Asian-Aust. J. Anim. Sci.
Vol. 24, No. 9 : 1303 - 1313
September 2011

www.ajas.info
http://dx.doi.org/10.5713/ajas.2011.10412

Effect of Antler Development Stage on the Chemical Composition of Velvet Antler in Elk (*Cervus elaphus canadensis*)*

Byong Tae Jeon, Sun Hee Cheong, Dong Hyun Kim, Jae Hyun Park, Pyo Jam Park, Si Heung Sung, David G. Thomas[1], Kyoung Hoon Kim[2] and Sang Ho Moon**

Korea Nokyong Research Center, Konkuk University, Chungju, 380-701, Korea

ABSTRACT : This study was conducted to provide the basic information to allow improved scientific assessment of velvet antler's quality by investigating the change of chemical composition during different antler growth stages in elk (*Cervus elaphus canadensis*). Twenty four antlers were harvested from elk stags (aged 4-5 years) on 65 days (VA65), 80 days (VA80) and 95 days (VA95) after button casting, and the chemical composition of each antler was determined in five sections (top, upper, middle, base, and bottom). Crude protein and ether extract content was the highest in the top section, whereas ash content was the highest in the bottom section in all groups ($p<0.05$). Glycosaminoglycan (GAG) content was higher in the VA65 group than in the VA95 group in the upper section of antler ($p<0.05$). The collagen content was higher in the VA65 group compared to the VA95 group in the middle and bottom sections ($p<0.05$), and increased downward from the top to the bottom section. The proportions of certain amino acids, including aspartic acid, glutamic acid and isoleucine were higher ($p<0.05$), whereas proline and glycine were lower in the top section of antler compared to all other sections ($p<0.05$). The proportion of linoleic acid, 11,14,17-eicosatrienoic acid, total ω-3 and ω-6 fatty acids and polyunsaturated fatty acids (PUFAs) for all sections in the VA65 group was higher than in the VA95 group ($p<0.05$). These results suggested that the quality of velvet antler is strongly influenced by antler development stage. (**Key Words :** Elk (*Cervus elaphus canadensis*), Chemical Composition, Growth Period, Velvet Antler)

INTRODUCTION

Antlers are unique among animal bones in that they grow and are cast every year. They grow very fast and are covered with 'velvet' a thick periosteum well supplied with blood vessels (Li, 2003). Velvet antler differentiates rapidly, showing a sequential development from the tip to the base, and then becomes hardened because of progressive mineralization and occlusion of blood vessels (Kay et al., 1982; Fletcher, 1986). Therefore, the chemical composition of velvet antler may vary greatly with both the antler portion and the stage of antler development.

Velvet antler is a well-known traditional oriental medicine, which has been used clinically in East Asia for thousands of years in the treatment of various diseases and as a tonic (Zhang et al., 1992). Recently, velvet antler consumption has increased worldwide, with consumers interested in the quantity, chemical composition and quality of velvet antler (Jeon and Moon, 2006). Several studies have been reported that the quality of velvet antler partially depends on the age, breed, growth stage, feeding condition and nutrition level of stag (Ha et al., 2003; Moon et al., 2004). In addition, some evidence suggests that the chemical composition of velvet antler varies with species, antler region, portion of the antler and the stage of antler development (Chapman, 1975; Landete-Castillejos et al., 2007). Numerous researchers have investigated the chemical composition of entire velvet antler and found it to be composed of a variety of minerals, proteins, collagens, omega-3 fatty acids, glycosaminoglycans, and prostaglandins in varying concentrations (Sunwoo et al., 1995; Sunwoo et al., 1997; Choi et al., 2006). Moreover, a number of recent animal studies have linked velvet antler consumption with an enhanced sense of well being and vitality, improved musculoskeletal function, enhanced resistance to disease and immune system modulation,

* This work was supported by Konkuk University in 2011.
** Corresponding Author : S. H. Moon. Tel: +82-43-840-3527, Fax: +82-43-851-8216, E-mail: moon0204@kku.ac.kr
[1] Institute of Food, Nutrition and Human Health, Massey University, Palmerston North, New Zealand.
[2] National Institute of Animal Science, RDA, Suwon, 441-706, Korea.
Received November 12, 2010; Accepted April 8, 2011

increased blood flow and blood pressure modulation, and promotion of rapid healing in tissues and bones (Elliott et al., 1996; Hemmings and Song, 2004; Moreau et al., 2004).

Although some studies on the chemical components and bioavailability of entire velvet antler have been carried out, there has been little research activity on standardization of farmed velvet antler, in particular, the chemical content of each section of velvet antler at different cutting times. Therefore, the aim of the present study was to provide basic information to allow the improved assessment of velvet antler quality and standardization of farmed velvet antler by investigating the change in chemical composition depending on antler development stage in elk (*Cervus elaphus canadensis*).

MATERIALS AND METHODS

Sample collection

Twenty four samples of velvet antler harvested on 65 days (VA65), 80 days (VA80) and 95 days (VA95) after casting were collected from randomly-selected deer farms in Korea, from June to August, 2007. Antlers were harvested from 24 elk (*Cervus elaphus canadensis*) stags, 4-5 years old, and one of the antler pair from each stag was used as a sample for analysis. All stags were healthy with no clinical signs of disease. Each antler sample was divided into five equal sections (top, upper, middle, base and bottom) along the main beam. Samples of each section were sliced with a bone slicer, freeze-dried and ground by a sample mill (KNIFETEC 1095 Sample Mill) to pass a 0.1 mm screen. Ground samples were stored in a freezer (-40°C) until chemical analysis.

Chemical analysis

The antler samples were analyzed for crude protein, crude fat (ether extract), crude fiber, total ash and mineral content using the methods of the Association of Official Analytical Chemists (AOAC, 1990). Collagen content was calculated by multiplying the hydroxyproline content by 7.25 (Cross et al., 1973). Hydroxyproline content was determined by the method of Bergman and Loxley (1962).

For glycosaminoglycan (GAG), uronic acid and sialic acid analysis, 50 mg of velvet antler sample was decalcified in 1 ml 0.05 M Na_2EDTA (pH 7.4, including 0.5 M Tris) for 2 days at 4°C. After decalcification, samples were centrifuged at 12,000 rpm for 10 min at 4°C to obtain a precipitate. A 3 ml sample of 0.1 M phosphate buffer (pH 6.5, containing 0.05 M cystein hydrochloride and 0.005 M Na_2EDTA) was mixed with 20 mg crude papain, and incubated for 30 min at 65°C to activate the enzyme. The activated enzyme was then mixed with the decalcified samples. The reaction was carried out for 16 h at 65°C, after which the upper liquid layer was removed.

