Studies on the physicochemical characteristics of the New Zealand deer's tail, *Cervus elaphus* var. *scoticus* (III)

Nam Kyung Lee¹ · Kyoung Hwa Jang¹ · Jong Tae Lee¹ · Jun Bae Kim¹ · Sung Tai Han¹ · Gyo In¹

Received: 9 August 2018 / Revised: 2 January 2019 / Accepted: 14 January 2019 / Published online: 2 March 2019
© The Korean Society of Food Science and Technology 2019

Abstract This study was designed to determine the nutritional profile and functional components of the NZT (New Zealand deer's tail, *Cervus elaphus* var. *scoticus* Lönnberg). Twenty-nine fatty acids, eighteen amino acids, twenty-five minerals, chondroitin, and phospholipids were detected by the auto-fatty acid analyzer, auto-amino acid analyzer, inductively coupled plasma optical emission spectrophotometer, absorbance measurements, and by weighing after separating, respectively. 7-Ketocholesterol was isolated from alcohol extract by silica gel column chromatography analysis. Four steroid hormones (androstene-3,17-dione, β -estradiol, testosterone, and dehydroepiandrosterone), one base and seven nucleosides, and *N*-acetylneuraminic acid were detected by a HPLC-photodiode array and HPLC-fluorescence detector. As a result, NZT was composed of many nutritional and functional ingredients found in New Zealand deer's antler (NZA) which was one of deer co-products, and it was

considered that NZT could be a novel health food resource such as NZA.

Keywords New Zealand deer's tail · Special byproducts of deer · *Cervus elaphus* var. *scoticus* · Physicochemical property

Abbreviations

AAA	Automatic amino acid analyzer
AE	Alcohol extract
Ala	Alanine
Arg	Arginine
Asp	Aspartic acid
CC	Calibration curve
CCR ²	Correlation coefficient
Cys	Cysteine
DHEA	Dehydroepiandrosterone
DMB	1,2-Diamino-4,5-methyleneoxybenzene
FLD	Fluorescence detector
Glu	Glutamic acid
Gly	Glycine
His	Histidine
Hypro	Hydroxyproline
Ile	Isoleucine
KMFDAS	Korea ministry of food and drug safety
Leu	Leucine
LOD	Limit of detection
LOQ	Limit of quantitation
LRE	Linear regression equation
Lys	Lysine
Met	Methionine
NANA	<i>N</i> -Acetylneuraminic acid
NZA	New Zealand deer's antler
NZT	New Zealand deer's tail
ODS	Octadecyl-silica

✉ Nam Kyung Lee
leenamkyung@kgc.co.kr; yhlisa@hanmail.net

✉ Gyo In
20109042@kgc.co.kr

Kyoung Hwa Jang
khjang@kgc.co.kr

Jong Tae Lee
bigbell@kgc.co.kr

Jun Bae Kim
20100067@kgc.co.kr

Sung Tai Han
next102@kgc.co.kr

¹ Laboratory of Fundamental Research, R&D Headquarters, Korea Ginseng Corp., 30, Gajeong-ro, Yuseong-gu, Daejeon 34128, Korea

Phe	Phenylalanine
PDA	Photodiode array
Pro	Proline
RE	Regression equation
RM	Raw material
RSD	Relative standard deviation
RT	Retention time
SD	Standard deviation
Ser	Serine
SH	Steroid hormone
Thr	Threonine
Tyr	Tyrosine
Val	Valine
WE	Water extract

Introduction

Red deer, sika, and elk, inhabit New Zealand, Russia, China, Japan and Canada. In traditional medicine books such as Dong-Ui-Bo-Gam (東醫寶鑑), various kinds of deer byproducts such as deer's antler (鹿茸), deer's blood (鹿血), deer's bone (鹿骨), deer's muscles (鹿筋), deer's head (鹿頭), deer's brain (鹿髓), deer's kidney (鹿腎), deer's hoof (鹿蹄肉), deer's fat (鹿脂), and deer's tail (鹿尾) have long been used as folk remedies (Chang, 2003). Deer's antler (鹿茸) refers to the whole cartilaginous antler that is in a precalcified stage on growing antlers. Antler is a popular traditional medicine, and many of its ingredients have been studied and used mainly as a growth tonic (Hattori et al., 1989; Kim et al., 1977; Lee et al., 2014).

The deer's tail (鹿尾) is almost entirely produced in New Zealand and is exported to Asia. The exterior of the deer's tail is covered with leather, and the inside is made up of meat and tailbone. In addition, it is known to the public that there is efficacy in its treatment of andropausal symptoms, and many men eat it as a health food. However, there are few studies on its active ingredients, efficacy or physicochemical properties (Bakke and Figenschou, 1983).

Deer byproducts are known to contain protein, fat, minerals, sialic acid, base and nucleosides, chondroitin, steroid hormones (SH) and so on. N-Acetylneuraminic acid (NANA) is a type of sialic acid that was identified in New Zealand deer's antler (NZA) (Lee et al., 2014). Sialic acids also play an important role in the treatment of several human viral infections (Drew, 1992; Varki and Gagneux, 2012). Chondroitin is an important structural component of cartilage and provides much of its resistance to compression (Baurle et al., 2009). 7-Ketocholesterol is a steroid found from NZA (Lee et al., 2014). Testosterone, which is one of the SHs, or its precursor component is expected to

relieve andropausal symptoms because it can increase testosterone levels (Lund et al., 1999; Noh et al., 2012). The above ingredients vary depending on the species of deer, and even within the same deer, the kinds and levels of ingredients vary depending on the byproducts from the particular species of deer.

This study was investigated and analyzed the New Zealand deer tail (NZT)'s physicochemical characteristics of functional compounds and nutrients using various analytical instruments. In addition, these results were suggested that NZT could be a novel source for manufacturing health foods, and various raw data, including the levels of the above ingredients could be provided as basic data for manufacturing health foods including NZT.

Materials and methods

Deer's tail material

Dried deer's tail of *Cervus elaphus* var. *scoticus* was imported from Rokland Corp. Ltd. (Christchurch, New Zealand) in 2013 and used as the raw material (RM). The materials were confirmed taxonomically by KGC raw materials headquarters, Korea Ginseng Corp., Daejeon, Korea, and the voucher specimen CEST-2013 was deposited at the R&D headquarters of Korea Ginseng Corp., Daejeon, Korea.

The NZT (12–24 g/tail, sex not distinguished) were ground into powder using a mill, and this was used as the RM. The RM (1 kg) was refluxed for 4 h 4 times with 70% ethanol/water, dried under vacuum, evaporated and lyophilized to obtain the alcohol extract (AE, 167 g). Additionally, the RM (1 kg) was refluxed for 4 h 4 times with water, dried under vacuum, evaporated and lyophilized to yield the water extract (WE, 448 g).

