Serum Biochemical Values during Antler Growth in Sika Deer (*Cervus nippon*)*

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ABSTRACT : Serum biochemical values were measured in blood samples collected from 8 fasted stags from both jugular and femoral veins at 18-day intervals during antler growth. Samples were analyzed for blood substrate, enzyme activity values, minerals and electrolyte. There were no significant differences in total protein, albumin, urea, creatinine, triglyceride, glucose or cholesterol concentration between veins or sampling dates. However, total-bilirubin concentration in the jugular vein on the casting date was three times higher than on the other sampling dates (p<0.05). There were no significant differences in alkaline phosphatase, aspartate aminotransferase, and alanine aminotransferase with progressing antler growth. The highest level of alkaline phosphatase concentration was on day 55 after casting. There were no significant differences in inorganic phosphorus, sodium and chloride concentration between jugular and femoral samples. Calcium concentration was significantly higher in the femoral vein on the cutting date (55 day) than in the jugular vein (p<0.05). There were few changes in serum biochemical values. However, some electrolytes and minerals had differences during antler growth. It is suggested that despite such a big event as antler growth, blood biochemical values are not variable if feeding conditions are consistently maintained as was the case in this study. (**Key Words :** Antler Growth, Enzyme Activity, Minerals, Serum Biochemical Value, Sika Deer)

INTRODUCTION

Commercial deer farming for antler production has depended on sika deer (*Cervus nippon*), red deer (*Cervus elaphus*) and elk (*Cervus canadensis*) in many countries throughout the world. Deer antlers are bony organs that are cast and fully regenerate each year (Li, 2003). Growing antlers are referred to as a velvet antler. Velvet antlers are mainly cartilage differing from horns found on bovidae (Haigh and Hudson, 1993).

Antler production is affected by several factors such as feeding condition, climate, age, health, nutrition and genetics (Blaxter et al., 1974; Zhou and Wu, 1979; Haigh and Hudson, 1993). Blood carries nutrients for cell metabolism into antler tissues during growth. It is assumed

that blood biochemical constituents have an influence on velvet antler growth. The chemical composition of blood in wild deer, including reindeer, has been widely studied in relation to nutrition, season, species and physiological status (Waid and Warren, 1984; Soveri et al., 1992; Säkkinen et al., 1999). There are many reports on the blood biochemical values of various domestic and wild deer species (Franzmann and LeResche, 1978; Reid and Towers, 1985; Chapple et al., 1991). Blood biochemical values are influenced by age, sex, climate, nutrition, disease and sampling methods in deer (Karesh et al., 1986; Catley et al., 1990; Maeda et al., 1990). However, only a limited number of reports of blood biochemical values in farmed deer are available (Wilson and Pauli, 1982; 1983; Chapple et al., 1991). Little research has been conducted in blood biochemical values during the antler growth period. Schams et al. (1991) and Choi et al. (1998) presented data from single blood samples collected from farmed deer at casting or cutting time of the antler.

The objective of this study was to investigate the serum biochemical values at regular intervals during the antler growth period in stags of sika deer (*Cervus nippon*) comparing samples from jugular and femoral veins.

Table 1. The chemical composition of experimental diets (%DM)

Item[1]	DM	CP	EE	CF	ADF	NDF	Ash	Ca	P
Alfalfa hay	88.0	16.4	1.8	34.3	41.2	56.3	8.0	1.3	0.2
Concentrate	89.4	16.0	4.0	18.6	11.7	36.7	2.3	0.7	0.4

[1] DM: Dry matter; CP: Crude protein; EE: Ether extract; CF: Crude fiber; ADF: Acid detergent fiber; NDF: Neutral detergent fiber.

