

## Summary

Antibiotic residues in milk and other foodstuffs of animal origin are a great concern for public health, as they could lead to toxicological effects in sensitive consumers and contribute to the generation of bioresistance. Moreover, drug residues could also have negative implications for the dairy industry, affecting the fermentation processes required to make related products such as yogurt and cheese, the main destination of goat milk production. To guarantee the safety of milk and derivatives, Maximum Residues Limits (EU-MRL) for different veterinary drugs in raw milk have been established by European legislation. However, the transfer of these substances from milk to cheese, has been poorly studied and, therefore, the impact of the use of raw milk containing admissible amounts of antibiotics on the cheese-making and the cheese safety is thus far unknown.

The aim of this thesis was to evaluate the transfer of the most widely used antibiotics used in dairy goats, from milk to fresh and matured cheese, as well as their effect on the cheese-making process and the quality characteristics of the cheeses during ripening. To this end, several studies were carried out using the experimental herd of Murciano-Granadina breed goats, and the pilot plant of Universitat Politècnica de València (Spain).

In the first study, antibiotic-free raw goat milk spiked individually with seven antibiotics (amoxicillin, benzylpenicillin, cloxacillin, erythromycin, ciprofloxacin, enrofloxacin and oxytetracycline) at EU-MRL equivalent antibiotic concentration, was used to make mature Tronchón cheeses, which were analyzed at different ripening times (0, 30, and 60 days) to determine antibiotic residues, pH, chemical composition, proteolytic and lipolytic activities, color, and textural properties. A sensory evaluation of 60 days ripened cheeses was also carried out. The cheese-making process was unaffected by the presence of most antibiotics in milk. Only erythromycin and oxytetracycline significantly increased the time required for cheese production ( $122\pm 29$  and  $108\pm 25$  min, respectively). Regarding cheese characteristics, the few differences found were related to the free fatty acid concentration, color and textural properties, which remained mostly undetected by the sensory analysis panelists. However, variable amounts of antibiotics, ranging from 7.4 to 68 %, were transferred from milk spiked with antibiotics at concentrations equivalent to the MRL, to cheese. Oxytetracycline and quinolones presented the highest retention rates as well as persistence along maturation, with high concentrations of quinolones (enrofloxacin:  $148\pm 12$   $\mu\text{g}/\text{kg}$ , ciprofloxacin:  $253\pm 24$   $\mu\text{g}/\text{kg}$ ) and oxytetracycline residues ( $20\pm 5.7$   $\mu\text{g}/\text{kg}$ ) in cheeses after 60 days of ripening.

Given that oxytetracycline is one of the most employed antibiotics in dairy goats, a similar study was carried out using different antibiotic concentrations closely related to the MRL (0, 50, 100, and 200 µg/kg). As described above, the presence of the oxytetracycline increased the time required for cheese production, the delay in acidification being antibiotic dose-dependent (26±7.2; 117±23.6; 217±28.4 min, respectively). The presence of this antibiotic hardly modified the organoleptic characteristics of the ripened cheeses such as texture, color and microstructure, whose differences were almost undetectable, even though the milk initially used contained twice the MRL allowed. Yet, the oxytetracycline concentration did not affect the volatile profile of the Tronchón cheeses, which was compared on a fortnightly basis during a 60-day period being was modified by the ripening time only. However, oxytetracycline was widely transferred from milk to cheese, with residue concentrations being 3.5-4.3 times higher than the drug concentration in raw milk used for cheese production. Oxytetracycline residues diminished along ripening in all cases, however, variable amounts of this substance remained in the ripened 60-day cheeses (6.6-79 µg/kg), which could be of concern for public health.

On the other hand, the off-label use of antibiotics with a legally established minimum safety period of seven days is a common practice in dairy goats due to the limited availability of drugs registered for this species. Macrolide antibiotics are widely applied in an off-label manner to treat mastitis and other infectious diseases like contagious agalactia in endemic areas, thus increasing the risk of drug residues in milk, as the required elimination period is not always known. An *in vivo* experiment to verify if the exceptional use of macrolides (erythromycin, tylosin, and spiramycin) in dairy goats generates residues in milk and cheeses was carried out. Ripened cheeses were made from bulk milk obtained before drug administration, 24 hours after treatment, and at the end of the legal withdrawal period. Residual amounts of erythromycin (234.9±52.7 µg/kg), tylosin (198.7±57.8 µg/kg) and spiramycin (1,539.8±469.4 µg/kg), widely exceeding their legal EU-MRLs established were found in milk collected 24 hours after treatment, making the cheese production in most cases impossible. After the seven-day period, only spiramycin was detected in goat milk (79.6±19.2 µg/kg) although no antibiotic residues were found in the cheeses. Results herein suggest that a withdrawal period of seven days seems suitable to guarantee milk safety after the off-label administration of erythromycin and tylosin without any negative effects neither on the milk nor on the cheese properties. For spiramycin applications, an extended withdrawal period should be evaluated.

Finally, in the last study, the objective was to evaluate the presence of drug residues in pasteurized fluid milk and fresh cheeses obtained from goat milk containing antibiotics (amoxicillin, benzylpenicillin, cloxacillin, neomycin, erythromycin, ciprofloxacin, enrofloxacin and oxytetracycline) at safety levels (EU-MRLs). The safety margin of these dairy products for consumers was also evaluated. Results showed that high amounts of antibiotics, between 71-100% of the initial concentration in raw milk, remained in pasteurized goat milk and were transferred to cheese to a high extent, with retention percentages ranging from 37.5 to 75%. Regarding the safety margin of goat milk products, calculated taking into account different age groups (children, teenagers and adults), and the published negative effects of such antibiotics on consumer health, results indicate that the minimum safety margin of pasteurized milk was obtained for ciprofloxacin, enrofloxacin and erythromycin in the group of children. Regarding fresh cheese, an elevated safety margin was obtained for all antibiotics and age groups considered.

In summary, from the studies carried out, it can be concluded that the cheese-making process and the quality properties of the 60-days ripened Tronchón cheeses were slightly affected by the presence of antibiotics in goat milk at equivalent EU-MRL concentration. However, large amounts of highly stable substances such as quinolones could remain in the final products. Similarly, it is important to emphasize that relatively high concentrations of antibiotics could remain in pasteurized fluid goat milk and related products such as fresh cheese and cheeses of a short ripening period. The presence of these antibiotics in dairy products might contribute to the development and spread of antimicrobial resistance, which is considered an important public health concern worldwide.

The result of this research could serve the public health authorities to assess if current control systems of antibiotics in milk and dairy products are adequate or have to be revised. Considering the differences in the milk composition from different species, and the great variety of existing cheeses, it would be advisable to continue the study of traceability of antibiotics in order to increase the safety margin of dairy products and to guarantee public health.