Reagents

The HPLC solvents were from JT Baker (Phillipsburg, NJ, USA). Androstene-3,17-dione, estradiol, testosterone, dehydroepiandrosterone (DHEA), 14% BF₃ in methanol, triundecanoin and undecanoic acid methyl ester were purchased from Sigma Chemical (St. Louis, MO, USA). HCl was purchased from Showa (Tokyo, Japan). The ninhydrin reagent and buffer solution were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Uracil, uridine, deoxyuridine, inosine, guanosine, deoxyguanosine, adenosine, deoxyadenosine, formic acid, sodium borate and hydrogen peroxide were purchased from Sigma-Aldrich (St. Louis, MO, USA). D-Glucuronolactone, Carbazole, ethyl ether, acetone, n-hexane, ethyl acetate,

methanol, acetonitrile, HNO_3 , NaOMe, iso-octane, NaCl were purchased from Merck (Darmstadt, Germany).

Proximate analysis

The proximate analysis (moisture, ash, crude protein, crude fat, and carbohydrate) of the NZT sample was determined by the standard methods of the Association of the Official Analytical Chemists (AOAC, 1995). The moisture level was determined by heating the fresh sample at 105 °C for 3 h until it reached a constant weight. The ash level was weighed from the residue obtained after cremation at 550 °C for 8 h. The crude protein level ($\text{N} \times 6.25$) was determined using the Kjeldahl method. The crude fat level was measured by Soxhlet extraction with petroleum ether as a solvent.

Fatty acid composition

Fatty acids were prepared with fatty acids methyl esters (FAMES) by the Metcalfe method (Metcalfe et al., 1966). RM powder (25 mg) was precisely taken in a glass tube, and triundecanoin reagent (1 mg)/iso-octane (1 mL) was added as an internal standard substance. Subsequently, 0.5 N NaOMe reagent (1.5 mL) was added, and the mixture was heated for 5 min in a heating block at 100 °C. After cooling, 14% BF_3 in methanol (2 mL) was added to the mixture, followed by filling with nitrogen, it was heated at 100 °C for 30 min. After cooling to 30 °C, iso-octane (1 mL) was added and nitrogen was filled, and covered with vigorous shaking for 30 s. Immediately, a saturated NaCl/water (5 mL) was added, nitrogen was filled, covered and shaken. After cooling to room temperature, the iso-octane layer separated from the NaCl/water layer was dehydrated with anhydrous sodium sulfate to prepare a test solution.

In addition, the reference material is prepared by dissolving 37 fatty acid methyl esters (standard stock solution: 37 component FAME mixture, Supelco™, Bellefonte, PA, USA) and undecanoic acid methyl ester, which is an internal standard substance, in iso-octane so as to have a concentration of 0.5 mg/mL, respectively.

The level of fatty acid was determined using a gas chromatography flame ionization detector (GC-FID) system equipped with a capillary column (0.20 μm , 0.25 mm $\times$ 100 m, Agilent 7890B, Sigma-adrich, Supelco™ SP-2560, Bellefonte, PA, USA). The oven temperature was programmed as follows (from 130 °C for 5 min to 180 °C at 3.0 °C/min, from 180 °C to 240 °C at 2.0 °C/min). The sample (1 μL) was injected with a 1:10 split ratio. Peak identification was conducted by comparison with internal standards. Quantification was accomplished by comparison of integrated peaks with calibration curves of the FAMES

using a software (Agilent open LAB chem station data analysis). The carrier gas was helium, and it was allowed to flow at a 1.0 mL/min rate.

In addition, AE and WE powders were also analyzed by GC-FID after preparation of fatty acid analysis samples by the same procedure as above RM.

Amino acid composition

The level of amino acids was determined by using an automatic amino acid analyzer L-8900 (AAA, Hitachi, Tokyo, Japan) equipped with an ion-exchange column (26224sc-PF, 4.6 mm $\times$ 60 mm, Kanto Chemical Co., Inc., Tokyo, Japan). The sample (100 mg) was precisely weighed, and 6 N HCl (5 mL) was added. After filling with nitrogen, the sample was sealed and subjected to acid hydrolysis at 110 °C for 22 h. The reactant was concentrated under reduced pressure at 55 °C by evaporation, dissolved in 0.02 N HCl, and passed through a 0.45 μm filter to prepare the analytical samples. The filtrate (20 μL) was loaded onto the speed amino acid analyzer. Concentrations of individual free amino acids were determined using an AAA. The amino acids were separated using ion-exchange chromatography with post column derivatization (injection pump pressure 0–19.6 MPa), flow rate (0.05–0.99 mL/min), auto-sampler, column oven, electrothermal cooling (30–70 °C), photometer (wavelength 570 nm, 440 nm), column oven temp. (reaction coil temp. 135 °C), mobile phase (pump 1, buffer PH-1, PH-2, PH-3, PH-4, PH-RG), (pump 2, ninhydrin), flow rate (pump 1, 0.4 mL/min), (pump 2, 0.35 mL/min), and detector (ultraviolet and visible colorimetric detection). Amino acids were detected at 570 nm with the exception of proline (Pro) and hydroxyproline (Hypro), which were detected at 440 nm. The results were recorded using a Minichrom data handling system.

Mineral composition

The mineral level was determined using an inductively coupled plasma optical emission spectrophotometer (ICP-OES) flow injection mercury system and cyclonic chamber in a dual-view method (Falcó et al., 2006; Sahan et al., 2007). The sample (1.0 g) was accurately weighed into a Teflon micro digestion vessel (100 mL), and 30% H_2O_2 (2 mL) and 65% HNO_3 (5 mL) were added, and the micro digestion vessels were allowed to digest overnight. The tubes were sealed with a Teflon cap and digested in a microwave oven digester (Milestone Ethos Sel, Sorisole, Italy). The sample was digested at 100 °C for 10 min, brought up to 140 °C at 8 °C/min, held at 140 °C for 6 min, allowed to cool, and then brought up to volume with 25 mL of deionized water. The sample was filtered with 0.45 μm

filters before analysis. Mineral analysis was performed using a Perkin Elmer® Optima TM 7300 DV ICP-OES instrument (Perkin Elmer Inc., Shelton, CT, USA). The instrument was calibrated using an ICP multi-element standard solution (Merk, Darmstadt, Germany) in 2% HNO₃. The validity of the calibration was monitored by the quality control check module within Win Lab32 for ICP software.

Chondroitin level

The chondroitin level was determined by the standard methods of the Korea Ministry of Food and Drug Safety (KMFDS) (KMFDS, 2012). The sample (0.3 g) was placed in a flask, (100 mL) and distilled water was added to the top of the flask. The sample solution (4 mL) was put in a flask (20 mL). The line was filled with distilled water. After filtration, 0.5% sodium borate solution (5 mL) was taken from each of the two colorless tubes. The colorimetric tube was sufficiently cooled with ice-cold water. D-Glucuronolactone (1 mL) and sample solution was added to each of the two colored tubes containing the above sodium borate, followed by cooling and mixing. Each tube was heated continuously to 100 °C for 10 min and immediately cooled with ice-cold water. Carbazole reagent (0.125%, 0.2 mL) was added to each of the two color tubes and mixed. The sample was heated in a water bath for 15 min and cooled to room temperature with ice-cold water. For the control, distilled water (1 mL) was used instead of the sample, and the absorbance at 530 nm was obtained after the reaction. The level of chondroitin that is contained in the sample is calculated as follows: chondroitin (mg/g)=glucuronic acid $\times$ 2.593.

