Changes of Chemical Composition in Blood Serum during the Antler Growth Period in Spotted Deer (*Cervus nippon*)*

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ABSTRACT : The aim of this study was to provide basic haematological information to allow improved nutritional management for velvet production in spotted deer (*Cervus nippon*) by investigating biochemical changes in blood values during the antler growth period. Blood samples, obtained from the jugular vein of twenty-five deer, were taken every 10 days from casting (day 0) to harvesting (day 50) of velvet antler. Negligible changes were found in the concentrations of total protein, albumin, and creatinine during the antler growth period, but there were significant changes in the concentrations of urea ($p < 0.05$) and uric acid ($p < 0.01$). The concentration of triglyceride was significantly higher ($p < 0.05$) during the antler growth period compared to casting time, while serum high-density lipoprotein concentrations were low and remained unchanged during the antler growth period. Serum glucose concentration increased ($p < 0.05$) significantly and was slightly changeable during antler growth. The serum concentrations of Ca and P did not fluctuate during antler growth, while those of Na, K and Cl showed slight differences between the time of casting and the rest of the antler growth period. No significant changes in concentrations of AST, ALT, amylase, CK, GGT and LDH were detected during the antler growth period. However, the concentration of ALK-P increased during antler growth reaching its peak on day 50 after casting. We found a significant difference in the concentration of ALK-P between the time of casting and the rest of the antler growth period ($p < 0.01$). Consequently, antler growth was associated with mild changes in measured serum biochemical values with the exception of ALK-P activity in spotted deer. (**Key Words :** Antler Growth, Blood Values, Enzyme Activity, Minerals, Spotted Deer)

INTRODUCTION

The annual cycle of casting and regeneration of antlers in deer is a unique phenomenon in mammalian species, in that it constitutes the regeneration of a complete organ. The antler cycle of stag, especially in temperate species, is an annual series of events with antler being grown, mineralized, and cast in a well-defined sequence (Goss, 1983). This phenomenon is generally caused by physiological, environmental and nutritional factors particularly, since the antler cycle is closely related to endocrine and blood constituents (Brown et al., 1983; DelGiudice et al., 1987;

Sempere et al., 1989; Garcia et al., 1997). While endocrine has been intensively studied, blood values of deer, particularly during the antler growth season have lacked investigation. Blood values are known to differ in breeding type, stress, nutrition, season and physiological condition (Blum et al., 1981; Axelrod and Reisine, 1984; Chatterton, 1990; Rick, 1992; Zomborszky et al., 1996; Lee et al., 2004). However, these studies mostly come from other animals and wild deer living in extreme conditions. Recently, we have seen some reports obtained from domesticated deer under intensive feeding conditions, but most of these are no more than the results of partial measurements of blood constituents. Thus, knowledge of the change of blood values during the antler growth period is scarce. It is thought that intensive feeding methods become either a temporary or permanent cause of stress to deer, unlike in the wilderness, which can have a changing effect on blood properties (Zomborszky et al., 1996). The development pattern of the maturation of antlers is similar to the calcification of cartilage in other mammals (Banks et al., 1983). The outer shell of long bone is usually composed of cortical bone, thick and hard calcic tissue, while its inner

Table 1. Chemical composition of the experimental diet

Constituent	DM[1]	CP	EE	CF	Ash	NDF	ADF
	----------------------- % in DM -------------------------						
Diet	61.3	20.5	3.0	30.6	7.0	53.4	34.7

[1] DM: dry matter, CP: crude protein, EE: ether extract, CF: crude fiber. NDF: neutral detergent fiber, ADF: acid detergent fiber.

