BIBLIOGRAPHIC REVIEW

THRICOLOGY FOR IDENTIFYING MAMMAL SPECIES AND BREEDS: ITS USE IN RESEARCH AND AGRICULTURE

TRICOLOGIA PARA IDENTIFICAÇÃO DE RAÇAS E ESPÉCIES DE MAMÍFEROS: APLICAÇÃO NA PESQUISA E NA AGROPECUÁRIA

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ADDITIONAL KEYWORDS

Cuticle. Medulla. Morphometry. Hair.

PALAVRAS CHAVE ADICIONAIS

Cuticula. Medula. Morfometria. Pelos.

SUMMARY

The microscopic structure of animal hair is species-specific. This allows not only the identification of species but also discrimination among breeds. Trichology is widely used for species identification in taxonomy, ecology, paleontology, archaeology and even forensic sciences and food quality control. However, its use in livestock production is still incipient. Getting to know this methodology and to disseminate this technique in livestock investigation opens perspectives for research concerning animal genetic resources (AnGRs). Many advantages are listed such as the facility of sampling and processing, a great reliability of the results and a low cost. Therefore, trichology is an important tool for local breed studies in Brazil considering that characterization may help elucidate characteristics such as hardiness, prolificity, resistance, that warrant conservation and breeding efforts of local breeds. This review was carried out to discuss the use, the application and the potential use of microscopic analysis of mammal hair in livestock research and production.

RESUMO

A estrutura microscópica de pelos é espécie-específica. Ela permite não apenas a identificação de espécies, mas também a identificação de raças. A tricologia é amplamente utilizada para identificação de espécies em pesquisas taxonômicas, ecológicas, paleontológicas, arqueológicas, como controle de qualidade de alimentos e forenses, mas são pouco exploradas na agropecuária. O conhecimento, o estudo e, principalmente, a divulgação desta técnica abre a perspectiva para a pesquisa em diversas áreas da produção animal principalmente nos estudos relacionados aos recursos genéticos animal (RGAs). Do ponto de vista zootécnico a utilização de uma metodologia de fácil execução com baixo custo de realização e confiável é uma alternativa de grande importância. A tricologia é uma importante ferramenta para estudos sobre raças locais no Brasil, considerando que a caracterização dos pelos pode elucidar características como rusticidade, prolificidade, resistência à endo e ectoparasitas e adaptação às condições adversas que justificam os programas de conservação de tais recursos genéticos animais. Esta revisão foi redigida com o objetivo de discutir o uso da análise microscópica de pelos de mamíferos, sua aplicação na pesquisa científica e a importância para a produção e pesquisa agropecuária.

INTRODUCTION

Among the animals, hair is inherent

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only in mammals (Dreyer, 1966; Chernova, 2002). Mammals acquired the hair during phylogeny and this characteristic is not evolutionarily related to any skin appendages of other animals (Chernova, 2002). Trichology (hair study) is widely used for species identification in taxonomy, ecology, paleontology, archaeology and even forensic sciences and food quality control. However, its use in livestock production is still incipient. This review was carried out to discuss the application and the potential use of qualitative and quantitative microscopic characteristics of hairs for species and breeds identification as well as for livestock research and production.

The hair is a filamentous, keratinized structure, which protrudes from the epidermal surface of the skin (Gartner and Hiatt, 2003; Ingberman and Monteiro Filho, 2006). Its structure is polymorphous and some of its features have diagnostic value in the identification of studied samples. Its structure can vary significantly in phylogenetically related species, subspecies and breeds, as well as among different developmental stages of the same individual (Chernova, 2002).

Two distinct types of hair constitute the coat of most mammals: an outer coat of thick hair and an undercoat of shorter, thinner and softer hair (Dreyer, 1966). However, Teerink (1991) suggests that the hair should be divided into two broad categories: guard coat (*overhair*) and undercoat (*underhair*).The transition from the guard to the undercoat occurs gradually, forming four main groups, three of these belonging to the guard group (GH0, GH1 and GH2) and one to the undercoat (UH) (Teerink, 1991).

The development of the two layers varies with the climate of the environment where the animal is found; it also underwent modifications due to the domestication process (Dreyer, 1966). The undercoat is shorter, thinner, wavy and numerous, it contributes to thermoregulation and protects against water penetration (Vanstreels *et al.,* 2010). The guard coat is longer, thicker and less numerous, it stands out and contributes mainly to mechanoreception, camouflage in the environment and general pattern determination of coat coloration (Teerink, 1991; Martin *et al.,* 2009).

