mg/ml. The results indicate that there is a relatively broad dose range over which the extract improves the rate of wound closure and that there were no adverse reaction to the extract.

Conclusion

Wound healing is a complex multi-phased process, in which the growth of new blood vessels (angiogenesis) plays an important role. The results of the research outlined above (Clark *et al*., 2004) indicate that deer velvet contains potent factors that are able to enhance the growth and migration of endothelial cells, as would be expected from its phenomenal growth rate. These factors are amenable to extraction utilising a novel patented procedure, and they appear to be very stable. Furthermore, they show excellent wound healing ability *in vivo* as well as high activity *in vitro*. Potentially, deer velvet products suitable for use in a variety of wound healing applications could be developed from this research. These high value products would be expected to overcome the price/benefit barrier for velvet.

FIGURE 4: Graphs showing the results of rat wound healing trials investigating the effect of dose of the specific LMW velvet extract on the rate of wound closure. The wounds were treated with 25 µl of either saline solution (Control) or with the LMW velvet extract in saline solution (Treated). The velvet extract was applied at (a) 0.1 mg/ml (b) 2 mg/ml (c) 10 mg/ml (d) 100 mg/ml. Doses were given on days 0, 2, 4, 6, 8 and 10 except for 100 mg/ml which had no day 10 application. Data presented are mean wound sizes on days following wounding, as percentages of the original wound sizes. Error bars shown at days 2, 4, 6, 8, 10, 12, 14 and 16 are the standard errors of the differences between means. The significance levels indicated with asterisks are: *P < 0.05, **P < 0.01, ***P < 0.001.



PRODUCTION IMPROVEMENT

There is a clear opportunity to improve velvet size and grade by strategic feeding at critical phases during the stag's annual antler cycle. Suttie *et al*. (1996) have shown that stags are most responsive, in terms of increased velvet weight, to strategic feeding after the rut and again during the velvet growing period.

We set out to develop a concentrate feed ration and test it during autumn (post-rut) and during the velvet antler growing period to determine the effects on velvet antler weight and grade.

Method

Concentrate feed rations were developed as shown in Table 1. The additives included a protected fat supplement as well as copper and selenium.

TABLE 1: Composition of diets fed to stags (%).

	Starter	Main
	A	B
Barley	43.6	43.6
Full fat soya	30.0	27.5
Broll	20.0	20.0
Additives	6.4	8.9

One hundred and forty six mixed age and 141 rising 2-year-old red deer stags were allocated to treatment within age group in a crossover design. During a 40 day period in autumn from May 20 to June 30, about half of each age group of stags was allocated to a diet of 500 g/head/day of the strategic supplement and 75 were allocated to 100 g/head/day of barley. For the first 14 days diet A, that contained 2.5% of protected fat, was fed and for the remainder diet B, that contained 5.0% protected fat, was fed. The stags were returned to standard winter supplementary feeding until 14 days from the anticipated date of first antler casting, on August 25 for the mixed age, and September 28 for the rising 2-year-old stags. The stags were then reallocated, within the age groups, such that approximately half of the group previously fed the strategic supplement were fed barley and *vice versa*. The remainder were fed the same diet as in autumn. This yielded 4 experimental groups. The stags were fed at the same level as before until velvet antler removal. Diet A was fed for 14 days and diet B for the remainder of the period.

Measurements

The stags were weighed once during mid winter. The antlers were weighed and graded after removal. For the mixed age stags the weight of the previous velvet antler was also recorded.

Data analysis

The effect of diet treatment on antler weight was examined by ANOVA with number of days of growth, mid winter live weight and previous velvet antler weight (mixed age stags only) added to the model as appropriate. The effect of diet on live weight in winter (after the first period of treatment) was also analysed with ANOVA. Multinomial logit functions were calculated to examine the effect of dietary treatment on the number of stags cutting antler of each grade in each treatment.

Results

For the 2-year-old stags there were no overall significant effects of treatment on live weight or antler weight (Table 2), but some interesting interactions were detected. The groups fed strategic supplement in the autumn/winter and spring had heavier antlers than those fed no supplements at either period ($P < 0.06$) and the group fed strategic supplements in both periods had significantly lower antler weight than predicted by the regression equation ($P < 0.03$). There was no overall significant effect of treatment on live weight but, as for antler weight, a significant interaction was observed with the group fed strategic supplements in both periods lighter than expected ($P < 0.009$).

There were no significant differences in grade due to treatment in the 2-year-old stags (Table 3), but there was a trend for a higher percentage of treated stags cutting C grade. In the mixed age stags there were no significant differences in live weight, antler weight or grade but there was a trend that treated stags cut more A grade velvet.

TABLE 2: Mean live weight and antler weight (kg) of 2-year-old and mixed age stags following feeding with or without supplements in spring and autumn/winter.