Phospholipid level

The level of the acetone-insoluble phospholipids was determined by the standard methods of the Korea Ministry of Food and Drug Safety (KMFDS) (KMFDS, 2012). After adding ethyl ether (40 mL) to (W₁) mg of the sample and refluxing for 10 min, the sample was filtered, and this process was repeated twice. The ethyl ether extract was concentrated under reduced pressure, dried and weighed (W₂ g). After dissolving the sample in ethyl ether (3 mL), acetone (15 mL) was added, and the sample was shaken, placed in ice-cold water, and left for 15 min. Acetone (50 mL) was added, and the mixture was cooled to 0 °C and allowed to stand on ice for 15 min. This mixture was centrifuged at 3000 rpm for 10 min using a centrifuge, and then, the supernatant was removed. Acetone (50 mL) was added to the precipitate, and the mixture was mixed well, shaken, and centrifuged in the same manner as before the supernatant was removed. The two supernatants were

combined, concentrated under reduced pressure, dried and weighed (W₃ g). Therefore, the amount of phospholipid, as acetone-insoluble matter, that is contained in the sample is (W₂–W₃)/W₁.

Isolation of 7-ketocholesterol

AE (20 g) was chromatographed on a silica gel column chromatography and eluted with a gradient of n-hexane $\rightarrow$ n-hexane : ethyl acetate=50: 1 $\rightarrow$ 20: 1 $\rightarrow$ 10 :1 $\rightarrow$ 5: 1 $\rightarrow$ 2:1 $\rightarrow$ 1: 1 $\rightarrow$ 1: 2 $\rightarrow$ 1: 5 $\rightarrow$ ethyl acetate $\rightarrow$ methanol in 8 fractions (TA-1 (1.856 g), TA-2 (0.786 g), TA-3 (4.275 g), TA-4 (0.825 g), TA-5 (0.408 g), TA-6 (0.414 g), TA-7 (0.787 g), and TA-8 (3.663 g)). TA-3 (4.27 g) was subjected to normal phase HPLC eluting with a silica gel column (n-hexane: ethyl acetate=5:1, RI detector) to create 7-ketocholesterol (8 mg).

SH composition

The level of 4 SHs (androstene-3,17-dione, β -estradiol, testosterone, and DHEA) were determined by HPLC–PDA. Analytical HPLC was conducted on a Waters Alliance liquid chromatography system (Waters, Milford, MA, USA) with an octadecyl-silica (ODS) column (Waters Spherisorb® S5 ODS2 PSS 831915 ODS, 5 μ m, 4.6 mm id $\times$ 250 mm, Milford, MA, USA). The mobile phase (60% methanol/H₂O containing 0.1% formic acid) showed good resolution and response between the SHs and interfering constituents. The flow rate of the mobile phase was 0.7 mL/min. The column eluate was detected with a PDA at 205 (DHEA), 238 (androstene-3,17-dione, testosterone), and 280 (β -estradiol) nm. The identification of the SHs in the eluate was determined from peaks with the same RT (retention time, min) and accordance of the PDA spectrum (λ) to the standard steroids. The SH level in the sample was determined using calibration curves (CCs) generated with authentic standards in the range of 0.01–100 μ g/mL. The level of each analyte was subsequently obtained from the corresponding CCs. Data acquisition and processing were performed using Empower 3 software (Waters, Milford, MA, USA). The LOD and LOQ for each analyte were determined at a S/N ratio of approximately 3 and 10, respectively (Table 1). Intra- and interlay variations were chosen to determine the precision of the method. For the intraday variability test, the standard solution was analyzed 3 times within 1 day, while for the interday variability test, the sample was examined each day for 3 consecutive days (Table 2). Variations were expressed by their relative standard deviations. The recovery was performed by adding a known amount of the SH standard (Table 3).

Table 1 Linear regressions, LODs, and LOQs of steroid hormones, base and nucleosides

Compound	Range (µg/ml)	Regression equation (n=3)	R ²	LOD (ng/ml)	LOQ (ng/ml)
Androstene-3,17-dione	0.01–100	$y = 4.00 \times 10^6 x + 2.22 \times 10^6$	0.9999	1.3	4.36
β-Estradiol	0.01–100	$y = 5.07 \times 10^5 x + 6.75 \times 10^4$	0.9998	1.2	3.96
Testosterone	0.01–100	$y = 3.71 \times 10^6 x + 1.41 \times 10^6$	0.9999	1.95	6.46
Dehydroepiandrosterone	0.01–100	$y = 9.96 \times 10^5 x + 3.12 \times 10^5$	0.9999	9	29.7
Uracil	0.001–10,000	$y = 1.45 \times 10^4 x + 1.19 \times 10^7$	0.9891	1.01	3.34
Uridine	0.001–10,000	$y = 3.90 \times 10^3 x + 6.99 \times 10^6$	0.9935	0.55	1.83
Deoxyuridine	0.001–10,000	$y = 1.11 \times 10^6 x + 8.25 \times 10^6$	0.9942	0.62	2.05
Inosine	0.001–10,000	$y = 1.08 \times 10^6 x + 7.98 \times 10^6$	0.9926	0.60	1.98
Guanosine	0.001–10,000	$y = 1.15 \times 10^6 x + 8.24 \times 10^6$	0.9862	0.64	2.11
Deoxyguanosine	0.001–10,000	$y = 1.05 \times 10^6 x + 7.59 \times 10^6$	0.9859	0.59	1.94
Adenosine	0.001–10,000	$y = 1.20 \times 10^6 x + 9.01 \times 10^6$	0.9936	0.67	2.22
Deoxyadenosine	0.001–10,000	$y = 1.16 \times 10^6 x + 8.69 \times 10^6$	0.9939	0.65	2.17

LOD limit of detection, LOQ limit of quantitation, R² R(regression coefficient)-square

Table 2 Intraday and interday precisions of steroid hormones, base and nucleosides (n=3)

Compound	Intraday RSD (%)			Interday RSD (%)
	Day 1	Day 2	Day 3	
Androstene-3,17-dione	0.56	0.59	0.62	0.67
β-Estradiol	0.73	0.87	0.83	0.75
Testosterone	0.35	0.43	0.28	0.32
Dehydroepiandrosterone	0.31	0.17	0.23	0.17
Uracil	1.62	1.45	1.63	1.51
Uridine	1.12	1.57	1.22	1.17
Deoxyuridine	1.01	1.24	0.92	1.09
Inosine	1.22	1.72	1.28	1.24
Guanosine	1.39	1.67	1.77	1.68
Deoxyguanosine	1.76	1.54	1.98	1.72
Adenosine	1.17	1.29	0.85	1.15
Deoxyadenosine	1.21	1.67	1.08	1.13

