

NUTRITIONAL COMPOSITION IN LEG BONE EXTRACTS FROM JEJU CROSSBRED HORSES AT DIFFERENT AGE

Hee-Jin Kim¹, Ji-Yeol Yoon¹, Juae Gil¹, Dongwook Kim¹, Hyun-Seok Chae² and Aera Jang¹

¹Department of Animal Products and Food Science, Kangwon National University, Chuncheon 200-701, Korea,

²National Institute of Animal Science, RDA, Jeju 690-150, Korea

Abstract –The effects of horse age on the proximate composition, collagen content, fatty acid profile, amino acid content and mineral contents of horse leg bone extracts(HLBE) were investigated. Crude protein and crude ash have the highest value HLBE at 38 month age, but crude fat showed the highest mean content in HLBE at 28month. The 32 and 38 month at HLBE showed higher levels ($p<0.05$) of collagen content than the 28 month. The major fatty acids of HLBE were palmitic acid and oleic acid. Essential fatty acid (C18:2n6, C18:3n6 and C18:3n3), USFA and MUFA have the highest fatty acid content in HLBE of 38 month. The 38 month at HLBE showed higher levels ($p<0.05$) of mostly amino acid content than the 28 month. Age had no effect on Ca content of HLBE. K, Zn and Fe value of HLBE were significant increased ($p<0.05$) during the raising term.

Key Words – Horse, Bone extraction, Leg bone, Nutritional composition, Jeju.

I. INTRODUCTION

In Korea, horses are used for the purpose of riding, food, etc. Horse products are commercially available in various forms such as horse meat, horse powder, extracts, tablets and etc. In recent years, population of Jeju cross bred horses were increased. But, some of horse are used for race and most of them are used food [10].

Bone extracts have been consumed as a source of nutrition for humankind throughout the ages. The mineral contents of bone are composed mostly of calcium and phosphorus. Calcium prevents osteoporosis by increasing bone formation and inhibiting the bone loss [11]. And there is also cartilage with bone. The fiber ingredient is collagen and in the cartilage. The chondroitin sulfate content that is in mammalian cartilage and bone decreased with a get older [4].

A study found that the horse bone extracts effect longitudinal bone growth in adolescent

male rats [9]. Kim *et al.* [7] suggest that anti-oxidation and anti-wrinkling effect were observe in horse leg bone hydrolysates. However, there is still lack of information about horse bone. Also, there is a lack of research of nutritional composition in HLBE. Therefore, the aim of the present work was to evaluate and compare the the nutritional composition in HLBE from jeju between 28, 32 and 38 month age .

II. MATERIALS AND METHODS

After slaughtering 4 horses of 28 month age, 32 horses of 38 month age and 5 horses of 15 month old based on the age of Jeju cross bred horses. After washing horse leg bone, leg bone boiled the horse leg bone with 3 times of water, which was subsequently discarded. Then, extraction goes for eight hours with water weighing six times of horse leg bone, and this kind of extraction would be done three times to getlatin. The horse leg bone extract liquid was freeze-dried. Collagen contents of extracts from Jeju native horse leg bone was determined by method of Kolar *et al.*[8]. Crude protein, crude fat, crude ash, moisture analysis was performed by combustion using the AOAC method [1]. Mineral contents, Amino acid composition, Fatty acid composition of extracts from Jeju native horse leg bone was determined by method of AOAC[1]. For the statistical analysis of all results data, an analysis of variance (ANOVA) of one way using General Linear Model (GLM) procedure SAS software (ver. 9. SAS Institute Inc., USA) was performed for all variables considered in the study. When treatment effects were significant ($P < 0.05$) mean values were compared with Duncan's multiple range test.

III. RESULTS AND DISCUSSION

Table 1 Proximate composition (%) of extracts from Jeju native horse leg bone

Age (month)	Moisture	Crude protein	Crude fat	Crude ash
28	93.05±0.044 ^a	5.20±0.194 ^b	1.65±0.019 ^a	0.10±0.000 ^c
	92.82±0.347 ^a	6.09±0.562 ^a	0.93±0.043 ^b	0.16±0.044 ^b
38	92.75±0.225 ^a	6.42±0.254 ^a	0.61±0.090 ^c	0.22±0.034 ^a

^{a-c}Mean±SE within same column with different superscript letters differ significantly at $p<0.05$.

