Article

Nutritional composition of equine meat and degree of substitution of bovine for equine meat in stores in Mexico City

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Abstract:

The degree of substitution of bovine meat for equine meat in different points of sale of the different boroughs of Mexico City was evaluated, and the benefits or similarities with bovine meat as a strong alternative of nutrients were identified. One hundred sixty-one samples of bovine meat were collected, which were subjected to near-infrared reflectance spectroscopy (NIR's – Food Scan) to determine nutritional composition (moisture content, protein, fat and collagen), meat color (Hunter Lab L*, a* and b*) and polymorphism of repeated sequences to characterize the bovine or equine origin by polymerase chain reaction in agarose gels (PCR). The variables of nutritional composition and meat color were analyzed in a completely randomized design. It was found that nine of the samples were positive for equine meat and 152 samples were positive for bovine meat; resulting in that

5.59 % of bovine meat was replaced by equine meat in the commercialization centers in Mexico City. Likewise, the moisture, protein, fat and collagen content fluctuated between the samples from 73.1 to 75.1 %, from 22.0 to 23.5 %, from 2.0 to 2.3 %, and from 1.3 to 1.4 mg g⁻¹, respectively; observing a slight increase (P<0.05) in the concentration of moisture and protein in bovine meat compared to equine meat. The L* (luminosity) of meat between animal species was different (P<0.05); while in the indicators of meat color a* (from red to green) and b* (from yellow to blue), it fluctuated from 29.80 to 37.50 and 15.00 to 16.20 (P>0.05). It is concluded that the percentage of substitution of bovine meat for equine meat (5.59 %) is considered low and constitutes consumer fraud. However, equine meat has the potential to be a viable alternative for human consumption, as the nutritional composition was similar to bovine meat.

Key words: Meat, Bovine, Equine, PCR, Substitution.

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Introduction

In recent years, molecular techniques for the identification of the species origin of meat and its by-products have been developed^(1,2). Especially, the use of techniques of polymorphism of repeated sequence and polymerase chain reaction (PCR), which have been effective in differentiating between wild Asian ass (*Equus hemionus*) meat and domestic horse (*Equus domesticus*) meat from markets in the city of Ulaanbatar, Mongolia⁽³⁾. Also, the PCR technique has been used to determine the origin of different animal species in concentrates, flours or meat by-products destined to food or animal food^(4,5,6).

Therefore, it is important to note that meat is considered one of the main sources of nutrients to meet nutritional requirements in human food; due to its high content of protein of high biological value, its contribution of minerals, vitamins⁽⁷⁾, essential fatty acids and fat-soluble vitamins⁽⁸⁾. In this sense, meat for human consumption comes mostly from bovine, porcine and avian species, and to a lesser extent meat from sheep, goats, fish and some wild species⁽⁹⁾. However, socioeconomic, environmental and nutritional trends have generated in the last decade a growing interest in alternatives to replace bovine meat⁽¹⁰⁾. Regarding the above, an alternative that has been used to replace bovine meat is equine meat, which supplies 0.25 % of world meat production⁽¹¹⁾.

Mexico is one of the main producers of equine meat and during 2013 contributed 11.2 % (83,500 t) of world production (745,966 t), exporting 17.3 % of national production⁽¹²⁾. While 82.7 % of equine meat production remains in the country and is mainly used to supply the pet and zoo animal food industry. However, the level of acceptance and consumption of equine meat for humans is not favorable because its commercialization is not known to the consumer⁽¹³⁾. This allows a fraudulent commercialization route that could promote the substitution of bovine for equine meat and a price premiun, since the economic cost of selling equine meat is well below bovine meat in butcher shops, and stores of this item in the large cities of the country⁽¹⁴⁾.

On the other hand, equine meat has nutritional values equivalent to other conventional meats⁽¹⁵⁾. However, it can present clear risks to human health, as they are not reared for food production, they are treated or injected with various chemicals, dangerous for humans, many of which are prohibited for use in farmed animals⁽¹⁶⁾. For this reason, it is important that the consumer is informed about the origin of the meat, nutritional quality and the market price. It is therefore important to evaluate the degree of substitution of bovine for equine meat in different points of sale of the different boroughs of Mexico City and to identify the benefits or similarities with the bovine meat as a strong alternative of nutrients.