RSD relative standard deviation

Base and nucleoside composition

The levels of one base (uracil) and seven nucleosides (uridine, deoxyuridine, inosine, guanosine, deoxyguanosine, adenosine, and deoxyadenosine) were determined by HPLC–PDA. The analytical HPLC analysis was conducted on a Waters Alliance liquid chromatography (Waters, Milford, MA, USA) with an ODS column (Waters Spherisorb® S5 ODS2 PSS 831915 ODS, 5 µm, 4.6 mm × 250 mm, Milford, MA, USA). The column eluate was detected with a PDA at 254 nm for the one base and seven nucleosides. The mobile phase consisted of a gradient from 0 to 100% methanol in water. The standard stock solution was separated using a gradient mobile phase consisting of water (mobile phase A) and methanol (mobile

phase B). The gradient elution was carried out as follows: 0–25 min, 90% A; 25–30 min, linear gradient to 0% A; 30–40 min, linear gradient to 0% A; and 40–60 min, 10% A. The flow rate was set at 0.7 mL/min, and the injection volume was 10 µL. The identification of analytes was based on their RTs when coinjected with standards. Standard stock solutions were diluted to appropriate concentrations for the plotting of the CCs. Each concentration of the standard solution was injected in triplicate, and the CCs were drawn by plotting the peak areas versus the concentrations of each analyte. The quantity of each analyte was subsequently obtained from the corresponding CCs. Data acquisition and processing were performed using Empower 3 software (Waters, Milford, MA, USA). The LOD and LOQ for each analyte were determined at a

Table 3 Recovery of steroid hormones, base, and nucleosides

Compound	Recovery (%)		
	5 µg/ml	10 µg/ml	20 µg/ml
Androstene-3,17-dione	100.2	100.3	101.5
β-Estradiol	98.7	98.5	98.2
Testosterone	99.5	99.9	102.1
Dehydroepiandrosterone	99.9	101.7	100.7
Uracil	99.9	100.7	101.6
Uridine	99.8	998.0	102.8
Deoxyuridine	98.9	99.8	100.9
Inosine	99.5	99.6	99.2
Guanosine	97.8	100.6	102.6
Deoxyguanosine	99.2	100.9	104.3
Adenosine	98.5	103.9	101.4
Deoxyadenosine	99.2	96.4	99.6

S/N ratio of approximately 3 and 10, respectively (Table 1). Intra- and interday variations were chosen to determine the precision of the method. For the intraday variability test, the standard solution was analyzed 3 times within 1 day, while for the interday variability test, the sample was examined each day for 3 consecutive days (Table 2). Variations were expressed by their relative standard deviations. The recovery was performed by adding a known amount of base or nucleoside standard (Table 3).

NANA level

The level of NANA (Sigma, A0812, St. Louis, MO, USA) was determined using an HPLC fluorescence detector (FLD) (Chen et al., 2011). The sample (100 mg) was treated with 50 mM HCl (35 mL) at 80 °C for 3 h. The NANA hydrolysate was converted into a fluorescent form by derivatization with 1,2-diamino-4,5-methyleneoxybenzene (DMB, Takara Bio-Inc., 4400, Shiga, Japan) followed by separation and analysis using an ODS column (Waters Spherisorb® S5 ODS2 PSS 831915 ODS, 5 µm, 4.6 mm × 250 mm, Milford, MA, USA) and a Waters Alliance 2695 HPLC-Waters 2475 multiwavelength FLD (Waters, Milford, MA, USA) with UV detection at 310 nm (AccQ-Tag method). The mobile phase was eluted with an isocratic condition of 7% methanol/9% acetonitrile/84% water for 30 min. The flow rate was set at 0.9 mL/min, and the injection volume was 10 µL. The level of NANA in the sample was determined using the calibration curve (CC) generated from an authentic standard in the range of 0.001–0.1 mg/mL. Data acquisition and processing were

performed using Empower 3 software (Waters, Milford, MA, USA).

Results and discussion

Proximate composition

As shown in Table 4, the proximate composition (weight %) of the NZT in the RM is composed of crude protein (71.92%), crude fat (7.48%), ash (3.44%), calculated carbohydrate (1.27%), and detected carbohydrate (galactose: 0%; glucose: 0.07%; fructose: 0.16%; sucrose: 0.11%; lactose: 0%; maltose: 0.03%). In WE extract, crude protein was 93.88%, ash was 2.55%, calculated carbohydrate (0.33%), and detected carbohydrate (galactose: 0%; glucose: 0.05%; fructose: 0.1%; sucrose: 0.1%; lactose: 0%; maltose: 0.04%), and crude fat was hardly found. In contrast to the above, AE consisted of approximately 47.09% crude fat, 34.18% crude protein, 4.51% ash, calculated carbohydrate (8.08%), and detected carbohydrate (galactose: 0%; glucose: 0.08%; fructose: 0.26%; sucrose: 0.27%; lactose: 0%; maltose: 0.04%).

According to the literature (USDA, 2018), venison's RM showed crude protein (86%), crude fat (10.64%), calcium (0.028%), and iron (0.011%). Therefore, the composition of the NZT did not vary much from the proximate properties of venison.

Fatty acid composition

As a result of the fatty acids that were identified in the RM of NZTs, 13 kinds of fatty acids are shown in Table 4. The relative abundance of fatty acids (total 38.29 mg/g) is in the following order: palmitic acid (C16:0)>stearic acid (C18:0)>palmitoleic acid (C16:1 Δ9c)>linoleic acid (C18:2 Δ9c, 12c)>oleic acid (C18:1 Δ9c)>heptadecanoic acid (C17:0)>myristic acid (C14:0)>myristoleic acid (C14:1 Δ9c)>elaidic acid (C18:1, Δ9t)>α-linolenic acid (C18:3 Δ9c, 12c, 15c)>arachidonic acid (C20:4 Δ5c, 8c, 11c, 14c)>pentadecanoic acid (C15:0)>heptadecenoic acid (C17:1, Δ10c). Among them, there are eight unsaturated fatty acids.

In addition, 29 fatty acids (total 289.94 mg/g) were detected in the AE, and the order is as follows: oleic acid (C18:1 Δ9c)>palmitic acid (C16:0)>stearic acid (C18:0)>palmitoleic acid (C16:1 Δ9c)>linoleic acid (C18:2 Δ9c, 12c)>myristic acid (C14:0)>heptadecanoic acid (C17:0)>myristoleic acid (C14:1 Δ9c)>tricosanoic acid (C23:0)>elaidic acid (C18:1, Δ9t)>α-linolenic acid (C18:3 Δ9c, 12c, 15c)>heptadecenoic acid (C17:1, Δ10c)<pentadecanoic acid (C15:0)>heneicosanoic acid (C21:0)>arachidic acid (C20:0)>behenic acid (C22:0)>

