Study on the Varying Patterns of Total Phospholipids, Selenium, Phosphorus, Reducing Sugar and Total Sugar, Hydrolyzed Amino Acids in the Velvet Antler of Northeast Sika Deer in Growth Period

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Abstract. In this study, the varying patterns of total phospholipids, selenium, phosphorus, reducing sugar and total sugar, hydrolyzed amino acids in the velvet antler of Northeast sika deer in growth period were evaluated. Eighteen Northeast sika deer were allocated into 6 groups according to the antler shedding time. Results indicated that there was significant difference of the selenium content between any two of the six groups ($P<0.05$) except that of Group 1 and Group 2 or Group 5 and Group 6. About the phosphorus there was significant difference between any two of the six groups ($P<0.05$) except that of Group 4 and Group 5 or Group 2 and Group 3 or Group 1 and Group 2. Group 6 had the lowest total Phospholipids content. Both of the reducing sugar and total sugar showed an increasing pattern initially and then decrease gradually.

1 Introduction

The velvet antler is the non-ossification horn of cervidae animals such as Northeast sika deer and red deer. It is a famous traditional Chinese medicine that owns many beneficial effects. Therefore, velvet antler was wildly used in the folk medicine [1]. Velvet antler contains protein, amino acids, peptide, trace elements, phospholipid, fatty acids, and so on [2]. In addition, it also contains some components like chondroitine, acidic polysaccharose, mucopolysaccharides [3, 4]. In this study, the varying patterns of total phospholipids, selenium, phosphorus, reducing sugar and total sugar , hydrolyzed amino acids in the velvet antler of Northeast sika deer in growth period were evaluated. And this may provide some theoretical foundations for the manufacture of velvet antler-health care products. [5, 6]

2. Experimental Section

2.1 Animals and sampling

The velvet antlers were collected from the deer farm of Dongfeng Pharmaceutical factory. Eighteen Northeast sika deer with an average age of four years. Velvet antler with a length of 1 cm was sampled after the animals were anesthetized. The blood in the velvet antler was drained with the vacuum pump [7]. The velvet antler was dried with the vacuum freeze drier and the weight was recorded.

2.2 Apparatus and methods

In this study, AFS-933 atomic fluorescence spectrophotometer, DK20 Multi-function digestion device, and Beck-man DU-7500 ultraviolet spectrophotometer were employed. Selenium, phosphorus, total phospholipids, reducing sugar, and total sugar were analyzed according to the followings methods, respectively (Table 1).

Table 1. The content of selenium and phosphorus

Items	Methods
Selenium	GB 5009.93
Phosphorus	GB12393
Total phospholipids	Duan et al., 1988 [8]
Reducing sugar	NY 318 1997-Ginseng products [9]
Total sugar	Lou et al., 2003 [10]
Hydrolyzed amino acids	GB/T5009.124

2.2.1 Selenium

Accurately weigh a certain amount of sample Weigh 0.5g ~ 2g sample, liquid sample lessons 1.00mL~ 10.00mL, placed in the digestion flask, add 10.0mL mixed acid and a few grains of glass beads, covered with a watch glass of cold digestion overnight. The next day on a hot plate heated and timely additional nitric acid. When the solution becomes clear and colorless with white smoke, the heating was continued to a residual volume of about 2mL, must not be evaporated to dryness. Cooling, plus

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5.0mL hydrochloric acid, and heating was continued until the solution becomes clear and colorless with white smoke appears, hexavalent selenium reduced to tetravalent selenium. Cooled and transferred to a 50 mL volumetric flask to volume and mix aside. While doing the blank test.

Instrument Reference conditions: negative high voltage: 340 V; lamp current: 100 m A; atomization temperature: 800℃; furnace height: 8mm; carrier gas flow rate: 500 mL/min; shielding gas flow rate: 1000 mL/min; measurement: standard curve method; reading mode: peak area; delay time: 1s; reading time: 15s; dosing time: 8s; injection volume: 2mL. setting the optimal conditions for the instrument, the furnace temperature was raised gradually to the desired temperature, start measuring stable after 10min~20 min. Continuous series of zero standard tube injection until after the reading has stabilized, the standard series of measurements into the standard curve. Into the sample measurement, the sample and blank samples were measured digestive, before each test different samples should be cleaned injector.

