Asian-Aust. J. Anim. Sci.
Vol. 20, No. 10 : 1546 - 1550
October 2007

www.ajas.info

Effects of Antler Development Stage on Fatty acid, Vitamin and GAGs Contents of Velvet Antler in Spotted Deer (*Cervus nippon*)*

S. R. Lee[1], B. T. Jeon, S. J. Kim, M. H. Kim, S. M. Lee[2] and S. H. Moon**

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

ABSTRACT : This study aimed to provide basic information as the foundation for further studies on the assessment of velvet antler quality by investigating the changes in fatty acid, vitamin A and E, minerals and GAGs contents by development stage of antler in spotted deer (*Cervus nippon*). Twelve stags (aged 4 to 5 years) were divided into two groups and velvet antler harvested 40 days (FDG) and 60 days (SDG) after casting of the buttons from the previous set. Total saturated fatty acid was lower in FDG than SDG ($p<0.05$). Total monounsaturated fatty acid, conjugated linoleic acid ($p<0.05$), polyunsaturated fatty acid and ω3 fatty acid were higher in FDG than SDG. The vitamin A content of FDG was higher than that of SDG, but the vitamin E content of FDG was lower than that of SDG ($p<0.05$). SDG had significantly higher calcium and phosphate content than FDG ($p<0.05$). The magnesium content showed a similar trend to the contents of calcium and phosphate, but there was no significant difference between SDG and FDG. Uronic acid content was higher in FDG than SDG but there was no significant difference. The contents of GAGs and sialic acid were significantly higher ($p<0.05$) in FDG than SDG. These results indicated that the longer stage of antler development had lower content of activating components and this lead to a decrease of antler quality. (**Key Words** : Spotted Deer, Fatty Acid, Vitamin, Minerals, GAGs, Velvet Antler)

INTRODUCTION

Velvet antlers are soft growing bony organs which are cast and fully regenerate every year (Li, 2003). The tissue of antler in velvet differentiates rapidly, showing a sequential development from tip to base (Kay et al., 1982). Therefore, the chemical composition of velvet antler may vary greatly depending on the stage of antler development.

Chemical analysis for velvet antler has been performed to determine available bioactive components that have medicinal efficacy. Although most of the published works reported on biomedical efficacy and chemical composition of velvet antler and changes of blood biochemical composition during the antler growth period (Miller et al., 1985; Sim et al., 1995; Sunwoo et al., 1995; Shin et al., 2000; Ha et al., 2005; Jeon et al., 2006a), only limited information is available to evaluate the quality of velvet antler around the world. Harvest time of velvet antler has been variable from 40 days to 100 days after the casting of buttons from the previous set (Haigh and Hudson, 1993). Chapman (1975) reported that the chemical composition of antlers depends on the portion of the antler analysed, the stage of antler development and the species of deer examined. Therefore, more detailed data is required on chemical composition including knowledge of variation that may exist between different stages of antler development and parts of antlers to assess antler quality exactly. It is also necessary to evaluate the harvesting time of velvet antler exactly not only by physical means, but also by chemical analysis. Hong et al. (1991) suggested that indicators of quality assessment by chemical means were calcium and iron. Some researchers suggested that the content of minerals (calcium, phosphorus and copper), the ratio of minerals (calcium/ash, calcium/phosphorus and calcium/iron) (Kang et al., 2000) and glycosaminoglycan and amino acid (lysine, glutamic acid, proline, and alanine) (Sunwoo et al., 2001) can be indicators to evaluate velvet antler quality. However, these indicators can be greatly affected by species.

* This work was supported by Konkuk University in 2006.

** Corresponding Author: S. H. Moon Department of Animal Science, College of Natural Sciences, Konkuk University, Chungju, 380-701, Korea. Tel: +82-43-8403527, Fax: +82-43-8518216, E-mail: moon0204@kku.ac.kr

[1] Dept. of Anim. Sci. and Env., Konkuk University, Seoul, 150-143, Korea.

[2] Dept. of Anim. Sci., Sangju National University, Sangju, Korea.

Received November 9, 2006; Accepted April 25, 2007

Table 1. Chemical composition of experimental diet components[1]

Component	DM	CP	EE	CF	Ash	Ca	P
	% in DM						
Alfalfa bale	80.0	16.4	1.8	34.3	8.0	1.3	0.2
Concentrate	94.7	16.0	4.0	18.6	2.3	0.7	0.4

[1] DM = Dry matter; CP = Crude protein; EE = Ether extract; CF = Crude fiber.

age and nutrition of deer and growth period of velvet antler. Consequently, complete indicators, which are established by a scientific basis, are of great concern to farmers, traders, customers and researchers. Further, the relationship of traditional and scientific methods for assessment of velvet antler quality must be investigated, and more progressive studies must be carried out at the same time.