The guard coat can be further subdivided into primary and secondary, presenting, along its length, two main parts, the shaft and the shield **(figure 1)**.The first one is the portion that follows the bulb, it is narrower and straighter. The second one is wider and lays between the shaft and the distal extremity of the hair (Day, 1966; Quadros and Monteiro-Filho, 2006a). Not all coats present characteristic features (Day, 1966; Ingberman and Monteiro Filho, 2006); however, the guard coat is more useful for specific identification by means of the microstructure of its cuticle

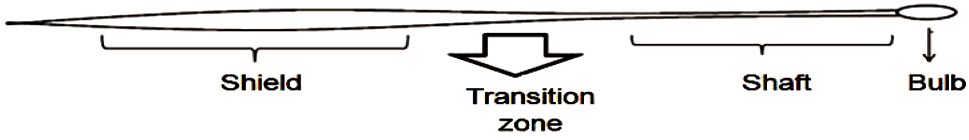

Figure 1. *Schematic structure of the guard hair*. (Estrutura esquemática do cabelo guarda).

and medulla, which represent morphological patterns characteristic of each species (Teerink, 1991; Chernova, 2003).

The hair is formed by three concentric layers of keratinized cells: the cuticle (outer layer), the cortex (intermediate layer) and the medulla (inner layer) (Teerink, 1991; Gartner and Hiatt, 2003; Stravisi, 2007).

The cuticle

The cuticle of the hair originates from a single epithelium layer (Meyer *et al.,* 2002). It represents the outermost layer of the hair being formed by overlapping transparent keratin scales (Keogh, 1983; Teerink, 1991). The pattern determined by these scales **(figure 2)** along the length of the hair, their shapes, sizes and types of margins have been recognized and used for species identification (Keogh, 1979; Stravisi, 2007).

The cortex

The cortex is composed of non-nucleated spindle cells, concentrically arranged and filled with α rigid keratin, seen by means of electronic microscopy (Hausman, 1920), but not by transmission or reflection in light microscopy (Keogh, 1983). The number of melanin granules in cortical cells determines the color of the hair, and these granules can be seen, in some cases, by light microscopy (Keogh, 1979).