Glycosaminoglycan content was determined by a microfilter plate adaptation of a dimethylmethylene blue assay of Farndale et al. (1982). Uronic acid content of the antler was determined by the method of Scott (1960) and Kosakaki and Yosizawa (1979). Sialic acid content was determined by the method of Warren (1959). Reagent A (40 μl) was added to the samples, standards and controls (80 μl) and mixed well. The mixture was left at room temperature for 20 min. Reagent B (400 μl) was then added and the tubes were shaken vigorously to remove the yellow-colored iodine. The tubes were left for a further 5 min at room temperature. Reagent C (1.2 ml) was then added before the tubes were shaken and heated at 100°C for 15 min. Samples were cooled rapidly to room temperature, and two liquid layers formed: one was red in color and the other was transparent. The red-colored solution was extracted and centrifuged for a few minutes to properly separate the two layers. The upper cyclohexanone layer was determined at the absorbance level of 549 nm.

For amino acid analysis, antler samples (80 mg) were combined with 10 ml of 6 N HCl. After N_2 gas was used to purge the samples in the test tube, the samples were hydrolysed in a dry oven at 110°C for 24 h. The hydrolysed samples were then evaporated and a sodium-distilled buffer (pH 2.2) was added. Samples were then filtered through a syringe filter (0.45 μm) and analyzed using an amino acid autoanalyzer (Pharmacia Biotech Biochrom 20, Ninhydrin Method). Amino acids were determined by absorbance at 440 and 570 nm.

Fatty acid analysis of the antler samples was carried out using the following procedure. Samples were first methylated using the Park and Goins (1994) method for fatty acid analysis. In brief, 3 ml of sample was transesterified to fatty acid methyl esters in benzene using 0.5 M NaOH/methanol for 10 min at 100°C. After cooling, the mixture was neutralized with HCl/methanol and then reheated. Fatty acid and methyl esters were extracted with hexane and measured by gas-liquid chromatography (HP 5890 II Series, Hewlett-Packard, Atlanta, USA) using a capillary column (Agilent-INNOWax, 30 mm×0.32 mm× 0.25 μm). The initial column temperature was programmed to 150°C and gradually increased to 200°C at 5°C/min. The components detected were identified by comparison with a standard mixture of fatty acid methyl esters (lipid standard and linoleic acid methyl esters, *cis*/*trans*-isomers, Sigma Ltd., St. Louis, USA). Composition of the free fatty acid fraction was expressed as a weight percentage of the total fatty acids.

Statistical analysis

All data are presented as means and standard deviation. Analysis of variance with Duncan's multiple range test was performed to evaluate the differences among the groups,

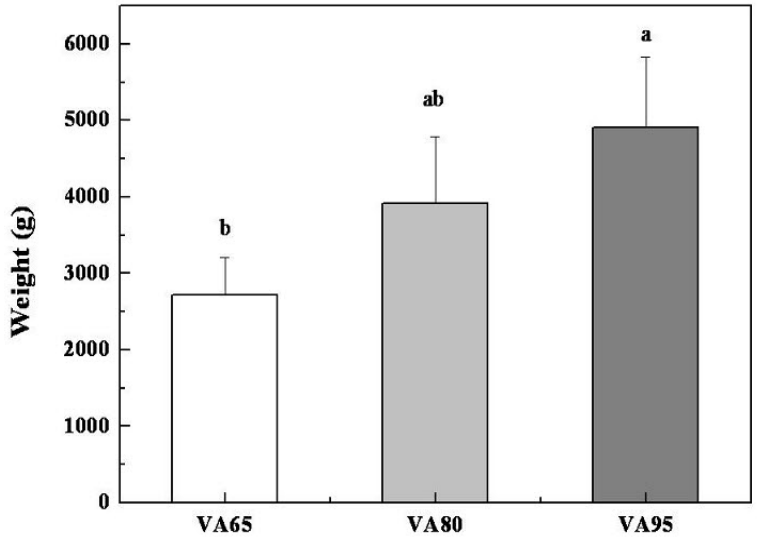

Figure 1. Velvet antler production at different cutting times in elk. VA65: group harvested 65 days after casting, VA80: group harvested 80 days after casting, VA95: group harvested 95 days after casting. [a, b] Means with different superscript among the groups are different ($p<0.05$).

using the Statistical Analysis System version 6.0 (SAS Institute, Cary, NC, USA). Statistical significance was defined as $p<0.05$.

RESULTS AND DISCUSSION

Velvet antler production

The production of velvet antlers at different cutting times is shown in Figure 1. In general, it is known that antlers constitute 1 to 5% of body weight (Weladji et al., 2005), and antler elongation has a typical S-shaped growth curve (Goss, 1983). In the present study, antler production of the VA65 group was lower compared to that of the VA95 group ($p<0.05$). It appeared that the velvet antlers had grown constantly from 65 days until 95 days after casting.

Chemical composition

The ash, crude protein, ether extract (crude fat), calcium and phosphorus content of velvet antlers is shown in Table 1. The crude protein and ether extract content was the highest in the top section of antler and decreased markedly in the other sections further down the antler ($p<0.05$). A similar pattern was observed in all groups. The ash content was the highest in the bottom section and markedly increased downward in all groups ($p<0.05$). The ash content in the base and bottom sections in the VA95 group was higher than in the same section in the VA65 group ($p<0.05$). Ullrey (1983) reported that protein and ash accounted for 80% and 20% of dry matter, respectively, in whole velvet antler from white-tailed deer. Although there was no difference between groups, in calcium (Ca) and phosphorus (P) content, it did tend to increase from the top to the bottom of the antler. These results indicated that velvet antlers were actively growing in the VA65 group, and were beginning to calcify in the VA95 group. Similar results were reported in Iberian red deer where the base section had greater ash content, and more Ca and P (Landete-Castillejos et al., 2007). Currey (1999) also reported that the mineral content of antlers was higher in the base section than in the tip. Miller et al. (1985) reported that Ca and P content of unskinned middle sections of White-tailed deer antlers was 0.19% and 0.10% of dry weight, respectively. These values were much lower than ours, and therefore indicate that our samples were ossified to a greater extent than those analyzed by Miller et al. (1985). However, it has also been reported that antler mineral content (including Ca, P, and Na) increase as deer age (Schultz et al., 1994). In another study, it was reported that antler ash, including Ca and P, increased throughout the antler growth period, and antler protein levels declined slightly during the last week of the velvet shedding period (Moen and Pastor, 1998). Therefore, it is possible that the longer the antler growth period, the greater the progress of mineralization, which leads to a decrease in the protein level and the ether extract content and an increase in the ash content in the VA95 group.