This study aimed to provide basic information as the foundation for further studies on the assessment of velvet antler quality by investigating the changes of fatty acid, vitamin A and E, minerals and GAGs contents, which are indicated as the main bioactive components in velvet antler, by section and growth period of velvet antler in spotted deer (*Cervus nippon*).

MATERIALS AND METHODS

Animals and their management

A feeding trial was conducted at HANA Deer Research Institute, from March to July 2004. Twelve stags of spotted deer (*Cervus nippon*) aged 4-5 years were used in the trial. All stags were healthy with no clinical signs of disease and the mean body weight was 86.7±5.3 (SD) kg at the beginning of the experiment. Stags were assigned to two groups having similar antler productivity based on the production record of the previous year (2003). Stags were housed outside in large wire netting pens with free access to water. All stags were allowed to access a basal diet of alfalfa bales *ad libitum* and commercial concentrate at the level of 1% of body weight on a fresh matter basis. The experimental diet was provided twice daily at 8 AM and 6 PM in equal portions. Chemical composition of the experimental diet is presented in Table 1.

Analytical methods

Velvet antler sample collection and preparation : Velvet antlers were harvested from stags of each group on the 40th day (FDG) and 60th day (SDG) after casting of the button from the previous set. Antlers were harvested using mechanical restraint after anesthesia with injection of muscular relaxant (Xylazine HCl, 1.5 mg/10 kg BW) by a veterinarian. Samples of each section were sliced with a bone slicer, freeze-dried and ground by sample mill (KNIFETEC 1095 Sample Mill) to pass a 0.1 mm screen. Ground samples were stored in a deep freezer (-40°C) for later chemical analysis.

Analysis of fatty acid

Total lipid of velvet antler was extracted with a mixture of chloroform and methanol (2:1, v/v) by the method of Floch et al. (1957). Lipid extracts obtained were methylated according to the methods of Takenoyama et al. (1999) with some modification. In brief, about 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 turbid preparation was neutralized with HCl/methanol and then reheated. Fatty acid and methyl esters were extracted with hexane and mastered by gas-liquid chromatography (HP 5890 II Series, Hewlett-Packard, Atlanta, USA) using a 0.32 mm I.D.×60 m capillary column (SUPELCOWAX-10, Supelco Ltd. Pennsylvania, USA). The initial column temperature was programmed at 170°C and increased to 220°C at 2°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-isomer*, Sigma Ltd., St. Louis, USA). Composition of the free fatty acid fraction was expressed as a weight percentage of the total fatty acids.

Analysis of vitamin A and E

Vitamin A (β-carotene) and Vitamin E (α-tocopherol) contents in velvet antler were determined respectively at 320 nm and 254 nm by the methods of the Association of Official Analytical Chemists (AOAC, 1990) using HPLC (model no 616/626, Waters, Millford, MA) equipped with a C18 column (Nova-pak 3.9×300 mm, 40°C) and UV detector (model no 486, Waters). Mobile phase was methanol (70) : acetonitle (30)+ PIC based. Flow rate was 1.0 ml/min.

Analysis of glycosaminoglycans (GAGs), uronic acid and sialic acid

Sample preparation : 50 mg of velvet antler sample was decalcified by 0.05 M NA_2EDTA 1 ml (pH 7.4, included 0.5 M Tris) for two days at 4°C. After decalcification, samples were centrifuged at 12,000 rpm for 10 min at 4°C to collect the precipitate. After addition of 3 ml 0.1 M phosphate buffer (pH 6.5, included 0.05 M cysteine hydrochloride and 0.005 M Na-EDTA) and mixing with crude papain (20 mg), the enzyme was activated for 30 min at 65°C. The activated enzyme was mixed with the decalcified sample and reacted for 16 h at 65°C, and then the upper layer was removed.

Glycosaminoglycans assay : Glycosaminoglycans were

Table 2. Fatty acid contents of velvet antler at different cutting times in spotted deer (mean±SE)

