VELVET ANTLER RESEARCH TOWARDS QUALITY AND ADDED VALUE

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INTRODUCTION

Since the beginning of deer farming in New Zealand growing antler (velvet) has been removed and sold as a key component of Asian Traditional Medicines. Production of velvet has steadily increased in New Zealand but financial returns have been variable. This is due to a number of factors including competition from Russian and Chinese velvet exports and the economic status of the major markets such as South Korea. It became apparent in the late 1980's that some research was necessary to establish the quality of NZ velvet, develop quality assurance standards and develop new added value products. In 1990 a research programme funded by the NZ Game Industry Board began at Invermay, with the aim of supplying research information to the NZ velvet industry that would allow it to develop and improve international market access for NZ velvet. It was considered that more diverse markets would improve the dollar returns from NZ velvet and substantially improve the long term stability and viability of the NZ Deer Industry. Now, five years later, this research programme has expanded and developed along two main lines, Quality Assurance and Product Development, and has attracted a great deal of commercial interest. The aim of this manuscript is to describe and discuss the principle research findings from the programme.

QUALITY ASSURANCE

The traditional velvet on the international market came from Russia and China. At the outset of the research programme it was important to carry out comparative analysis of composition to determine whether or not NZ velvet resembled the Russian and Chinese antlers. In NZ antlers are typically removed just before the upper or crown tines (red deer) develop, because this is what the market requires. In addition a study took place to determine the consequences of earlier or later velvet removal on antler composition. Also, in response to information from Korea that specific chemical composition might be used for grading/quality determination, relevant analytical techniques were set up to measure and evaluate the specific components. Throughout the research programme a significant problem has frustrated research; namely, there is no objective definition of quality. The Koreans, among other criteria, use antler colour as an indicator of quality and consequently we have recently studied factors that affect antler colour.

COMPARATIVE COMPOSITION

Antler composition

Antlers grow extremely rapidly and it is to be expected that variations in chemical composition will take place both with time and also with position in the antlers. Studies were carried out to determine the effect of species, section of antler and time on chemical composition. The studies were carried out in two parts: firstly, mineral composition and total lipid levels were determined and secondly, the levels of specific lipids and free amino acids were measured.

Mineral composition and total lipid

Dried velvet antler from NZ born and bred adult red deer, wapiti and fallow deer, Russian wapiti, Chinese malu (wapiti) and Chinese meihualu (sika) were available for analysis.

On receipt the dried velvet antlers were weighed, the hair was burned off using a gas burner, and the antlers were reweighed after being cleaned lightly with a damp cloth. Each antler was then cut into 4 main sections for analysis, as outlined in Figure 1. Bez tines were excluded from all analyses. Complete analyses were carried out for all main antler sections. In all cases the dermis was peeled off the velvet antler before analysis. The complete analysis included dry matter (DM, by incubation at 105°C for 4 hours freeze drying), ash (by incineration in a muffle furnace at 550°C overnight), total lipid (by petroleum ether extraction for 7 hours), nitrogen (by Kjeldahl extraction) and determinations of the following minerals: calcium, phosphorus, sulphur, magnesium, potassium, sodium, manganese, zinc, copper, iron, selenium and cobalt. The nitrogen and mineral analyses were carried out using standard methods for analysis of plant material (Anon 1979).

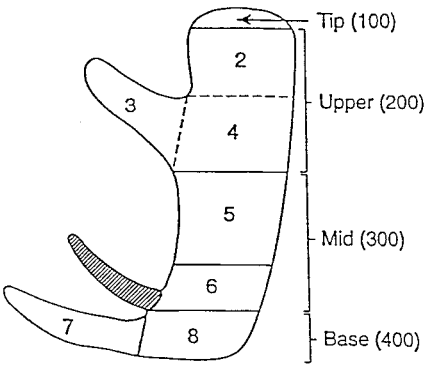

Figure 1. Antler sections used for compositional analysis.

Adult red deer top grade antler

Table 1 presents selected analytical data for the 4 main antler sections for the NZ red antlers. The 3 lower sections all comprised approximately equal portions of the antler, with the tip making up only an average 2.7% of the dry weight. The ash and calcium contents of the 4 sections reflect the very low calcification in the tip of the antler and the increasing degree of calcification towards the base. The lipid (which is the major component of pantocrin) concentration was highest in the tip but, with the tip being only a very small proportion of the antler, the overall lipid content was determined largely by the other sections.