part consists of sponge bone 75-85% full of marrow (Riggs and Melton, 1986). It is postulated that bone development and calcification are related to the concentration of osteocalcin and increased activation of osteoblast due to activated alkaline phosphatase (ALK-P) (Brown et al., 1983; Eiben and Fischer, 1984; Eems et al., 1988). Fast growth and maturing antlers require large quantities of minerals, which at least are partially supplied from bone. Consequently, deer develop physiologically temporary osteoporosis (Banks et al., 1968a; b). Osteoblast, osteoprogenitor cells, chondroblasts and chondrocytes have unique receptors of 1,25 hydroxychorecalciferol which play a role in forming and activating the osteoblast of antlers (Chen et al., 1979; Manolagas et al., 1980). It stimulates osteoblast due to the increase of ALK-P synthesis and osteoblastic cells, and directly affects division and revelation of chondrocytes. It ultimately affects the formation of antler properties (Fritsch et al., 1985; Takigawa et al., 1988). Therefore, the concentration level of ALK-P in the blood is a good index of antler growth. As a result, various reports continue to be written on the relationship between the concentration level of ALK-P in blood and the development cycle of the antler (Graham et al., 1962; Morris and Bubenik, 1983; Gao et al., 1988). In reference to antler development, blood constituents, minerals and enzymes seem to be closely related to the growth of antler. However, we have few reports on the changes in their parameters during the development of antlers. Therefore, this study was carried out to provide basic information to lay the foundation for further studies on velvet antler production in deer farming.

MATERIALS AND METHODS

Experimental animals

This experiment took place in the Hana Deer Research Institute located in Chungju, Chungbuk Province, Korea from April to August 2004. Twenty-five male spotted deer with the mean live weight of 78.5 kg at the beginning of the experiment were used in the trial. These animals were selected and divided into 5 unreplicated groups of five on the basis of body weight and previous record of antler production for the convenience of management. Deer in each group were housed outside in an opened fence of 6×8 m pens with free access to water and commercial mineral blocks. Throughout the experiment, forest by-product silage, alfalfa bale, lupin seed, and concentrate were supplied *ad*

libitum to each deer as an experimental diet formulated with a ratio of 40, 5, 31 and 24% on a fresh weight basis. Deer were fed equal amounts of meal twice a day in the morning and late afternoon. The chemical composition of the experimental diet is given in Table 1.

Blood sampling

In order to analyze and compare the biochemical values of blood during the antler growth, blood samples were taken from resting deer after an overnight fast on the 0, 10th, 20th, 30th, 40th and 50th day after the casting of antler buttons from the previous set. Blood samples were drawn from the jugular veins using disposable syringes of 20 ml volume and were kept in a container with heparin to prevent coagulation. By using a centrifugal separator, drawn samples separated serum on the spot and were kept in a deep freezer at -80°C until later analysis.

Blood analysis

Frozen serum was used for the assay of biochemical values, total-protein, albumin, urea, uric acid, triglyceride, high-density lipoprotein, cholesterol, glucose, total-bilirubin, direct-bilirubin, creatinine, alkaline phophatase (ALK-P), aspartate aminotransferase (AST), alanine aminotransferase (ALT), amylase, creatine kinase (CK), GGT, lactate dehydrogenase (LDH), and inorganic elements such as Ca, P, Na, K, and Cl. Serum were measured by Epx. Abbott Spectrum (Abbott Laboratories, USA) at the Medical Center of Konkuk University.

Statistical analysis

Statistical analysis was performed with the SAS package (SAS Institute, 1995) and the significance of differences was done by Duncan's multiple range test using the Linear General Model after dispersed analysis.

RESULTS AND DISCUSSION

Chemical constituents of blood serum

Table 2 shows the changes in serum constituents during antler growth after the casting of antler buttons from the previous set in spotted deer. Negligible changes were found in the contents of total protein, albumin, and creatinine during the antler growth period, but there were significant changes ($p < 0.05, 0.01$) in the contents of urea and uric acid. The content of triglyceride was significantly high during the antler growth period compared to casting time and the content of high-density lipoprotein was low with no significance during the antler growth period. The serum content of glucose greatly increased ($p < 0.05$) and varied slightly during the antler growth period.