Material y methods

Sampling sites

Meat samples were collected in 69 commercialization centers, including the main distribution centers such as La Merced, Calle 7, Rastro Viejo, Central de Abastos and Ferrería, located in the different boroughs of Mexico City.

Sampling design

One hundred sixty-one (161) samples of bovine meat were collected; 23 samples were from butcher shops in large distribution centers and 138 of borough markets, sampling a total of 69 butcher shops that correspond to 22.5 % of total butcher shops in Mexico City (307).

The sampled bovine meat commercialization points were identified on the portal of the Institute for Access to Public Information and Protection of Personal Data (IPPDP, for its acronym in Spanish) of Mexico City. Considering the following assumptions: (a) the economic level (high, medium and low) of the region and (b) that the sampling be carried out randomly, distributed homogeneously in all the borough regions (North, South, East and West). The number of samples were determined with the central limit theorem (CLT) with a standard deviation of 8.9 % according to preliminary tests⁽¹⁷⁾, being random the selection of the market. Likewise, that of the establishments, two markets were selected for each borough (Table 1), being representative of the total number of points of sale under the CLT at a standard deviation of 7.3% in the sampling variables.

Mexico City				
Demonsh	Butcher shop			
Borough	Total	Sampled	Samples collected	
Álvaro Obregón	15	3	6	
Azcapotzalco	20	5	10	
Benito Juárez	16	4	8	
Coyoacán	17	4	8	
Cuajimalpa	5	1	2	
Cuauhtémoc	34	8	16	
Gustavo A. Madero	49	11	22	
Iztacalco	17	4	8	
Iztapalapa	20	5	10	
La Magdalena	5	1	2	
Miguel Hidalgo	19	4	8	
Milpa Alta	9	1	2	
Tláhuac	19	4	8	
Tlalpan	18	4	8	
Venustiano Carranza	33	8	16	
Xochimilco	11	2	4	
Centros de comercialización	-	-	23	

Table 1: Number of markets, sampled markets and number of samples per borough of

Sample size

The sample of raw meat was 250 g obtained from the cut called sirloin steak (*Biceps femoris*) and simulating the usual conditions of the consumers. To reduce the risk of contamination during the sampling period, these were kept in identified and sealed plastic bags, which were kept inside an isothermal container at a temperature of 4 °C. Once the samples of a day of sampling were collected, they were taken to the Laboratory of the Postgraduate Degree in

Animal Production for separation into subsamples and storage in freezing at -20 °C until their several analysis.

Nutritional composition of meat

The moisture, protein, fat, and collagen contents of the meat were determined by nearinfrared reflectance spectroscopy (NIR's) in the Foodscan Meat Analyzer (FOSS®, Denmark). One hundred eighty grams of sample was weighed and a grinding was carried out with a Picalica food processor (Moulinex®, France) for 30 sec (two series of 15 sec) according to the methodology proposed by the AOAC⁽¹⁸⁾.

Determination of meat color

The color of the meat sample was determined in the Miniscan (Hunterlab®, USA), performing five readings per sample in an upper, lower, right, left and center quadrant according to the methodology proposed by the International Commission of L'Eclairage (1976)⁽¹⁹⁾.