Table 1 Composition of adult NZ red deer velvet (n = 17) in each of the 4 main sections of the antler and the combined total

	Total Dry Weight %	Lipid %	Nitrogen %	Ash %	Calcium %
Tip	2.7	5.6	12.2	6.6	0.3
Upper	35.3	2.7	9.1	28.4	9.3
Mid	29.8	2.0	8.1	37.8	13.5
Base	32.5	2.6	7.6	38.8	14.7
Total	100	2.5	8.4	34.0	12.1

The effect of stage of growth on composition in NZ red two year old antler

Antlers were removed from 2 year old NZ red deer stags (n = 12) as follows. One antler was removed 55 days after casting of the previous hard antler from each stag and the opposite was removed from 43 to 67 days after casting. The data are illustrated (Figure 2) relative to the antler removed at day 55. There were marked effects of the stage of growth (days after casting) on the comparative composition of velvet antler as reflected in the regression relationships between components and days after casting. The relative contents of ash (total minerals), calcium and phosphorus increased with days of growth while lipid, sulphur, sodium, potassium and selenium decreased.

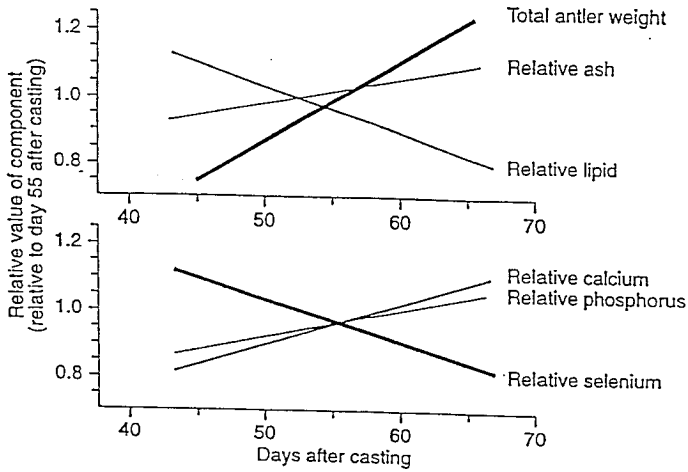

Figure 2. Relative contents of antler constituents with days of growth.

Comparisons between species

Both fallow and wapiti antlers were higher in nitrogen than the red deer (Table 2) and the fallow antlers were lower in calcium. The Russian antlers were lower in total lipid than red deer and the Chinese meihualu were higher in ash and calcium.

Table 2 Comparisons of mean values in the complete antler between species and NZ red deer (* P<0.05, ** P<0.01, *** P<0.001; otherwise comparisons are not significantly different)

	n	Lipid %	Nitrogen %	Ash %	Calcium %
NZ Red	17	2.5	8.4	34.0	12.1
NZ Wapiti	6	2.2	9.4***	34.0	12.0
NZ Fallow	6	2.7	9.5 ***	32.6	10.5 **
Russian	6	1.5 **	9.0 *	35.5	12.9
Chinese Malu	6	2.7	9.3 ***	34.9	12.1
Chinese Meihualu	6	2.2	8.8	36.8 **	13.1 *

Acting on information received from Korea that ganglioside and free amino acid levels might form part or all of quality assurance regulations for imported antler (Hong 1991), relevant assays were set up. The aim was to analyse NZ velvet antler and characterise variability with respect to grade and section.

Gangliosides/Sphingomyelins

Gangliosides are animal glycosphingolipids occurring mainly in the central nervous system but also distributed in other tissues. Within tissues they are located in the outer surface of cell membranes and in the synaptic membranes of the central nervous system. Gangliosides consist of fatty acids, sphingosine, simple sugars (galactose and glucose), galactosamine and sialic acid. They are thought to be involved with cell metabolism and growth.