Sialic acid, glycosaminoglycans (GAGs) and uronic acid content

The sialic acid, GAGs and uronic acid content of velvet antlers at different cutting times in elk are shown in Table 2. The sialic acid and uronic acid content increased markedly from the bottom to the top section ($p<0.05$), but there was no difference among three groups across the different cutting times. Scott and Hughes (1981) reported that the

Table 1. Crude protein, ether extract, crude ash and mineral content (DM %) of each section of velvet antler at different cutting times in elk

Item[1]		VA65[2]	VA80	VA95
CP	Top	66.41±12.83^{A}	69.92±6.03^{A}	65.58±2.39^{A}
	Upper	60.73±6.93^{AB}	62.96±6.95^{AB}	63.27±3.60^{A}
	Middle	56.60±2.56^{BC}	60.86±7.64^{ABC}	58.21±3.89^{B}
	Base	55.65±3.61^{BC}	56.73±7.12^{BC}	54.12±3.27^{BC}
	Bottom	52.15±3.35^{C}	52.14±5.02^{C}	50.72±2.82^{C}
EE	Top	2.74±0.45^{A}	3.03±0.93^{A}	2.66±0.43^{A}
	Upper	1.76±0.18^{B}	2.00±0.24^{B}	1.89±0.31^{B}
	Middle	1.54±0.10^{abB}	1.71±0.12^{aBC}	1.46±0.24^{bC}
	Base	1.45±0.16^{B}	1.45±0.10^{BC}	1.33±0.10^{C}
	Bottom	1.42±0.38^{B}	1.31±0.20^{C}	1.17±0.14^{C}
Ash	Top	23.36±4.11^{D}	29.49±7.79^{D}	29.45±3.50^{D}
	Upper	37.95±2.02^{aC}	34.13±2.06^{bC}	36.15±2.31^{abC}
	Middle	39.18±2.25^{BC}	37.50±2.69^{BC}	40.45±2.50^{B}
	Base	41.75±1.46^{bB}	41.86±3.37^{bB}	46.18±1.85^{aA}
	Bottom	44.94±1.72^{bA}	47.42±1.62^{aA}	48.43±0.85^{aA}
Calcium	Top	3.43±0.83^{C}	4.32±3.08^{B}	4.44±0.64^{B}
	Upper	8.20±1.74^{B}	6.58±3.13^{AB}	6.83±1.71^{AB}
	Middle	7.53±1.80^{B}	6.87±4.52^{AB}	4.73±0.81^{AB}
	Base	9.02±1.42^{AB}	8.07±4.85^{AB}	7.33±2.11^{A}
	Bottom	10.65±2.32^{A}	10.54±4.83^{A}	7.32±2.99^{A}
Phosphorus	Top	2.59±0.54	2.66±1.60^{B}	2.58±0.28
	Upper	5.03±1.18	3.76±1.53^{AB}	3.74±0.87
	Middle	4.54±1.06	3.87±2.33^{AB}	2.60±0.54
	Base	5.24±0.70	4.58±2.41^{AB}	3.95±1.09
	Bottom	6.00±1.37	6.01±2.25^{A}	4.02±1.65

Data are mean±SD values (n = 8 per group).

$^{A, B, C, D}$ Means with different superscripts in the same column (cutting time) are different ($p<0.05$).

$^{a, b}$ Means with different superscript in the same row are different ($p<0.05$).

[1] CP = Crude protein, EE = Ether extract.

[2] VA65 = Group harvested 65 days after casting, VA80 = Group harvested 80 days after casting, VA95 = Group harvested 95 days after casting.

uronic acid content of deer antler was 0.3%, which was much lower than our results in all sections. Glycosaminoglycans have been shown in animal models to have an antioxidant effect which could reduce joint erosion, stimulate biosynthesis of cartilaginous tissue or inhibit its degradation, and also serve as potent regulators of synoviocytes, which control the integrity of joint fluids (Allen et al., 2008). In the present study, the GAGs content of the upper section of antler was higher in the VA65 group than the VA95 group ($p<0.05$). In all groups the GAGs content was the highest in the top section of antler and decreased markedly in the other sections further down the antler ($p<0.05$). Several studies have reported similar results with uronic acid, sulfated GAGs and sialic acid content decreasing on moving down from the upper antler sections to the base sections (Sunwoo et al., 1995; Ha et al., 2003). Anionic molecules of the GAGs chondroitin sulfate in the growth plate have been reported to have important roles as ion exchangers in endochondral bone formation (Hunter, 1991). Chondroitin sulfate is the major antler GAGs component comprising 88% of the total uronic acid content and may be a potentially important carbohydrate in the antler (Sunwoo et al., 1995; Ha et al., 2005). Our results indicate that the sialic acid, GAGs and uronic acid content of the antler is decreased by the extent of mineralization in velvet antler.

Collagen content

The collagen content of velvet antler at different cutting times in elk is shown in Figure 2. Collagen is a major protein in velvet antler (Goss, 1983), and numerous researchers have demonstrated that the growth plate of

Table 2. Uronic acid, GAGs and sialic acid content (DM %) of each section of velvet antler at different cutting times in elk

Items		VA65[1]	VA80	VA95
Sialic acid	Top	0.44±0.12^{A}	0.50±0.16^{A}	0.47±0.06^{A}
	Upper	0.39±0.12^{AB}	0.38±0.08^{AB}	0.40±0.06^{B}
	Middle	0.36±0.11^{AB}	0.34±0.05^{B}	0.36±0.04^{BC}
	Base	0.32±0.07^{AB}	0.31±0.03^{B}	0.32±0.02^{C}
	Bottom	0.31±0.06^{B}	0.28±0.02^{B}	0.30±0.02^{C}
GAGs	Top	2.97±0.28^{A}	2.60±0.16^{A}	2.25±0.75^{A}
	Upper	0.57±0.18^{aB}	0.43±0.09^{abB}	0.38±0.07^{bB}
	Middle	0.37±0.05^{C}	0.36±0.05^{BC}	0.35±0.05^{B}
	Base	0.35±0.04^{C}	0.32±0.06^{BC}	0.32±0.08^{B}
	Bottom	0.31±0.06^{C}	0.26±0.05^{C}	0.29±0.07^{B}
Uronic acid	Top	1.28±0.19^{A}	1.26±0.22^{A}	1.11±0.51^{A}
	Upper	0.72±0.19^{B}	0.68±0.10^{B}	0.67±0.14^{B}
	Middle	0.63±0.16^{BC}	0.60±0.06^{BC}	0.56±0.24^{B}
	Base	0.54±0.15^{BC}	0.52±0.12^{BC}	0.48±0.14^{B}
	Bottom	0.48±0.14^{C}	0.46±0.10^{C}	0.43±0.13^{B}

