Chemical Composition of Antlers from Wapiti (*Cervus elaphus*)

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Antlers, the fast growing structures of cervids, are fascinating tissues that are useful as a model for the study of bone growth and metabolism. They are also used for oriental medicine and tonics. However, little is known about their chemical composition. Four sets of fresh velvet antlers were obtained at 65 days after button casting from four 4-year-old wapiti, and the chemical composition of each antler was determined in four sections (tip, upper, middle, and base). The contents of dry matter, collagen, ash, calcium, phosphorus, and magnesium increased ($p < 0.05$), and those of protein and lipid decreased ($p < 0.05$) downward from the tip to the base. The concentrations of uronic acid, sulfated glycosaminoglycan, and sialic acid decreased ($p < 0.05$) downward. Amino acid and fatty acid contents, expressed as percentage of total protein and lipid, respectively, also varied ($p < 0.05$) among sections. The tip section, which is the growth center of the antler, had highest proportions of tyrosine and isoleucine and lowest proportions of glycine and alanine. The C18:3ω6 fatty acid was found in the tip section only.

Keywords: *Antlers; wapiti; medicine; chemical composition*

INTRODUCTION

Velvet antlers have been an important traditional oriental medicine for many years. With increasing demand for velvet antler, deer farming is becoming a rapidly growing industry as an alternative form of animal agriculture in many parts of the world, including North America, Europe, and New Zealand (Sim, 1987). World wide, ≈2.5 million deer are farmed, most of which serve this demand. Each year, over 4000 tons of antler enter international markets to supplement local production in Eastern Pacific Rim Countries. The use of velvet antlers as a food supplement is growing rapidly in Europe and North America. The international market demands antlers of specific size, shape, and mineralization. Antlers grow in length by endochondral ossification, and in diameter by intramembranous ossification (Banks, 1974). They are suggested to be a useful model for the study of bone growth and metabolism (Goss, 1983). Wapiti antlers are removed at ≈60 days of growth (June in northern photoperiods) when the main beam just begins to bulb at the fourth tine. Antlers can be divided into four sections, tip, upper, middle, and base. Various sections are used differently to treat diseases such as anemia, arthritis, hypercholesterolemia, and cancer or to promote health as traditional oriental tonics (Fennessy, 1991). For food researchers, antlers with various biological activities could be a useful ingredient to develop a new food product that can modulate physiology of our body and protect us against diseases. Such a function of food is important in addition to the well-known functions for food of nutrition and palatability.

Despite high demand, limited information is available concerning the chemical composition of antlers (Sim and Hudson, 1991; Sim et al., 1995). Such information is important to understand the growth and development of antlers as well as their function as nutritional supplements or pharmaceutical agents. The present study was undertaken to determine protein, amino acid, carbohydrate, and fatty acid contents in the four sections of wapiti antler.

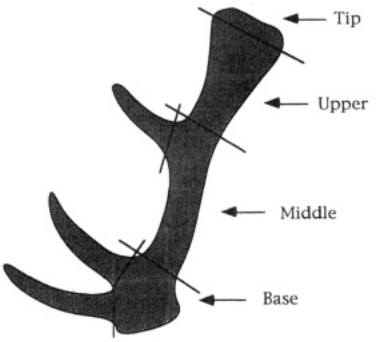

Figure 1. Drawing of velvet antlers of wapiti showing four sections used in analysis of chemical composition.

MATERIALS AND METHODS

Preparation of Antlers. Velvet antlers were obtained from four 4-year-old wapiti stags averaging 365 kg that were maintained at the University of Alberta's Ministik Research Station. These animals were kept on a high-quality pelleted diet with 17.9% crude protein, 0.84% calcium, and 0.44% phosphorus provided *ad libitum*. Velvet antlers (≈65 days after casting of the buttons from the previous set) were harvested from each stag under local or general anesthesia by a veterinarian, and each antler was divided with a butcher knife into four sections (tip, upper, middle, and base) of the main beam (Figure 1). Samples of each section were skinned, weighed, and homogenized with a meat grinder. Because antler growth depends on endochondral ossification and intramembranous ossification (Banks, 1974), the velvet skin, which is not the tissue where ossification takes place, was not included in the present analysis. This omission was thought to provide analytical data more specific to the growing antler tissue than those with unskinned antler. However, we are planning to analyze velvet skin in the near future. Homogenized samples were freeze-dried and stored at −20 °C until analyzed.