Proximate composition of extracts from Jeju native horse leg bone for different age is given in Table 1. The percentage of moisture (92.75 - 93.05%) did not differ between the treatments. The crude protein content in HLBE of 28 month age (5.20%) was lowest ($p<0.05$). The crude ash of HLBE was significant increased ($p<0.05$) during the raising term, but the Crude fat of HLBE was significant decreased ($p<0.05$), during the raising term. Duerr and Earle[2] show that fat and ash of beef bone extracts were 1.14-1.42% and 0.14-0.2% respectively. This result of fat and ash is similar with our result crude fat and crude ash.

Table 2 Collagen contents (g/100ml) of extracts from Jeju crossbred horse leg bone

Age (month)	Extraction					
	1 st	2 nd	3 rd	4 th	5 th	6 th
28	1.59±0.057 ^{ba}	1.16±0.021 ^{bc}	1.12±0.027 ^{bd}	1.20±0.016 ^{ab}	0.77±0.019 ^{be}	0.57±0.007 ^{bf}
	1.79±0.097 ^{aA}	1.59±0.144 ^{aB}	1.31±0.197 ^{aC}	1.15±0.080 ^{aCD}	1.21±0.110 ^{aCD}	1.09±0.103 ^{aD}
38	1.54±0.101 ^{ba}	1.48±0.089 ^{aA}	1.42±0.097 ^{aAB}	1.35±0.039 ^{aBC}	1.31±0.072 ^{aC}	1.25±0.059 ^{aC}

^{a-b}Mean±SE within same column with different superscript letters differ significantly at $p<0.05$.

^{A-F}Mean±SE within same row with different superscript letters differ significantly at $p<0.05$.

Collagen contents of extracts from Jeju native horse leg bone for different age and number of extraction is given in Table 2. HLBE of 32 month age at 1st Extraction was the highest with 1.79 g/100ml. The collagen content has highest in the first extraction at all treatment groups, and has lowest in the sixth extraction at all treatment groups. Because of their excellent bioactivity, good biocompatibility and non-irritation of the body, collagen is used as important active components[6].

Table 3 Fatty acid composition of extracts from Jeju cross breed horse leg bone

Fatty acid (%)	Age (month)		
	28	32	38
C14:0	1.79±0.014 ^a	1.97±0.660 ^a	1.36±0.064 ^a
C16:0	32.73±0.141 ^a	30.92±0.746 ^b	28.83±0.389 ^c
C16:1n7	4.46±0.134 ^b	4.62±0.599 ^b	5.82±0.402 ^a
C18:0	6.46±0.078 ^a	6.10±0.351 ^a	4.95±0.428 ^b
C18:1n9	31.55±0.311 ^{ab}	30.09±1.118 ^b	32.23±0.318 ^a
C18:1n7	3.52±0.247 ^a	3.09±0.235 ^b	2.33±0.093 ^c
C18:2n6	14.30±0.212 ^b	17.15±0.866 ^a	18.12±0.259 ^a
C18:3n6	0.00±0.000 ^b	0.00±0.000 ^b	0.02±0.016 ^a
C18:3n3	4.21±0.120 ^b	5.11±0.748 ^{ab}	5.41±0.177 ^a
C20:1n9	0.25±0.021 ^b	0.24±0.061 ^b	0.44±0.050 ^a
C20:4n6	0.51±0.007 ^a	0.50±0.042 ^a	0.33±0.027 ^b
C20:5n3	0.05±0.007 ^a	0.04±0.004 ^a	0.04±0.004 ^a
C22:4n6	0.12±0.057 ^{ab}	0.15±0.039 ^a	0.04±0.003 ^b
C22:6n3	0.06±0.021 ^a	0.03±0.008 ^b	0.06±0.002 ^a
SFA	40.98±0.212 ^a	38.98±0.836 ^b	35.14±0.672 ^c
USFA	59.02±0.212 ^c	61.02±0.836 ^b	64.86±0.920 ^a
w3/w6	0.29±0.000 ^a	0.29±0.051 ^a	0.30±0.009 ^a
MUFA	39.78±0.042 ^{ab}	38.04±1.129 ^b	40.81±0.689 ^a

^{a-c}Mean±SE within same row with different superscript letters differ significantly at $p<0.05$.