Data are mean±SD values (n = 8 per group).
$^{A, B, C}$ Means with different superscripts in the same column (cutting time) are different ($p<0.05$).
$^{a, b}$ Means with different superscript in the same row are different ($p<0.05$).
[1] VA65 = Group harvested 65 days after casting, VA80 = Group harvested 80 days after casting, VA95 = Group harvested 95 days after casting.

velvet antler contains collagen type II, IX and XI (Wardale and Duance, 1993; Sunwoo, 1998). Collagen type X is found exclusively within the zone of hypertrophic chondrocytes, which are actively involved in the mineralization process (Rucklidge et al., 1996). In the present study, the collagen content of the middle and bottom sections of antler was higher in the VA65 group than the VA95 group ($p<0.05$). Also, the collagen content increased markedly on moving down from the top to the bottom section of antler in all groups ($p<0.05$). Similarly, Sunwoo et al. (1995) reported that the proportion of collagen in velvet antlers of elk increased downward from the upper sections to the base sections (approximately 1.4, 2.5 and 3.2 times higher in the upper, middle and base section, respectively, than in the tip section) with a concomitant increase in mineral content. Antler collagen appears to be

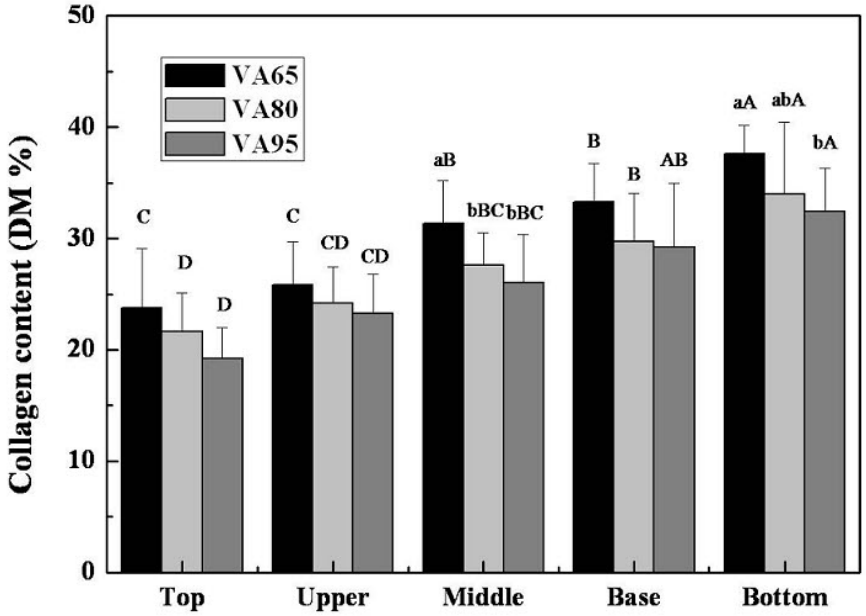

Figure 2. Collagen content by each section of velvet antler at different cutting times in elk. VA65: group harvested 65 days after casting, VA80: group harvested 80 days after casting, VA95: group harvested 95 days after casting. $^{A, B, C, D}$ Means with different superscripts in the same group are different ($p<0.05$). $^{a, b}$ Means with different superscript among the groups are different ($p<0.05$).

involved as an organic element, reinforcing the mineralized tissue structure and provide mechanical strength to the tissue (Sunwoo et al., 1995).

Amino acid composition

The composition of amino acids in velvet antlers at different cutting times is shown in Table 3. There were no differences in the individual amino acid composition of the

Table 3. Amino acid composition (DM %) of each section of velvet antler at different cutting times in elk

		VA65[1]	VA80	VA95
Asp	Top	5.01±0.33^{aA}	4.52±0.16^{abA}	4.09±0.96^{b}
	Upper	4.08±0.47^{B}	4.17±0.38^{AB}	4.20±0.25
	Middle	3.93±0.50^{B}	3.98±0.63^{B}	3.65±0.23
	Base	4.41±0.81^{AB}	4.09±0.39^{AB}	3.88±0.83
	Bottom	4.5±1.00^{aAB}	4.21±0.51^{bAB}	3.95±0.19^{a}
Thr	Top	1.58±0.12^{b}	1.66±0.20^{aA}	1.68±0.13^{abA}
	Upper	1.43±0.19^{b}	1.55±0.19^{aAB}	1.42±0.10^{bBC}
	Middle	1.27±0.09	1.33±0.28^{B}	1.47±0.09^{AB}
	Base	1.51±0.34	1.56±0.27^{AB}	1.37±0.13^{D}
	Bottom	1.54±0.43	1.55±0.26^{AB}	1.31±0.11^{CD}
Ser	Top	3.02±0.12^{aA}	2.64±0.13^{b}	2.91±0.28^{aA}
	Upper	2.47±0.25^{B}	2.44±0.24	2.55±0.18^{BC}
	Middle	2.39±0.32^{B}	2.35±0.36	2.22±0.14^{D}
	Base	2.70±0.52^{AB}	2.43±0.23	2.77±0.30^{AB}
	Bottom	2.76±0.63^{AB}	2.46±0.28	2.40±0.13^{CD}
Glu	Top	8.28±1.09^{aA}	6.68±1.22^{b}	6.73±0.48^{bA}
	Upper	5.80±0.53^{B}	5.68±0.57	5.84±0.29^{B}
	Middle	5.67±0.43^{B}	5.32±0.47	5.13±0.17^{C}
	Base	6.41±1.07^{B}	5.73±0.61	6.43±0.56^{A}
	Bottom	6.54±1.40^{B}	5.78±0.56	5.57±0.33^{BC}
Pro	Top	3.11±0.99^{bB}	3.67±1.19^{abB}	5.02±1.01^{a}
	Upper	4.09±0.21^{bAB}	4.55±0.52^{aA}	5.34±0.61^{a}
	Middle	4.75±0.42^{bA}	5.22±0.48^{bA}	5.68±0.37^{a}
	Base	3.81±1.88^{AB}	4.17±0.46^{B}	5.17±0.82
	Bottom	3.91±1.94^{bB}	4.82±0.27^{abA}	5.87±0.46^{b}
Gly	Top	4.27±2.39^{bB}	4.72±1.93^{abC}	7.24±1.22^{aC}
	Upper	7.18±0.26^{bA}	6.82±0.49^{bAB}	8.83±0.77^{bA}
	Middle	6.99±3.04^{A}	7.95±0.22^{A}	8.61±0.59^{AB}
	Base	8.21±0.58^{aA}	6.08±1.37^{bBC}	7.60±0.92^{aBC}
	Bottom	8.32±0.47^{abA}	7.25±0.78^{bAB}	8.91±0.89^{aA}
Ala	Top	3.03±0.56^{B}	3.06±0.53^{BC}	3.40±0.25^{B}
	Upper	3.59±0.35^{AB}	3.50±0.17^{A}	3.70±0.18^{A}
	Middle	4.80±2.64^{A}	3.64±0.34^{AB}	3.37±0.11^{B}
	Base	3.94±0.47^{aAB}	3.25±0.16^{bC}	3.39±0.28^{bB}
	Bottom	4.00±0.58^{AB}	3.62±0.17^{A}	3.58±0.17^{AB}
Val	Top	2.63±0.26	2.30±0.19	2.58±0.29^{A}
	Upper	2.46±0.36	2.18±0.26	2.31±0.21^{ABC}
	Middle	2.33±0.37	2.08±0.56	1.92±0.21^{BC}
	Base	2.65±0.58	2.20±0.19	2.42±0.16^{AB}
	Bottom	2.70±0.69	2.18±0.25	1.79±0.94^{C}
Ile	Top	1.74±0.32^{aA}	1.28±0.42^{b}	1.26±0.13^{b}
	Upper	1.00±0.13^{B}	0.92±0.25	0.93±0.08
	Middle	0.90±0.09^{B}	0.76±0.10	0.76±0.04
	Base	1.09±0.29^{B}	0.99±0.23	1.16±0.18
	Bottom	1.11±0.35^{B}	0.92±0.25	1.25±0.86