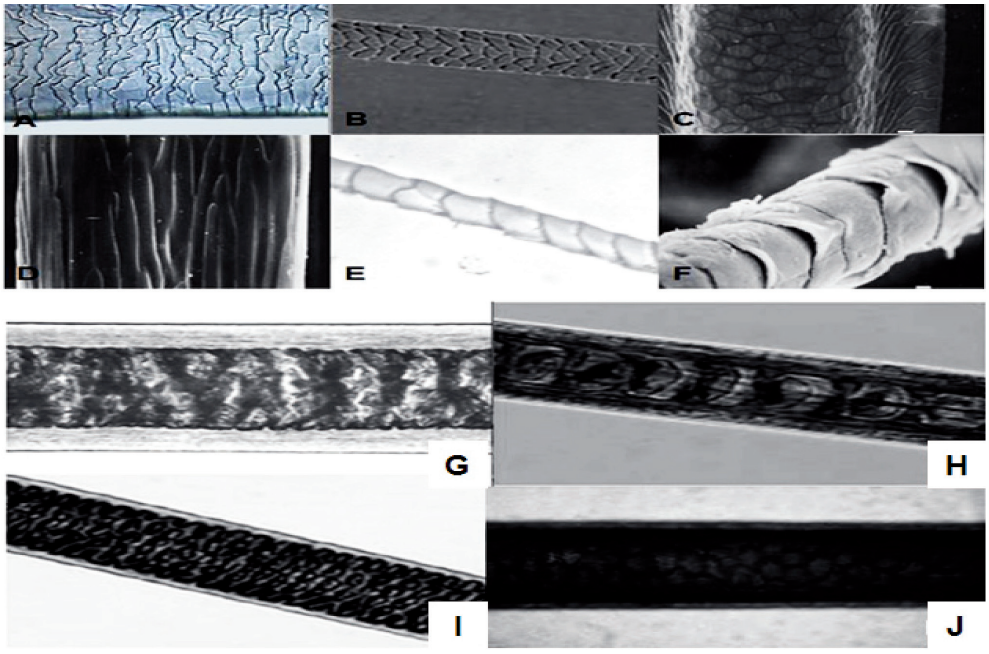

Figure 2. *Cuticular patterns of different species A) Cuticular pattern* Bos gaurus *(400x); B) Cuticular pattern* Puma yagouaroundi *(200x); C) Cuticular pattern* Leopoldamys edwardsi *(400x); D) Cuticular pattern* Lepus tolai *(400x); E) Cuticular pattern* Micoureus paraguayanus *(400x); F) Cuticular pattern* Oreotragus oreotragus *(5,000x). Medullary patterns of different species G) Medullary pattern* Lutra lutra *(400x); H) Medullary pattern* Monodelphis domestica; *I) Medullary pattern* Holochilus brasiliensis *(400x); J) Medullary pattern* Sus scrofa. (Padrões cuticulares de diferentes espécies A) padrão cuticular *Bos gaurus* (400x); B) padrão cuticular *Puma yagouaroundi* (200x); C) padrão cuticular *Leopoldamys edwardsi* (400x); D) padrão cuticular *Lepus tolai* (400x); E) padrão cuticular *Micoureus paraguayanus* (400x); F) padrão cuticular *Oreotragus oreotragus* (5,000x). Padrões medulares de espécies diferentes G) padrão medular *Lutra lutra* (400x); H) padrão medular *Monodelphis domestica;* I) medular padrão *Holochilus brasiliensis* (400x); J) padrão medular *Sus scrofa.*

Source: De Marinis and Asprea (2006); Kuhn (2009); Quadros and Monteiro-Filho (2010); Chernova (2002); Sahajpal *et al.* (2009); Quadros and Monteiro-Filho (2010); Abreu *et al.* (2011).

The amount and type of melanin (eumelanin and pheomelanin) present in the hair as well as the presence or absence of air bubbles in the medulla determines the color one sees macroscopically (Quadros and Monteiro-Filho, 2006a). The cortex has limited use in the identification keys; however, its size relative to the medulla in longitudinal view of the hair as well as its size and shape in transverse sections can be used in hair identification (Keogh, 1979; Teerink 1991).

The medulla

The medulla **(figure 2)** is composed of soft β keratin in the early stages of its development (Keogh, 1983). However, the cuticle and the cortex may grow faster than the medulla, which results in the air spaces within (Keogh, 1979). The medulla is formed of narrowly adjacent dead cells, but, unlike the cortex, they can be distinguished (Keogh, 1979).

Although medulla dead cells may contain pigment, they are often transparent (Keogh, 1979).The air cavities in medulla are black under the microscope, which may obscure the medulla structure itself. The meaning of these medullary cavities may be related to thermoregulation; however, if the air is expelled, the various arrangements of the medulla can be easily viewed and these arrangements are used for classification and taxonomic criteria (Keogh, 1979).

Fundamentals of trichology

Trichology (*thricos* = hair and *logos* = study), a noninvasive method for identifying mammals. Consists in the analysis of the morphology of the hair and it has been used since the early twentieth century. By combining the main features presented by the cuticle, cortex and medulla, the researchers created dichotomous keys to identify mammal species (Quadros and Monteiro-Filho, 1998). The combination of these three layers show morphologic patterns that, together, give any species specific morphological traits (Quadros and Monteiro-Filho, 2006a) used for taxonomic identification in ecology, morphology, textile testing, forensics, among other areas (Amman *et al.,* 2002; Chernova, 2003). Thus, these anatomical structures are of great value in species identification (Quadros and Monteiro-Filho, 2006a; Pech-Canché *et al.,* 2009). The comparison of hair from different origins (either using taxidermic animals, fecal samples, gastrointestinal contents, carcasses, etc.) can be performed due to keratinization, which gives them great strength, regardless of the type of process they have been submitted to, whether chemical (taxidermy and digestion) or mechanical, such as mastication and weathering (Quadros and Monteiro-Filho, 1998b; Quadros and Monteiro-Filho, 2006a).

The analyzed hair must be collect in the dorsal region of the body of mammals (Kuhn, 2009). However, studies conducted by Day (1966); Dreyer (1966); Riggott and Wyatt (1980), focused on the differences of the structure of hair collected from different parts of the body and demonstrated that they can be compared, without jeopardizing the identification, as well as the hair of animals of different sexes and ages, but the authors reported that there are exceptions to the extremities as ears, head, tail, neck and limbs.