SFA: Saturated fatty acid, MUFA: Monounsaturated fatty acid, PUFA: Polyunsaturated fatty acid

The fatty acid composition of extracts from Jeju cross breed horse leg bone for different age is presented in Table 3. The three most abundant fatty acids in HLBE were palmitic acid, oleic acid and linoleic acid. The C16:0 values of HLBE were significant decreased ($p<0.05$) during the raising term. The C18:1n9 value was higher ($p<0.05$) in HLBE of 38 month than in HLBE of 32 month. The HLBE of 38 month have a value of C18:2n6, C18:3n6 and C18:3n3, they are essential fatty acid, is the highest. ($p<0.05$).

Amino acid contents of extracts from Jeju cross breed horse leg bone for different age is presented in Table 4. Amino acid contents of HLBE were rich glycine, proline, glutamic acid, alanine, and arginine.

Table 4 Amino Acid contents of extracts from Jeju cross breed horse leg bone (% in sample)

Amino Acid	Age (month)		
	28	32	38
Aspartic acid	3.81±0.050 ^c	4.07±0.025 ^b	4.82±0.005 ^a
Threonine	1.15±0.065 ^b	1.22±0.000 ^b	1.44±0.025 ^a
Serine	1.92±0.045 ^c	2.15±0.010 ^b	2.54±0.005 ^a
Glutamic acid	6.70±0.050 ^c	7.14±0.015 ^b	8.53±0.010 ^a
Proline	6.99±0.030 ^b	7.01±0.000 ^b	8.55±0.090 ^a
Glycine	13.17±0.030 ^c	13.84±0.040 ^b	17.40±0.060 ^a
Alanine	4.96±0.125 ^b	5.17±0.010 ^b	6.35±0.005 ^a
Valine	1.92±0.085 ^b	2.10±0.010 ^b	2.47±0.025 ^a
Isoleucine	0.87±0.010 ^b	0.92±0.010 ^b	1.05±0.020 ^a
Leucine	2.25±0.020 ^c	2.40±0.015 ^b	2.71±0.035 ^a
Tyrosine	0.09±0.020 ^b	0.34±0.045 ^a	0.39±0.045 ^a
Phenylalanine	1.36±0.040 ^b	1.53±0.035 ^{ab}	1.73±0.060 ^a
Histidine	1.22±0.020 ^a	1.06±0.035 ^b	1.02±0.020 ^b
Lysine	2.25±0.010 ^c	2.41±0.015 ^b	2.98±0.045 ^a
Arginine	4.62±0.165 ^b	4.71±0.035 ^b	5.86±0.090 ^a

^{a-c}Mean±SE within same row with different superscript letters differ significantly at $p<0.05$.

Golan and Jelen [5] suggest that amino acid contents of beef bone were abundant glycine, glutamic acid, alanine and arginine. They value of HLBE were significant increased ($p<0.05$) during the raising term. The HLBE of 38 month was highest content at essential amino acids that are threonine, valine, isoleucine, leucine, phenylalanine and lysine (1.44, 2.47, 1.05, 2.71, 1.73, 2.92%).

Table 5 Mineral contents of extracts from Jeju cross breed horse leg bone (mg/ml)

Mineral contents	Age (month)		
	28	32	38
Mg	9.86±0.106 ^a	8.77±0.764 ^b	8.26±0.204 ^b
P	46.52±1.067 ^b	54.22±4.256 ^b	92.35±9.284 ^a
K	110.53±9.041 ^c	199.23±14.016 ^b	268.92±6.487 ^a
Ca	56.28±3.894 ^a	55.81±3.982 ^a	49.92±1.526 ^a
Mn	>0.0012 ^b	>0.0012 ^b	0.81±0.000 ^a
Zn	1.25±0.240 ^c	1.90±0.327 ^b	8.62±0.059 ^a
Cu	1.19±0.211 ^{ab}	1.88±0.902 ^a	0.52±0.145 ^b
Se	>0.0915 ^b	>0.0915 ^b	0.28±0.112 ^a
Na	558.54±28.376 ^c	659.97±49.363 ^b	736.60±14.631 ^a
Fe	3.69±0.251 ^c	5.44±1.003 ^b	17.50±0.284 ^a

^{a-c}Mean±SE within same row with different superscript letters differ significantly at $p<0.05$.