Table 4 The levels of nutritional and functional ingredients

Ingredients	Raw material	Water extract	Alcohol extract
Moisture (%)	15.89±0.59	3.23±0.13	6.14±0.30
Fat (%)	7.48±0.25	ND	47.09±1.87
Protein (%)	71.92±2.52	93.88±3.68	34.18±1.31
Ash (%)	3.44±0.13	2.55±0.09	4.51±0.18
Carbohydrate (calculated %)	1.27	0.33	8.08
Lauric acid (C12:0, mg/g)	ND	ND	0.62±0.04
Myristic acid (C14: 0, mg/g)	1.67±0.12	ND	11.02±0.84
Myristoleic acid (C14:1 Δ9c, mg/g)	1.13±0.08	ND	8.17±0.69
Pentadecanoic acid (C15:0, mg/g)	0.36±0.01	ND	1.88±0.06
10-Pentadecenoic acid (C15:1 Δ10c, mg/g)	ND	ND	0.20±0.01
Palmitic acid (C16:0, mg/g)	11.33±0.85	ND	59.38±3.87
Palmitoleic acid (C16:1 Δ9c, mg/g)	5.77±0.42	ND	30.72±2.61
Heptadecanoic acid (C17: 0, mg/g)	1.90±0.06	ND	9.42±0.81
10-Heptadecenoic acid (C17:1 Δ10c, mg/g)	0.35±0.01	ND	2.12±0.15
Stearic acid (C18:0, mg/g)	6.93±0.52	ND	31.31±2.63
Elaidic acid (C18:1 Δ 9t, mg/g)	0.69±0.02	ND	3.91±0.18
Oleic acid (C18:1 Δ 9c, mg/g)	2.07±0.15	ND	85.21±6.29
Linolelaidic acid (C18:2 Δ 9t, 12t, mg/g)	ND	ND	0.45±0.02
Linoleic acid (C18:2 Δ 9c, 12c, mg/g)	4.99±0.27	ND	28.63±1.99
γ-Linolenic acid (C18:3 Δ 6c, 9c, 12c, mg/g)	ND	ND	0.30±0.01
α-Linolenic acid (C18:3 Δ 9c, 12c, 15c, mg/g)	0.57±0.02	ND	2.48±0.15
Arachidic acid (C20:0, mg/g)	ND	ND	1.22±0.08
11-Eicosenoic acid (C20:1 Δ11c, mg/g)	ND	ND	1.08±0.07
11,14-Eicosadienoic acid (C20:2 Δ11c, 14c, mg/g)	ND	ND	0.60±0.02
8,11,14-Eicosatrienoic acid (C20:3 Δ8c, 11c, 14c, mg/g)	ND	ND	0.70±0.02
11,14,17-Eicosatrienoic acid (C20: 3c Δ11c, 14c, 17c, mg/g)	ND	ND	0.36±0.01
Arachidonic acid (C20:4 Δ5c, 8c, 11c, 14c, mg/g)	0.54±0.01	ND	0.39±0.01
5,8,11,14,17-Eicosapentaenoic acid (C20: 5 Δ5,8,11,14,17, mg/g)	ND	ND	0.46±0.01
Heneicosanoic acid (C21: 0, mg/g)	ND	ND	1.85±0.05
Behenic acid (C22:0, mg/kg)	ND	ND	1.15±0.09
4,7,10,13,16,19-Docosahexaenoic acid (C22: 6 Δ4c, 7c, 10c, 13c, 16c, 19c, mg/g)	ND	ND	0.46±0.01
Tricosanoic acid (C23:0, mg/g)	ND	ND	4.80±0.24
Lignoceric acid (C24:0, mg/g)	ND	ND	0.73±0.03
Nervonic acid (C24:1 Δ9c, mg/g)	ND	ND	0.33±0.01
Asp (mg/g)	69.55±2.76	75.40±2.69	12.80±0.48
Glu (mg/g)	65.40±2.61	86.20±2.43	23.70±0.92
Gly (mg/g)	40.40±1.57	82.70±3.12	12.80±0.42
Lys (mg/g)	40.40±1.25	43.10±1.73	12.80±0.34
Pro (mg/g)	37.90±1.52	79.10±3.16	19.30±0.63
Ala (mg/g)	33.80±1.36	46.70±1.56	17.20±0.64
Leu (mg/g)	29.50±1.07	23.40±0.92	23.70±0.95
Thr (mg/g)	29.49±0.77	30.60±1.25	10.70±0.43
Tyr (mg/g)	27.30±1.08	21.60±1.06	2.10±0.06
Ser (mg/g)	25.20±1.89	25.10±0.94	10.70±0.34
Arg (mg/g)	25.20±0.98	32.30±1.26	4.30±0.13
Ile (mg/g)	23.80±0.96	18.00±0.72	12.80±0.36
Val (mg/g)	23.80±0.92	16.10±0.64	15.00±0.45
Phe (mg/g)	21.20±0.84	12.60±0.74	10.70±0.28

Table 4 continued

Ingredients	Raw material	Water extract	Alcohol extract
Hypro (mg/g)	14.70±0.65	55.70±2.12	1.90±0.07
Met (mg/g)	10.60±0.32	10.70±0.32	0.40±0.12
His (mg/g)	10.60±1.42	14.40±0.56	2.10±0.04
Cys (mg/g)	8.30±0.33	5.40±0.20	1.30±0.04
Ca (µg/kg)	8,962,537.00±35,850.15	624,036.40±18.73	24,600.47±12.54
K (µg/kg)	5,614,600.00±24,574.00	6,049,271.00±241,970.84	13,846,130.00±553,845.20
Na (µg/kg)	2,826,025.00±1130.41	2,788,374.00±11,153.50	6,674,366.00±3769.55
Mg (µg/kg)	553,501.40±2214.00	516,689.10±2066.76	9088.00±79.46
Fe (µg/kg)	93,615.50±854.23	51,875.58±155.64	ND
Ti (µg/kg)	57,829.03±2313.11	4515.86±186.00	ND
Ag (µg/kg)	20,330.52±513.24	2893.46±115.72	10.65±0.32
Rb (µg/kg)	10,890.50±435.80	11,305.16±452.30	32,378.01±1295.14
Sr (µg/kg)	8738.56±349.56	1519.07±60.76	ND
Ba (µg/kg)	8322.44±332.90	3968.17±119.04	74.58±3.66
Al (µg/kg)	4684.34±187.45	3596.16±107.88	532.71±15.97
Cu (µg/kg)	3531.09±141.24	23,612.69±944.48	1832.52±73.28
Cr (µg/kg)	2437.29±97.49	661.36±26.44	383.55±11.32
As (µg/kg)	535.01±21.36	268.68±8.04	21.31±0.85
Se (µg/kg)	396.51±13.84	361.68±11.01	479.44±19.16
Ga (µg/kg)	392.34±14.72	155.01±5.33	ND
Pb (µg/kg)	368.57±14.74	4340.19±130.20	74.58±2.61
Hg (µg/kg)	344.79±11.76	454.69±181.60	383.55±15.49
Li (µg/kg)	356.68±14.25	103.34±4.12	127.85±3.08
V (µg/kg)	309.12±12.37	62.00±2.48	10.65±0.32
B (µg/kg)	273.45±10.92	6427.61±193.81	95.89±2.85
Cs (µg/kg)	95.11±3.80	124.01±3.65	ND
Cd (µg/kg)	95.11±3.42	310.01±12.35	330.28±12.45
In (µg/kg)	83.22±2.09	103.34±3.08	31.96±0.67
Zn (µg/kg)	ND	3079.47±9.24	ND
Phospholipid (mg/g)	8.80±0.35	9.30±0.21	111.00±3.44
Chondroitin (mg/g)	12.50±0.05	12.00±0.36	13.50±0.04
7-ketocholesterol (isolated, mg/g)	0.067	ND	0.4
Androstene-3,17-dione (µg/g)	4.01±0.08	ND	23.85±0.54
β-Estradiol (µg/g)	4.15±0.21	ND	24.36±0.32
Testosterone (µg/g)	4.06±0.12	ND	24.17±0.54
Dehydroepiandrosterone (µg/g)	4.38±0.26	ND	28.64±0.84
Uracil (mg/g)	1.72±0.05	0.33±0.01	3.31±0.14
Uridine (mg/g)	3.63±0.14	1.75±0.05	6.72±0.26
Deoxyuridine (mg/g)	0.51±0.02	0.06±0.03	0.76±0.05
Inosine (mg/g)	0.95±0.03	0.43±0.02	1.61±0.05
Guanosine (mg/g)	1.08±0.03	0.72±0.03	1.63±0.04
Deoxyguanosine (mg/g)	0.57±0.02	0.22±0.01	1.30±0.03
Adenosine (mg/g)	0.04±0.01	0.01±0.01	0.04±0.01
Deoxyadenosine (mg/g)	0.04±0.01	ND	0.02±0.01