One of the pioneering studies of the microstructure of the hair was performed by Hausman (1920), whose aim was to verify the authenticity of the fibers used in fur coats. After this work, several other studies on the microstructure of the hair have been carried out in taxonomic, ecological, paleontological and forensic researches, producing keys of identification for different species (Mayer, 1952; Dreyer, 1966; Keogh, 1979; Meng and Wyss, 1997; Fernández and Rossi, 1998; Amman *et al.,* 2002; Lungu *et al.,* 2007; Sahajpal *et al.,* 2009; Quadros and Monteiro-Filho, 2010; Sato *et al.,* 2010; Abreu *et al.,* 2011; Anwar *et al.,* 2012). Although there are numerous studies, this technique is still not exploited in agriculture.

According to Barker (1999) the goal of conservation is to maintain the maximum genetic diversity of each species, the conservation of

diversity within species. Therefore, studies of phenotypic and genetic characterization are key to conservation programs of animal genetic resources (AnGR), because they allow previous identification of poulations isolated in their environment for a long time (Mariante *et al.,* 2009).

Method of trichological analysis

The following method of trichological analysis was recommended by Quadros and Monteiro-Filho (2006b).

Material collection

One must obtain a sample, directly with fingers, of the region of the intersection of the median line with the line of the girdle on the back of the specimen to be studied. After that, the guard coat containing the bulb and the apex must be separated, with the aid of a magnifying glass, if necessary, and washed in ethanol and dried in commercial paper towel.

Preparation of slides for cuticular print observation

After washing and subsequent drying of the hair on paper, a thin layer of colorless nail polish must be spread on a clean glass slide. The hair is placed on a blade after the nail polisher dries at room temperature for 15 to 20 minutes.

The slide containing the hair must be placed on a piece of wood and covered with another coating with transparent tape forming a sandwich. The assembly must be pressed by a vise or a rectangular arm press; after opening the vise, the slide with the hair must be separated from the rest of the assembly.

After complete drying of the nail polisher, around 30 minutes, the hair is removed by the distal end, by gently rubbing with a fingertip, producing cuticle prints (outer layer of the hair), which should be stored and protected from dust for subsequent observation under optical microscope. Drying times referred to are variable according to the humidity and temperature at the moment and location of slide preparation.

Preparation of slides for medulla observation

The hair that was removed from the cuticular print slide is placed in commercial, 30-volume hydrogen peroxide cream, of cosmetic use, for 80 minutes. Thick hair is cut in the shield one to three times for that step. After that, it is washed in water and dried on paper towels.

Then, the permanent slides are mounted, with transparent synthetic mounting medium and coverslip or temporary slides, with water or glycerol and coverslips. The reading of the microscope slides should be performed under light microscopy, with an increase of 100x to 400x, depending on the size of the hair, because it is necessary that the entire thickness of the hair fits within the visual field.

Morphological patterns of hair microstructure

The first work that developed a naming system for 166 species was Hausman (1920). It provides the diagnosis of eight cuticle patterns and eight medulla patterns for these species. Other studies have been carried out in various regions of the world; however, these patterns are often difficult to interpret due to the lack of a standardized nomenclature and descriptors accompanied by illustrations. Only in 2006 a study was conducted in Brazil (Quadros and Monteiro-Filho, 2006a), describing the morphology and microstructure of the hair cuticle and medulla of 64 mammal species found in Brazil with classification and taxonomy in Portuguese.

Cuticular patterns

Quadros and Monteiro-Filho (2006a) defined 15 cuticular patterns **(figure 3)** through a combination of six characters: edges overlapping, scales shape, scales size, scales orientation, scales ornamentation and continuity of scale margins.

