

# Innovation for Sustainability in Sheep and Goats

Edited by:

R. Ruiz, A. López-Francos, L. López Marco



## OPTIONS méditerranéennes

SERIES A: Mediterranean Seminars  
2019 – Number 123



CIHEAM

OPTIONS  
méditerranéennes



# CIHEAM

**Centre International de Hautes Etudes  
Agronomiques Méditerranéennes**

**International Centre for  
Advanced Mediterranean Agronomic Studies**

**Président / President: Mohammed SADIKI  
Secrétaire Général / Secretary General: Plácido PLAZA**

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

Le Centre International de Hautes Etudes Agronomiques Méditerranéennes (CIHEAM) a été créé, à l'initiative conjointe de l'OCDE et du Conseil de l'Europe, le 21 mai 1962. C'est une organisation intergouvernementale qui réunit aujourd'hui treize Etats membres du bassin méditerranéen (Albanie, Algérie, Egypte, Espagne, France, Grèce, Italie, Liban, Malte, Maroc, Portugal, Tunisie et Turquie).

Le CIHEAM se structure autour d'un Secrétariat général situé à Paris et de quatre Instituts Agronomiques Méditerranéens (IAM), localisés à Bari (Italie), Chania (Grèce), Montpellier (France) et Saragosse (Espagne).

Avec au cœur de son action trois missions fondamentales (formation, recherche, coopération), le CIHEAM s'est progressivement imposé comme une référence dans ses domaines d'activité : l'agriculture, l'alimentation et le développement rural durable en Méditerranée.

Founded in 1962 at the joint initiative of the OECD and the Council of Europe, the International Centre for Advanced Mediterranean Agronomic Studies (CIHEAM) is an intergovernmental organisation comprising thirteen member countries from the Mediterranean Basin (Albania, Algeria, Egypt, France, Greece, Italy, Lebanon, Malta, Morocco, Portugal, Spain, Tunisia and Turkey).

CIHEAM is made up of a General Secretariat based in Paris and four Mediterranean Agronomic Institutes (IAM) located in Bari (Italy), Chania (Greece), Montpellier (France) and Zaragoza (Spain).

In pursuing its three central missions (education, research and cooperation), CIHEAM has become a reference in its fields of activity: Mediterranean agriculture, food and sustainable rural development.

## **IAM Instituts Agronomiques Méditerranéens Mediterranean Agronomic Institutes Bari - Chania - Montpellier - Zaragoza**

### **IAM-Bari**

Dir.: Maurizio RAELI  
Via Ceglie, 9  
70010 Valenzano, Bari, Italy  
Tel.: (+39) (080) 4606 111 - Fax: (+39) (080) 4606 206  
iamdir@iamb.it  
www.iamb.it

### **IAM-Montpellier**

Dir.: Pascal BERGERET  
3191, Route de Mende  
34093 Montpellier Cedex 5, France  
Tel.: (+33) (0)4 67 04 60 00 - Fax: (+33) (0)4 67 54 25 27  
pascal.bergeret@iamm.fr et/and sciuto@iamm.fr  
www.iamm.fr

### **IAM-Chania**

Dir.: Giorgios BAOURAKIS  
P.O. Box 85  
73100 Chania, Crete, Greece  
Tel.: (+30) 28210 35000 - Fax: (+30) 28210 35001  
baouraki@maich.gr  
www.maich.gr

### **IAM-Zaragoza**

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

# Validation of a microbiological inhibition system based on Eclipse Farm 3G coupled with e-Reader to screening $\beta$ -lactam and tetracycline antibiotics in goat's cheese whey

J. Giraldo<sup>1</sup>, R. Cabizza<sup>2</sup>, L. Mata<sup>3</sup> and M.P. Molina<sup>1</sup>

<sup>1</sup>Universitat Politècnica de València, Institute of Animal Science and Technology, Camino de Vera, 14, 46022 Valencia (Spain)