Table 4 continued

Ingredients	Raw material	Water extract	Alcohol extract
N-acetylneuraminic acid (mg/g)	3.01±0.09	2.73±0.11	0.11±0.01

Values represent the mean±S.D. (standard deviation) of three independent experiments

ND not detected, *Ala* alanine, *Arg* arginine, *Asp* aspartic acid, *Cys* cysteine, *Glu* glutamic acid, *Gly* glycine, *His* histidine, *Hypro* hydroxyproline, *Ile* isoleucine, *Leu* leucine, *Met* methionine, *Phe* phenylalanine, *Pro* proline, *Ser* serine, *Thr* threonine, *Tyr* tyrosine, *Val* valine

11-eicosenoic acid (C20:1 Δ11c)>lignoceric acid (C24:0)>8, 11, 14-eicosatrienoic acid (C20:3c Δ8c, 11c, 14c)>lauric acid (C12:0)>11, 14-eicosadienoic acid (C20:2 Δ11c, 14c)>C-5, 8, 11, 14, 17-eicosapentaenoic acid (C20:5 Δ5c, 8c, 11c, 14c, 17c)=4, 7, 10, 13, 16, 19-docosahexaenoic acid (C22:6 Δ4c, 7c, 10c, 13c, 16c, 19c)>linolelaidic acid (C18:2 Δ9t, 12t)>arachidonic acid (C20:4 Δ5c, 8c, 11c, 14c)>C-11, 14, 17-eicosatrienoic acid (C20:3 Δ11c, 14c, 17c)>nervonic acid (C24:1 Δ9c)>γ-linolenic acid (C18:3 Δ6c, 9c, 12c)>pentadecenoic acid (C15:1, Δ10c). Among them, there are eighteen unsaturated fatty acids. The total fatty acid level in AE was about 7.5 times higher than that in RM, and sixteen of fatty acids (lauric acid, C12:0; 10-pentadecenoic acid, C15:1 Δ10c; linolelaidic acid, C18:2 Δ 9t, 12t; γ-linolenic acid, C18:3 Δ 6c, 9c, 12c; arachidic acid, C20:0; 11-eicosenoic acid, C20:1 Δ11c; 11,14-eicosadienoic acid, C20:2 Δ11c, 14c; 8,11,14-eicosatrienoic acid, C20:3 Δ8c, 11c, 14c; 11,14,17-eicosatrienoic acid, C20: 3c Δ11c,14c,17c; 5,8,11,14,17-eicosapentaenoic acid, C20: 5 Δ5,8,11,14,17; heneicosanoic acid, C21: 0, mg/g; behenic acid, C22:0; 4,7,10,13,16,19-docosahexaenoic acid, C22: 6 Δ4c, 7c, 10c, 13c, 16c, 19c; tricosanoic acid, C23:0; lignoceric acid, C24:0; nervonic acid, C24:1 Δ9c) were contained only in AE. However, no fatty acid was detected in the water layer.

According to the literature (Lee et al., 2003), the total level of fatty acid in NZT is much higher than that in Korean deer antler (*Cervus Canadensis*, Elk). The levels of palmitic acid (C16:0) and stearic acid (C18:0) in NZT were much higher than that in Korean deer antler. However, the level of eicosenoic acid in Korean deer antler was much higher than that in NZT. In addition (Manley and Forss, 1979), the level of palmitoleic acid in NZT was much higher than that in venison. However, the levels of other fatty acids were not significantly different from those of venison.

Amino acid composition

Eighteen amino acids were detected from NZTs by AAA. As shown in Table 4, the levels of the amino acids in the

RM were in the following order: aspartic acid (Asp)>glutamic acid (Glu)>glycine (Gly)>lysine (Lys)>proline (Pro)>alanine (Ala)>leucine (Leu)>threonine (Thr)>tyrosine (Tyr)>serine (Ser)>arginine (Arg)>isoleucine (Ile)>valine (Val)>phenylalanine (Phe)>hydroxyproline (Hypro)>methionine (Met)>histidine (His)>cysteine (Cys). The order in the WE is Glu>Gly>Pro>Asp>Hyp>Ala>Lys>Arg>Thr>Ser>Leu>Tyr>Ile>Val>His>Phe>Met>Cys. The order in the AE was Leu and Glu>Pro>Ala>Val>Asp, Gly, Ile, Lys, and Arg>Thr, Ser, and Phe>Arg>Tyr and His>Hypro>Cys>Met. The 12 amino acids (Asp, Thr, Ser, Glu, Gly, Ala, Met, Lys, His, Arg, Hypro, and Pro) were in the same order, where the relative levels in WE>RM>AE. The 5 amino acids (Cys, Val, Ile, Tyr, and Phe) were in the same order, where the relative levels in RM>WE>AE. Leu showed the same order, where RM>AE>WE. As a result, the WE showed a higher level than that of the AE for amino acids such as Gly, Ala, Arg, HyPro, and Pro, which were water-soluble amino acids constituting the collagen in the RM.

As shown in Table 4, the levels of amino acids constituting the collagen were shown from the NZT-WE (amino acid, mg/g; Gly, 82.7; Ala, 46.7; Arg, 32.3; HyPro, 55.7; Pro, 79.1). However, the levels of amino acids constituting the collagen were isolated from the NZA- middle-WE (amino acid, mg/g; Gly, 102.0; Ala, 58.8; Arg, 36.0; HyPro, 81.7; Pro, 98.8) (Lee et al., 2014). Therefore, the levels of NZT-WE's amino acids constituting the collagen were much lower than that of the NZA-middle-WE.

Mineral composition

The 25 minerals constituting the NZTs were detected by ICP-OES. As a result of analyzing 25 kinds of inorganic elements using ICP, 24, 25, and 19 inorganic elements were detected from the RM, WE, and AE, respectively. However, the total mineral level was 1.82% in RM, 1.01% in WE and 2.06% in AE. The total mineral level of AE was higher than that of WE due to the increased presence of minerals such as K and Na. As shown in Table 4, based on concentration, the order of the minerals in the RM is Ca>

K>Na>Mg>Fe>Ti>Ag>Rb>Sr>Ba>Al>Cu>Cr>As>Se>Ga>Pb>Hg>Li>V>B>Cs>Cd>In. The order of the mineral levels in WE was K>Na>Ca>Mg>Fe>Cu>Rb>B>Ti>Pb>Ba>Al>Zn>Ag>Sr>Cr>Hg>Se>Cd>As>Ga>Cs>Li>In>V. The order of the mineral levels in the AE was K>Na>Ca>Mg>Rb>Cu>Al>Se>Hg>Cr>Cs>Li>B>Ba>Pb>In>As>V>Ag.