It is well known that the biochemical values of blood are influenced by diet. It has been reported that the

Table 2. Changes in the concentrations of serum constituents during antler growth in spotted deer

Constituents[1]	Days after casting*					
	0	10	20	30	40	50
T-P (g/dl)	7.26±0.13[a]	7.21±0.0 [a]	7.45±0.19 [a]	7.47±0.17 [a]	7.38±0.1 [a]	7.35±0.16 [a]
Alb (g/dl)	3.43±0.05 [a]	3.15±0.03 [ab]	3.32±0.06 [ab]	3.29±0.12 [ab]	3.31±0.03 [ab]	3.17±0.04 [b]
Urea (mg/dl)	28.5±1.3 [ab]	30.3±6.4 [a]	30.7±6.2 [a]	29.7±3.7 [ab]	23.0±2.7 [b]	23.3±0.96 [b]
Creat (mg/dl)	1.35±0.08 [a]	1.01±0.15 [a]	1.05±0.23 [a]	1.20±0.22 [a]	1.37±0.15 [a]	1.28±0.08 [a]
UA (mg/dl)	0.75±0.26 [a]	0.62±0.23 [a]	0.31±0.12 [b]	0.27±0.07 [b]	0.45±0.18 [a]	0.49±0.12 [a]
Trig (mg/dl)	22.8±1.3 [b]	33.7±11.9 [a]	30.7±2.2 [a]	32.5±3.2 [a]	28.5±4.3 [a]	34.5±9.2 [a]
HDL (mg/dl)	39.2±4.2 [a]	33.6±0.3 [a]	35.2±3.5 [a]	37.5±4.1 [a]	33.3±2.3 [a]	34.1±2.0 [a]
Chole (mg/dl)	82.3±6.8 [a]	72.7±2.4 [a]	76.7±6.4 [a]	78.3±9.3 [a]	71.3±4.4 [a]	81.1±5.6 [a]
Gluc (mg/dl)	62.5±5.8 [b]	149.7±68.7[a]	115.3±30.8[a]	139.0±9.1 [a]	137.7±43.4[a]	122.3±13.2[a]

* Values are means±SE.
[1] T-P: Total protein, Alb: Albumin, Creat: Creatinine, UA: Uric acid, Trig: Triglyceride,
  HDL: High density lipoprotein, Chole: Cholesterol, Gluc: Glucose.
[a, b, c] Within a row, means not sharing a common superscript letter are significantly different at $p < 0.05$.

Table 3. Serum concentrations of calcium (Ca), phosphorus (P), potassium (K), sodium (Na), and chloride (Cl) during antler growth in spotted deer

Constituent	Days after casting*					
	0	10	20	30	40	50
Ca (mg/dl)	9.17±0.15[b]	9.77±0.23 [a]	9.77±0.18 [a]	9.90±0.32 [a]	9.70±0.17 [a]	8.90±0.32 [b]
P (mg/dl)	5.84±0.32 [a]	5.27±1.88 [a]	5.33±0.19 [a]	6.30±1.42 [a]	5.63±1.09 [a]	5.61±0.28 [a]
Na (mEq/L)	144.7±0.4 [a]	140.3±1.4 [b]	143.7±2.1 [ab]	139.7±3.7 [b]	135.3±1.7 [c]	143.1±1.0[ab]
K (mEq/L)	4.37±0.09 [b]	4.73±0.12 [ab]	4.67±0.32 [ab]	5.03±0.22 [a]	4.73±0.43 [ab]	4.58±0.10 [ab]
Cl (mEq/L)	103.0±0.8 [b]	107.0±2.6[ab]	108.3±1.7 [qb]	110.3±3.7 [ab]	112.7±5.3 [a]	104.9±1.6 [ab]

* Values are means±SE.
[a, b, c] Within a row, means not sharing a common superscript letter are significantly different at $p < 0.05$.

concentrations of total protein and albumin of deer ranged from 6.1-6.9 g/dl and 3.3-3.7 g/dl depending upon deer species (Reid and Towers, 1985; Chapple et al., 1991; Audige, 1992). In this study, the changes in the concentrations of total protein and albumin in stags of spotted deer during the antler growth period showed a similar pattern. However, the concentration of protein was slightly higher than the reference range. The reindeer that were fed lichen only which was winter feed from a natural habitat showed low concentration levels of total protein and albumin (Nieminen and Heiskari, 1989). This was caused by a deficiency of protein in the diet. It also shows that total protein and albumin in serum are correlated and are closely connected to the protein levels of the food which was ingested. Therefore, it is thought that the high level of serum total protein could be attributed to the feeding condition in this experiment. Because the animals were consistently fed a diet having a high level of protein during the antler growth period (Table 1), the serum contents of total protein and albumin, which are easily modified by feeding conditions, were comparatively stable during antler growth. On the other hand, according to several researchers, the concentrations of other blood values are known to be closely related to diet (Hyvarinen et al., 1975; Valtonen and Eriksson, 1977; Nieminen, 1980; DelGiudice et al., 1987). In this experiment, few changes could be considered clinically meaningful in the concentrations of other