Table 2. Mean (±SE) blood values in samples drawn from jugular and femoral veins during antler growth in sika deer

Item	Day after casting			
	0	18	36	55
T-protein (g/dl)				
Jugular	8.24±0.37	7.67±0.03	7.77±0.34	7.57±0.73
Femoral		7.60±0.24	7.77±0.34	7.65±0.61
Albumin (g/dl)				
Jugular	3.77±0.12	3.47±0.23	3.42±0.20	3.45±0.23
Femoral		3.42±0.15	3.42±0.20	3.45±0.17
Creatinine (mg/dl)				
Jugular	1.32±0.17	1.22±0.29	1.17±0.22	1.27±0.38
Femoral		1.49±0.22	1.12±0.17	1.30±0.37
Urea (mg/dl)				
Jugular	25.3±0.48	22.2±0.59	23.1±0.17	25.25±0.26
Femoral		20.85±0.09	23.35±0.49	25.52±0.09
Uric acid (mg/dl)				
Jugular	0.2±0.48	0.22±0.59	0.15±0.17	0.27±0.26
Femoral		0.32±0.09	0.28±0.4	0.37±0.09
TG[1] (mg/dl)				
Jugular	14.5±0.95	16.0±0.56	10.75±6.07	10.75±6.84
Femoral		14.25±5.12	12.0±8.64	11.25±7.5
CL[2] (mg/dl)				
Jugular	60.75±15.6	51.0±23.98	53.0±22.44	61.25±20.8
Femoral		56.5±22.35	53.0±22.58	61.25±19.8
Glucose (mg/dl)				
Jugular	123.25±31.41	139.25±51.11	125.25±30.66	93.75±6.84
Femoral		141.75±49.11	111.75±1.78	92.25±16.47
T-bilirubin (mg/dl)				
Jugular	1.05±0.45[A]	0.5±0.39[B]	0.22±0.05[B]	0.3±0.2[B]
Femoral		0.85±0.5[a]	0.2±0.16[b]	0.3±0.2[ab]

[1] Tri-glyceride. [2] Cholesterol.
[A,B or a,b] Within a row, means not sharing a common superscript letter are significantly different at $p < 0.05$.

MATERIALS AND METHODS

Experimental animals and management

The investigation was carried out at HANA deer research institute (36.9°N), Chungju, Chungchongbuk-do, Korea, from March to August, 2003. Eight stags of sika deer (*Cervus nippon*) aged 4 and 5 years were used for collection of blood samples. All stags were healthy with a mean body weight of 77.7±5.2 (SD) kg at the start of the experiment. Stags were individually maintained in an outdoor 5×4 m pen during the experimental period. Stags were allowed access to alfalfa hay *ad libitum* and concentrate at the level of 1% of body weight on a fresh matter basis as the experimental diet. Feed was provided at 9 am with unlimited water. The chemical composition of the diet is presented in Table 1.

Blood samples

Blood samples were drawn from the jugular vein on day 0 and from both jugular and femoral veins on days 18, 36 and 55 into evacuated glass tubes with no anticoagulant at 18-day intervals from the antler buttons casting to the cutting date (55 days). Blood samples were periodically taken following sedation from resting stags at a regular time before feeding. Samples (15 ml) were immediately centrifuged at 3,000 rpm for 15 min to separate serum and stored at -40°C until analysis.

Biochemical analysis

Blood samples were analyzed using an auto blood analyzer (EPX: Abbott Spectrum, Abbott Labora Toried, USA). Items for analysis were blood substrates (total-protein, albumin, urea, creatinine, triglyceride, cholesterol, glucose, total-bilirubin), enzyme activities (alkaline

Table 3. Mean (±SE) enzyme activities in blood serum drawn from jugular and femoral veins during antler growth in sika deer