<sup>2</sup>Università degli Studi di Sassari, Dipartimento di Agraria, Viale Italia, 39, 07100 Sassari (Italy)

<sup>3</sup>Zeulab S.L., R&D Department. Bari, 25 dplp, 501970 Zaragoza (Spain)

**Abstract.** The presence of antimicrobial residues in milk and dairy products could cause negative technological effects and represents a risk for consumer health. During the cheese-making process, antibiotics could be retained in curd or eliminated in whey to a greater or lesser extent. Whey is a by-product used in the manufacture of foodstuffs for human consumption and animal feeding and therefore, the presence of antibiotic residues in this product should be controlled. Thus, it is crucial to develop an analytical strategy to screen antibiotics in whey. A new system for screening antibiotics in raw milk was recently developed, in which a microbial inhibitor tube test (Eclipse Farm) and a device (e-Reader) are coupled. The new system allows the incubation of the test and a continuous monitoring of the test colour change. The aim of this work was to study the performance of the new system for the detection of  $\beta$ -lactam and tetracycline residues in goat's cheese whey. A preliminary study demonstrated the necessity to include a one-hour diffusion period at room temperature. The performance was validated in agreement with European Commission Decision 2002/657/EC. Specificity of microbial test was evaluated by analysing one hundred and thirty whey samples. Results showed a low percentage of false-positive results. The detection limits for amoxicillin, benzilpenicillin, cephalexin and oxytetracycline in goat's cheese whey were calculated and the detection capabilities (CC $\beta$ ) were below or at the MRL levels, with the exception of amoxicillin, which was slightly above them. In conclusion, Eclipse Farm coupled to e-Reader represents an appropriate method to screen  $\beta$ -lactams and tetracycline residues in whey.

**Keywords.** Whey – Antibiotic –  $\beta$ -lactams – Tetracyclines – Screening test.

**Validation d'un système d'inhibition microbiologique basé sur Eclipse Farm 3G couplé avec un lecteur e-Reader pour le dépistage des antibiotiques  $\beta$ -lactam et tétracycline dans le lactosérum de fromage de chèvre**

**Résumé.** La présence de résidus d'antibiotiques dans le lait et les produits laitiers peut entraîner des problèmes technologiques et un risque pour la santé des consommateurs. Pendant le processus de fabrication du fromage, les antibiotiques peuvent rester attachés au caillé ou peuvent être éliminés dans le sérum. Le lactosérum est un sous-produit utilisé dans la fabrication de denrées alimentaires destinées à la consommation humaine et à l'alimentation animale et, par conséquent, la présence de résidus d'antibiotiques dans ce produit doit être bien contrôlée. Il est donc essentiel de mettre en place une stratégie analytique pour l'analyse des antibiotiques dans le lactosérum. Un nouveau test d'inhibition microbienne (Eclipse Farm) couplé avec un dispositif d'incubation et de lecture (e-Reader) a été récemment développé pour le dépistage d'antibiotiques dans le lait cru. Le nouveau système permet l'incubation et la surveillance continue du changement de la couleur du test. L'objectif de ce travail consistait à étudier la performance du nouveau système pour la détection des résidus de  $\beta$ -lactames et de tétracyclines dans le lactosérum du fromage de chèvre. Une étude préliminaire a démontré la nécessité d'inclure une étape de diffusion d'une heure à température ambiante. Le système a été validé conformément aux procédures décrites dans la décision 2002/657/CE de la Commission Européenne. La spécificité du test a été évaluée en analysant 130 échantillons de lactosérum. Les résultats ont montré un faible pourcentage de résultats faussement positifs. Les limites de détection pour l'amoxicilline, la benzilpenicilline, la cé-

*phalexine et l'oxytétracycline dans le lactosérum du fromage de chèvre ont été calculées, ainsi que les capacités de détection (CC $\beta$ ), toutes en dessous ou au même niveau que les Limites de Résidus Maximum (LRM), sauf pour l'amoxicilline, avec une limite légèrement supérieure. En conclusion, Eclipse Farm couplé à e-Reader représente une méthode appropriée pour la détection des résidus des  $\beta$ -lactames et tétracyclines dans le lactosérum de chèvre.*