Table 3. Amino acid composition (DM %) of each section of velvet antler at different cutting times in elk (Continued)

		VA65[1]	VA80	VA95
Leu	Top	4.37±0.22^{aA}	3.91±0.13^{bA}	3.84±0.45^{bA}
	Upper	3.55±0.50^{AB}	3.60±0.30^{A}	3.47±0.28^{ABC}
	Middle	3.33±0.62^{B}	3.30±0.78^{B}	2.92±0.37^{BC}
	Base	3.84±0.90^{AB}	3.51±0.43^{AB}	3.60±0.29^{AB}
	Bottom	3.92±1.03^{AB}	3.61±0.49^{AB}	2.74±1.24^{C}
Tyr	Top	1.64±0.17^{aA}	1.40±0.18^{b}	1.16±0.33^{bA}
	Upper	1.09±0.15^{B}	1.11±0.21	1.00±0.12^{AB}
	Middle	0.94±0.13^{B}	0.87±0.18	0.77±0.09^{C}
	Base	1.14±0.32^{B}	1.17±0.25	1.06±0.28^{A}
	Bottom	1.17±0.38^{B}	1.10±0.25	0.96±0.12^{AB}
Phe	Top	2.43±0.27^{A}	2.14±0.18^{A}	2.06±0.38
	Upper	1.95±0.27^{B}	2.01±0.18^{A}	2.02±0.16
	Middle	1.88±0.31^{B}	1.89±0.42^{B}	1.75±0.16
	Base	2.12±0.43^{AB}	1.90±0.16^{AB}	1.95±0.32
	Bottom	2.17±0.50^{AB}	2.03±0.26^{A}	1.88±0.14
His	Top	1.66±0.09	1.53±0.12^{A}	1.84±0.40^{A}
	Upper	1.38±0.24	1.42±0.15^{A}	1.38±0.14^{BC}
	Middle	1.29±0.26	1.35±0.46^{B}	1.18±0.22^{C}
	Base	1.51±0.38	1.40±0.23^{AB}	1.63±0.28^{AB}
	Bottom	1.54±0.44	1.43±0.23^{AB}	1.27±0.13^{C}
Lys	Top	3.21±0.37	3.03±0.31	3.28±0.27^{A}
	Upper	3.08±0.34	3.06±0.21	3.07±0.14^{AB}
	Middle	2.96±0.41	2.86±0.49	2.63±0.23^{C}
	Base	3.31±0.62	2.94±0.24	3.12±0.21^{AB}
	Bottom	3.38±0.74	3.08±0.32	2.88±0.18^{BC}
Arg	Top	3.51±0.51^{ba}	3.13±0.38^{b}	3.84±0.42^{aAB}
	Upper	3.54±0.25^{b}	3.30±0.18^{b}	3.96±0.32^{aA}
	Middle	3.74±0.30^{a}	3.43±0.27^{b}	3.51±0.08^{abB}
	Base	3.98±0.42^{a}	3.20±0.33^{b}	3.83±0.34^{aAB}
	Bottom	4.05±0.57	3.41±0.13	3.80±0.32^{AB}

Data are mean±SD values (n = 8 per group).
$^{A, B, C, D}$ Means with different superscripts in the same column (cutting time) are different ($p<0.05$).
$^{a, b}$ Means with different superscript in the same row are different ($p<0.05$).
[1] VA65 = Group harvested 65 days after casting, VA80 = Group harvested 80 days after casting, VA95 = Group harvested 95 days after casting.

antlers among the groups. It has been reported that aspartic acid, glutamic acid, proline, glycine and arginine are the predominant amino acids in velvet antler, and account for approximately 32.5-37.2% of the total amino acids (Jeon et al., 2009). In the present study, the proportions of the individual amino acids, such as aspartic acid, threonine, serine, glutamic acid, isoleucine, leucine, tyrosine, phenylalanine, histidine and lysine, were higher in the top and upper sections compared to the other sections ($p<0.05$). Within the top section of antler, the levels of glutamic acid, isoleucine, leucine and tyrosine were higher in the VA65 group compared to the VA80 and VA95 groups ($p<0.05$). Sunwoo et al. (1995) also reported that the amino acid content of Wapiti velvet antler decreased downward from the tip to the base section. However, in the current study, proline and glycine levels were lowest in the top section ($p<0.05$) and increased from the top to the bottom sections in all groups. In addition, levels of these amino acids were higher within the top antler section in the VA95 group than the VA65 group ($p<0.05$). This effect could be related to large amounts of collagen in the younger velvet antler compared to the older more mineralized antler. From these results, it is clear that amino acid content, which may be one of the factors representative of the quality of velvet antler, is influenced by the mineralization and elongation of antlers.