Chemical Analyses. Antler samples were analyzed for protein, ash, calcium, phosphorus, and magnesium by the methods of the Association of Official Analytical Chemists (1984). For amino acid analysis, samples were hydrolyzed

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Table 1. Weights and Chemical Analysis of Four Sections of Antler[a]

item	antler section			
	tip	upper	middle	base
weight (g)	38.37 ± 3.65[a]	834.32 ± 43.73[b]	656.48 ± 27.31[c]	427.23 ± 11.97[d]
dry matter (%)	14.36 ± 0.19[a]	24.95 ± 0.86[b]	31.35 ± 0.49[c]	42.05 ± 0.66[d]
protein (%)	69.08 ± 0.88[a]	61.50 ± 0.77[b]	57.13 ± 0.41[c]	49.27 ± 1.08[d]
collagen (%)	10.01 ± 0.52[a]	14.35 ± 1.38[b]	25.83 ± 0.84[c]	31.99 ± 1.26[d]
lipid (%)	18.94 ± 1.00[a]	2.67 ± 0.07[b]	1.02 ± 0.05[c]	0.50 ± 0.04[cd]
uronic acid (%)	1.24 ± 0.17[a]	1.36 ± 0.11[a]	0.16 ± 0.02[b]	0.11 ± 0.01[b]
sulfated GAG[b] (%)	3.73 ± 0.47[a]	4.67 ± 0.27[b]	0.34 ± 0.03[c]	0.26 ± 0.03[c]
sialic acid (%)	0.61 ± 0.01[a]	0.30 ± 0.06[b]	0.25 ± 0.03[b]	0.09 ± 0.02[c]
ash (%)	9.40 ± 0.27[a]	34.45 ± 0.61[b]	39.87 ± 0.60[c]	48.04 ± 0.40[d]
calcium (%)	0.42 ± 0.01[a]	3.32 ± 0.07[b]	11.77 ± 0.23[c]	16.50 ± 0.36[d]
phosphorus (%)	0.39 ± 0.02[a]	1.84 ± 0.05[b]	6.95 ± 0.07[c]	8.59 ± 0.12[d]
magnesium (%)	0.04 ± 0.00[a]	0.08 ± 0.00[b]	0.19 ± 0.01[c]	0.29 ± 0.01[d]

[a] All values for chemical analysis are on a dry matter basis. Means in rows with different superscripts are significantly different ($p < 0.05$). [b] GAG, glycosaminoglycan.

with 6 N HCl at 110 °C for 24 h. Hydroxyproline content in the hydrolysate was determined by the method of Stegemann and Stalder (1967). The content of collagen was calculated by multiplying the content of hydroxyproline by 7. The separation and quantification of amino acids other than hydroxyproline were accomplished by HPLC (model 5000, Varian Associates, Inc., Sunnyvale, CA) equipped with a Varian fluorochrome detector (Jones and Gilligan, 1983). The precolumn derivatization was carried out by mixing the sample with a fluoraldehyde reagent, *o*-phthaldialdehyde, in a 1:1 ratio, using a Varian autosampler that injects the resulting derivative into the column with a delay time of 12 s. The analytical column (4.6 × 150 mm) was packed with 3-μm Supelcosil LC-18 (Supelco Canada, Mississauga, ON) reversed phase. The guard column (4.6 × 50 mm) was packed with a 20–40-μm Supelcosil LC-18 reversed phase. Chromatographic peaks were recorded and integrated with a Shimadzu Ezchrom Chromatography Data System (Shimadzu Scientific Instruments, Inc., Columbia, MD).

Total lipids were determined by extracting samples with chloroform:methanol (2:1, v/v) according to Folch et al. (1957). The lipid samples were dried under nitrogen and methylated with boron trifluoride methanol according to the method of Metcalfe and Pelka (1961). The fatty acid methyl esters of total lipids were analyzed by a Varian (model 3700) gas liquid chromatograph with an SP2330 capillary column (30 m × 0.5 mm, inside diameter). The initial column temperature was 120 °C for 1 min. The temperature was programmed to increase by 5 °C/min until 190 °C. The final temperature of 190 °C was held for 5 min. The gas flow rates were as follows: nitrogen, 34 mL/min; hydrogen, 30 mL/min; and air, 300 mL/min. Chromatogram peaks were integrated with the same equipment as for the amino acid analysis.