DNA extraction from meat tissue

For the identification of the species of bovine or equine meat, the extraction of deoxyribonucleic acid (DNA) from the initial sample of 250 g was carried out, 500 mg of meat were taken, placed in microtubes and frozen at -80 °C for 48 h in deep-freezing (ThermoSientific®, model 2186). Subsequently, the samples were lyophilized (Labconco®, model Freezone 4.5) for 5 d and the dried tissue was ground with the help of a TissueLyser II® cell disruptor (Qiagen, Germany). Once the sample was ground, the extraction procedure of DNA was performed, placing in each microtube 1 ml of lysis solution [Tris base (C4H₁₁NO₃) 50mM pH8, EDTA (C₁₀H₁₆N₂O₈) 0.1M, SDS (NaC₁₂H₂₅SO₄) 0.5%, 7 μ l of proteinase K] and they were incubated at 50 °C for 2 continuous hours. Afterwards, 500 μ l of Phenol:Chloroform:Isoamyl (C₆H₅OH: CHCl₃:C₅H₁₂O) (12:24:1) was added and it was centrifuged at 10,000 rpm for 10 min (Centrifugue eppendorf 5810 R). The supernatant was transferred to another microtube, adding 1 ml of 70 % ethanol (C₂H₅OH) at a temperature of -20 °C, mixing by inversion until DNA precipitated. Finally, it was

centrifuged at 10,000 rpm for 10 min, a pellet formed and it was dried in a vacuum centrifuge (Vacufuge plus). To resuspend the DNA pellet, 50 μ l of molecular grade H₂O was used.

PCR test for determination of the species of the meat

Prior to DNA amplification by PCR, it was verified that the extracted DNA was sufficiently pure and free of protein contamination. To measure the concentration of DNA, a Nanodroop® spectrophotometer (Thermo Scientific, model ND-100) was used under the following indicators: DNA has a maximum absorbance at 260 nm (50 μ g/mL have an OD at 260=1), while proteins have it at 280 nm. DNA purity was calculated, considering absorption between A260/A280 (1.9 and 1.7).

Table 2 shows the sequences selected from the literature of oligonucleotides used for the amplification of specific DNA fragments by animal species⁽¹⁴⁾; these consisted of a forward universal primer and the equine and bovine species-specific reverse oligonucleotides. Amplification of the specific fragments was performed by conventional PCR. DNA amplification by PCR was carried out considering a final volume of 50 μ L [5 μ L of 10 x PCR buffer, 1 μ L of 2 0 μ M of dNTPs, 2 μ L of universal oligonucleotide [10 pmol/ μ L], 2 μ L of equine oligonucleotide [10 pmol/ μ L], 2 μ L of bovine oligonucleotide [10 pmol/ μ L], 0.25 μ L of Taq polymerase (Roche®), 250 ng of DNA and 32. 75 μ L of nuclease-free PCR-grade water]. For the amplification of the selected sequences, a Maxygen (Axygene®) thermocycling program was used, which consisted of: an initial denaturation phase, in which the reaction mixture was maintained at 94 °C for 30 sec so that the two template DNA strands separated. The reaction mixtures were then subjected to 35 cycles of three stages each [alignment (60 °C for 30 sec), extension (72 °C for 30 sec) and refrigeration (4 °C)].

Table 2: Sequence of pairs of oligonucleotides for the determination of fresh meat cuts for bovine and equine from different borough markets of Mexico City

Species	Primer	Sequence	Size
Universal	Forward	GAC CTC CCA GCT CCA TCA AAC ATC TCA TCT	NA
Bovine	Reverse	CTA GAA AAG TGT AAG ACC CGT AAT ATA AG	274 bp
Equine	Reverse	CTC AGA TTC ACT CGA CGA GGG TAG TA	439 bp

NA=Not reported.

Electrophoresis gel

Once the reactions were finished, 5 μ L amplified fragments (amplicons) of the PCR products of the samples were taken to be analyzed by conventional electrophoresis. Five microliters of the amplicon was mixed with 3 μ L of 5x loading buffer, placing 8 μ L in each well of agarose gel [Seakem (Lonza®) 3 % (P/V) in 1,500 ml TAE 1x, with 25 μ L of Ethidium Bromide (Invitrogen®)]. It was run at 100 Volts for 45 min to later perform the reading of amplicons in a UV light photodocumenter. The similarity of the selected sequences was analyzed by applying the BLAST® software (http://www.ncbi.nlm.nih.gov/BLAST).