Fatty acid composition

The fatty acid profiles of velvet antler at different cutting times are shown in Table 4. In this study, the proportions of individual and total saturated fatty acids (SFAs) were not different between the five antler sections.

Table 4. Fatty acid composition (DM %) of each section of velvet antler at different cutting times in elk

		VA65[1]	VA80	VA95
Myristic acid	Top	3.040±0.120	2.973±0.081	2.947±0.071
C14:0	Upper	2.917±0.228	3.005±0.083	3.040±0.142
	Middle	2.964±0.128	2.976±0.061	2.903±0.300
	Base	3.074±0.043	3.082±0.357	3.044±0.306
	Bottom	2.952±0.123	3.022±0.098	3.015±0.071
Palmitic acid	Top	30.165±0.410^{ab}	30.799±0.709^{a}	29.886±0.564^{bB}
C16:0	Upper	29.635±0.919^{a}	30.341±0.561^{b}	30.146±0.686^{abAB}
	Middle	30.359±1.613	30.456±0.339	29.963±0.379^{B}
	Base	30.301±0.720	30.233±0.909	30.793±0.824^{A}
	Bottom	30.454±1.440	29.789±0.805	30.337±0.751^{AB}
Stearic acid	Top	12.208±0.616	12.383±0.265	12.437±0.688
C18:0	Upper	13.128±0.755	11.964±0.235	12.471±0.571
	Middle	12.865±0.977	12.915±1.002	12.673±0.564
	Base	13.067±0.815	12.409±0.849	12.497±0.773
	Bottom	12.612±0.637	12.523±0.835	12.492±0.837
Arachidic acid	Top	2.596±0.433	2.565±0.107^{AB}	2.666±0.415
C20:0	Upper	2.542±0.448	2.817±0.258^{A}	2.630±0.443
	Middle	2.702±0.326	2.528±0.228^{AB}	2.317±0.403
	Base	2.643±0.315	2.628±0.292^{AB}	2.345±0.409
	Bottom	2.698±0.192^{a}	2.430±0.164^{bB}	2.688±0.164^{a}
Total SFA	Top	48.008±0.506	48.719±0.496	47.935±0.722^{AB}
	Upper	48.222±0.825	48.126±0.731	48.287±0.689^{AB}
	Middle	48.891±1.394	48.875±1.243	47.857±0.596^{B}
	Base	49.085±0.606	48.351±1.344	48.679±0.579^{A}
	Bottom	48.715±0.650	47.763±0.907	48.531±0.549^{AB}
Palmitoleic acid	Top	0.884±0.165	0.655±0.104	0.745±0.253
C16:1ω7	Upper	0.782±0.031	0.808±0.120	0.855±0.216
	Middle	0.829±0.164	0.695±0.208	0.817±0.231
	Base	0.947±0.367	0.703±0.109	0.854±0.287
	Bottom	0.834±0.156	0.721±0.108	0.830±0.414
Oleic acid	Top	12.977±0.945	12.557±0.265	13.646±0.904
C18:1ω9	Upper	12.849±0.785	12.276±0.990	12.823±0.740
	Middle	13.024±0.856	12.663±0.212	13.092±0.658
	Base	13.425±1.446	12.288±0.597	12.970±0.865
	Bottom	12.920±0.690	12.974±0.974	12.940±0.756
Erucic acid	Top	7.064±0.227^{a}	6.864±0.704^{a}	5.912±0.537^{b}
C22:1ω9	Upper	6.939±0.222	6.398±0.422	6.287±0.780
	Middle	6.299±0.825	6.407±0.425	5.712±0.450
	Base	6.517±0.557	6.523±0.301	6.065±0.314
	Bottom	6.626±0.966	6.365±0.555	6.018±0.526

Table 4. Fatty acid composition (DM %) of each section of velvet antler at different cutting times in elk (Continued)

		VA65[1]	VA80	VA95
Nervonic acid	Top	0.789±0.156	0.618±0.085	0.648±0.142
C24:1ω9	Upper	0.672±0.087	0.753±0.099	0.711±0.134
	Middle	0.754±0.217	0.789±0.111	0.680±0.130
	Base	0.698±0.091	0.705±0.090	0.698±0.131
	Bottom	0.804±0.158	0.753±0.166	0.693±0.173
Total MUFA	Top	21.713±1.297	20.694±0.947	20.951±1.001
	Upper	21.242±0.966	20.233±0.925	20.676±1.411
	Middle	20.907±1.340	20.554±0.333	20.300±0.384
	Base	21.587±1.551	20.218±0.552	20.587±0.770
	Bottom	21.184±1.195	20.812±0.783	20.482±0.808
Linoleic acid	Top	7.178±0.149^{a}	6.396±0.254^{b}	6.550±0.538^{b}
C18:2ω6	Upper	7.060±0.162^{a}	6.445±0.785^{b}	6.043±0.189^{b}
	Middle	6.964±0.970	7.292±0.647	6.304±0.592
	Base	7.381±0.303^{a}	6.596±0.877^{ab}	6.302±0.714^{b}
	Bottom	7.169±0.722^{a}	6.713±0.303^{ab}	6.157±0.194^{b}
Arachidonic acid	Top	0.675±0.104	0.653±0.072^{B}	0.732±0.126
C20:4ω6	Upper	0.638±0.090	0.738±0.119^{AB}	0.775±0.092
	Middle	0.779±0.187	0.848±0.069^{A}	0.766±0.090
	Base	0.786±0.115	0.830±0.161^{AB}	0.732±0.142
	Bottom	0.792±0.228	0.830±0.158^{AB}	0.760±0.056
Total ω-6	Top	7.853±0.189^{a}	7.048±0.195^{bB}	7.282±0.529^{b}
	Upper	7.698±0.144^{a}	7.182±0.702^{abB}	6.818±0.210^{b}
	Middle	7.743±1.033	8.139±0.605^{A}	7.070±0.617
	Base	8.167±0.279^{a}	7.426±0.760^{abAB}	7.033±0.780^{b}
	Bottom	7.961±0.799^{a}	7.543±0.386^{abAB}	6.917±0.221^{b}
Linolenic acid	Top	0.638±0.080	0.632±0.080	0.610±0.091
C18:3ω3	Upper	0.616±0.092	0.610±0.090	0.702±0.125
	Middle	0.668±0.071	0.695±0.168	0.648±0.048
	Base	0.701±0.029^{a}	0.600±0.032^{b}	0.678±0.075^{a}
	Bottom	0.682±0.078	0.665±0.059	0.695±0.050
11,14,17-Eicosatrienoic acid	Top	0.988±0.086^{a}	0.895±0.062^{a}	0.728±0.138^{b}
C20:3ω3	Upper	0.921±0.123^{a}	0.905±0.174^{a}	0.698±0.110^{b}
	Middle	0.886±0.055^{a}	0.893±0.074^{a}	0.680±0.086^{b}
	Base	0.990±0.163^{a}	0.898±0.103^{a}	0.650±0.071^{b}
	Bottom	0.943±0.179^{a}	0.868±0.043^{a}	0.614±0.178^{b}
Total ω-3	Top	1.626±0.142^{a}	1.527±0.085^{a}	1.338±0.088^{b}
	Upper	1.537±0.204^{a}	1.515±0.261^{a}	1.400±0.172^{b}
	Middle	1.554±0.052^{a}	1.588±0.170^{a}	1.328±0.091^{b}
	Base	1.692±0.190^{a}	1.498±0.121^{b}	1.328±0.092^{b}
	Bottom	1.626±0.119^{a}	1.533±0.041^{a}	1.309±0.187^{b}
ω-6/ω-3	Top	0.207±0.017^{ab}	0.217±0.017^{a}	0.185±0.022^{b}
	Upper	0.200±0.029	0.214±0.052	0.206±0.028
	Middle	0.204±0.028	0.196±0.022	0.190±0.028
	Base	0.207±0.026	0.203±0.024	0.191±0.027
	Bottom	0.205±0.019	0.203±0.009	0.190±0.031