Mineral contents of extracts from Jeju cross breed horse leg bone for different age is

presented in Table 5. Na is the predominant element, and has the highest mean content ($p<0.05$) in HLBE of 38 month age(736.60 mg/ml) and the lowest mean content in HLBE of 38month age (558.54 mg/ml). Ca showed no significant difference among other month age. K is an essential mineral that is essential for the homeostatic balance of body fluids [3]. K has the highest value in HLBE of 38 month age. Zn and Fe values of HLBE were significant increased ($p<0.05$) during the raising term. HLBE of 38 month age (92.35 mg/ml) contained significantly higher P content compared to HLBE of 28 and 32 month age (46.52 and 54.22 mg/ml). But most of mineral contents has the highest in HLBE of 38 month age.

IV. CONCLUSION

This study investigated comparative analysis of nutritional composition in HLBE from Jeju Crossbred Horses between 28, 32 and 38 month. Crude protein, crude ash, collagen have the highest mean content in HLBE of 38 month, but crude fat has the lowest mean content in HLBE of 38 month . HLBE of 38 month contained significantly higher C18:2n6, C18:3n6 and C18:3n3 acid content compared to HLBE of 28 month. It can clearly be seen that the horse age has a marked effect on the amino acid content at HLBE. The 38 month was found to have higher essential amino acid than the 28 month. P, K, Mn, Zn, Se, Na and Fe have the highest in HLBE of 38 month age. Therefore, the HLBE 38 month old age has been shown to have better nutritional composition than the HLBE of 28 and 32 month old age.

ACKNOWLEDGEMENTS

This work was carried out with the support of the “Cooperative Research Program for Agriculture Science & Technology Development (Project No. PJ009417)” Rural Development Administration, Republic of Korea.

REFERENCES

1. AOAC (1990). official methods of analysis (15th ed, 1st suppl.). Arlington, VA: Association of Official Analytical Chemistry.

2. Duerr, P. E. and Earle, M. D. (1974) The extraction of beef bones with water, dilute sodium hydroxide and dilute potassium chloride. *Journal of the Science of Food and Agriculture* 25: 121–128.
3. FAO (Food, Nutrition and Agriculture), Alimentation, nutrition et agriculture. Alimentación, nutrición y agricultura, (2009) www.fao.org/docrep/x2650T/x2650T00.htm.
4. Gilbreath, R.L., Di Marco, G. R. and Vander Noot, G. W. (1971). Age and muscle-related differences of acid mucopolysaccharides in bovine muscle tissue. *J. Anim Sci.* 32(4): 620-3.
5. Golan, A. and Jelen, P. (1979) Nutrition evaluation of low temperature alkaline extracts from beef bone. *Journal of food sciences.* 44(2): 332.
6. Kamara, M. T., Zhu, K., Amadou, I., Tarawalie, F. and Zhou, H. (2009). Functionality, in vitro digestibility and physicochemical properties of two varieties of defatted foxtail millet protein concentrates. *Int. J. Mol. Sci.* 10: 5224–5238
7. Kim, D. W., Kim, H. J., Chae, H. S., Park, N. G., Kim, Y. B. and Jang A. R. (2014). Anti-oxidation and anti-wrinkling effects of Jeju horse leg bone hydrolysates. *Korean J. Food Sci. An.* 34:844-851..
8. Kurt, K. (1990). Colorimetric determination of hydroxyproline as measure of collagen content in meat and products: NMKL Collaborative Study. *J. Assoc. Off. Anal. Chem.*, 73(1): 55.
9. Lee, S. N., Choi, H., Son, J. B., Jeong, J.S., Kim, Y.J., Kang, D.P., Park, S.Y., Kang, S.C. and Leem, K.H. (2009). Effects of Horse Bone Powder Extract on Longitudinal Bone Growth in Adolescent Male Rats *Kor. J. Herbology.* 24(3) : 69-77.
10. Lee, C.E., Seong, P. N., Oh, W. Y. and Kim, K. I. (2005). Effects of Castration on Growth and Meat Quality in Finishing Male Jeju Horses. *J. Anim. Sci. and Technaol.(Kor.)*. 47(3):391-396
11. Reid, I. R. and Ibbertson, H. K. (1986). Calcium supplements in the prevention of steroid-induced osteoporosis. *Am. J. Clin.Nutr.* 44: 287-290.