2.2.2 Phosphorus

Accurately weigh a certain amount of sample Weigh 0.1~ 0.5g in the 100m L Kjeldahl flask, 3m L sulfuric acid, 3m L perchloric acid - nitric acid digestion, placed on digestion furnace, beginning to brown-black liquid bottle. When the solution turned to be colorless or slight yellow clear liquid that is completely digested. The solution was allowed to cool, after the transfer plus 20m L, cooled to 100m L flask, washed several times with water Kjeldahl flask washings were combined into the volumetric flask, add water to volume, and mix. This solution is a liquid sample measurement. digested sample taken with the same amount of sulfuric acid, perchloric acid - nitric acid digestion, according to the same method blank solution. phosphorus standard curve Imbibe phosphorus.standard solution 0,0.5,1.0,2.0,3.0,4.0,5.0 mL, were placed 20mL stoppered test tube, followed by adding 2mL molybdic acid solution shake and let stand a few seconds. Join 1m L sodium sulfite solution, 1m L hydroquinone solution shake. Add water to volume, and mix. Standing 0.5h later absorbance was measured at 660nm wavelength spectrophotometer. To measure the absorbance of the standard curve for phosphorus content.

Imbibe the measured liquid 2m blank solution L and the same amount of samples were placed 20m L stoppered test tube, and the remaining steps with the standard curve to measure the absorbance Richard unknown liquid phosphorus content standard curve.

2.2.3 Total phospholipids

Weigh 2g sample in a stoppered Erlenmeyer flask, Folch reagent (chloroform - methanol 2: 1) 20m L, said that given the weight, ultrasound extraction 1h, placed at room temperature, weighed and the weight, plus Folch reagent re-fill, 3000r · min - 1, centrifugal 5min, the supernatant was filtered to 25m L flask, with Folch reagent to the mark, as the test solution.

2.2.4 Reducing sugar

Accurately weighed sample 2g, set in the 100mL beaker, added 85 ring ethanol 50mL, mixing, holding 30 min at 500C in a water bath, filtered, and the residue was then 85% ethanol solution was extracted twice, and the filtrate were combined, the ethanol was evaporated, add a small amount of distilled water and a total volume of 100 mL.

After each tube and mix prepared standard curve method of operating a measured absorbance of each tube, to identify the appropriate reducing sugar content in the standard curve, the samples reducing sugar.

2.2.5 Total sugar

Weigh 1g sample in a test tube, added 10mL, 15mL hydrochloric acid solution and distilled water mixed and heated for 30 min, a solution of iodine with potassium iodide to check the degree of hydrolysis in a boiling water bath, if it has completely hydrolyzed, was not blue color (of starch), cooled I added drops of phenolphthalein indicator, with a 10% sodium hydroxide solution to reddish, filtered and set the volume to 100mL. And then to learn the exact solution 10ml, set people 100mL volumetric flask with distilled water to the mark.

After each tube and mix prepared standard curve method of operating a measured absorbance of each tube, to identify the appropriate reducing sugar content in a standard curve of the samples to the total sugar content.

2.2.6 Hydrolyzed amino acids

Accurately weigh a certain amount of sample to the nearest 0 .0001g, sample weight within 10~20 mg, saying that a good sample put in the hydrolysis tube. Hydrolysis: Add in the hydrolysis tube 6.0 mol/L HCl 15.00mL, was added thioglycolic acid 1.00 ml (for protection), cover stopper with vacuum pump to vacuum, tap into 0 psi, this hydrolysis tube and placed in the oven thermostat seal 110℃ ± 1℃ after 24h of incubation, remove the cooling. Open hydrolysis tube stopper, the hydrolyzate was filtered and washed constant volume to 50.00ml volumetric flask. 1.00 ml draw in the filtrate was 25 ml beaker, evaporated to dryness on a water bath, the residue was dissolved in water 1 ~ 2 ml, then dried up again evaporated to dryness three times, and finally the residue was 0.02mol/L HCl 1.00mL dissolved machine measurement.