Table 4. Fatty acid composition (DM %) of each section of velvet antler at different cutting times in elk (Continued)

		VA65[1]	VA80	VA95
Total PUFA	Top	9.479±0.264^{a}	8.575±0.152^{bB}	8.620±0.499^{b}
	Upper	9.235±0.127^{a}	8.697±0.480^{bB}	8.218±0.222^{c}
	Middle	9.297±1.046^{a}	9.727±0.669^{aA}	8.398±0.574^{b}
	Base	9.859±0.310^{a}	8.924±0.788^{bAB}	8.362±0.752^{b}
	Bottom	9.586±0.851^{a}	9.076±0.406^{aAB}	8.225±0.209^{b}
PUFA/SFA	Top	0.198±0.007^{a}	0.176±0.003^{bB}	0.180±0.010^{b}
	Upper	0.192±0.004^{a}	0.181±0.009^{bB}	0.170±0.005^{c}
	Middle	0.191±0.025^{a}	0.199±0.010^{aA}	0.175±0.012^{b}
	Base	0.201±0.006^{a}	0.185±0.018^{abAB}	0.172±0.017^{b}
	Bottom	0.197±0.017^{a}	0.190±0.011^{aAB}	0.170±0.006^{b}

Data are mean±SD values (n = 8 per group).
$^{A, B}$ Means with different superscripts in the same column (cutting time) are different ($p<0.05$).
$^{a, b, c}$ Means with different superscript in the same row are different ($p<0.05$).
[1] VA65 = Group harvested 65 days after casting, VA80 = Group harvested 80 days after casting, VA95 = Group harvested 95 days after casting.

The proportion of palmitic acid (C16:0) was lower in the upper section ($p<0.05$), and arachidic acid (C20:0) was higher in the bottom section ($p<0.05$) in the VA65 group compared to the VA80 group. The proportions of individual and total mono-unsaturated fatty acids (MUFAs) were largely similar in all groups, with oleic acids (C18:1ω7 and 9) accounting for more than half of total MUFA. The only MUFA to differ in level between the groups was erucic acid (C22:1ω9) which was higher in the top section ($p<0.05$) of the VA65 and VA80 groups compared to the VA95 group. Sunwoo et al. (1995) only detected linolenic acid (C18:3ω6) in the top section of antler from Wapiti. In the VA80 group, the levels of arachidonic acid (C20:4ω6) and total ω-6 fatty acid were higher in the middle section compared to the top sections ($p<0.05$). On the other hand, the proportions of linoleic acid (C18:2ω6) and total ω-6 fatty acid for the top, upper, base and bottom sections in the VA65 group were higher than in the VA80 and/or VA95 group ($p<0.05$). Although there were no differences in the proportions of the individual and total ω-3 fatty acids in the different sections of antler in each group, 11,14,17-eicosatrienoic acid (C20:3ω3) and total ω-3 fatty acids were increased in the VA65 and VA80 groups compared to the VA95 group in all antler sections ($p<0.05$). Omega-3 fatty acids are reported to have anti-inflammatory effects, and have been found to be effective in reducing symptoms in patients with rheumatoid arthritis (Berbert et al., 2005; Leeb et al., 2006). In the current study, the proportions of total polyunsaturated fatty acids (PUFAs) and the PUFA to SFA ratio were higher in the VA65 group than the VA95 group for all antler sections ($p<0.05$). The variation in velvet antler nutrient composition, including sialic acid, uronic acid, collagen, amino acids and fatty acids, appeared to be related to the faster ossification accompanying the growth and development of antlers. Based on the results of this research, it is considered that the lower content of bioactive components in the later antler development stage (VA95) leads to a decrease of whole antler quality for nutritional supplements or pharmaceutical agents.

REFERENCES

Allen, M., K. Oberle, M. Grace, A. Russell and A. J. Adewale. 2008. A randomized clinical trial of elk velvet antler in rheumatoid arthritis. Biol. Res. Nurs. 9:254-261.

AOAC. 1990. Official methods of analysis. 15th edn. Association of Official Analytical Chemists, Arlington, Virginia.

Berbert, A. A., C. R. Kondo, C. L. Almendra, T. Matsuo and I. Dichi. 2005. Supplementation of fish oil and olive oil in patients with rheumatoid arthritis. Nutr. 21:131-136.

Bergman, I. and R. Loxley. 1962. Two improve and simple methods for the spectrophotometric determination of hydroxyproline. Anal. Chem. 35:1961-1965.

Chapman, D. I. 1975. Antlers-bones of contention. Mamm. Rev. 5:121-172.

Choi, H. K., K. H. Kim, K. H. Kim, Y. S. Kim, M. W. Lee and W. K. Whang. 2006. Metabolomic differentiation of deer antlers of various origins by HNMR spectrometry and principal components analysis. J. Pharm. Biomed. Anal. 4:1047-1050.

Cross, H. R., Z. L. Carpenter and G. C. Smith. 1973. Effect of intramuscular collagen and elastin on bovine muscle tenderness. J. Food Sci. 38:998-1003.

Currey, J. D. 1999. The design of mineralized hard tissues for their mechanical functions. J. Exp. Biol. 202:3285-3294.