**Mots-clés.** Lactosérum – Antibiotique –  $\beta$ -lactames – Tétracyclines – Test de dépitage.

---

## I – Introduction

The wide use of antimicrobials in food-producing animals, to prevent and to treat diseases, can constitute a problem in milk and dairy products due to the presence of antibiotic residues. In fact, their presence in milk could cause negative technological effects (Berruga *et al.*, 2008). Moreover the presence of antibiotics in milk products could have direct health implications for the consumers with allergic reactions and lead to an increase of resistant bacteria with serious consequences in human and animal health (WHO, 2014). To guarantee the consumer health, the European Union established the Maximum Residue Limits (MRL) in milk but up to now, no limits has been established for dairy products, as cheese and whey (EC, 2010).

Whey is a by-product of cheese-making process with many applications in food industry and livestock and therefore, possible presence of drug residues could have direct negative effects for human and animal health and in dairy sector by affecting fermentation processes. During cheese-making process, antibiotics present in milk, could be retained in cheese or carried in whey depending of the chemical nature of each substance (Cabizza *et al.*, 2016). For all these reasons, the availability of an analytical strategy to screen antibiotics in whey seems necessary.

In last years the strategy to control of antibiotics in milk has been introduced at different levels of the chain. Nowadays, not only central laboratories but dairies and farmers are aware with the control of antibiotics. Thus, the availability of automatic screening methods has become a real need. A new system for the screening of antibiotics in raw milk was recently developed (Mata *et al.*, 2016). This new system combine a microbial inhibitor tube test (Eclipse Farm 3G) and a device (e-Reader) that allows the incubation of the test and the continuous monitoring of the color change. The aim of this work was to study the performance of this new system for detection of  $\beta$ -lactams and tetracyclines in goat's cheese whey.

## II – Material and methods

### 1. Whey Samples

Whey samples were produced by the application of a laboratory scale cheese-making, as described by Giraldo *et al.* (2017). Raw milk was obtained from the experimental flock of healthy and untreated Murciano-Granadina goats at Universitat Politècnica de Valencia (Valencia, Spain). Whey samples were analyzed for chemical composition using MilkoScan 6000 (Foss, Hillerød, Denmark) and the pH value was valued by a pH-meter (Crison, Barcelona, Spain).

### 2. Antibiotics

Four antibiotics representative of the most used molecules in veterinary were chosen. The molecules tested were amoxicillin, benzilpenicillin, cephalixin, and oxytetracycline. All antibiotics were purchased from Sigma-Aldrich Química, S.A. (Madrid, Spain). Stock solutions (1 mg/mL) were prepared in according to International Dairy Federation (ISO/IDF, 2003). Working dilutions were prepared daily.

### 3. Performance of Eclipse Farm 3G Test and e-Reader

To assay the detections of antibiotics in whey, Eclipse Farm 3G test (ZEULAB, Zaragoza, Spain) coupled to the e-Reader detector were used. The test procedure was carried out following manufacturer recommendations, doing a preliminary study to improve the system for the analysis of milk whey samples. This modification of original instructions was necessary to eliminate gradients of color (purple and yellow). For this, 10 goat's milk whey samples were tested with different treatments: centrifugation (3000 rpm, 10 minutes), heating (80°C, 10 minutes), centrifugation and heating (under the same conditions) and with pre-diffusion for one hour at room temperature.

Previously, cut-off level for Eclipse Farm coupled to e-Reader was calculated following indications by Mata *et al.* (2016). Seventy-five goat's milk whey samples were used, obtaining their mean and standard deviation. The cut-off for microbiological test was the result of mean value plus three times the standard deviation.

