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Morphological Characterization of Donkeys (*Equus asinus*) and Molecular Identification of its Meat Produced in Korea

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Abstract: This study aimed to investigate the morphological characteristics of donkey (*Equus asinus*) bred in South Korea. To prevent the sale of donkey meat as horse meat, we developed a method to differentiate horse meat from donkey meat in Korea. The results of physical characterization of 79 domestic donkeys revealed that the ear length was 17-28 cm (mean, 23 cm); body height was 90-135 cm (mean, 118.3 cm); body length was 109-150 cm (mean, 131.2 cm) and body color was white, chestnut, gray, black and brown. We obtained 689 bp polymerase chain reaction products from donkey meat and 620 bp products from horse meat (6 horses were used for analysis) in order to differentiate the meat.

Key words: Donkey, Korea, meat differentiation, morphological characteristics, chain reaction, products from

INTRODUCTION

The Equidae family includes a single genus Equus which is classified into four subgenera having eight species. The subgenus Equus includes *E. caballus* (domestic horses and Przewalski's or Mongolian wild horse), subgenus Asinus includes *E. asinus* (donkey), *E. hemionus* (Onager, Asian wild asses) and *E. kiang* (Kiang). The subgenus Dolichohippus includes only *E. grevyi* (Grevy's zebra) and the subgenus Hippotigris includes *E. zebra* (mountain zebra), *E. burchelli* (Burchell's or plains zebra) and *E. quagga* (Quagga) (Nowak and Paradiso, 1983).

The morphologically distinct feature of wild *E. asinus* is chestnut coloration only on the forelimb. The other features include a long, thin scruffy mane; thin dorsal stripe, usually traces of stripes on the legs (at least fetlocks) and a restricted white (or pale) region on the underside. This species has a very long cranium, short palate long, diastema large, pterygopalatine fossa and a squared, upturned occipital crest. The nasal end of the pre-maxilla is narrow and insinuated into a corner of the narial notch. The orbit is high, rounded and not anteroposteriorly elongated. Post-cranially, very short, narrow distal phalanges, translating externally to small, narrow hooves are present. *E. africanus* (African wild ass) turns reddish in Summer and greyer in Winter with contrasting white legs and a less contrasting whitish underside; the white wedges behind the shoulder and in front of the haunch, highly prominent in hemiones are evanescent. *E. africanus* is classified into three subspecies. *E. africanus* africanus (Nubian wild ass, cross-shaped black stripe) is approximately 115-121 cm in size. A dorsal stripe is always present and is nearly always complete from the mane to tail tuft; it is crossed by a usually thin, fairly short shoulder stripe. Leg stripe where present are restricted to a few bands at the fetlocks. The diastema is relatively short and the post-orbital constriction is well marked. The nasal process of the pre-maxilla ends bluntly. A bridge is never present between the metaconid and metastylid in the lower premolars and molars. The Nubian wild ass is probably not the ancestor of the domestic donkey. *E. africanus* somaliensis (Somali wild ass) have short ears 188-200 mm with height of 120-125 cm; the dorsal stripe is often absent and when present is often incomplete, broken at some point along the dorsum. Shoulder cross is often absent. Leg stripes are present from the hooves to above the carpus and tarsus. Diastema is relatively long and the post-orbital constriction is less marked. A thickened bar of bone is present behind the orbits, marking the highest point on the profile. The nasal process of the pre-maxilla is thin and pointed. At least a trace of a bridge is always present between the metaconid and metastylid in the lower premolars and molars. Somalia asses seem to have longer legs and shorter

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bodies than Nubian ones. There are three known kinds including *E. africanus* subsp. (Saharan wild ass) (Groves and Ryder, 2000).

The domestication of *E. asinus* has been assumed to have occurred in around 6,000 BC in North Africa (Egypt area) from the Nubian and Somali wild asses (Beja-Pereira *et al*., 2004; Rossel *et al*., 2008).