Elliott, J. L., J. M. Oldham, G. W. Asher, P. C. Molan and J. J. Bass. 1996. Effect of testosterone on binding of insulin-like growth factor-I (IGF-I) and IGF-II in growing antlers of fallow deer (*Dama dama*). Growth Regul. 6:214-221.

Farndale, R. W., C. A. Sayer and A. J. Bsrett. 1982. A direct spectrophotometric assay for sulfated glycosaminoglycans in cartilage cultures. Connect. Tiss. Res. 9:247-248.

Fletcher, T. J. 1986. Reproduction: seasonality. In: Management and Diseases of Deer (Ed. T. L. Alexander). Veterinary Deer Society, London. pp. 17-18.

Goss, R. J. 1983. Developmental anatomy of antlers. In: Deer Antlers: Regeneration, Function and Evolution (Ed. R. J. Goss). Academics Press, New York. pp. 133-171.

Ha, Y. W., B. T. Jeon, S. H. Moon and Y. S. Kim. 2003. Biochemical components among different fodders-treated antlers. Kor. J. Pharmacogn. 34:40-44.

Ha, Y. W., B. T. Jeon, S. H. Moon, H. Toyoda, T. Toida, R. J. Linhardt and Y. S. Kim. 2005. Characterization of heparin sulfate from the unossified antler of *Cervus elaphus*. Carbohydr. Res. 340:411-416.

Hemmings, S. and X. Song. 2004. The effects of elk velvet antler consumption on the rat: Development, behaviour, toxicity and the activity of liver gamma-glutamyltranspeptidase. Comp. Biochem. Physiol. 138:105-112.

Hunter, G. A. 1991. Role of proteoglycan in the provisional calcification of cartilage. A review and reinterpretation. Clin. Orthop. Rel. Res. 262:256-280.

Jeon, B. T., S. J. Kim, S. M. Lee, P. J. Park, S. H. Sung, J. M. Kim and S. H. Moon. 2009. Effect of antler growth period on the chemical composition of velvet antler in sika deer (*Cervus nippon*). Mamm. Biol. 74:374-380.

Jeon, B. T. and S. H. Moon. 2006. A review on feeding system for deer production. JIFS. 3:39-44.

Kay, R. N. B., M. Phillio, J. M. Suttie and G. Wenham. 1982. The growth and mineralization of antlers. J. Physiol. 322:4(Abstr.).

Kosakaki, M. and Z. Yosizawa. 1979. A partial modification of the cartilage method of Bitter and Muir for quantization of hexuronic acids. Anal. Biochem. 93:295-298.

Landete-Castillejos, T., A. Garcia and L. Gallego. 2007. Body weight, early growth and antler size influence antler bone mineral composition of Iberian Red Deer (*Cervus elaphus hispanicus*). Bone 40:230-235.

Leeb, B. F., J. Sautner, I. Andel and B. Rintelen. 2006. Intravenous application of omega-3 fatty acids in patients with active rheumatoid arthritis. The ORA-1 trial. An open pilot study. Lipids 41:29-34.

Li, C. 2003. Development of deer antler model for biochemical research. Rec. Adv. Res. Updates 4:255-274.

Miller, K. V., R. L. Marchinton and J. R. Beckwith. 1985. Variations in density and chemical composition of white-tailed deer antlers. J. Mamm. 66:693-701.

Moen, R. and J. Pastor. 1998. Simulating antler growth and energy, nitrogen, calcium and phosphorus metabolism in caribou. Rangifer, Special Issue 10:85-97.

Moon, S. H., S. K. Kang, S. M. Lee, M. H. Kim and B. T. Jeon. 2004. A study on the seasonal comparison of dry matter intake, digestibility, nitrogen balance and feeding behavior in spotted deer fed forest by-product silage and corn silage. Asian-Aust. J. Anim. Sci. 17:57-65.

Moreau, M., J. Dupuis, N. H. Bonneau and M. Lecuyer. 2004. Clinical evaluation of a powder of quality elk velvet antler for the treatment of osteoarthritis in dogs. Can. Vet. J. 45:133-139.

Park, P. W. and R. E. Goins. 1994. *In situ* preparation of fatty acid methyl esters for analysis of fatty acid composition in foods. J. Food. Sci. 59:1262-1266.

Rucklidge, G. J., G. Milne, K. J. Bos, C. Farquharson and S. P. Robins. 1997. Deer antler does not represent a typical endochondral growth system: immunoidentification of collagen type X but little collagen type II in growing antler tissue. Comp. Biochem. Physiol. 118B:303-308.

Schultz, S. R., M. K. Johnson, S. E. Feagley, L. L. Southern and T. L. Ward. 1994. Mineral content of Louisiana white-tailed deer. J. Wildl. Dis. 30:77-85.

Scott, J. E. 1960. Aliphatic ammonium salts in the assay of acidic polysaccharides from tissues. Methods Biochem. Anal. 8:145-197.

Scott, J. E. and E. W. Hughes. 1981. Chondroitin sulfate from fossilized antlers. Nature 291:580-581.

Sunwoo, H. H. 1998. Isolation, characterization and localization of glycosamines in growing antlers of wapiti (*Cervus elaphus*). Comp. Biochem. Physiol. Part B. 120:273-283.

Sunwoo, H. H., T. Nakano, R. J. Hudson and J. S. Sim. 1995. Chemical composition of antlers from Wapiti (*Cervus elaphus*). J. Agric. Food Chem. 43:2846-2849.

Sunwoo, H. H., L. Y. M. Sim, T. Nakano, R. J. Hudson and J. S. Sim. 1997. Glycosaminoglycans from growing antlers of wapiti (*Cervus elaphus*). Can. J. Anim. Sci. 77:715-721.

Wardale, R. J. and V. C. Duance. 1993. Characterization of porcine articular and growth plate collagens. J. Cell. Sci. 105:975-984.

Warren, L. 1959. The thiobarbituric acid assay of sialic acids. J. Biol. Chem. 234:1971-1975.

Weladji, R. B., O. Holand, G. Steinheim, J. E. Colman, H. Gjostein and A. Kosmo. 2005. Sexual dimorphism and intercohort variation in reindeer calf antler length is associated with density and weather. Oecologia 145:549-555.

Zhang, Z. Q., Y. Zhang, B. X. Wang, H. O. Zhou, Y. Wang and H. Zhang. 1992. Purification and partial characterization of anti-inflammatory peptide from pilose antler of *Cervus Nippon Temminck*. Acta Pharmacol. Sin. 27:321-324.