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Evaluation of hippuric acid content in goat milk as a marker of feeding regimen

A. Carpio,* D. Bonilla-Valverde,† C. Arce,‡ V. Rodríguez-Estévez,‡[1] M. Sánchez-Rodríguez,‡ L. Arce,* and M. Valcárcel*

*Department of Analytical Chemistry, Institute of Fine Chemistry and Nanochemistry, University of Córdoba, Annex C3 Building, Campus of Rabanales, E-14071 Córdoba, Spain
†Centro de Investigación y Calidad Agroalimentaria de Los Pedroches (CICAP) Research, Apdo. Correos 105, Ctra. de la Canaleja s/n, 14400 Pozoblanco, Córdoba, Spain
‡Department of Animal Production, University of Córdoba, Campus of Rabanales, E-14071 Córdoba, Spain

ABSTRACT

Organic producers, traders, and consumers must address 2 issues related to milk: authentication of the production system and nutritional differentiation. The presence of hippuric acid (HA) in goat milk samples has been proposed as a possible marker to differentiate the feeding regimen of goats. The objective of this work is to check the hypothesis that HA could be a marker for the type of feeding regimen of goats by studying the influence of production system (conventional or organic) and feeding regimen (with or without grazing fodder). With this purpose, commercial cow and goat milk samples (n = 27) and raw goat milk samples (n = 185; collected from different breeds, localizations, and dates) were analyzed. Samples were grouped according to breed, feeding regimen, production system, and origin to compare HA content by ANOVA and honestly significant difference Tukey test at a confidence level of ≥95%. Hippuric acid content was obtained by analyzing milk samples with capillary electrophoresis. This method was validated by analyzing part of the samples with HPLC as a reference technique. Sixty-nine raw goat milk samples (of the total 158 samples analyzed in this work) were quantified by capillary electrophoresis. In these samples, the lowest average content for HA was 7 ± 3 mg/L. This value corresponds to a group of conventional raw milk samples from goats fed with compound feed. The highest value of this group was 28 ± 10 mg/L, corresponding to goats fed compound feed plus grass. Conversely, for organic raw goat milk samples, the highest concentration was 67 ± 14 mg/L, which corresponds to goats fed grass. By contrast, the lowest value of this organic group was 26 ± 10 mg/L, which belongs to goats fed organic compounds. Notice that the highest HA average content was found in samples from grazing animals corresponding to the organic group. This result suggests that HA is a good marker to determine the type of goats feeding regimen; a high content of HA represents a diet based mainly or exclusively on eating green grass (grazing), independently of the production system. Hence, this marker would not be useful for the actual organic policies to distinguish organic milk under the current regulations, because organic dairy ruminants can be fed organic compound feed and conserved fodder without grazing at all.

Key words: authentication, organic farming, capillary electrophoresis, high performance liquid chromatography

INTRODUCTION

In recent decades, debate has been growing about the ethical aspects of production and trade. The International Federation of Organic Agriculture Movements defines organic livestock production as a system based on the harmonious relationship between land, plants and livestock, respect to the physiological and behavioral needs of livestock, and using organically grown foodstuffs or natural resources as fodders (IFOAM, 2002). Consumers associate organic farming with grazing animals (fodder diet, freedom, and welfare); however, the standards for organic livestock farming detailed by the organic farming regulations of the European Community (Council of the European Union, 2007) allow an intensive open air production, without grazing and feeding animals with organic compound feeds adding green or conserved fodder (e.g., silage, hay, straw, and so on). Producers, traders, and consumers of organic food regularly use the concept of the natural (naturalness) to characterize organic farming and organic food, in contrast to the unnaturalness of conventional farming. Critics sometimes argue that such use lacks any

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[1]Corresponding author: pa2roesv@uco.es

rational (scientific) basis and only refers to sentiment (Verhoog et al., 2003).

On the basis of a systematic review from a total of 52,471 articles about nutritional quality of foods, Dangour et al. (2009) identified 162 studies (137 crop and 25 livestock products) dealing with organic food; only 55 were of satisfactory or higher quality. Those authors established that the analysis of the very limited database on livestock products found no evidence of a difference in nutrient content between organically and conventionally produced livestock products. Hence, organic producers, retailers, and consumers must address 2 issues related to food: authentication of the production methods and nutritional differentiation.