Statistical analysis

The variables of nutritional quality of bovine and equine meat (moisture, protein, fat, collagen and color) were analyzed by a completely randomized design⁽²⁰⁾, the treatments were the meat type factor, equine meat had 9 repetitions and bovine meat 152 repetitions. A general linear model was used with the SAS statistical package⁽²¹⁾. The means of the treatments were analyzed using Tukey's multiple comparison test, and results were considered significant when $P < 0.05^{(20)}$. The following mathematical model was used:

 $Yij = \mu + Ti + Eij$

Where:

Yij were the nutritional characteristics;
μ corresponds to the value of the mean of the respective variables;
Tj represents the effect of the species;
Eij represents the experimental error.

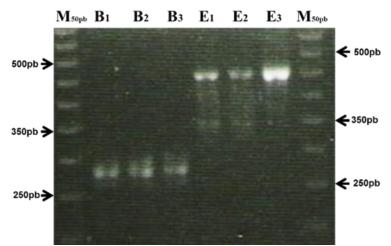
Also, a Chi-square (X^2) test was carried out in order to compare the results of the meat DNA amplicons with those expected, so the hypothesis was that all the meat samples acquired from butcher shops will be of bovine species.

Results

Species identification

The amplicons, product of conventional PCR tests, had fragments of 439 and 274 base pairs (pb) that correspond to the molecular weight of the DNA of bovine and equine meat, respectively (Figure 1). Resulting in a total of 152 positive samples for bovine meat and 9 of the samples were positive for equine meat (Figure 2).

Figure 1: Electrophoresis gel of the PCR test of the samples of bovine and equine meat from stores in Mexico City



M_{50pb}= Standard of 50pb; +B₁₋₃ Bovine positive control (274pb); +E₁₋₃ Equine positive control (439pb).

Figure 2: Electrophoresis gel of the PCR test of the samples of bovine and equine meat from stores in Mexico City

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- Negative control, +B Bovine positive control, +E Equine positive control, and sample number.

Nutritional composition

A slight increase (P < 0.05) was observed in the concentration of moisture and protein in bovine meat compared to equine meat (Table 3). While the fat and collagen content showed no differences (P > 0.05) between species and fluctuated from 22.0 to 23.5 %, from 2.0 to 2.3 %, from 73.1 to 75.1 % and from 1.3 to 1.4 mg g⁻¹. On the other hand, the color of bovine meat was higher (P < 0.05) compared to equine meat in terms of luminosity (L) (Table 4); while no differences (P > 0.05) were observed in meat colors of a* (red to green) and b* (yellow to blue) and they fluctuated from 29.80 to 37.50 and 15.00 to 16.20, respectively.

in Mexico City				
Species	Protein ^a	Fat ^a	Moisture ^a	Collagen ^b
Bovine	23.51 ± 0.11	2.3 ± 0.05	75.13 ± 0.19	1.46 ± 0.02
Equine	22.00 ± 0.50	2.0 ± 0.44	73.16 ± 0.69	1.38 ± 0.09
Pr>F	0.001	0.25	0.01	0.32

 Table 3: Nutritional chemical composition of equine and bovine meat samples from stores

^a Values expressed as a percentage; ^b values expressed in mg g⁻¹.

Table 4: Color indicators (L, a and b) of samples of equine and bovine meat from stores in

 Mexico City

Species	L	а	b
Bovine	37.50 ± 0.22	16.20 ± 0.01	14.90 ± 0.01
Equine	29.80 ± 0.88	15.00 ± 0.11	13.40 ± 0.11
Pr>F	0.0001	0.27	0.26

L=luminosity; a= red/green color; b=yellow/blue color.