The height of *E. asinus's* body varies considerably between 61 cm (Sicilia *E. asinus*) and 168 cm (American *E. asinus*) depending on the species; it has been reported to be approximately 102 cm on average. The coat color also varies such as white, gray and black and a black stripe is present from the mane to the tail and another stripe is located sideways on the shoulders. The mane is short and straight up and the shape of the tail with long hairs only at the tip is closer to that in a cow than in a horse. The ears are very long and the base and end are black.

Immediately after the domestication of *E. asinus*, the hybridization between horse and *E. asinus* was performed in around 5,000 BC (Allen and Short, 1997; Short, 1997). *E. asinus* has been used by humans for centuries and its most important role is transportation. *E. asinus* still remains an important work stock animal in poor territories (Polidori and Vincenzetti, 2012).

Approximately 5.9 million heads of work stock *E. asinus* occur globally and most of them are essential sources of economy for poor communities in 3rd world countries. Unlike for other whole-hoofed animals, studies on *E. asinus* are not sufficient. They are domestically bred mostly in Gyeonggi-do, Jeonla-do, Gyeongsang-do and Gangwon-do regions. The purposes of breeding are mostly for experience, tourist carriage or meat but no basic data for the utilization of domestically bred donkeys and preservation of blood are available. Although, the social interest or the value of donkey as a genetic resource is increasing as well as considering the mildness and obedience of donkey, superiority to horse, ease of handling and management specification and health administration easier than those for horses, relatively very few research studies have been performed on donkeys.

To the knowledge, this is the first study to investigate the morphological characteristics of donkey (*E. asinus*) and molecular identification of its meat produced in South Korea.

MATERIALS AND METHODS

Animals: About 79 mixed breed donkeys (21 males and 58 females; mean age, 2.7 years) reared for leisure and meat production from four farms (Yeongweol, Icheon, Sunchang and Changwon) were investigated for their morphological characteristics (ear size, body height and body length, coat color and stripe). The analysis for meat differentiation was conducted using two donkeys, two Thoroughbred horses, one mule and one Mongolian horse.

Investigation of morphological characteristics: Morphological characteristics measured were ear length, body height and body length. The body height was measured from the ground to withers; the body length was from the chest to the hip area and the ear measurement was from the beginning area of the ear to the edge. The characteristics of coat color and black stripes in donkey were investigated.

DNA extraction and polymerase chain reaction: Meat differentiation was performed using Polymerase Chain Reaction (PCR) according to the method by Franco *et al*. (2016) with slight modification. Genomic DNA was extracted from the blood and meat by using a MagExtractor System MFX-2000 (Toyobo, Osaka, Japan) according to the manufacturer's protocols (Tozaki *et al*., 2001). PCR was performed using 50 ng genomic DNA in 20 μL PCR mix (1 U Taq DNA polymerase, 800 μM dNTP mix, 1.25 mM $MgCl_2$ and 7.5 pmol each primer) by using the following conditions: 95°C for 3 min, 36 cycles of 95°C for 30 sec, 50°C for 30 sec and 72°C for 1 min, followed by 72°C for 10 min. The forward primer was designed to hybridize with a conservative region of the mitochondrial DNA D-loop of horse and donkey (5′-CTGGCATCTGGTTCTTTCTT-3′). The reserve primers were designed to hybridize a polymorphic region of D-loop in horse (5′-GGTTTGGCAAGATTGTGTTG-3′) and donkey (5′-GTGTGTGAGAGTTAGGCTTC-3′) which allowed the amplification of specific PCR fragments (Franco *et al*., 2016). PCR products were identified using Mupid-2 Plus Electrophoresis (Optima JP, Tokyo, Japan) on a 2.5% agarose gel at 100 V for 30 min.