We had, from an early stage in this programme, concerns that the Korean researchers may not have been measuring true gangliosides. After much research, it is now clear that the two components quantified by the Korean researchers are sphingomyelins (Sm), which are phospholipids. Sm, which are also called ceramide phosphocholines, are structural component of cell membranes. It appears that Sm are involved in complex bioregulatory pathways; for example they are potent inhibitors of protein kinase C which plays an important role in signal transduction in cells. Consequently there are grounds to believe that Sm are biologically active, and it may be that the Sm are very important for velvet antler quality.

The assay method chosen for use was a thin layer chromatography (TLC) based system, similar to that used by the Korean scientists for the assay of "gangliosides" in velvet antler (Hong, 1991).

Sm components were present in higher concentrations in upper sections of NZ red deer antlers compared with lower sections (Table 3). These results indicate that higher quality sections of antler have higher absolute levels of Sm than lower quality sections.

Table 3 **Effect of antler section on sphingomyelin levels in adult NZ red deer velvet antlers (n = 4). SED is the standard error of the difference**

	Section						
	1	2	3	4	7	8	SED
Sphingomyelin (mg/g)	2.92	1.78	1.70	1.35	0.57	0.36	0.15

Antlers from Russian and Chinese deer were analysed for Sm levels (Table 4). Although there were consistent differences between Sections 2 and 8 for each species, there were no significant differences among the species.

Table 4 **Effect of species and section on sphingomyelin levels (mg/g). In all cases Sections 2 and 8 differ significantly (P<0.001). The species x section interaction is significant, P<0.05**

	Section	
	2	8
NZ Red	1.80	0.41
NZ Wapiti	2.05	0.65
Russian	1.51	0.48
Chinese Malu	1.48	0.62
Chinese Meihualu	1.63	0.64
SED (Red to Others)	0.14	
SED (Others to Others)	0.12	
SED (Species x Section)	0.18	

Free Amino Acids (FAA)

Velvet antler, as a dietary supplement or traditional medicine, might be expected to provide high levels of some or all of the essential amino acids. FAA were measured using a reversed phase high performance liquid chromatography procedure after derivatisation using phenyl isothiocyanate (PITC), and were determined in all samples analysed in the sphingomyelin assay.

The distribution of FAA in different parts of the antler was examined in four of the antlers (Table 5). When the main beam sections are compared (1, 2, 4 and 8), the levels of FAA decreased from tip to base.

Table 5: Effect of section on free amino acid levels (mmol/g) of NZ red deer antlers

	Section						
	1	2	3	4	7	8	SED
Total	111	83	92	51	37	15	4.3
Essential	25	26	29	20	13	4	1.7

The antlers from several species (same series as for Sm data) were analysed for FAA (Table 6). The FAA levels Chinese malu were significantly higher than the other species, particularly in Section 2. Both Chinese species were high relative to the other species for Section 8. The Russian antlers were similar to NZ red deer and wapiti in FAA composition.

Table 6 **Effect of species on free amino acid (m mol/g). In all species Section 2 and 8 differ significantly (P<0.001). The species and section interaction was not significant, P>0.05 (* P<0.05, ** P<0.01)**

	Section			
	2		8	
	Total	Essential	Total	Essential
NZ Red	82	26	17	5
NZ Wapiti	84	31	15	5
Russian	77	19	37	7
Chinese Malu	148	38	44	10
Chinese Meihualu	9	22	43	9
SED (NZ red to others)	13.8 ** (Total)		3.8 * (Essential)	
SED (Others to Others)	16.9 ** (Total)		4.7 * (Essential)	

Appraisal of spingomyelins and FAA as quality criteria

In red deer there was a strong linear relationship between total Sm level and total FAA within the antler (Figure 3). This means that parts of the antler which are regarded as being in general of higher quality have higher levels of the components selected by the Koreans as potential quality assurance criteria. Upper parts of the antler have higher levels of Sm and FAA. Red deer and wapiti antlers and those from Russia and China had similar Sm levels. FAA levels were enigmatically high for Chinese deer but otherwise similar in other species. In this study several important variables were not studied: these include stag nutrition, species outside NZ, processing technique and stage of development at harvest. It may be that the SM/FAA data are only relevant in a gross sense as quality criteria but insensitive to some important variable of quality.