Uronic acid contents were determined by the carbazole reaction (Kosakai and Yoshzawa, 1979) after digesting samples with twice crystallized papain (Sigma) (Scott, 1960). Samples of sections 2–4, which contained bony tissues of varying amounts were decalcified with 10% Na_2 EDTA containing 0.05 M Tris (pH 7.4) before papain proteolysis. The digests were also assayed for sulfated glycosaminoglycan (GAG) by the dimethylmethylene blue dye binding method (Farndale et al., 1982). Sialic acid was determined by the method of Warren (1959) after hydrolysis of samples in 0.1 N sulfuric acid at 80 °C for 1 h. The chromophore formed was extracted using 1-propanol (Nakano et al., 1994) instead of cyclohexanone used by Warren (1959).

Statistical Analyses. One-way ANOVA was used to determine the difference among means of antler weight, dry matter, contents of protein, collagen, uronic acid, glycosaminoglycan, sialic acid, lipid, ash, calcium, phosphorus, and magnesium, and amino acid and fatty acid profiles. When significant differences occurred ($p < 0.05$), Scheffe's F test was used to determine the difference between means (Steel and Torrie, 1980). All analyses were conducted using version 4.01 of StatView (Abacus Concepts, Inc., Berkeley, CA). Data are presented as means ± SEM.

RESULTS

Results of analysis of each section of skinned antlers are summarized in Table 1. The weights of antlers were highest ($p < 0.05$) at the upper section, higher ($p < 0.05$) in the middle than in the base, and lowest ($p < 0.05$) at the tip section. The contents of dry matter, collagen, calcium, phosphorus, and magnesium were lowest ($p < 0.05$) in the tip section, and increased downward (upper < middle < base). The proportion of collagen in total protein showed a similar changing pattern. In contrast, the contents of protein and lipid were highest ($p < 0.05$) in the tip, and decreased downward with no difference in the lipid value between the middle and the base sections. Uronic acid contents were higher ($p < 0.05$) in the tip and the upper sections than in the middle and base sections. Sulfated GAG contents decreased ($p < 0.05$) downward, with no difference between the middle and the base sections. Sialic acid contents were highest ($p < 0.05$) in the tip section and decreased with no difference between the upper and the middle sections.

The proportion of individual amino acids (Table 2) did not reflect the section-related variations of total protein with the exception of lowest ($p < 0.05$) arginine content in the base section and highest ($p < 0.05$) contents of tyrosine and isoleucine in the tip section. The variations in the contents of glycine and alanine, the amino acids present in relatively large amounts in collagen were, however, similar to those of collagen.

The fatty acid profiles of the four sections are shown in Table 3. Oleic acids (C18:1ω7 and 9) accounted for more than half of total fatty acids. The proportions of C16:0, C16:1ω7, and C20:2ω6 decreased ($p < 0.05$), whereas those of C18:0, C18:2ω6, C20:4ω6, C22:4ω6, and ω−6 increased ($p < 0.05$) downward. The fatty acid C18:3ω6 (0.16%) was found only in the tip section. The proportions of the remaining fatty acids (C14:0, C18:3ω3, C20:1ω9, C20:3ω6, C22:6ω3, SAFA, MUFA, ω−3, and ω−6) and the ω−6 to ω−3 ratio were similar among the four sections.

DISCUSSION

There is little information available concerning chemical composition of wapiti antlers. However, a limited number of studies have been published on antlers of other cervids. Ullrey (1983), in a study of whole velvet antlers from white-tailed deer, reported that dry matter accounted for 20% of wet weight, and protein and ash accounted for 80 and 20% of dry matter, respectively. In the present study, values calculated for the skinned whole antler were 31% for dry matter, 57% for protein,

Table 2. Analysis of Amino Acid in the Four Sections of Antler (Percent of Dry Matter)[a]

