# Innovation for Sustainability in Sheep and Goats

Edited by:
R. Ruiz, A. López-Francos, L. López Marco

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## International Centre for Advanced Mediterranean Agronomic Studies

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11, rue Newton, 75116 Paris, France
Tél.: +33 (0) 1 53 23 91 00 - Fax: +33 (0) 1 53 23 91 01 / 02
secretariat@ciheam.org
www.ciheam.org

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3191, Route de Mende
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pascal.bergeret@iamm.fr et/and sciuto@iamm.fr
www.iamm.fr

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Dir.: Javier SIERRA
Avda. Montañana, 1005
50059 Zaragoza, Spain
Tel.: (+34) 976 716000 - Fax: (+34) 976 716001
iamz@iamz.ciheam.org
www.iamz.ciheam.org

# Effect of the presence of neomycin in goat's milk on the making and characteristics of Tronchón cheese

**P. Quintanilla[1], M.C. Beltrán[1], B. Palau[1], M.I. Escriche[2] and M.P. Molina[1]**

[1]Institute for Animal Science and Technology
[2]Institute of Food Engineering for Development. Universitat Politècnica de València.
Camino de Vera, s/n. 46022, Valencia, Spain

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**Abstract.** Antibiotic residues in milk and dairy products are a potential problem for public health, and for the dairy industry. This study investigated the effect of the presence of neomycin in goat's milk on the production and characteristics of semi-hard Tronchón cheese. Cheeses were made, in duplicate, from raw goat's milk without antibiotics and with addition of neomycin at the Maximum Residue Limit (1500 µg/kg). Possible changes in the characteristics and the presence of antibiotic residues in the cheeses were evaluated at different time points along ripening (1, 30 and 60 days). Processing time was increased (48 ± 18 min) by the presence of neomycin in milk. Regarding cheeses, lower values of water activity as well as reduced free fatty acids and free amino-acid concentrations were detected in cheeses from milk with the antibiotic, suggesting a minor biochemical activity throughout ripening. Thus, a greater lightness and lower redness value were observed in this type of cheese, also presenting lower springiness. Moreover, high concentrations of neomycin were retained in the cheese and concentrated during ripening, leading to a concentration factor of 4.9, with respect to the initial concentration in the milk, potentially implying a risk to public health.

**Keywords.** Antibiotic – Neomycin – Goat milk – Cheese.

***Effet de la présence de néomycine dans le lait de chèvre sur la fabrication et les caractéristiques du fromage Tronchón***

***Résumé.*** *La présence de résidus d'antibiotiques dans les produits laitiers peut constituer un problème pour la santé publique et pour l'industrie laitière. Dans ce travail, nous avons évalué l'effet de la présence de néomycine dans le lait de chèvre sur la fabrication et les caractéristiques du fromage Tronchón. Les fromages ont été fabriqués, en double exemplaire, à partir du lait de chèvre cru sans antibiotiques et avec l'addition de néomycine à la limite maximale de résidu (1500 µg/kg). Nous avons étudié les changements dans les caractéristiques du fromage et la présence de résidus à différentes périodes de maturation (1, 30 et 60 jours). La durée du procédé de fabrication augmentait (48 ± 18 min) sous l'effet de la présence de néomycine dans le lait de chèvre. Concernant les caractéristiques du fromage, nous avons observé une diminution dans les valeurs de l'activité de l'eau ainsi qu'une réduction dans les concentrations d'acides gras libres et d'acides aminés libres dans les fromages avec l'antibiotique, en suggérant une activité biochimique mineure pendant la maturation. Nous avons aussi observé une plus grande légèreté, moins rougeur et une plus faible élasticité dans ce type de fromage. En plus, pendant la maturation, les concentrations de néomycine dans le fromage augmentaient 4,9 fois par rapport à la concentration initiale dans le lait, ce qui pourrait impliquer un risque pour la santé publique.*