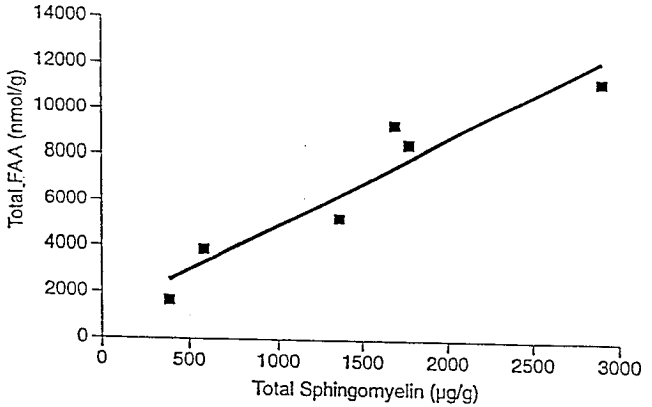

Figure 3. Relationship between total free amino acids (FAA) and sphingomyelin levels. The data are from sections 1, 2, 3, 4, 7, 8 from NZ red deer antlers.

PRODUCT DEVELOPMENT

Extract production from antlers

Considerable interest has been shown in New Zealand and internationally in development of extracts which concentrate biologically and clinically active substances from velvet antlers. Classically Russian pantocrine is made in a 50:50 v/v mixture of water and alcohol. At Invermay we have developed both aqueous and organic extraction systems. We have measured the effects of several antler variables on yield of these extracts.

The yield of extractable substances from both extracts is higher in the antler tips compared with the bases (Table 7). This is so even from dried velvet, probably reflecting an increase in soluble components in these sections. A and B graded antlers had similar yields for both extractions (Table 8). In contrast (Table 9) freeze dried C grade antlers had lower yield of extractables than B grade antlers, but there was no grade difference in antlers commercially processed. Commercial processing using conventional heat drying technology reduced the yield compared with freeze-drying for both aqueous and organic extracts. It is likely that heat used in commercial processes reduces soluble components.

Table 7 The effect of the section of NZ red deer velvet antlers on yields of extractables after aqueous or organic extraction (± standard deviation)

	Average yield (%) from dried NZ red deer velvet antlers											
	Section											
Extract	1		2		3		4		7		8	
Aqueous	28.4	(5.86)	27.5	(4.96)	22.0	(3.06)	28.8	(3.74)	13.9	(2.81)	12.6	(1.61)
	8.41	(2.61)	5.04	(0.75)	-	-	2.93	(0.74)	2.09	(0.28)	1.78	(1.30)
Organic												

Table 8 The effect of grade of NZ red deer velvet antler on the yield (%) of extracts with aqueous or organic extraction (± standard deviation)

		Section			
Extract	Grade	2		8	
Aqueous	A	39.2	(2.26)	14.4	(1.54)
	B	39.6	(1.77)	13.8	(2.55)
Organic	A	2.99	(0.23)	1.08	(0.16)
	B	3.05	(0.17)	0.93	(0.19)

Table 9 The effect of grade and process technique on the yield (%) of extract from dried NZ red deer antlers

		Section 2		Section 8	
		Grade		Grade	
Extract	Drying method	B	C	B	C
Aqueous	Freeze dried	35.5	31.5	11.8	7.7
	Commercial 1	25.5	26.5	9.3	6.3
	Commercial 2	22.6	22.9	7.6	6.6
Organic	Freeze dried	2.70	2.59	1.07	0.94
	Commercial 1	2.33	2.20	0.94	0.72
	Commercial 2	1.93	1.90	1.02	0.88

Although details of the processes cannot be presented, the data show that both the part of the velvet antler and processing technique affected yields. In contrast, for those examined, grade of antler did not substantially affect yield.

Research on biological effectiveness of organic extracts

Organic extracts from antlers have been tested in an antitumour assay. Samples of interest are incubated for 72 hours with P388 (murine leukaemia) cells. The concentration of sample required to reduce the P388 cell growth by 50% (compared to control cells) is determined. The results are expressed as an IC_{50} in mg of extract/ml of solvent (2:1 dichloromethanol/methanol).