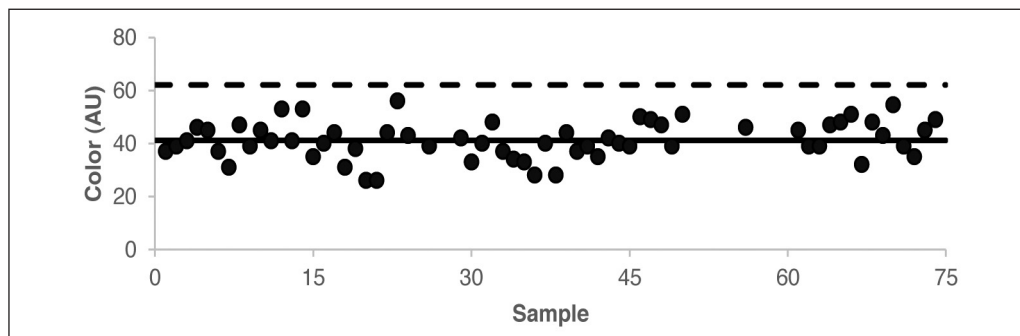
A study of false positive rate was also carried out and, in this case, 130 negative goat's milk whey samples were tested.

To determinate the detection capabilities ( $CC\beta$ ) with whey samples the method previously described by Mata *et al.* (2016) was applied according to the CRL guidelines (CRLs, 2010).

### III – Results and discussion

After evaluating several procedures to prepare samples for the analysis, adding a step of pre-diffusion for 1 hour at room temperature was selected as the best choice.

The cut-off was calculated by using the mean value from 75 negative samples (41.2) and the corresponding standard deviation (7.0). The result was of 62.1 (mean of the negative samples plus 3 times the standard deviation), as it is observed in Fig. 1. Thus, e-reader values equal or above 62 were considered as positive samples.



**Fig. 1. Results obtained by e-Reader for 75 goat's whey samples analysed with Eclipse Farm test. Solid line indicates the average value. Dashed line indicates the proposed cut-off level with a confidence interval higher than 99%.**

The composition of goat's whey samples is presented on the Table 1. A minimum false-positive rate was found for these goat's milk whey samples, which was equal to 2.3%. This percentage is in agreement with the false positive rate calculated by Beltrán *et al.* (2015) for goat's milk using different microbial inhibitor tests (BRT MRL, 1.4%; Delvotest SP-NT, 4.3%; Delvotest DA, 3.1%; Eclipse 100, 0.6%). Therefore, Eclipse Farm coupled to e-Reader continues being appropriated, on terms of false-positive results, when whey matrix is analyzed.

**Table 1. Quality parameters of goat's milk whey samples free of antimicrobials**

Parameters	Mean	SD <sup>a</sup>	Minimum	Maximum
pH	6.45	0.22	5.46	6.69
Fat	0.68	0.16	0.33	1.21
Protein	1.09	0.15	0.83	1.48
Lactose	4.91	0.10	4.63	5.10
Total solids	6.69	0.16	6.36	7.12

<sup>a</sup> SD: standard deviation.

The detection limits and detection capabilities goat's milk whey for 4 antimicrobials are summarized in Table 2.

**Table 2. Detection limits ( $\mu\text{g}/\text{kg}$ ) and detection capabilities of Eclipse Farm coupled to e-Reader for four representative  $\beta$ -lactams and tetracycline residues in goat's milk whey**

Antimicrobial	MRL <sup>a</sup> ( $\mu\text{g}/\text{kg}$ )	Detection limit <sup>b</sup> ( $\mu\text{g}/\text{kg}$ )	e-Reader value <sup>c</sup>	Detection capability <sup>b</sup> ( $\mu\text{g}/\text{kg}$ )	No. positive <sup>d</sup> / no. samples	e-Reader value <sup>d</sup>
Amoxicillin	4	4	71.4 $\pm$ 5.3	5	20/20	106.8 $\pm$ 11.8
Benzilpenicillin	4	3	107.0 $\pm$ 15.2	3	40/40	117.2 $\pm$ 24.4
Cephalexin	100	>50	63.2 $\pm$ 18.4	60	39/40	108.4 $\pm$ 19.2
Oxytetracycline	100	>60	67.8 $\pm$ 12.3	100	60/60	94.9 $\pm$ 12.2

<sup>a</sup> MRL: EU Maximum Residue Limit ( $\mu\text{g}/\text{kg}$ ).