Item[1]	Day after casting			
	0	18	36	55
AST (U/L)				
Jugular	70.75±17.1	86.2±33.4	54.3±13.2	52.2±10.7
Femoral		57.3±4.0	53.5±12.6	50.0±11.7
ALT (U/L)				
Jugular	34.75±3.8	28.5±2.8	28.7±2.2	28.7±4.5
Femoral		27.3±4.6	28.7±2.2	30.0±5.4
ALK-P (U/L)				
Jugular	348.0±82.7	788.5±625.0	970.5±452.1	1,361.3±588.4
Femoral		403.3±97.2	947.8±460.4	1,056.0±557.5
Amylase (U/L)				
Jugular	74.74±28.8	62.7±21.5	66.7±15.2	64.0±12.8
Femoral		5.7±20.0	61.7±17.9	63.0±10.6
LDH (U/L)				
Jugular	378.5±35.6	491.0±240.2	317.5±46.5	321.0±32.7
Femoral		279.0±35.1	312.5±46.6	304.3±35.4
CK (U/L)				
Jugular	293.7±63.8	229.7±47.1	247.5±26.2	280.0±20.4
Femoral		256.0±70.6	284.3±6.1	226.5±23.1

[1] AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; ALK-P: Alkaline phosphatase; LDH: Lactate dehydrogenase; CK: Creatine kinase.

phosphatase (ALK-P), aspartate aminotransferase (AST), alanine aminotransferase (ALT)), electrolyte (Cl) and minerals (Ca, P, Na). The content of crude protein (CP), ether extract (EE), crude fiber (CF) and ash, including Ca and P, in the diet was determined according to the methods of the Association of Official Analytical Chemists (AOAC, 1990). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) content of the diet were analyzed according to the procedures of Van Soest et al. (1991).

Statistical analysis

Data were expressed as sampling date group means with standard error. Serum parameters were compared between sampling dates by one-way analysis of variance. If the time effects were statistically significant (p<0.05), the Tukey test was used for pair-wise comparison of the group means and paired t-tests were used to determine statistically significant differences between veins at the level of p<0.05. The statistical analyses were performed using the SAS package program (SAS, 1988).

RESULTS

Serum constituents

Mean serum constituents obtained in jugular and femoral veins are shown in Table 2. Serum total protein concentration on the casting date was a little higher than those of other sampling dates in the jugular vein. There were, however, no significant differences between blood samples collected during the antler growth period. There were no significant changes between veins. Serum albumin concentrations showed no significant differences between

blood samples collected from the jugular vein during the antler growth period, and were at a similar level to those in the femoral vein during the antler growth period. There was little difference in serum albumin concentration between veins.

Serum urea concentration showed no significant differences although there was a little change in blood samples drawn from the jugular vein. There was no significant difference by sampling date in blood samples drawn from the femoral vein. On the other hand, serum creatinine concentration did not change much during the antler growth period in blood samples drawn from the jugular and the femoral veins. There were no significant differences in triglyceride, glucose and cholesterol concentrations between sampling dates and veins. Although there were no significant differences, triglyceride and glucose concentrations tended to decrease similarly with progressing antler growth. Total bilirubin concentration showed much variation and was significantly higher on the casting date than on day 18 after casting in the jugular vein (p<0.01). There was no significant difference in the femoral vein during antler growth. The total bilirubin concentration was a lot higher on the casting date than on the other sampling dates and was stable from day 36 to day 55 after casting.

Changes of enzyme activities

After casting, changes of serum enzyme activities in blood samples drawn from the jugular and femoral veins at 18-day intervals in stags of spotted deer are shown in Table 3. There were no significant differences in the aspartate aminotransferase (AST), alanine aminotransferase (ALT)

Table 4. Mean (±SE) electrolyte and mineral concentrations in blood serum drawn from jugular and femoral veins during antler growth in sika deer

Item[1]	Day after casting			
	0	18	36	55
Ca (mg/dl)				
Jugular	8.07 ± 0.67^A	7.32 ± 0.28^{AB}	7.65 ± 0.43^{AB}	6.8 ± 0.52^B
Femoral		7.62 ± 0.28	7.57 ± 0.62	7.72 ± 0.41
P (mg/dl)				
Jugular	7.65 ± 1.5	6.12 ± 1.06	6.67 ± 0.75	6.77 ± 0.63
Femoral		7.27 ± 2.4	7.15 ± 0.9	7.32 ± 0.8
Na (mmon/L)				
Jugular	157.0 ± 8.6^A	145.7 ± 2.0^B	143.2 ± 0.8^B	142.0 ± 3.4^B
Femoral		148.0 ± 2.1	143.0 ± 0.5	144.5 ± 1.7
Cl (mmon/L)				
Jugular	101.0 ± 3.8^A	94.7 ± 3.6^{AB}	93.0 ± 3.7^{AB}	92.3 ± 5.1^B
Femoral		95.5 ± 1.9	94.2 ± 2.2	95.2 ± 4.2