Biological activity in the P388 assay decreases from upper sections to lower sections (ie, it requires a higher concentration of extract to have the same inhibitory effect; Table 10). Processing technique also influences activity (Table 11). These results indicate that there is potent anti-tumour activity in organic extracts from NZ red velvet antler and this is influenced by section of the antler, grade and processing technique. Data, however, are highly variable both between and within antlers. It is now important to carry out further studies on the reasons for this variability and whether stags consistently produce antlers of higher activity than others. Ways of handling and processing antlers to maximise activity must also be sought and optimal systems developed.

Table 10 **The effect of antler section (of C grade NZ red velvet antler) on the activity (IC_{50}) of organic antler extracts in the P388 assay (SED = standard error of the difference between means)**

	Section				
	2	4	7	8	SED
IC_{50} dose (mg/ml)	35	32	378	409	77

Table 11 The effect of drying method and antler section on the activity of organic extract from NZ red velvet antler. Results expressed as IC_{50} (mg/ml) in the P388 assay. Results are not corrected for differences in dry matter content or extract yield

	Freeze dried	Commercial 1	Commercial 2	SED
Section 2	860	197	195	148
Section 8	720	318	469	696

Research on biological effectiveness of aqueous extracts

The aqueous extracts described above have been evaluated for biological effectiveness by measuring their effects on growth of antler cells in culture. The method used for the antler cell culture has been published (Sadighi *et al* 1994).

In the first series of studies extracts were made from whole combined A and B grade NZ red velvet antlers. The dose response of the extracts on mitogenicity of antler cells from the x and y lines (Figure 4) revealed that there was a peak at extract concentrations of 1.25 - 2.5 mg/ml after which mitogenicity declined.

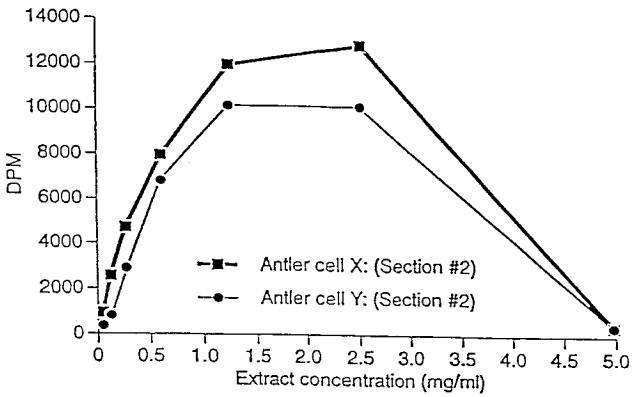

Figure 4. Mitogenicity of antler extracts: the relationship between cell growth response of antler X and Y cell lines (uptake of tritiated thymidine) and the concentration of aqueous velvet antler extracts in the medium (extracts from NZ red deer velvet antler).

These results, taken together, reveal that there are potent stimulators of cell division in aqueous antler extracts.

Further experiments (data not shown) with 3T3 mouse fibroblast cells have shown that the aqueous velvet extracts are also potent stimulators of growth in non-antler derived cells.

HARVEST TECHNOLOGY AND COLOUR

In the Korean marketplace New Zealand velvet is noted for its rich dark red colour which contrasts to the browner hue of Russian velvet. We have consequently begun a preliminary investigation into the factors that influence colour of processed velvet antler. While these studies are not sufficiently complete to present detailed results it must be stated that removal technique appears to play only a minor role in determining colour. Much more important is the processing technique, particularly the processing temperature. We consider in the future that antler processing will become more specific and techniques will vary according to the requirements of end users. Traditional users of cut antler slices may require a very different process compared with makers of the various antler extracts.

CONCLUSIONS

The compositional studies have shown that NZ antler is very similar to that from Russia and China, which compete in the international market place with NZ product. This information has already been disseminated to NZ marketers to aid promotional efforts. The data from the specific compositional studies (Sm and FAA) have revealed some interesting comparisons but no convincing tool to measure quality. Our colour studies reveal that we are some way off determining an objective measure of antler quality.

The group has been successful in making extracts and developing techniques for their objective efficacy analysis. These extracts are becoming available to NZ producers. The concept that specific processing techniques, and indeed specific antler grades, might be used for extract production to make specific oriental medicines is a serious challenge for the industry in the near future.

Overall the programme is progressing well towards its goal of improving market access for NZ antler and increasing markets for New Zealand products in the East.

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