Discussion

Identification of equine meat in different points of sale in Mexico City

The PCR technique and the sequence of oligonucleotide pairs reported by Matsunaga⁽⁴⁾ were useful for the identification in cuts of fresh meat for bovine and equine in the present study⁽²²⁾, and to detect that 5.59 % of the meat sampled in stores in Mexico City belonged to the equine species. The substitution of bovine for equine meat represents a consumer fraud, since the price of one meat and another, at national level, differs by almost 100 %, which is neither

ethically nor commercially acceptable⁽²³⁾. Although equine meat is a viable alternative for human consumption, similar to other types of meat obtained from traditional species such as bovine, porcine and poultry⁽¹⁰⁾, the acceptance in Mexico is limited due to cultural reasons⁽⁹⁾, since in other countries such as Italy, Belgium, Russia and Germany, it is perfectly accepted. On the other hand, the sale of equine meat in the domestic market represents a potential risk to health, since this activity is not regulated by government agencies, and there may be the presence of drugs used in production that can leave hazardous residues in meat, as evidenced by Rubio's study⁽²³⁾, where it was found that 9.93 % of meat samples analyzed in different states of Mexico tested positive for horse meat and 93.10 % of the selected samples exceeded the maximum residue limits (LMR) for clenbuterol –established by FAO⁽²⁴⁾– and 100 % – according to the zero tolerance limit of Mexican laws, which confirms that there is a latent health risk for national consumers.

Nutritional composition of bovine and equine meat

The nutritional composition of equine meat is similar to bovine $meat^{(25,26)}$. Moisture constitutes about 70 %, protein 22 %, intramuscular fat ranges from 0.5 to 6 %, and minerals account for about 1.5 %⁽²⁷⁾. In this sense, the findings on moisture content were higher (P<0.05) for bovine meat compared to equine meat, and it could be related to the type of muscle, age at slaughter and sex of the animals⁽⁹⁾. The type of muscle significantly influences the moisture content of bovine and equine meat, being greater in the semimembranosus muscle⁽²⁷⁾. These results were similar to those reported by Lorenzo and Pateiro⁽²⁸⁾, who, when evaluating the influence of muscle type on the nutritional value of calf meat, observed moisture content values from 53 to 77 %, respectively. While Tateo *et al*⁽²⁹⁾ presented meat samples from males and females of the "Heavy Draft Italian" breed with moisture values similar to those reported in the present study (70 and 73 %, respectively).

On the other hand, the concentration of protein in bovine and equine meat are in the values reported as ideal (15 to 23 %) for human consumption^(11,29). Bovine meat showed an increase in protein concentration, which could be mainly related to factors such as sex, age, muscle type and production system^(8,9). While the protein concentration of equine meat was similar to those reported by other authors^(9,27,29), who observed levels ranging between 20 and 22 % and who conclude that the factors that influence the concentration of protein are similar to those that affect cattle.

It is important to note that the fat / protein ratio is a key characteristic of the healthy qualities of meat for human consumption⁽³⁰⁾. The low intramuscular fat content of equine meat is due to the fact that they tend to store adipose tissue subcutaneously⁽³¹⁾. For this reason, some

authors call it "healthy meat"⁽³²⁾. This characteristic is included in commercialization strategies. Mainly for people trying to keep their weight under control⁽³³⁾; since the World Health Organization⁽³⁴⁾ recommends that only 30 % of the daily energy intake of the human being from the diet should be originated by the concentration of fat, so equine meat seems to be a good source of protein with low fat content⁽¹⁰⁾.

The importance of color as a characteristic of physical assessment and quality of meat allows showing the variations of the chemical state (degree of oxidation) of the pigment of a certain moment of the meat and the physical state of the meat, the structure of the muscle fibers and the amount of light reflected (L*a*b)⁽¹⁹⁾. In this sense, the lower luminosity (L) of equine meat may be due to the amount of oxygenation of myoglobin that is related to the value of $a^{*(35)}$. In this sense, equine meat has a higher concentration of myoglobin in adult life⁽³⁶⁾. In addition, the value of a^* increases and the value of L* reduces, and it tends to a darker color⁽³⁷⁾, which explains the similarity values of the meat color for the species in the present study.

Conclusions and implications

The degree of substitution of bovine meat for equine meat (5.59 %) in the commercialization centers in Mexico City is low. However, if equine meat is produced and handled according to the regulations applied to bovine meat, it has great potential as an alternative meat for the national consumer, since the nutritional composition was similar to bovine meat.

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Conflict of interest statement

The authors declare that they have no conflict of interest for the publication of this scientific paper.

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