3. Results and Analysis

3.1 The contents of selenium and phosphorus in the velvet antler

Results indicated that there was significant difference of the selenium between any two of the six groups ($P<0.05$) except that of Group 1 and Group 2 or Group 5 and

Group 6. About the content of phosphorus there was significant difference between any two of the six groups (P<0.05) except that of Group 4 and Group 5 or Group 2 and Group 3 or Group 1 and Group 2 (Table 2).

Table 2. The contents of selenium and phosphorus in the velvet antler

Group	Selenium (ng/g)	Phosphorus (%)
1	211.41a	5.05d
2	221.34a	5.86cd
3	186.24b	6.57c
4	150.44c	7.5b
5	124.01d	7.86b
6	131.31cd	8.81a

Selenium is an important ingredient of glutathione peroxidase and many health care products were wildly consumed because of their high content of this element. As we known, velvet antler was rich in selenium too. Results in this study indicated the highest content of selenium was at the 40 days after the antler shedding. Phosphorus is vital to the life because it is not only the ingredient of nucleic acid and ATP, but also makes up a lot of enzymes with the other elements. Such results Antler Growth Law emergence are inseparable. Deer stag in mid-April after a year in late fall to early May faceplate, antler began to rapidly grow, reached a peak of about 70 d, before the peak of the growth, the growth rate is greater than the speed of ossification, ossification and then faster than the growth rate, and then dried skin off bone horn, bones, horns last fall, the end of an antler growth cycle. Velvet deposited nutrients in addition to the outer part of the minerals by food intake, and thus this body needs to absorb a large number of deer nutrients for the growth of the supply of velvet. Phosphorus involved in energy metabolism, the body if the lack of Phosphorus, the bone marrow, tooth development is not normal. Osteoporosis, softening, easy to fracture or suffer from rickets in children, loss of appetite, muscle weakness.

The content of phosphorus was increased as the shedding time of velvet antler prolonged. Phospholipids can not only facilitate the cell renewal and maintain the metabolism, but also promote the immunity and regenerative capacity of human beings [11, 12].

3.2 The contents of total phospholipids, reducing sugar and total sugar in the velvet antler

Table 3. The contents of total phospholipids, reducing sugar and total sugar in the velvet antler

Groups	Total phospholipids (%)	Reducing sugar (%)	Total sugar (%)
1	2.45ab	0.24d	3.61e
2	2.39ab	0.39c	4.42d
3	2.54a	0.54a	5.04c
4	2.62a	0.50ab	6.15a
5	2.67a	0.47b	5.61b
6	2.18b	0.32c	5.73ab

Generally, Group 5 had the highest total phospholipids content (P<0.05) whereas Group 6 had the lowest (P<0.05).

The sugar is an important organic compound which is widely distributed in the natural world. It is one of the primary sources for life sustaining. Both of the reducing sugar and total sugar showed an increasing pattern initially and then decrease gradually (Table 3).

The experiment measured plum velvet herbs total phospholipid content, slightly lower than the reported in the literature, may be related to Antler taken herbs processing methods; about Deer Antler total phospholipid content has not been reported. Determination of the total phospholipid content of common mainly colorimetry and HPLC. Phospholipid assay is converted to inorganic phosphorus colorimetrically to calculate the total phosphorus content of the phospholipid content. The HPLC method is generally measured total phospholipid content of each phospholipid component, summing the total phospholipid content of each phospholipid content, this method has the instrument detects a bit fast, but many phospholipid components, each group is not easy to points are measured completely [13], and the price is relatively expensive Ao, measured in terms of total phospholipid higher costs. The colorimetric test solution by a standard phosphorus as a low cost reference and measurement method is simple, the application is also more practical significance to promote [14].