<sup>b</sup> Positive results are defined as an e-Reader value higher than 62.

<sup>c</sup> Mean  $\pm$  SD (n = 5).

<sup>d</sup> Mean  $\pm$  SD.

According to the Table 2, all the obtained detection limits are below or equal to their corresponding MRLs. However, in the case of detection capabilities, in which a higher number of samples is tested, amoxicillin has shown a higher value ( $\text{CC}\beta = 5 \mu\text{g}/\text{kg}$ ) than their legal limit. It is observed that when a low e-Reader value is determined for the detection limit (amoxicillin and oxytetracycline) the corresponding  $\text{CC}\beta$ s are higher but with high e-Reader values (Table 2). These results suggest that  $\text{CC}\beta$ s for both molecules are close to detection limits but statistically do not reach the same level. Moreover, standard deviations were relatively high, suggesting that there is an important variability in the results between repetitions of fortified whey samples.

Comparing our results with those reported by other authors in milk, some differences are found. Beltrán *et al.* (2015) obtained  $\text{CC}\beta$  higher than the calculated in this study using different microbiological tests (BRT MRL, Delvotest SP-NT, Delvotest DA and Eclipse 100) in sheep and goat's milk. In the case of goat's milk, in general, all detection capabilities were lower than the obtained for cheese whey samples, with the exception of the cephalixin, whose  $\text{CC}\beta$  for BRT MLR and Delvotest SP-NT was around or above their MRL (>100  $\mu\text{g}/\text{kg}$  and 75  $\mu\text{g}/\text{kg}$  respectively) and oxytetracycline could not be detected at MRL by any test used in this study.

Using Eclipse Farm coupled to e-Reader to analyze cow's milk samples, Mata *et al.* (2016) studied detection limits and detection capabilities for these same antimicrobials and they obtained results too similar to this study. Regarding detection capabilities, the e-Reader values in cow's milk were lower than in goat's cheese whey for amoxicillin ( $\text{CC}\beta = 4 \mu\text{g}/\text{kg}$ ) and cephalixin ( $\text{CC}\beta = 50 \mu\text{g}/\text{kg}$ ), but for oxytetracycline, detection capability was the same for both matrices.

Finally, these results could be compared with the obtained by Giraldo *et al.*, (2016), who tested also these same antibiotics in goat's milk whey, using Eclipse 100 (ZEULAB, Spain), a microbiological

test with similar characteristics than the used in this study. Detection capabilities were slightly lower than the calculated in this study for amoxicillin ( $CC\beta = 3.9 \mu\text{g}/\text{kg}$ ), benzylpenicillin ( $CC\beta = 2.6 \mu\text{g}/\text{kg}$ ) and cephalexin ( $CC\beta = 48.4 \mu\text{g}/\text{kg}$ ). On the contrary, for oxytetracycline, the  $CC\beta$  was above the MRL of this molecule ( $116.8 \mu\text{g}/\text{kg}$ ).

## IV – Conclusions

Eclipse Farm coupled to e-Reader allow to detect benzipenacillin, cephalexin and oxytetracycline to concentrations below or equal to their MRLs, with a minimum rate of false-positive results. Thus, this method could be a good alternative to screening of  $\beta$ -lactams and tetracyclines residues in cheese whey.

## Acknowledgments

This work is part of the AGL-2013-45147-R funded by the Ministry of Science and Innovation (Madrid, Spain).