	Spring							
	No Supplements (SEM)				Supplements (SEM)			
	Live weight		Antler weight		Live weight		Antler weight	
Autumn/Winter	2-year-olds	mixed age	2-year-olds	mixed age	2-year-olds	mixed age	2-year-olds	mixed age
No supplements	109.1 (1.8)	153.1 (1.9)	1.44 (0.05)	2.93 (0.06)	111.5 (1.6)	151.8 (2.2)	1.56 (0.05)	2.94 (0.07)
Supplements	109.5 (1.6)	157.8 (2.2)	1.57 (0.05)	2.97 (0.07)	106.6 (1.6)	156.9 (2.0)	1.47 (0.05)	3.00 (0.07)

TABLE 3: Antler grades (%) for 2-year-old and mixed age stags following different levels of feed supplementation.

	No supplements		**Autumn/Winter supplements**		**Spring supplements**		**Supplements Autumn/ Winter and Spring**	
	2-year-old	mixed age	2-year-old	mixed age	2-year-old	mixed age	2-year-old	mixed age
A	-	17.4	-	20.7	-	25.7	-	33.8
B	2.9	69.8	2.7	63.8	2.7	57.1	-	54.1
C	27.5	16.3	43.2	17.1	42.9	17.1	37.1	9.5
D	62.3	-	43.2	-	50.0	-	50.0	-
E	7.3	-	10.8	-	5.4	-	10.0	-

Conclusion

Neither trial produced a significant positive result on antler weight. However, in both age groups there was a trend that antler grades of supplemented animals were higher than those fed barley only in both periods. Subsequent economic analysis revealed that in most years, feeding the concentrate ration gave a value increasing benefit, primarily by raising grades.

BREEDING FOR VELVET

Genetic potential has a large influence on velvet production; therefore selection for velvet yield receives significant emphasis in velvet production systems. Heritability estimates for velvet antler weights have ranged from 0.43 for 2-year-old stags through to 0.85 for 8-year-old stags (van den Berg & Garrick, 1997) and coefficients of variation in velvet weight are also high, ranging from 22% to 29% in the same study. These results indicate that although velvet weight is highly heritable, there is a significant additive genetic variation to select on and a strong response to selection for velvet weight might be expected. Industry breeding programmes have capitalised on this, and genetic trends analysed in several leading industry breeding herds have shown strong genetic trends in velvet weight, in the order of 15-30 g/year (unpublished data). These improvements in velvet have been made both through selection within existing populations and importation of new genotypes with superior velvet potential. Selection has until recently (past 5 years) been entirely on phenotypic performance, without utilising tools such as those provided by objective genetic evaluation. However, with highly heritable traits responses to mass selection based on phenotype are generally strong, as the phenotypic performance of an individual is a good predictor of genetic merit.

While velvet weight is highly heritable with significant variation, selection programs are limited by generation intervals and the sex-limited expression of the trait. Velvet antler weight of spiker (one-year-old males) is generally not considered by deer farmers to be a good indicator of velvet production as older ages, primarily because spiker velvet does not often show the potential development of tines and antler conformation, which only appears at 2 years and over. Therefore, most selection of replacement stags for a velveting herd is based on 2-year-old velvet weight, while selection of velveting sires is often based on velvet weights at later ages still. This is in part a result of a perception among deer breeders that 2-year-old velvet weight is only a moderate predictor of later velvet production potential, and that variation in maturity patterns mean that superior stags at 2 years will not always retain their advantage as older ages. Estimates of genetic correlations between velvet weights at different ages range between 0.76 and 0.99, indicating that while the correlations are not unity, they are strong and so selection on 2-year-old velvet will lead to a strong correlated response in velvet weight at older ages. Selection based on two-year-old velvet carries a significant penalty in terms of extending the generation interval and reducing annual genetic gain compared to selection for other traits measured on young stags such as body weight for venison production. Selection of stags based on velvet weight at 3 years and older further increases the generation interval, and is unlikely to compensate with increased accuracy of selection for lifetime velvet production.

Sex-limited expression of velvet weight also reduces genetic gain, as hinds can only be evaluated via performance of male relatives. However, the impact on genetic gain is less than if the trait were expressed in females only, as is the case for many traits in other livestock production systems. However, use of genetic evaluation technology has the potential to significantly aid selection for velvet weight, particularly for selecting replacement hinds.

Both the sex-limited expression of velvet weight and the age of expression in males mean that an indirect indicator trait for velvet production, able to be measured on young males and females, would be of significant benefit in increasing annual genetic gain for velvet production. Live weight has been shown to have a moderate to high genetic relationship with velvet production, and might be a suitable selection aid to improve velvet weight. However, live weight also tends to be highly correlated with feed costs, and so use of live weight as an indirect criterion to improve a breeding objective which accounts for feed costs, is unlikely to be as favourable as the genetic correlations with velvet weight initially suggest. Few if any other correlated indicators of velvet weight currently exist. Genetic markers for velvet production would potentially lead to rapid increases in genetic progress, but the cost-benefit analysis would need to be sound to justify the significant investment in research and development required to identify suitable markers. In the mean time, selection based on soundly designed phenotypic recording programmes together with genetic evaluation based on phenotype and pedigree still has potential for increasing genetic progress in velvet production.

OVERALL SUMMARY

Deer velvet is an ancient traditional medicine but suffers some growing pains in adapting to the needs of the Western dietary supplement industry. Clear benefits have been shown and production has been supported by the research presented in this paper.

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