variables during antler growth owing to the consistent feeding of a similar diet, although there were some differences depending on sampling dates. Also, the concentrations of blood values were within reference ranges (Larsen et al., 1985; Klinger et al., 1986; DelGiudice et al., 1987; Shin, 1987; Soveri et al., 1992; Zomborszky et al., 1996) and showed relatively stable fluctuations during the antler growth period. Therefore, it is suggested that while antler growth is a big event which demands a lot of nutrients, blood values do not change remarkably when feeding conditions are consistently maintained.

Minerals and electrolytes

The changes in serum minerals and electrolytes during antler growth of spotted deer are shown in Table 3. The concentration of Ca slightly increased until day 40 after casting and then showed a slight decrease on day 50 when antler growth slowed down. The serum concentration of P was 5.84 mg/dl at the time of casting. During the antler growth period, the concentration did not fluctuate around that value. The concentration of Na gradually decreased (p<0.01) until day 40 after casting and then slightly increased on the day 50. The concentration of K in blood serum was 4.37 mEq/L at the time of casting. It showed high concentrations during the antler growth period compared to the time of casting. The concentration of Cl in blood serum of spotted deer was 103 mEq/L, 107.0 mEq/L,

Table 4. Changes in serum enzyme activities during antler growth in spotted deer

Enzyme[1]	Days after casting[*]					
	0	10	20	30	40	50
ALK-P (U/L)	155.9±24.5[b]	242.6±24.5[a]	243.3±7.9[a]	264.7±24.7[a]	260.7±31.7[a]	329.8±41.0[a]
AST (U/L)	72.5±4.3[ab]	79.0±13.5[ab]	78.7±17.6[ab]	97.0±45.0[a]	58.7±10.5[b]	72.2±4.5[ab]
ALT (U/L)	49.7±2.9[a]	53.7±4.5[a]	53.3±1.8[a]	50.7±6.5[a]	47.0±2.5[a]	46.6±1.8[a]
Amyl (U/L)	289.7±23.4[a]	262.7±35.5[a]	278.7±31.2[a]	276.3±38.7[a]	267.0±38.6[a]	290.2±22.1[a]
CK (U/L)	284.8±57.1[a]	306.0±102[a]	203.0±7.5[a]	223.0±50.9[a]	216.0±45.7[a]	269.0±52.3[a]
GGT (U/L)	19.1±2.1[a]	20.0±3.4[a]	24.0±4.0[a]	23.7±3.7[a]	18.3±1.2[a]	20.1±1.3[a]
LDH (U/L)	328.7±17.1[a]	430.0±32.2[a]	354.7±44.7[a]	426.0±67.6[a]	389.3±61.2[a]	370.4±18.5[a]

[*] Values are means±SE.
[1] ALK-P: alkaline phosphatase, AST: aspartate aminotransferase, ALT: alanine aminotransferase.
  Amyl: amylase, CK: creatine kinase, GGT: γ-glutamyl transferase, LDH: lactate dehydrogenase.
[a, b, c] Within a row, means not sharing a common superscript letter are significantly different at $p<0.05$.