Antler is a valuable traditional Chinese medicine, complex composition, in recent decades, and found a variety of active ingredients, such as amino acids, fatty acids, chondroitin sulfate, hypoxanthine, uracil, and phospholipids polyamine compounds [15]. Wherein the phospholipid is considered to be one of the active ingredients [16], in addition to inhibition of monoamine oxidase activity action outside, stimulate growth and hematopoietic function, enhance the regeneration process and improved neuromuscular function of the system antler etc, velvet phospholipids effect is closely related to. Antler existing literature on total phospholipid segmented detection, content decreasing from top to bottom [17], but did not find them in the diversity of the statistical analysis report.

3.3 Results of hydrolyzed amino acids

Table 4 The content of hydrolyzed amino acids

Group	Amino acid at the middle of velvet antler (%)	Amino acid at the top of velvet antler (%)
1	47.55a	57.15ab
2	45.35ab	59.62a
3	40.25c	50.86c
4	40.13c	50.67c
5	39.05c	51.78bc
6	41.35bc	62.95a

Note: the different superscripts of the data on the same row means significant difference (P < 0.05 or P < 0.01)

It can be indicated from this Table 4 that among the hydrolyzed amino acids of velvet antler of Northeast Sika Deer in different growth periods, there are significant difference of amino acids content at the middle of velvet antler between group 1 and 3, 4, 5, 6 (p＜0.05), and

between group 2 and 3, 4, 5, 6 ($p<0.05$). There are significant difference of amino acids content at the top of velvet antler between group 1 and 3, 4, 6, between group 2 and 3, 4, 5, and between group 6 and 3, 4, 5 ($p<0.05$).

Amino acids are organic component velvet topped the content of nutrients, the highest glycine; amino acids are the basic components of living organism tissue cells for life events play a pivotal role. If the body lacks any kind of essential amino acids, it can cause physiological dysfunction, affecting the normal antibody metabolism [18], leading to disease. Similarly, if the body lacks certain non-essential amino acids, it will produce antibodies metabolic disorders. Arginine and citrulline formation of urea is very important; cystine intake will cause reduction of insulin, blood sugar. Another example is the post-traumatic cystine and arginine requirements increase [19], such as the lack of adequate energy even if you still can not synthesize proteins. Thus, the presence of amino acids in the human body, not only provides an important raw material for the synthesis of proteins, but also for the promotion of growth and normal metabolism, provides the material basis of life-sustaining

4. Conclusions

Selenium is an important ingredient of glutathione peroxidase and many health care products were wildly consumed because of their high content of this element. As we known, velvet antler was rich in selenium too. Results in this study indicated the highest content of selenium was at the 40 days after the antler shedding. Phosphorus is vital to the life because it is not only the ingredient of nucleic acid and ATP, but also makes up a lot of enzymes with the other elements. The content of phosphorus was increased as the shedding time of velvet antler prolonged. Phospholipids can not only facilitate the cell renewal and maintain the metabolism, but also promote the immunity and regenerative capacity of human beings [20]. Generally, Group 5 had the highest total phospholipids content ($P<0.05$) whereas Group 6 had the lowest ($P<0.05$). The sugar is an important organic compound which is widely distributed in the natural world. It is one of the primary sources for life sustaining [21]. Both of the reducing sugar and total sugar showed an increasing pattern initially and then decrease gradually. These data may provide some theoretical foundations for the manufacture of velvet antler-health care products.

Amino acid is the basic unit composing the protein. Some physiological activities in velvet antler are directly correlated to the amino acid. The amino acid in human body provides the important material for protein synthesis, but also provides the material basis for promoting growth, normal metabolism and maintaining the life [22]. The content of hydrolyzed amino acids increases along with time, while the change is not regular but some groups of hydrolyzed amino acids show significant difference ($p<0.05$), which may be correlated to the accumulation of amino acids at different time. All the results provide the scientific basis for collection and utilizaiton of velvet antler.

5. Summary

These data may provide some theoretical foundations for the manufacture of velvet antler-health care products.

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