[1] Ca: Calcium; P: Phosphorus; Na: Sodium; Cl: Chloride.
A, B Within a row, means not sharing a common superscript letter are significantly different at $p < 0.05$.

and alkaline phosphatase (ALK-P) in serum between sampling dates and veins. AST in serum was at the highest activity on the 18th day after casting and slightly decreased on the 36th day after casting, and thereafter remained stable in the jugular vein. On the other hand, AST maintained a steady range in the femoral vein. On the casting date, ALT in the jugular vein tended to be higher on the casting date and then remained constant until day 55, which was similar to results in the femoral vein.

Mean amylase, LDH and CK did not change significantly during antler growth, whereas mean serum LDH was higher on day 18 after casting than on the other sampling dates.

Changes of electrolyte and minerals values

After casting, changes of serum electrolyte and minerals in blood samples drawn from the jugular and femoral veins of spotted deer are shown in Table 4. Calcium (Ca) concentration was highest on the casting date and lowest on day 55 after casting (p<0.05) in the jugular vein, but there was no significant difference in the femoral vein. However, it was noted that when compared in both veins on day 55 after casting, calcium concentration was significantly higher in the femoral vein than in the jugular vein (p<0.05). There were no significant differences in P concentration between sampling dates or veins in this study.

Sodium (Na) concentration in serum was significantly higher on the casting date than on day 55 after casting in the jugular vein (p<0.001), and a similar pattern was observed in the femoral vein (p<0.001). Chloride (Cl) was significantly higher on the casting date than on day 55 after casting in the jugular vein (p<0.05).

DISCUSSION

The growth of velvet antlers is influenced by nutrition,

especially, protein and minerals. French et al. (1956) reported that poor nutrition resulted in weak and small antler production. Wallmo et al. (1977) also concluded that it was necessary for feed to contain 17% protein for optimal antler production. In contrast, Liang et al. (1993) and Jeon and Moon (2000) argued that sika deer under intensive farming had to receive feed containing 20-23% protein for high velvet production. It appears that deer usually need above 16% dietary protein for optimal antler production. Also, bone minerals were mobilized during the antler growth. Ullrey et al. (1973; 1975) stated that about 0.38-0.40% calcium and 0.30% phosphorus (dry matter basis) in feed was adequate for antler development in white-tailed fawns. In this experiment, in excess of 16% crude protein was provided *ad libitum* to all stags. Therefore, nutritional values, as shown in Table 1, ensured optimal growth of antler in this experiment.

It is well known that blood values are affected by nutritive condition. Franzmann and LeResche (1978) reported that serum total protein and albumin reflect the nutritive state of the Alaskan moose. Soveri et al. (1992) reported that protein and albumin concentrations were lower at levels of 5.37-5.62 g/dl and 3.0-3.5 g/dl, respectively, in reindeer fed on lichens in winter, which were the natural winter food of the reindeer, containing mainly carbohydrates but being poor in protein, minerals and vitamins (Nieminen and Heiskari, 1989). In another study (Reid and Towers, 1985) serum concentration of protein was 5.0-7.8 g/dl and albumin was 3.1-4.4 g/dl during the summer in red deer. Soveri et al. (1992) also reported that total protein concentration decreased when the animal lost weight and this decrease in serum protein concentration was associated with a decrease in both plasma albumin and globulin concentrations. These results greatly suggested that the nutritive level of the food fed to deer was closely related with serum protein and albumin

concentrations and closely agrees with our observation in this study. The findings of this study indicate that all stags were given enough protein for optimal antler growth. Furthermore, the concentrations of other serum constituents including urea, creatinine, glucose and cholesterol were not changeable during antler growth, were not different between veins and were within the reference range reported by Jeon et al. (2006).