## References

- Beltrán M.C., Berruga M.I., Molina A., Althaus R.L. and Molina M.P., 2015.** Performance of current microbial tests for screening antibiotics in sheep and goat milk. In: *International Dairy Journal*, 41, p. 13-15.
- Berruga M.I., Battacone G., Molina M.P., Román M. and Molina A., 2008.** Influence of  $\beta$ -lactams on Manchego cheese manufacture. In: *IDF special issue: 5th international symposium on the challenge to sheep and goats milk sectors*, part 3, p. 222-224.
- Cabizza R., Rubattu N., Salis S., Pes M., Comunian R., Paba A., Addis M., Testa C. and Urgeghe P.P., 2016.** Transfer of oxytetracycline from ovine spiked milk to whey and cheese. In: *International Dairy Journal*, 70, p. 12-17.
- CRLs, 2010.** Guidelines for the validation of screening methods for residues of veterinary medicines. Community Reference Laboratories for residues. In: [http://ec.europa.eu/food/food/chemicalsafety/residues/lab\\_analysis\\_en.htm](http://ec.europa.eu/food/food/chemicalsafety/residues/lab_analysis_en.htm)
- EC, 2010.** Commission Regulation (UE) No 37/2010 of 22 December 2009 on pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin. In: *Official Journal of the European Union*, L15, p. 1-72.
- Giraldo J., Althaus R.L., Beltrán M.C. and Molina M.P., 2017.** Antimicrobial activity in cheese whey as an indicator of antibiotic drug transfer from goat milk. In: *International Dairy Journal*, 69, p. 40-44.
- ISO/IDF, 2003.** Milk and milk products- Guidelines for a standardized description of microbial inhibitor tests. In: *IDF Standard 183*, International Dairy Federation. Brussels, Belgium:
- Mata L., Sanz D. and Razquin P., 2016.** Performance of Eclipse Farm Test Coupled to e-Reader for Antibiotic Residues Detection in Raw Milk. In: *Food Analytical Methods*, 9, p. 519-527.
- WHO, 2014.** Antimicrobial resistance. In: *Bulletin of the World Health Organization*, 61, p. 383-394.



# CIHEAM

Centre International de Hautes Etudes  
Agronomiques Méditerranéennes

International Centre for  
Advanced Mediterranean Agronomic Studies

---

**Conseil d'Administration / Governing Board**  
**Président / President: Mohammed SADIKI**

## **Délégués des Pays Membres / Member Country Delegates**

Albanie / Albania: Irfan TARELLI

Algérie / Algeria: Saida ZOUGGAR

Egypte / Egypt: Mohammed SOLIMAN MOHAMED

Espagne / Spain: Sebastián FRAILE ARÉVALO

France: Valérie VADUEL

Grèce / Greece: Charambalos KASIMIS

Italie / Italy: Teodoro MIANO

Liban / Lebanon: Wafaa DIKAH HAMZE

Malte / Malta: Justin ZAHRA

Maroc / Morocco: Mohammed SADIKI

Portugal: Nuno FIGUEIRA BOAVIDA CANADA

Tunisie / Tunisia: Mahmoud Elies HAMZA

Turquie / Turkey: Nihat PAKDIL

---

## **Comité Scientifique Consultatif / Scientific Advisory Committee**

### **Membres / Members:**

**Maher Abdel-Mohsen ABDEL-HAMID** (Cairo University – EGYPT)

**Tomás GARCIA AZCARATE** (CSIC – SPAIN)

**Lamiae GHAOUTI** (IAV Hassan II – MOROCCO)

**Giulio MALORGIO** (Universita di Bologna – ITALY)

**Apostolos PAPADOPOULOS** (Harokopio University of Athens – GREECE)

**Suat ŞENSOY** (Yuzuncu Yil University – TURKEY)

**Carole SINFORT** (Montpellier SupAgro – FRANCE)



# OPTIONS

## méditerranéennes

SERIES A: Mediterranean Seminars  
2019 – Number 123

### Innovation for Sustainability in Sheep and Goats

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

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.

OPTIONS  
méditerranéennes



Food and Agriculture Organization  
of the United Nations

Prix: 76,22 Euro

ISBN: 978-2-85352-590-9

ISSN: 1016-121-X