amino acid	antler section			
	tip	upper	middle	base
aspartic acid	6.64 ± 0.23	7.46 ± 0.37	6.76 ± 0.29	6.78 ± 0.24
glutamic acid	10.52 ± 0.36	9.99 ± 0.39	10.07 ± 0.28	11.20 ± 0.25
serine	3.04 ± 0.20[a]	3.73 ± 0.16[b]	3.29 ± 0.16[ab]	3.51 ± 0.12[ab]
histidine	2.11 ± 0.15[a]	2.84 ± 0.19[b]	1.87 ± 0.20[a]	1.57 ± 0.07[a]
glycine	7.63 ± 0.66[a]	9.70 ± 0.23[b]	12.23 ± 0.68[c]	17.42 ± 0.14[d]
threonine	3.29 ± 0.15[ab]	3.62 ± 0.17[a]	3.07 ± 0.19[ab]	2.90 ± 0.10[b]
arginine	5.15 ± 0.36[a]	5.51 ± 0.12[a]	5.97 ± 0.16[a]	7.26 ± 0.16[b]
alanine	5.74 ± 0.25[a]	6.99 ± 0.21[b]	7.50 ± 0.21[b]	8.87 ± 0.20[c]
tyrosine	2.11 ± 0.10[a]	1.92 ± 0.09[ab]	1.60 ± 0.10[b]	1.62 ± 0.04[b]
valine	4.34 ± 0.15[ab]	4.75 ± 0.26[a]	3.92 ± 0.23[ab]	3.69 ± 0.12[b]
phenylalanine	3.34 ± 0.17	3.90 ± 0.23	3.19 ± 0.21	3.18 ± 0.08
isoleucine	2.37 ± 0.08[a]	1.77 ± 0.08[b]	1.69 ± 0.06[b]	1.76 ± 0.04[b]
leucine	6.42 ± 0.29[ab]	7.22 ± 0.41[a]	5.67 ± 0.38[b]	5.11 ± 0.17[b]
lysine	3.87 ± 0.24	4.33 ± 0.12	3.79 ± 0.21	3.96 ± 0.13

[a] Means in rows with different superscripts are significantly different ($p < 0.05$).

Table 3. Fatty Acid Composition (Percent of Total Fat) of Four Sections of Antler[a]

fatty acid	antler section			
	tip	upper	middle	base
C14:0	1.18 ± 0.03	1.15 ± 0.01	0.87 ± 0.04	1.06 ± 0.23
C16:0	16.27 ± 0.66[a]	15.10 ± 0.12[a]	14.44 ± 0.44[ab]	12.19 ± 0.58[b]
C16:1ω7	4.26 ± 0.35[a]	2.09 ± 0.03[b]	1.19 ± 0.07[bc]	0.83 ± 0.15c
C18:0	7.79 ± 0.21[a]	7.58 ± 0.18[a]	9.28 ± 0.09[b]	9.61 ± 0.54[b]
C18:1ω7 and 9	55.72 ± 1.69	59.31 ± 0.63	54.81 ± 1.12	57.40 ± 2.54
C18:2ω6	2.58 ± 0.09[a]	3.22 ± 0.13[b]	5.20 ± 0.09c	4.61 ± 0.12d
C18:3ω6	0.16 ± 0.00[a]	0.00 ± 0.00[b]	0.00 ± 0.00[b]	0.00 ± 0.00[b]
C18:3ω3	0.86 ± 0.20	0.64 ± 0.05	1.02 ± 0.03	0.87 ± 0.05
C20:1ω9	0.19 ± 0.03	0.22 ± 0.05	0.46 ± 0.10	0.18 ± 0.03
C20:2ω6	1.98 ± 0.15[a]	1.28 ± 0.04[b]	0.43 ± 0.2c	0.45 ± 0.02c
C20:3ω6	0.73 ± 0.13	0.79 ± 0.01	0.80 ± 0.07	0.64 ± 0.05
C20:4ω6	2.99 ± 0.19[a]	3.40 ± 0.03[a]	5.28 ± 0.06[b]	5.62 ± 0.52[b]
C22:4ω6	0.39 ± 0.02[a]	0.51 ± 0.12[a]	0.78 ± 0.03[b]	0.82 ± 0.09[b]
C22:6ω3	1.29 ± 0.03	1.30 ± 0.02	1.36 ± 0.03	1.37 ± 0.07
SAFA[b]	27.15 ± 0.89	25.79 ± 0.29	26.82 ± 0.53	25.12 ± 1.29
MUFA[c]	61.89 ± 0.93	63.07 ± 0.23	58.33 ± 0.69	60.50 ± 1.96
ω−3[d]	2.15 ± 0.21	1.94 ± 0.06	2.38 ± 0.05	2.25 ± 0.12
ω−6[e]	8.82 ± 0.24[a]	9.20 ± 0.21[a]	12.48 ± 0.33[b]	12.14 ± 0.58[b]
ω−6:ω−3	4.19 ± 0.47	4.75 ± 0.07	5.25 ± 0.25	5.41 ± 0.04