***Mots-clés.*** *Antibiotique – Néomycine – Lait de chèvre – Fromage.*

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## I – Introduction

Goat's milk demand has increased in recent years as it is easier to digest and is an ideal substitute for cow milk in the diet of children, the elderly and people with allergies to cow milk (Haenlein, 2004). Traditionally, goat's milk production was intended for the manufacture of dairy products, especially cheese. In dairy goats, the use of antibiotics to treat mastitis and other animal health conditions is a common practice that might contaminate the milk supply if appropriate measures are not taken. Among antibiotics, the aminoglycosides (gentamicin, neomycin and streptomycin, among others) are usually applied in to treat infectious diseases caused by gram-negative bacteria (Davies and Wright, 1997). The presence of antibiotic residues in milk can affect consumer health, either generating resistance to antibiotics or causing allergies (EFSA, 2016), as well as the production, thus affecting the growth of microorganisms responsible for fermentation or biochemical reactions (Novés *et al.,* 2015). For consumer protection, the European Union (EU Regulation 37/2010), established Maximum Residue Limits (MRLs) in products of animal origin such as milk, but so far, they have not been established for dairy products, such as butter, yogurt or cheese. It has been shown that the presence of antimicrobial residues in milk may result in the retention of varying amounts of these substances in cheese (Adetunji, 2011; Cabizza *et al.,* 2016). Also, the presence of antibiotics might have negative effects on cheese quality parameters. However, related studies are very scarce. Thus, the aim of this study was to analyse the effect of the presence of neomycin, on the production and the characteristics of Tronchón cheeses along ripening.

## II – Materials and methods

### 1. Cheese manufacture

Tronchón cheeses were made at the Universitat Politècnica de València (UPV) pilot plant using raw milk from the experimental flock of Murciano-Granadina goats of UPV. Cheese manufacture was performed in duplicate from goat's milk without antibiotics (control) and with neomycin (N1876, Sigma-Aldrich Química, SA, Madrid, Spain) at an equivalent concentration to the MRL (1,500 µg/kg) in a 50 l vat, following the traditional Tronchón cheese-making procedure. After moulding, the pressed rennet curd was salted and ripened for 60 days.

### 2. Cheese analysis

The physicochemical, colour and texture properties of the Tronchón cheeses were evaluated at 1, 30 and 60 days of ripening. Cheese composition (moisture, fat, protein and salt content) was determined in duplicate using a FoodScan infrared device (Foss, Foss Iberia, Barcelona). The water activity of samples (aw) was measured in duplicate by using a dew point hygrometer (Decagon Devices Inc., Aqualab 4TE, USA). Regarding pH, a pH-meter with a penetration electrode (Crison Instruments, S.A., Basic 20, Spain) was employed, making 6 measurements per sample. The free amino-acid (FFA) concentration, as indicator of proteolysis in cheeses, was determined by the method reported by Folkertsma and Fox (1992). The free fatty acid (FFA) concentration, as indicator of lipolysis level, was performed following the methodology described by Nuñez *et al.* (1986). The colour of the cheeses was measured using a Minolta spectrocolorimeter, model CM-3600D (Minolta, Japan), considering the coordinates of the CIE colour space L* a* b*, which were obtained by reflectance, with illuminant D65 and the observer 10º. The texture was determined through a texture profile analysis (TPA) using a TA.XT.plus texturometer (Stable Micro Systems, UK). A double compression analysis was performed with a cylindrical probe of 45 mm diameter (P/45), with a deformation percentage of 50% and a holding time of 5 seconds. 9 measurements were made per cheese in cylinders 2 cm in diameter and 1 cm thick. The concentration of residual neomycin in cheeses was analysed in the Laboratory of Public Health of Galicia (Spain), using a High Performance Liquid Chromatography (HPLC-MS-MS) method.

### 3. Statistical analysis

Data generated by this study were subjected to a two-way analysis of variance (ANOVA), including the effect of antibiotic concentration (0 and 1500 µg/kg) and ripening time (1, 30 and 60 days) as variation factors. The least significant differences were calculated using the LSD test (least significant difference) and a significance level of $\alpha$=0.05, using Statgraphics Centurion XVI (version 16.2.04; Statpoint Technologies, Inc. Warrenton, Virginia, VA, USA).