RESULTS AND DISCUSSION

Morphological characteristics of donkey: The morphological characteristics of 79 donkeys bred in South Korea are shown in Table 1. The ear length was 17-28 cm (average, 23 cm); body height, 90-135 cm (average, 118.3 cm) and body length, 109-150 cm (average, 131.2 cm). The distribution of coat colors was white (7/79, 8.9%), chestnut (25/79, 31.6%), gray (29/79, 36.7%), black (13/79, 16.5%) and brown (5/79, 6.3%) with dorsal stripes.

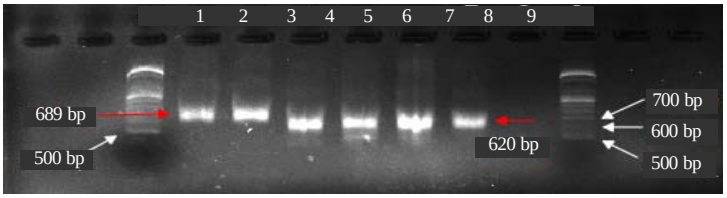

Fig. 1: Agarose gel electrophoresis of Polymerase Chain Reaction (PCR) amplicons from four representative animals Lanes 1and 9, molecular marker, 100 bp ladder; donkey meat and blood (lanes 2 and 3); Mongolian horse blood (lane 4); Thoroughbred horse meat and blood (lanes 5 and 6); mule blood (lane 7) and PCR negative control (lane 8)

Table 1: Physical characterization of 79 donkeys

Ear length (mean) (cm)	Body height (mean) (cm)	Body length (mean) (cm)	Coat colors	Remarks
17-28 (23)	90-135 (118.3)	109-150 (131.2)	White, gray, chestnut, black, brown	Dorsal stripe

Differentiation of meats by using PCR: The results of differentiation of meats using the PCR technique by using 6 horses are shown in Fig. 1. All genotyped animals (donkeys, horses and mules) presented D-loop amplicons of the expected size in 100% accordance with their breeding records. Donkey meat yielded 689 bp PCR products and could be differentiated from other horse meats that yielded 620 bp PCR products.

To meet the demands of domestic donkey markets and ensure better quality donkey resources and meat, it is most important to secure donkeys that are well adapted to the domestic feeding environment and having excellent blood characteristics. However, very few studies have investigated the breeding scale and other characteristics of domestically bred donkeys.

The results of morphological characterization and coat color of domestically bred donkeys were similar to those reported by several previous studies (Groves and Ryder, 2000). Thus, most of the domestically bred donkeys might have originated from the northern regions such as China and Mongolia as revealed by studies performed using microsatellite markers and mitochondrial DNA.

Horse meat and horse by-products is considered as Taboo in South Korea. Horses are usually utilized for racing or riding, therefore, they are treated as companion animals and almost not consumed in inland, except the Jeju Island area. However, some donkeys are used for experience or tourist carriage but usually bred for the purpose of eating rather than as companion animals.

Donkey meat attracts attention in the future stock-breeding market as advanced meat along with horse meat and Korean beef. Its texture is soft, sweet and clean. Currently, it is consumed at some fine dining restaurants in Asian markets such as China and Hong Kong.

Donkeys are expected to become a high-value genetic resource in the future in South Korea. In addition, donkey meat might be disguised as horse meat. To monitor this, protecting breeding farms by developing a donkey meat traceability system such as that for cattle is necessary. The method of differentiating meat by using PCR suggested in this study might be useful for the differentiation of donkey meat in the future. We believe that this method might permit a rapid, cheap and easy way to differentiate donkey and horse meat. Further, the demands of domestic donkey market can be met and better-quality donkeys and donkey meat can be produced by breeding donkeys with excellent bloodlines and formulating and enhancing the donkey breeding technology. Efforts should be undertaken to promote the utilization of donkey and help increase farmer's income by conducting more studies on domestically bred donkeys in the future.

CONCLUSION

In conclusion, the morphological characteristics of donkey (*E. asinus*) and molecular identification of its meat produced will be useful to promote the utilization of donkeys in South Korea.

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