Fatty acid [1]	FDG[2]	SDG[3]
	----------- % of total lipid ----------------	
C14:0	0.625±0.031	0.670±0.045
C15:0	2.383±0.726	3.835±1.423
C16:0	18.648±1.217	19.563±1.872
C17:0	1.260± 0.315	2.768±1.873
C18:0	11.673±0.388	11.395±0.606
C20:0	0.898±0.363	1.205±0.497
C22:0	0.463±0.135	0.615±0.189
C24:0	0.530±0.199	0.765±0.311
C14:1 ω 5	0.013±0.006	0.023±0.006
C16:1 ω 7	1.890±0.185	1.567±0.215
C18:1 ω 9	17.093±0.889 ^{a}	13.903±1.631 ^{b}
t-C18:1 ω 9	4.087±0.432	3.520±0.375
C20:1 ω 9	0.490±0.052	0.407±0.065
C22:1 ω 9	0.307±0.035 ^{a}	0.230±0.026 ^{b}
C24:1 ω 9	0.193±0.025 ^{a}	0.160±0.020 ^{b}
C18:2 ω 6	4.938±0.742	4.335±0.664
C20:2 ω 6	1.715±0.789 ^{a}	0.388±0.279 ^{b}
C20:3 ω 6	0.625±0.070 ^{a}	0.455±0.044 ^{b}
C20:4 ω 6	4.220±0.301 ^{a}	3.500±0.286 ^{b}
C18:3 ω 3	0.140±0.014	0.128±0.062
C22:6 ω 3	0.248±0.050	0.195±0.044
C22:3 ω 3	0.013±0.005	0.015±0.013
cis9-tans11 CLA	2.830±0.390 ^{a}	1.680±0.395 ^{b}
tran10-cis12 CLA	0.037±0.006 ^{a}	0.013±0.012 ^{b}
CLA1	0.007±0.012	0.007±0.012
CLA2	0.165±0.030	0.163±0.040
Total SFA	36.483±1.415 ^{b}	40.815±1.272 ^{a}
Total MUFA	24.073±1.397 ^{a}	19.810±2.183 ^{b}
Total PUFA	11.898±1.830	9.015±0.835
Total ω 6	11.498±1.774 ^{a}	8.678±0.822 ^{b}
Total ω 3	0.400±0.068	0.338±0.057
Total CLA	3.039±0.423 ^{a}	1.863±0.427 ^{b}

[a, b] Within the same row, means not sharing a common superscript letter are significantly different at p<0.05.
[1] CLA = Conjugated linoleic acid, SFA = Saturated fatty acid, MUFA = Monounsaturated fatty acid, PUFA = Polyunsaturated fatty acid.
[2] 40-day group. [3] 60-day group.

determined by a microtiter plate adaptation of the dimethylmethylene blue assay of Farndale et al. (1982).

Uronic acid assay : Uronic acid content of velvet antler was determined by the method of Scott (1960) and Kosakai and Yosizawa (1979).

Sialic acid assay : Sialic acid was determined by the method of Warren (1959); samples, standards and controls (80 μl) were added to 40 μl reagent A and mixed well. These were left at room temperature for 20 min. 400 μl of reagent B was added and then the tubes shaken vigorously to expel the yellow colored iodine. These were left for a further 5 min at room temperature. After addition of 1.2 ml of reagent C, the tubes were shaken and heated at 100°C for 15 min. Samples were cooled rapidly to room temperature. These were divided into two layers, one being colored red and the other was transparent. The red colored solution was extracted and centrifuged for a few minutes in order to properly separate the two layers and the absorbance at 549 nm of the upper cyclohexanone layer was determined.

Table 3. Vitamins, minerals, uronic acid, GAGs and sialic aicd contents of velvet antler at different cutting time in spotted deer. (mean±SE)[1]

Item	FDG[2]	SDG[3]
Vitamin A (μg/g)	0.21±0.03	0.19±0.02
Vitamin E (μg/g)	0.52±0.09 ^{b}	0.87±0.06 ^{a}
Ca (% DM)	9.14±2.45 ^{b}	12.20±0.40 ^{a}
P (% DM)	4.78±0.61 ^{b}	6.07±0.51 ^{a}
Mg (% DM)	0.27±0.01	0.29±0.01
Uronic acid (%DM)	0.80±0.14	0.55±0.05
GAGs (% DM)	1.07±0.18 ^{a}	0.48±0.11 ^{b}
Sialic acid (% DM)	0.20±0.02 ^{a}	0.12±0.01 ^{b}

[a, b] Within the same row, means not sharing a common superscript letter are significantly different at p<0.05.
[1] 40-day group. [2] 60-day group.

Statistical analysis

The main effects between groups were subjected to ANOVA using the general linear model procedure of SAS (2002, Version 9.0) and significant differences were determined by t-test (LSD) at the level of $p<0.05$.

RESULTS

The fatty acid composition of velvet antler at different cutting times in spotted deer is presented in Table 2. Total saturated fatty acid (SFA) was lower in FDG than SDG ($p<0.05$). On the other hand, total monounsaturated fatty acid (MUFA), conjugated linoleic acid (CLA, $p<0.05$), polyunsaturated fatty acid (PUFA) and ω3 fatty acid were higher in FDG than SDG. With progressing antler development, SFA was increased and unsaturated fatty acids (MUFA and PUFA) were decreased. Palmitate, stearate, oleate, linoleate, and arachidonate were the predominant fatty acids of velvet antler in spotted deer.