## III – Results and discussion

The cheese-making process was moderately affected by the presence of neomycin in goat's milk which inhibit the starter culture activity and increased by 48 ± 18 min the time required to reach the final pH of the cheeses (pH = 5.3) during the pressing stage. Antibiotics affecting kinetic acidification in the manufacture of fermented products have been reported by other authors (Novés *et al.,* 2015; Cabizza *et al.,* 2017). Regarding the characteristics of the cheeses, Table 1 shows the parameters evaluated along with the results of the analysis of variance. As shown in Table 1, a lower water activity ($p<0.001$) as well as shorter concentrations of FFA ($p<0.05$) and FAA ($p<0.01$) were found in the cheeses made from spiked goat's milk, suggesting that the microbial metabolism was also reduced along ripening by the presence of the residual amounts of neomycin in the curd. Thus, the lower degree of biochemical reactions in cheeses with the antibiotic could be related to the higher lightness ($p<0.001$) observed in this type of cheese (Rohm and Jaros, 1997), which also presented a lower redness value ($p<0.05$), especially, in the second month of ripening ($p<0.001$). Regarding texture analysis, the presence of the antibiotic significantly affected the springiness of the cheeses, being lower ($p<0.05$) in cheeses with neomycin. Cohesiveness also declined drastically during the first month of ripening ($p<0.01$) in cheeses made from contaminated milk.

**Table 1. Average values of parameters analysed in Tronchón cheese and ANOVA F-ratio for each of the two factors: neomycin concentration (C) and ripening time (T) and their interaction (C*T)**

| Parameters | Neomycin concentration (µg/kg) | | | Ripening time (days) | | | | ANOVA F-ratio | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 1,500 | SE[1] | 1 | 30 | 60 | SE[1] | C | T | C*T |
| pH | 5.29 | 5.27 | 0.02 | $5.36^{b}$ | $5.26^{a}$ | $5.22^{a}$ | 0.02 | $0.47^{ns}$ | $11.02^{*}$ | $3.00^{ns}$ |
| Moisture (%) | 40.27 | 40.86 | 0.36 | $42.81^{c}$ | $40.64^{b}$ | $38.24^{a}$ | 0.44 | $1.38^{ns}$ | $27.29^{***}$ | $0.26^{ns}$ |
| Fat (%) | 32.56 | 32.11 | 0.26 | $31.72^{a}$ | $32.03^{a}$ | $33.25^{b}$ | 0.32 | $1.47^{ns}$ | $6.06^{*}$ | $0.22^{ns}$ |
| Protein (%) | 22.24 | 21.79 | 0.19 | $20.76^{a}$ | $21.85^{b}$ | $23.45^{c}$ | 0.23 | $3.02^{ns}$ | $37.32^{***}$ | $0.05^{ns}$ |
| NaCl (%) | 1.85 | 1.87 | 0.05 | $1.64^{a}$ | $1.85^{b}$ | $2.09^{c}$ | 0.06 | $0.07^{ns}$ | $11.62^{***}$ | $0.79^{ns}$ |
| Water activity | $0.97^{b}$ | $0.96^{a}$ | 0.00 | $0.98^{c}$ | $0.97^{b}$ | $0.96^{a}$ | 0.00 | $26.79^{***}$ | $432.7^{***}$ | $0.39^{ns}$ |
| FAA[2] | $2.76^{b}$ | $2.45^{a}$ | 0.06 | $0.76^{a}$ | $2.96^{b}$ | $4.09^{c}$ | 0.08 | $11.84^{**}$ | $426.2^{***}$ | $3.40^{ns}$ |
| FFA[3] | $2.67^{b}$ | $2.34^{a}$ | 0.08 | $1.64^{a}$ | $2.70^{b}$ | $3.18^{c}$ | 0.10 | $6.87^{*}$ | $56.28^{***}$ | $3.26^{ns}$ |
| L* | $86.72^{a}$ | $87.66^{b}$ | 0.18 | $90.69^{c}$ | $86.03^{b}$ | $84.84^{a}$ | 0.22 | $14.00^{***}$ | $198.9^{***}$ | $0.97^{ns}$ |
| a* | $-1.24^{b}$ | $-1.31^{a}$ | 0.02 | $-0.32^{c}$ | $-1.57^{b}$ | $-1.93^{a}$ | 0.03 | $5.19^{*}$ | $962.3^{***}$ | $8.23^{***}$ |
| b* | 11.83 | 11.50 | 0.13 | $10.74^{a}$ | $12.56^{c}$ | $11.71^{b}$ | 0.16 | $3.17^{ns}$ | $31.57^{***}$ | $1.62^{ns}$ |
| Hardness (N) | 23.36 | 22.58 | 0.52 | $30.28^{b}$ | $19.11^{a}$ | $19.53^{a}$ | 0.63 | $1.13^{ns}$ | $99.5^{***}$ | $0.85^{ns}$ |
| Adhesiveness (N*s) | -1.01 | -1.04 | 0.03 | $-0.58^{a}$ | $-1.24^{b}$ | $-1.26^{b}$ | 0.04 | $0.39^{ns}$ | $84.5^{***}$ | $2.16^{ns}$ |
| Springiness | $0.63^{b}$ | $0.60^{a}$ | 0.01 | $0.82^{c}$ | $0.56^{b}$ | $0.46^{a}$ | 0.01 | $5.80^{*}$ | $619.1^{***}$ | $2.81^{ns}$ |
| Cohesiveness | 0.43 | 0.42 | 0.00 | $0.68^{c}$ | $0.33^{b}$ | $0.27^{a}$ | 0.00 | $1.64^{ns}$ | $2442^{***}$ | $7.47^{**}$ |
| Chewiness (N) | 7.92 | 7.52 | 0.20 | $17.00^{c}$ | $3.64^{b}$ | $2.51^{a}$ | 0.25 | $1.99^{ns}$ | $1062^{***}$ | $0.02^{ns}$ |