In the case of organic milk, different techniques have been used for nutritional differentiation. Many authors have compared the composition and content of FA between organic and conventional milk (Ellis et al., 2006; Collomb et al., 2008; Molkentin, 2009, Molkentin and Giesemann, 2010). Ellis et al. (2006) found that organic milk had a higher proportion of PUFA and n-3 FA, and this effect of production system remained significant even after accounting for some potentially confounding management and nutritional factors in the analyses. Collomb et al. (2008) also found a higher content in PUFA, conjugated linoleic acids, and branched FA in organic milk with significantly higher levels of grasses and lower levels of concentrates in the fodder of organic farming.

Other authors have also carried out different comparisons in the composition and content of different analytes between organic and conventional milk samples to find suitable markers to certify the milk production system and avoid fraud. Molkentin (2009) studied the influence of the production system (organic or conventional) and the season by measuring the carbon-stable isotope ratio ($\delta^{13}C$) using isotope-ratio mass spectrometry, and the content of α-linoleic acid in milk using gas chromatography. These parameters were selected because both of them are measured in the easily accessible milk fat. Molkentin (2009) concluded that $\delta^{13}C$ and α-linoleic acid are not good markers to discriminate between organic and conventional milk; one of the main reasons being the seasonal variation showed by both components. They also investigated their applicability as markers for authentication of organic milk in Germany, due to its higher content in organic milk. However, they did not consider the feeding regimen of animals (only the production system).

Furthermore, Molkentin and Giesemann (2010) concluded that analyses involving the combination of threshold values for $\delta^{13}C$, $\delta^{15}N$, or C18:3n-3 content in milk components can improve the authentication of organic milk. Thus, multivariable analyses can increase robustness and reduce the number of exceptions in organic milk authentication.

After the determination of a mixture of organic acids (oxalic, citric, orotic, benzoic, uric, and hippuric acids) in milk samples, Carpio et al. (2010) found that only hippuric acid (**HA**) could be a marker to distinguish milk from goats fed on different production system. However, these authors concluded that it is necessary to check if the HA content comes from grazing fodder or from organic handling.

This initial hypothesis, highlighted by Carpio et al. (2010), is supported by the conclusions summarized by other authors. Some of the first articles that mentioned the natural presence of HA in cow milk were those presented by Karabinos and Dittiner (1943), Patton (1953), and Svensen (1974). According to Sieber et al. (1995), HA concentration of cow milk may be up to 50 mg/kg, although Patton (1953) found that HA concentration ranged from 31 to 64 mg/L in skim milk. Svensen (1974) observed a higher amount of HA in milk from grazing than from indoor forage feeding. Also, Besle et al. (2010) found a higher content of HA in milk from cows with a diet based on grazing grassland pasture in comparison to those with a diet based on different diets of concentrate and silage or hay forages. Besle et al. (2010) related milk HA to the presence of chlorogenic acid, neochlorogenic acid, or caffeoyl compounds in the diets based on grassland pasture (per Gonthier et al., 2003). Forages contain large amounts of aromatic compounds both in the insoluble cell wall and in the cellular content in the form of water and ethanol-soluble polyphenols specific to each plant taxon. These aromatic compounds, including HA, are partially degraded in the rumen and partly absorbed in the rumen and intestinal mucosa, following an immediate conjugation before they are transformed in the liver and excreted in urine or milk (Gatley and Sherratt, 1977; Scheline, 1991). Hence, an important factor in the variation of content of aromatic compounds in milk samples could be the feeding regimen and the fodder quality that goats have consumed, which are seasonally influenced by climatic conditions of the area and the month of sample collection (Peinado-Lucena et al., 1992).

The aim of the present work was, first, to check the hypothesis proposed by Carpio et al. (2010), that the evaluation of HA in goat milk samples is a possible marker to differentiate the type of feeding regimen supplied to goats; second, to validate capillary electrophoresis (**CE**) to determine HA content by a reference technique, such as HPLC; and third, elucidate if the differences in HA content between organic and conventional milk are due to feeding regimen more than production system (organic or conventional).