There are thirteen minerals (Ca, Mg, Fe, Li, Ti, V, Cr, Ga, As, Sr, Ag, Ba, and Al) that cannot be easily extracted into water and 70% ethanol and instead remain in the original material. Five minerals that are better extracted into 70% ethanol rather than into water are Na, K, Se, Rb, and Cs. In contrast, fourteen minerals (Ca, Mg, Zn, Fe, B, Ti, Cu, Ga, As, Ag, Cd, In, Ba, and Al) extract better into water than into 70% ethanol.

According to the literature (Manley and Forss, 1979), the order of the mineral levels in venison was K>Mg>Ca>Fe. On the other hand, the order of the mineral levels of NZT was Ca>K>Na>Mg>Fe. The reason for the higher Ca levels in NZT than those in venison is presumably because NZT contains tail bone.

Chondroitin level

As shown in Table 4, the chondroitin level was almost the same in RM, AE and WE.

No reports of chondroitin in venison have been found.

Phospholipid level

As shown in Table 4, the phospholipid level reported as acetone insolubles in AE was approximately 12 times higher than that in the WE or RM. This result was because phospholipid was better extracted in 70% ethanol than it was in water. The order was shown as AE (111 mg/g)>WE (9.3 mg/g)>RM (8.8 mg/g). However, lecithin was not detected.

The phospholipid level in venison was 4.5 mg/g (Kasai et al., 1999). Therefore, it was estimated that the NZT had approximately twice the phospholipid content as that in venison.

7-Ketocholesterol level

Amorphous white powder; FAB-MS (JMS-600 W/JEOL, m/z): 401 [M+H]⁺, 367, 264, 221, 147, 81, 55. ¹H-NMR (Bruker Biospin Avance II, 400 MHz, CDCl₃, δ): 0.61 (3H, s, 18-Me), 1.1 (3H, s, 19-Me), 0.92 (3H, d, 21-Me), 0.87 (3H, d, 6.6, 26-Me), 0.86 (3H, d, 6.6, 27-Me), 5.62 (1H, m, H-6), 3.60 (1H, m, H-3) and ¹³C-NMR (100 MHz, CDCl₃, δ_c): 202.4 (C-7), 165.2 (C-5), 126.1 (C-6), 70.5 (C-3), 54.8 (C-17), 50.0 (C-9, C-14), 45.4 (C-8), 43.1 (C-13), 41.8 (C-12), 39.5 (C-24), 38.7 (C-4), 38.3 (C-10), 36.3 (C-

1), 36.2 (C-22), 35.7 (C-20), 31.2 (C-2), 28.6 (C-16), 28.0 (C-25), 26.3 (C-15), 23.8 (C-23), 22.8 (C-26), 22.6 (C-27), 21.2 (C-11), 18.9 (C-21), 17.3 (C-19), 12.0 (C-18). 7-Ketocholesterol was identified using the various physicochemical data previously separated from New Zealand deer antler (Lee et al., 2014).

7-Ketocholesterol was isolated from the NZT-(RM: 0.067 mg/g; AE: 0.4 mg/g; WE: not detected). However, 7-ketocholesterol was also isolated from the NZA-mixture of tip, upple, middle, and base- (RM: 0.11 mg/g; AE: 2.89 mg/g; WE: not detected) (Lee et al., 2014). Therefore, the NZT-RM and NZT-AE levels of 7-ketocholesterol were much lower than that of the NZA-RM and NAA-AE, respectively.

SH composition

Four SHs (androstene-3,17-dione, 17β-estradiol, testosterone, and DHEA) in NZT were detected by HPLC-PDA (Fig. 1). As shown in Table 4, they were almost undetectable in the WE. SHs in the AE were approximately 6 times more abundant than those in the RM.

In addition, the testosterone level in the NZT's was much higher than that in cattle hair (approximately 6–15 ng/g) (Gleixner and Meyer, 1997). Eighteen SHs were detected from deer antler (Lu et al., 2013), but the level of each SH showed trace amounts. However, only four SHs were detected in NZTs, but the level of each SH was higher than that in deer antler (Lu et al., 2013).

Base and nucleoside composition

One base (uracil) and seven nucleosides (uridine, guanosine, inosine, deoxyguanosine, deoxyuridine, adenosine, and deoxyadenosine) were detected by HPLC-PDA from the NZT (Fig. 2). One base and seven nucleosides were identified by comparing the physicochemical data of the New Zealand deer antler's base and nucleosides (Lee et al., 2014). One base and seven nucleosides were more soluble in AE than they were in WE. As shown in Table 1, the order of the extract levels is AE>RM>WE. The order of the levels of one base and seven nucleosides was uridine>uracil>guanosine>inosine>deoxyguanosine>deoxyuridine>adenosine>deoxyadenosine.

In addition, one base and seven nucleosides were also isolated from the NZA-mixture of tip, upple, middle, and base-AE (base or nucleoside, mg/g; uridine, 5.53>guanosine, 3.95>adenosine, 3.42>uracil, 1.8>inosine, 1.58>deoxyguanosine, 0.79; deoxyuridine, 0.79>deoxyadenosine, 0.53) (Lee et al., 2014). Therefore, the levels of guanosine, adenosine, and deoxyadenosine were NZT<NZA. However, the levels of uracil, and uridine were NZA<NZT.

Fig. 1 The high-performance liquid chromatography-photodiode array (HPLC-PDA) chromatogram of steroid hormones. *DHEA* dehydroepiandrosterone

Fig. 2 The high-performance liquid chromatography-photodiode array (HPLC-PDA) chromatogram of one base and seven nucleosides

NANA level

Ganglioside is a derivative of N, O-substituted neuraminic acid, which is a compound that exhibits various activities by binding with sialic acid, a monosaccharide compound

with a backbone of nine carbon atoms, and various glycosphingolipids (Mocchetti, 2005). Representative sialic acids include NANA and 2-keto-3-deoxynonic acid.

No reports of NANA in venison have been found. However, the ganglioside literature on deer antler has been

reported extensively (Mocchetti, 2005). NANA, a partial structure of ganglioside was detected from NZA (Lee et al., 2014). As shown in Table 4, the order of the level of NANA was RM>WE>AE. As described above, the level of NANA was higher in WE.

Differentiation of each ingredient in RM, WE, and AE

The order of the ingredient levels in RM is crude protein, 719.2 mg/g (amino acids, 537.14 mg/g)>crude fat 74.8 mg/g (fatty acids, 38.30 mg/g)>ash 34.4 mg/g (minerals, 18.16 mg/g)>chondroitin, 12.5 mg/g>phospholipids, 8.81 mg/g>base and nucleosides, 8.49 mg/g>NANA, 3.01 mg/g>SHs, 97 µg/g>7-ketocholesterol, 67 µg/g.

The order of the ingredient levels in AE is crude fat, 470.9 mg/g>crude protein, 341.8 mg/g (amino acids, 194.0 mg/g)>fatty acids, 289.95 mg/g>phospholipids, 110.95 mg/g>ash 45.1 mg/g (minerals, 20.59 mg/g)>base and nucleosides, 15.4 mg/g>chondroitin, 13.5 mg/g>SHs, 1.181 mg/g>7-ketocholesterol, 0.4 mg/g>NANA, 0.11 mg/g.