[1] SE: standard error.

[2] FAA: Free amino acids (mg leucine/100 g cheese).

[3] FFA: Free Fatty Acids (meq/100 g milk fat).

a, b, c: Different letters in the same row indicate significant differences ($p< 0.05$); ***$P< 0.001$; **$P< 0.01$; *$P< 0.05$; ns: non-significant.

THPLC analysis indicated a high concentration of neomycin in the cheese at the beginning of maturation which increased along time (day 1: 5,590 ± 14 µg/kg; day 30: 6,420 ± 707 µg/kg; day 60: 7,415 ± 120 µg/kg), due to the loss of moisture occurring during this period (Table 1). Similar amounts of residues were obtained along ripening (p>0.05) when antibiotic concentrations are expressed on dry basis. Thus, although neomycin is water soluble (Papich, 2016) results herein indicate that this substance is largely retained in rennet curd and shows high stability under the maturation conditions. In fact, a concentration factor of 4.9 ± 0.1, with respect to the initial concentration of neomicyn in milk, was calculated in Tronchón cheese at the end of ripening. These results agree to those reported by Giraldo *et al.* (2017) when assessing the transfer of antibiotics from goat milk to whey in a laboratory scale study.

## IV – Conclusions

The presence of neomycin, at safety level, in goat's milk destined to the manufacture of ripened cheese may affect the development of biochemical processes during maturation leading to organoleptic shortcomings in the final product. Moreover, high concentrations of the antibiotic can be retained in mature cheese, this poses a potential risk for the consumer. It would be convenient to assess the retention mechanisms of this substance and other antibiotics in depth to avoid public health issues.

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# Innovation for Sustainability in Sheep and Goats

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Sheep and goat farming systems face a harsh present and an uncertain future, apparently compromised by a general lack of competitiveness stemming from poor technical and economic results, but also due to severe social and environmental challenges. Innovative solutions are needed to make the sheep and goat value chain more efficient, profitable and sustainable, but also more appealing for society, particularly to guarantee generational turnover for farms. Such innovations should be aimed at improving production techniques, labour organisation, equipment and infrastructures and developing collective programmes for selection or health campaigns. Innovation should also contribute to strengthening social forms of organisation such as product quality schemes or communal areas management. Also, innovative feeding strategies coupled with precision flock management practices that reduce gaps in production and adjust to the environmental challenges, hold promise to tackle the above mentioned objectives.

This publication compiles 81 contributions presented at the **joint Seminar of the FAO-CIHEAM Sub-Networks on Production Systems and Nutrition on Sheep and Goats, held in Vitoria-Gasteiz, Spain, in October 2017.** The Seminar was co-organised between the Department of Animal Production of Neiker-Tecnalia (the Basque Institute for Agricultural Research and Development) and the Mediterranean Agronomic Institute of Zaragoza (IAMZ-CIHEAM), with collaboration of the H2020 Project iSAGE (Innovation for Sustainable Sheep and Goat Production in Europe), the FAO, and support of the Department for Economic Development and Infrastructures of the Basque Government, the Municipality of Vitoria-Gasteiz, the Diputación Foral de Alava and the Idiazabal Denomination of Origin.

The articles are grouped into the four thematic sessions of the Seminar: (i) Innovation's conceptual and practical framework: application to the agro-food sector; (ii) Innovations to adapt sheep and goat feeding and production systems and industry to new societal demands; (iii) Precision farming and other technical innovations for increasing efficiency in sheep and goats; (iv) Success stories of innovations in the sheep and goat industry, with special focus on increasing consumption and adding value to products.

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