Medullary patterns

Quadros and Monteiro-Filho (2006a) defined 17 medullary patterns using six different

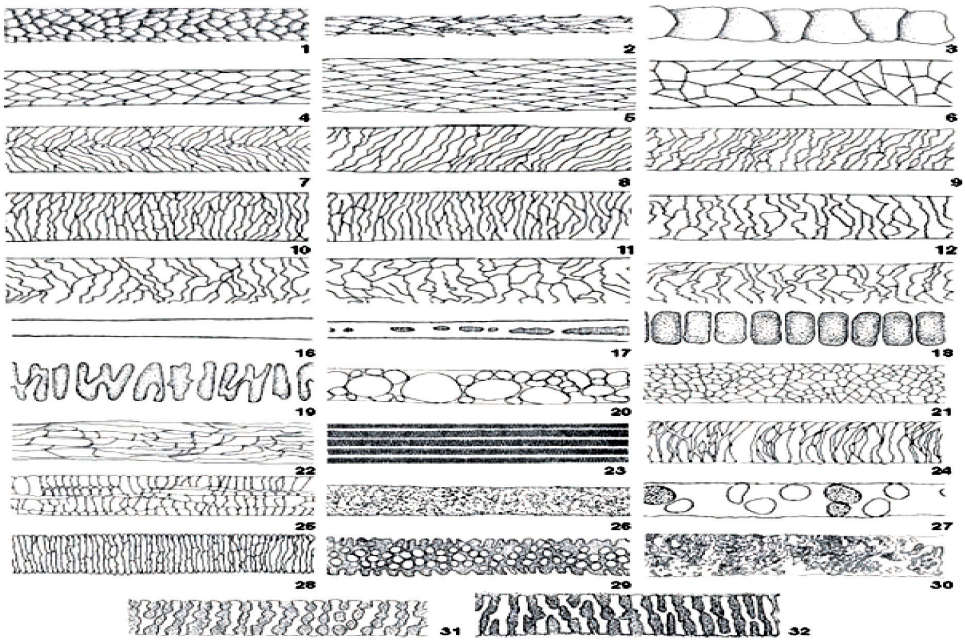

Figure 3. *Cuticular patterns of the shaft of 64 Brazilian mammal species (1) large leafy shape; (2) narrow leafy shape; (3) conoid shape; (4) large rhombus; (5) narrow rhombus; (6) mosaic; (7) double oblique wavy shape; (8) simple oblique wavy shape; (9) simple oblique wave shape with scales with ornamented edges; (10) transverse wavy shape; (11) transverse wavy shape with scales with incomplete edges; (12) transverse wavy shape with scales with ornamented edges; (13) irregular wavy shape; (14) irregular wavy shape with scales with incomplete edges; (15) irregular wavy shape with scales with ornamented edges. Medullary patterns of the shield of 64 Brazilian mammal species (16) absent; (17) discontinuous; (18) uniseriate ladder; (19) diagonal uniseriate; (20) anisocellular; (21) polygonal; (22) glandular; (23) string shape; (24) spindle shape, (25) corn shape; (26) amorphous; (27) matricial; (28) trabecular; (29) reticular; (30) sieved; (31) alveolar; (32) striped.* (Padrões cuticulares do eixo de 64 espécies de mamíferos brasileiros (1) forma grande de folhas; (2) forma de folhas estreitas; (3) forma conóide; (4) grande losango; (5) losango estreita; (6) em mosaico; (7) forma ondulada dupla oblíquo; (8) forma ondulada oblíqua simples; (9) forma de onda oblíquo simples com escalas com bordas ornamentadas; (10) transversal forma ondulada; (11) forma ondulada transversal com escalas com bordas incompletos; (12) forma ondulada transversal com escalas com bordas ornamentadas; (13) forma ondulada irregular; (14) forma ondulada irregular com escalas com bordas incompletos; (15) forma ondulada irregular com escalas com bordas ornamentadas. Padrões medular do protetor de 64 espécies de mamíferos brasileiros (16) ausente; (17) descontínuo; (18) escada uniseriate; (19) uniseriate diagonal; (20) anisocellular; (21) poligonal; (22) glandular; (23) forma cadeia; (24) forma de fuso, (25) a forma de milho; (26) amorfo; (27) matricial; (28) trabecular; (29) reticular; (30) peneirado; (31) alveolar; (32) listrado. Source: Quadros and Monteiro-Filho (2006a).

characters **(figure 3)**, as follows: presence of medulla, continuity, number of rows of cells, cells arrangement, cell shape and ornamentation of the margin.

Morphometric patterns of the hair microstructure

The use of computer techniques in trichology pattern recognition provides a means to

reduce the subjectivity of the conventional process, since manual techniques rely on the interpretation of an expert instead of mathematics quantitative measurements (Verma *et al.,* 2002; Moyo *et al.,* 2006). Another key steps are the use of digital imaging in the identification of specific taxonomic characteristics (Stravisi, 2007), and measurements of the statistical parameters that describe these characters and the relationships among them (Valentin *et al.,* 1994; Stravisi, 2007). Moyo et al. (2006) conducted the first study of classification of trichology patterns of African mammals through the application of pattern recognition techniques to hair scales, as detailed below.