[a] Means in rows with different superscripts are significantly different ($p < 0.05$). [b] Total saturated fatty acids include C12:0, C14:0, C15:0, C16:0, C17:0, C18:0, C20:0, and C24:0. [c] Total monounsaturated fatty acids include C15:1ω5, C16:1ω7, C18:1ω7, C18:1ω9, and C20:1ω9. [d] Total omega−3 fatty acids include C18:3ω3 and C22:6ω3. [e] Total omega−6 fatty acids include C18:2ω6, C18:3ω6, C20:2ω6, C20:3ω6, C20:4ω6, and C22:4ω6.

and 39% for ash. The apparent differences between the results of the two studies may be smaller if corrections were made for antlers with skin in the present data. The skin was not included in the present analysis (see Materials and Methods for explanation). Scott and Hughes (1981) reported that hydroxyproline and uronic acid concentrations in deer antler were 42 mg/g and 300 μg/g, respectively. The hydroxyproline value may be close to that (31.4 mg/g) calculated for skin-free whole antler in this study, assuming that the sample analyzed by Scott and Hughes (1981) had skin, although no information was given by these authors. Their uronic acid value was much lower than ours (7.4 mg/g after correction). Miller et al. (1985) reported that calcium, phosphorus, and magnesium concentrations in the unskinned middle section of all-white-tailed-deer antlers were 190.1, 101.3, and 10.94 mg/g of dry weight, respectively. The samples analyzed by Miller et al. (1985) were ossified to a greater extent than those analyzed in the present study.

Collagen has been suggested to be the major protein in the antler (Goss, 1983). However, little is known about the distribution of this protein in the antler. Our results indicated that the amount of collagen in dry tissue increases downward (≈1.4, 2.5, and 3.2 times higher in the upper, middle, and base sections, respectively, than in the tip section) with concomitant increase in mineral contents. The proportion of collagen of total protein also showed a similar trend. The major function of collagen is to provide mechanical strength to the tissue. It appears that proximal tissue (tissue closer to the center of the body), which sustains distal tissue, requires more collagen than does distal tissue, and that collagen is involved as an organic element to reinforce mineralized tissue structure.

The growth center of antler resembles the growth plate (physis) of long bone in that longitudinal growth depends on endochondral ossification (Banks, 1974). The proximal portion of tip section and the distal portion of upper section are the sites where endochondral ossification takes place. It has been proposed that anionic molecules of GAG chondroitin sulfate in the growth plate have important roles as ion exchangers in endochondral bone formation (Hunter, 1991). Our recent study showed that chondroitin sulfate is the major GAG in the four sections of wapiti antler (H. H. Sunwoo, T. Nakano, and J. S. Sim, unpulished results). Thus, the greater concentrations of chondroitin sulfate uronic acid or dimethylmethylene blue dye reactive GAG observed in the tip and the upper sections than in the remaining sections are not surprising. In addition to collagen and GAG contents, those of lipid, sialic acid, tyrosine,

isoleucine and C18:3ω6 fatty acid were also highest in the tip section. The biological importance of these compounds is unknown.

Antlers have been used for many centuries as important medicine and tonic in oriental countries such as China, Japan, and Korea. However, not much is known about the elements of antler that have beneficial effects on the physiological functions of our body. Zhao et al. (1992) reported presence of complement-activating proteoglycan containing chondroitin sulfates in antlers of *Cervus nippon* Temmick. Chondroitin sulfate may be a potentially important carbohydrate in the antler as a food. Oral administration of chondroitin sulfate resulted in reduction of pain in osteoarthritis patients (Gross, 1983; cited by Paroli et al., 1991). We have recently found increased hematocrit values in chickens fed wapiti antler powder (H. H. Sunwoo and J. S. Sim, unpublished results). Unpublished data from this laboratory also showed that intraperitoneal administration of wapiti antler extract to mice resulted in increased growth rate in the animals. This result suggests the presence of growth factors in the extract used. Further investigation of molecules with growth-promoting activity in wapiti antlers is under progress in our laboratory.

The present study provides previously unreported information of the overall chemical composition of four sections of wapiti antler. Quantitative variations were observed in proteins, carbohydrates, minerals, and lipids among the sections. These variations appeared to be related to the difference in function among sections. However, no satisfactory explanation of the role of each element is available at present. The present analysis also provides a basis for the study of wapiti antler composition in relation to animal age, nutrition, environment, and drying method, which should facilitate efficient production of high quality antlers as a food and as pharmaceutical agents.

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