The contents of vitamin A and E and minerals at different cutting times in velvet antler are shown in Table 3. The vitamin A content of FDG (63 IU/100 g, 0.21 μg/g) was higher than that of SDG (57 IU/100 g, 0.19 μg/g), but the vitamin E content of FDG (156 IU/100 g, 0.52 μg/g) was lower than that of SDG (216 IU/100 g, 0.87 μg/g) ($p<0.05$). Although vitamin A and E are fat soluble vitamins, this trend was contrary to each other.

The content of calcium (Ca), which was the main mineral in velvet antler, was 9.14% and 12.2% and phosphorus (P) content was 4.78% and 6.07% in FDG and SDG, respectively. SDG had significantly higher Ca and P contents than FDG ($p<0.05$). The magnesium (Mg) content

followed a similar trend to the contents of Ca and P, but there was no significant difference between SDG and FDG.

The contents of uronic acid, glycosaminoglycans (GAGs) and sialic acid of velvet antlers at different cutting times in spotted deer are shown in Table 4. Uronic acid content was higher in FDG than SDG but there was no significant difference between cutting times. The contents of GAGs and sialic acid were significantly higher ($p<0.05$) in FDG than SDG.

DISCUSSION

There is little information available concerning the chemical composition of antlers in cervids, especially in spotted deer (Jeon et al., 2006b). From previous studies (Ullrey, 1983; Sunwoo et al., 1995) in other species, it would be assumed that protein and ash are major components and lipid, carbohydrate and vitamins are minor components in antlers. However, many researchers have placed great emphasis on these minor components as a bioactive assessment of the quality of antler. Therefore, to determine the quality of antler by chemical means, it will be necessary to do exact quantitative analysis on these minor components in antlers. Especially, because velvet antlers are fastest growing tissues and are rapidly mineralized, it is thought that the chemical composition of antler varies greatly with the stage of antler development. In this study, the contents of fatty acid, vitamin A and E, minerals and carbohydrates were changed by the stage of antler development with higher contents in FDG and lower contents in SDG except for minerals.

Sunwoo et al. (1995), Kim (2002) and Lee et al. (2003) reported that total SFA content increased and total MUFA and PUFA contents decreased from the upper to base section, the latter being the more mineralized section of antler. Similarly, SFA content was higher and MUFA and PUFA contents were lower in SDG which was at a later stage of antler development than in FDG. Therefore, it was likely that fatty acid contents were affected by mineralization owing to elongation of velvet antler.

Only limited information is available on the content of vitamins in velvet antler around the world. Lee et al. (2003) reported that vitamin A content in velvet antler of elk was 16.40 IU/100 g and 12.32 IU/100 g for the upper and middle section, respectively. This means that the content of vitamin is decreased with progressive antler growth and mineralization. Thus, our study can hypothesize that content of vitamins in velvet antler is closely related to mineralization and elongation. However, there was insufficient information available to interpret the higher content of vitamin E in SDG.

The contents of Ca, P and Mg in antler were lower in FDG than in SDG and minerals also increased with extent of antler growth in this study. Generally, bone ash contents depend on the extent of mineralization (Pastrana et al., 1991). It is thought that mineral contents were affected by ossification accompanying antler development and thus, the contents of minerals in SDG were significantly higher than those in FDG. On the other hand, our observations for the contents of minerals are lower than the values of 22.1-23.4% Ca, 10.8-12.6% P and 0.73-0.80% Mg reported for hard antlers of swamp deer, axis deer and hog deer (Pathak et al., 2001). The differences are obviously because of the velvet antlers used in this study, which might have not undergone complete ossification, but are closer to the values for velvet antler of elk (Sunwoo et al., 1995).

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). Sunwoo et al. (1995) supposed that chondroitin sulfate may be a potentially important carbohydrate in the antler. Ha et al. (2003) and Sunwoo et al. (1995) reported that the contents of uronic acid, sulfated GAGs and sialic acid decreased from the upper to the base section. Thus, those contents are decreased by the extent of mineralization in velvet antler. In this study, the contents of GAGs, uronic acid and sialic acid were higher in FDG than in SDG. This variation appeared to be related to the faster ossification in SDG which accompanied the extension of the growth period. In conclusion, these results indicated that the longer stage of antler development lowered contents of bioactive components and this lead to a decrease of antler quality. It is expected that the quality of velvet antler would be decreased greatly by extension of the antler growth period.

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