Methodology description

The methodology described below was defined by Stravisi (2007). Each hair is photographed from the slide by a digital camera attached to the microscope. Digital photography allows enlarging the image, then increasing the contrast working directly on the image (Bayer *et al.,* 2001; Stravisi, 2007).

The reading of microscope slides should be performed under light microscopy, with an increase of 100 to 400x, depending on the hair dimension, because it is necessary that the entire thickness of the hair fits within the visual field. Subsequently, microscopy images are obtained in two distinct zones of the hair on each blade: on the distal third of the shaft and on the thicker part of the hair (Stravisi, 2007).

For each hair, four photographs should be selected, two of each zone (shield and shaft). Images must be saved in JPG format with a resolution of 2592x1944 pixels. For image analysis, ImageJ software - Open software should be used. ImageJ provides a rectangular area of regions of interest and this tool can be used to manually sample the scale pattern of the image (Moyo *et al.,* 2006).

The images can be converted to grayscale, to eliminate color effect. If necessary, the image can be improved by brightness and contrast. The software used allows the conversion of pixels to micrometers (μm) after setting the scale. Measures should be taken for both cuticular and medullar patterns (Stravisi, 2007).

For medullary patterns, the total thickness of the hair and the thickness of the medulla must be measured by means of straight lines corresponding to the diameters. As for the cuticular patterns, a freehand drawing of the outline of the cuticle scales must be performed for the measurement of area, perimeter, length, roundness of the shape, among other characteristics (Stravisi, 2007).

These measurements must be made to obtain the quantitative indices for cuticle, medulla and cortex characteristics. For this design, a graphic tablet (tablet) should be used along with the ImageJ software (Stravisi, 2007).

Applying trichological analysis in agriculture

This type of analysis has been used in conservation programs of native breeds of sheep in Italy in order to ensure the genetic origin of these animals. The studies were conducted by Razzara (2009) and Tormen (2011).

The object of the research conducted by Razzara (2009) was to evaluate the genetic purity of six animals (four females and two males) supposedly belonging to Padovana breed, as well as the genetic distance of the individuals. Therefore, genotypic and phenotypic analyzes of animals were performed and trichological analysis was one of them. To exclude possible contamination of the herd during periods of transhumance, the DNA was compared with the breeds Alpagota, Brogna, Lamon, Foza and Apennine, aiming at validation. The results of the trichological analysis showed that the six animals differed from the breeds to which they were compared. This information was confirmed by the results of genetic variability, which was very low among the six animals, but high between them and the other breeds.

The objective of Tormen´s study (2011) was to determine possible differences among

local sheep breeds. In the case of unidentified populations of the genus *Ovis*, the use of dichotomous keys based only on analyzes of trichological sections can induce to errors. For this reason, morphometric patterns of the four native Italian breeds, Alpagota, Brogna, Lamon and Foza, and the not regional breed, Appenninica were used.The results showed that the medians of the examined areas (cuticle and medulla) were statistically different, and the Appenninica breed differed the most from the other breeds ($p<0.01$). Thus, it was concluded that the method is feasible for the characterization of sheep breeds with the potential benefit for local mammal populations. In Brazil, a team of researchers from the Federal University of Goiás, Embrapa Pantanal and Embrapa Beef Cattle joined with researchers from the University of Padova in Italy in order to perform the morphological characterization of local Brazilian cattle breeds: Caracu, Curraleiro, and Pantaneiro and Nellore, through trichological morphometry. Currently, similar studies are not known in Brazil and it is expected to provide a quick and inexpensive technique for implementing racial identification of these animals helping the conservation efforts of those locally adapted breeds.

CONCLUSION

Qualitative and quantitative phenotipical and genetical characterization of animal breeds is of great importance to mantain the diversity of species, because it facilitates the selection process of specific characteristics inside herds and breeds. The reviwed technique is easy, reliable and economically applicable, demonstrating the feasibility of developing projects that search for species or racial identification. Studies of this nature are fundamental to animal improvement and open perspectives for research in specific areas of animal production, mainly in herds participating in conservation programs and origin certification processes, as well as other sorts of economic interests such as new materials with functional applicability.

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