108.33 mEq/L ($p<0.05$), 110.33 mEq/L ($p<0.01$), 112.67 mEq/L and 104.94 mEq/L on day 0, 10, 20, 30, 40, and 50 after casting, respectively. The concentration of Cl increased slightly during the growth period of antlers. It has been reported that there were significant differences in the concentrations of Ca among farmed deer: red deer had a range of 9.44-14.08 mg/dl (Knox et al., 1988) and 3.4-15.16 mg/dl (Wilson and Pauli, 1982), and rusa deer had a range of 8.0-11.32 mg/dl (Audige, 1992). Calcium concentrations generally differ in gender and age. Thus, the younger the animal, the higher the Ca concentration (Chapple et al., 1991), which means that growing animals require more calcium in their metabolism than those which have already finished growth. However, Ca concentration did not differ by season. The typical growth type of antlers shows a sigmoid curve (Muir et al., 1987). The decrease of Ca on day 50 after casting means that antler growth would be slowed down during that period. That is to say, at the time of strong growth, deer demand more Ca, thus increasing the uptake of Ca. However, after day 40, when antler growth slows down, the demand for Ca decreases and the metabolic rate of Ca decreases, therefore decreasing the concentration level of Ca in blood. Brown et al. (1983) reported that the concentrations of Ca and P in blood serum remained relatively stable throughout the year. In this study, we also found that the concentration of Ca and P did not change a lot despite the changing in physiological condition of antler growth, as long as nutrition was properly maintained. It is thought that more detailed comparisons between the concentration during antler growth and the concentration before and after casting will be needed. The serum concentration of Na in white-tailed deer was in the range of 137-143 mEq/L (DelGiudice et al., 1987) while that of farmed red deer was in the range of 70-220.0 mEq/L, and 109-154 mEq/L (Wilson and Pauli, 1982). It has been reported that the concentrations of Na increased in deer that were fed a low-protein diet (Seal et al., 1987). However, DelGiudice et al. (1987) reported that there had been no change in the concentration level even when the existing diet was replaced by high-protein and high energy feedstuff

(CP 13.0%) and low-protein and low-energy feedstuff (CP 6.9%), respectively. This coincides with a study by Anderson et al. (1972), within which they found that grasses eaten by deer have nothing to do with seasonal changes in the concentration of Na. In the present study, it is assumed that since the experimental diet remained constant, the serum concentration of Na had only negligible changes during the antler growth period. The reindeer whose weight loss was 13-25% during winter time showed a trend of lower concentration of K along with weight loss, staying in the concentration range of 3.2-4.5 mmol/L. It was reported that the low content of potassium, contained in lichen that was fed, created such a trend (Soveri et al., 1992). This result is consistent with the report that deer whose weight loss was 11% and 21% during winter time had a lower concentration level of K (Seal et al., 1972). Such phenomena suggest that the K content in the diet of ruminants directly affects the concentration of K in blood serum. When a high-energy diet was fed to white-tailed deer, the average concentration of K in blood serum was 3.6 mEq/L. We hypothesize that deer whose nutrition was properly managed, did not undergo any significant changes in the concentration of K, even during the antler growth period. The fluctuation in Na and Cl concentrations did not follow the same trend during antler growth. No specific pattern of changes was observed. We can draw from this result that deer whose nutrition is appropriately managed do not undergo any great changes in the mineral content in blood, even during the antler growth period.

Enzyme activities

Table 4 shows some of the serum enzyme activities in stags of spotted deer during the antler growth period. The serum activity of ALK-P was 155.92 U/L at the time of casting, but it gradually increased during the period of antler growth and ALK-P was highest ($p<0.01$) on day 50. The serum activities of AST and amylase were 72.58 U/L and 289.67 U/L at the time of casting, respectively, and although there was a negligible increase and decrease during antler growth, the differences were not significant

statistically. The serum activity of CK decreased after casting. There were no significant differences during antler growth, although the change of activity had a wide range. The concentration of LDH in blood serum was 328.75 U/L at the time of casting. It rapidly increased to 430.0 U/L (p<0.01) on day 10 after casting, but it quickly decreased to 354.67 U/L on day 20. It increased to 426.0 U/L on day 30, decreased to 389.33 U/L on day 40, and further to 370.38 U/L on day 50 after casting. Compared with the concentration at the time of casting, a significant difference was only observed on day 10 after casting. GGT did not change during the antler growth period.