In this study, enzyme activities did not change much during the antler growth period. In previous studies, Spraker (1993) and Marco and Lavin (1999) reported that the capture and immobilization of wild ungulates is likely to be one of the most stressful events, as is clearly indicated by the changes in some of their haematological and biochemical blood constituents that capture induces. Rehbinder and Edqvist (1981) observed that the activity of AST in blood of reindeer rose during handling and Cardinet (1989) reported that specific (CK) and non-specific (LDH and AST) muscle enzymes are released into the blood at times of muscular stress or disorders. The AST was 63.2 U/l in red deer captured by chemical means and this level doubled in animals captured by physical means. ALT was 31.2 U/l and doubled to levels of 55.1 U/L in deer captured by physical means (Marco and Lavin, 1999). Biarghov et al. (1976) also reported that enzyme activities were higher in deer fed a high nutritive diet than in deer fed a low nutritive diet. In this study, mild changes in enzyme activity during the antler growth period were most probably caused by consistent diet and handling.

AlK-P was 348.0 U/L on the casting date and elevated dramatically to 1,361.3 U/L on the cutting date in the jugular vein but there was no significant difference in the femoral vein during antler growth. By contrast, in June and July, the typical period for rapid antler growth, plasma concentration of ALK-P was significantly higher in antler veins than in the other veins (Bubenik et al., 1987). However, high ALK-P associated with velvet antler growth was reported by Eiben et al. (1984) in fallow deer and ALK-P increased during the antler growth. In adult deer, serum ALK-P levels were elevated during the antler growth period (Karen et al., 1988). In this study there was a similar trend. Thus ALK-P activity was probably associated with skeletal development and might be partly responsible for calcium absorption. When in velvet, the cartilage of an antler possesses the usual array of ALK-P found in cartilage elsewhere (Goss, 1983). In this study it was also shown that although there was no significant difference, ALK-P concentration increased with progressive antler growth. Further work is necessary to examine a more detailed correlation between ALK-P and antler growth.

Consistent with the results of Jeon et al. (2006), Ca concentration was lower (8.9 mg/dl) on day 50 after casting. Others reported a Ca concentration of 9.16-9.92 mg/dl in pregnant reindeer (Sakkinen et al., 1999) and 10.1 mg/dl in female reindeer (Soveri et al., 1992) fed on lichens in winter. The growing antler contains a number of necessary cells, including fibroblasts, chondroblasts, chondrocytes and osteocytes, which transform into cartilage. Later, the cartilage is turned to bone as calcium complex forms, as the antler growth stage is progressed to fibroblasts, cartilage, calcified cartilage and finally bone before the rut. This study supports the concept that Ca is sufficiently required during the antler growth period. Calcium has been found to be missing from thighbone, suggesting that bone mineral reserves that have been used during the antler development are replaced by the diet. However, further study is necessary to estimate ossification level related to different levels of feeding on Ca in the antler cycle and compare with ALK-P and vitamin D. It would also be necessary to determine dietary requirements for Ca in deer. There was a low concentration of P in reindeer fed lichen (Soveri et al., 1992). Hence, changes in serum P are reflective of differences in the intake of P in feed. Results from this study were higher than those of previous studies, but other findings suggest that P concentrations in this study were at the normal level required to maintain body homeostasis.

CONCLUSIONS

Blood biochemical values are important for assessing the nutritional and metabolic state of deer. The measurements which have been performed in this study clearly demonstrate that there were little differences in blood biochemical values for both jugular and femoral veins during the antler growth period, with the exception of some electrolyte and minerals, which would be expected to be higher during the antler growth period of stags. Despite a big event such as antler growth, because feeding conditions and handlings were consistently maintained this did not cause major variation in blood biochemical values in this study. However, more data is needed, since individual animals may show different responses, depending on factors such as handling method and feeding management.

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