The order of the ingredient levels in WE is crude protein 938.9 mg/g (amino acids 679.0 mg/g)>ash 25.5 mg/g>chondroitin 12.0 mg/g>minerals 10.1 mg/g>phospholipids 9.3 mg/g>base and nucleosides 3.46 mg/g>NANA 2.73 mg/g.

In conclusion, the major ingredients (protein, fat, mineral and chondroitin, phospholipid, sialic acid, base and nucleosides, and steroids) of NZTs were analyzed. NZT contained NANA, base and nucleoside, chondroitin, and 7-ketocholesterol, as did antler (Lee et al., 2014). However, 7 α -hydroxycholesterol and 7 β -hydroxycholesterol were not present in NZT. Additionally, the levels of testosterone and 17 β -estradiol are much higher in NZT than those in antler. The level of palmitoleic acid is much higher in NZT than that in venison. Additionally, the phospholipids were present at approximately twice the level than that in venison. In the case of protein, there are many constituent amino acids of collagen that are derived from leather part of NZT. Since the tail bone remained in the NZT, the Ca level was higher than that in venison.

Acknowledgments This work was funded by Korea Ginseng Corp.

Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

References

- AOAC. Official Methods of Analysis of AOAC. 16th ed. Association of Official Analytical Chemists, Arlington, VA, USA (1995)
- Baeurle SA, Kiselev MG, Makarova ES, Nogovitsin EA. Effect of the counterion behavior on the frictional-compressive properties of chondroitin sulfate solutions. *Polymer*. 50: 1805–1813 (2009)
- Bakke JM, Figenschou E. Volatile compounds from the red deer (*Cervus elaphus*) secretion from the tail gland. *J. Chem. Ecol.* 9: 513–514 (1983)
- Chang, IM. Treatise on Asian Herbal Medicines. Vol. I, Haksulpyun-sukwan press, Seoul, Korea. pp. 90–111 (2003)
- Chen HJ, Wang P, Chen Y, Li HW. Determination of total sialic acid content in human milk by HPLC-FLD. *Food Sci.* 32: 308–311 (2011) <http://www.spkx.net.cn/EN/> or <http://www.spkx.net.cn/EN/Y0/V1/I/308>
- Drew KR. Venison and other deer products. pp 225–226. In: The biology of deer. Brown RD (ed) Springer, New York, USA (1992)
- Falcó G, Llobet JM, Bocio A, Domingo JL. Daily intake of arsenic, cadmium, mercury, and lead by consumption of edible marine species. *J. Agric. Food Chem.* 54: 6106–6112 (2006)
- Gleixner A, Meyer HHD. Detection of estradiol and testosterone in hair of cattle by HPL/EIA. *Fresenius J. Anal. Chem.* 357: 1198–1201 (1997)
- Hattori M, Yang XW, Kaneko S, Nomura Y, Namba T. Constituents of the pilose antler of *Cervus Nippon* var. *mantchuricus*. *Jap. J. Pharmacogn.* 43: 173–176 (1989). <http://ci.nii.ac.jp/naid/110008908175>
- Kasai T, Sakai H, Ishijima Y, Hasegawa T. Proximate composition, properties of lipid and mineral contents in Yeso Sika deer (*Cervus nippon yesoensis*) meat. *Nippon Shokuhin Kagaku Kogaku* 46: 710–718 (1999). <https://doi.org/10.3136/nskkk.46.710>
- Kim YE, Lim DK, Shin SU. Biochemical studies on antler (*Cervus nippon* var. *taiouanus*) V. A study of glycolipids and phospholipids of antler velvet layer and pantocrin. *Korean Biochem. J.* 10: 153–164 (1977). www.newnonmun.com/article=10020017;http://www.ndsl.kr/ndsl/search/detail/article/articleSearchResultDetail.do?cn=JAKO197734755179946&SITE=CLICK
- KMFDS (Korea Ministry of Food and Drug Safety), Health Functional Food Testing Method. pp 249–250. In: 3.4.6. Chondroitin. III. KMFDS (ed), Cheongju, Korea (2012) http://www.mfds.go.kr/brd/m_218/view.do?seq=12711 Accessed Oct. 19, 2018.
- KMFDS (Korea Ministry of Food and Drug Safety), Health Functional Food Testing Method. pp 283–289. In: III. 3.5.2. Phospholipid. KMFDS (ed), Cheongju, Korea (2012) http://www.mfds.go.kr/brd/m_218/view.do?seq=12711 Accessed Oct. 19, 2018.
- Lee BY, Lee OH, Choi HS. Analysis of food components of Korean deer antler parts. *Korean J. Food Sci. Technol.* 30: 52–56 (2003)
- Lee NK, Shin HJ, Kim WS, Lee JT, Park CK. Studies on the chemical constituents of the New Zealand deer velvet antler *Cervus elaphus* var. *scoticus*-(I). *Nat. Prod. Sci.* 20: 160–169 (2014). <http://www.ksp.or.kr/>
- Lu C, Wang M, Mu J, Han D, Bai Y, Zhang H. Simultaneous determination of eighteen steroid hormones in antler velvet by gas chromatography–tandem mass spectrometry. *Food Chem.* 141: 1796–1806 (2013)
- Lund BC, Bever-Stille KA, Perry PJ. Testosterone and andropause: the feasibility of testosterone replacement therapy in elderly men. *Pharmacotherapy* 19: 951–956 (1999)
- Manley TR, Forss DA. Fatty acids of meat lipids from young red deer (*Cervus elaphus*). *J. Sci. Food Agric.* 30: 927–931 (1979)

- Metcalfe LD, Schmitz AA, Pelka JR. BF_3 -methanol procedure for rapid quantitative preparation of methyl esters from lipids. *Anal. Chem.* 38: 514 (1966)
- Mocchetti I. Exogenous gangliosides, neuronal plasticity and repair, and the neurotrophins. *Cell Mol Life Sci.* 62: 19–20, 2283–2294 (2005)
- Noh YH, Kim DH, Kim JY, Park J, Kim OH, Han D, Kim WY, Kim SS, Lee MY, Heo SH, Kim M, Lee WB, Jeong Y, Myung SC. Improvement of andropause symptoms by dandelion and rooibos extract complex CRS-10 in aging male. *Nutr. Res. Pract.* 6: 505–512 (2012)
- Sahan Y, Basoglu F, Gucer S. ICP-MS analysis of a series of metals (namely: Mg, Cr, Co, Ni, Fe, Cu, Zn, Sn, Cd and Pb) in black and green olive samples from Bursa, Turkey. *Food Chem.* 105: 395–399 (2007)
- United States department of agriculture (USDA), USDA food composition databases, <http://www.nal.usda.gov/fnic/foodcomp/search/%7CUSDA>. Nutrient Database, NDB Numbers 17348, 13434, 10023 and 17060 Accessed Jul. 13, 2018
- Varki A, Gagneux P. Multifarious roles of sialic acids in immunity. *Ann. N.Y. Acad. Sci.* 1253: 16–36 (2012)

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.