ALK-P is an enzyme related to bone growth, and when bone remodeling occurs the activity of ALK-P increases. Chapple et al. (1991) reported that the average concentration of ALK-P in the blood plasma of male chital deer was 219 U/L, but it increased greatly to 960 U/L on day 60 after casting. A similar seasonal change, related to the annual antler cycle, was also observed in other deer species (Brown et al., 1978; Morris and Bubenik, 1983). It is generally known that the average concentration of ALK-P in blood serum is higher in young animals than in mature ones. In our study, a close relationship between the serum activity of ALK-P and antler cycle was also found (Graham et al., 1962; Morris and Bubenik, 1983). The serum ALK-P activity and hydroxyproline levels in white-tailed deer increased during the growth period of antlers (Eems et al., 1988), showing a similar trend to the report of Graham et al. (1962). Their dissertation proved a correlation between antler growth and ALK-P concentration, since the concentration steadily increased during antler growth. The serum concentration of ALT and amylase was still within reference range (Chapple et al., 1991). CK is an enzyme that expedites synthesis and resolution of creatine phosphate, which is an energy source involved in muscular contraction. It exists in a high density form mainly in skeletal muscles, myocardium, brain and smooth muscle. That is to say, the activity of CK fluctuates greatly according to the kinds and extent of exercise. Thus, the activation of CK in the blood plasma of undomesticated deer is higher than that of domesticated ones (Chapple et al., 1991; Zomborszky et al., 1996). Therefore, the activity of CK differed according to feeding conditions and environment. However, as in this study, under the same feeding conditions, the serum activity of CK was not greatly different during the antler growth period. LDH is also an enzyme spread throughout tissues. It exists in various organs and has low peculiarity. These muscular enzymes are released to the blood by exercise, extreme stress at the time of being captured, physical disorder, and so on. The activation of chital male deer was higher than that of their female counterparts, and it was higher in deer that were growing than in deer that stopped growing (Chapple et al., 1991). We thought that since the spotted deer in this study were well tamed by good domestication and frequent handling before the experiment, the activation of CK and LDH would be relatively stable. However, we did not observe any correlations between antler growth and the activation of CK and LDH. GGT is synthetic enzyme that transfers γ-glutamyl of γ-glutamyl peptide into other amino acids or peptides. It plays a role in re-absorption, transportation of amino acids and hydrolysis of glutathione. It exists mostly in renal tube and brush border membranes in animal tissues (Lee and Lee, 1996). The activation of GGT in the blood serum of spotted deer was 19.08 U/L at the time of casting, increasing to 20.0 U/L on day 10 and 24.0 U/L on day 20 after casting. However, it then dropped to 23.67 U/L on day 30 and 18.33 U/L on day 40 after casting. On day 50, it shot up to 20.19 U/L. However, no significant differences during the antler growth period were found, compared with those at the time of casting.

CONCLUSION

We conducted this research in order to obtain basic data about the biochemical changes in blood values during antler growth from spotted deer fed intensively in the paddock, and to analyze the relevance to antler growth. In order to measure the change in blood values during antler growth, we randomly selected 25 heads of 4 to 6 year-old stag that had been fed in the paddock and drew blood samples from these stags every 10 days from casting to the 50th day. We measured the chemical constituents, minerals and enzyme activation in blood serum. Negligible changes in the contents of total protein, albumin, and creatinine were found during the antler growth period, but there were significant changes (p<0.05, 0.01) in the contents of urea and uric acid. The content of triglyceride was significantly high during the antler growth period compared to casting time while the content of high-density lipoprotein was low with no significance. The serum content of glucose greatly increased (p<0.05) and was slightly changeable during the antler growth period. The concentrations of Ca and P did not show any great fluctuations during antler growth, while those of Na, K and Cl showed slight differences between the time of casting and the rest of the antler growth period. No significant changes in concentrations of AST, ALT, amylase, CK, GGT and LDH were detected during the antler growth period. However, the concentration of ALK-P continued to increase as antlers grew reaching a peak on day 50 after casting. We found a significant difference in the concentration of ALK-P between the time of casting and the rest of the antler growth period (p<0.01). Antler growth was associated with mild changes in measured serum biochemical values in spotted deer.

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