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Interpretive Summary:

Title: Quantification of milk yield and composition changes as affected by subclinical mastitis along the current lactation in sheep

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Subclinical mastitis causes great economic losses in ovine dairy livestock due to the reduction of milk yield and the alteration of its chemical composition. In this paper the effect of subclinical mastitis on milk yield and composition has been quantified on the half-udder basis by a direct comparison between infected and uninfected glands. A compensation of milk loss in the infected gland by an increase of milk production in the uninfected one has been confirmed. Changes appeared in the very week of infection and remained within the current lactation.

Quantification of milk yield and composition changes as affected by subclinical mastitis along the current lactation in sheep

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ABSTRACT

The aim of this work was to quantify at half-udder level the changes of ewe milk yield and composition because of unilateral subclinical mastitis within the current lactation. Fluctuations due to production level, infection severity, time from the onset of infection and lactation curves were also researched. Yield and composition of milk from half-udders of unilateral infected ewes were compared between them and with a set of healthy halves using a mixed model. The experiment was completed with a whole-udder approach on the same animals. To test the effect of intramammary infection **(IMI)** in the following 7 weeks from the onset of infection, 20 ewes that acquired unilateral subclinical mastitis during lactation and 40 healthy ones were used. Another group of 20 ewes unilaterally infected from the first lactation week and other 40 healthy ones were studied to test the effect of IMI on the lactational milk yield and composition. The individual milk loss in ewes infected along lactation was of 15% for the following 7 weeks after the onset of infection, and a 6.6% more milk production by the uninfected half to compensate milk lost by the infected one was quantified. The lactational milk yield loss in ewes infected from post-partum week was of 17%. The changes on milk yield were noticed from the very week of infection diagnosis. The production level of animals influenced the milk yield changes caused by IMI in such a way that the more productive ewes lost more milk, although these losses were proportional to their production level. On the other hand, infection severity affected milk loss between glands, being more pronounced as SCC increased. A clear decrease of lactose content and of casein/protein ratio because of subclinical IMI was obtained and this reduction was not modified along the trial.

Key words: subclinical mastitis, ewe milk yield, ewe milk composition, lactation.

INTRODUCTION

Mastitis causes loss of milk yield and modification of main components as a result of damage in the mammary secretor tissue (Burriel, 1997; Burriel and Wagstaff, 1998). Previous studies on dairy sheep reported that subclinical intramammary infection (IMI) by CNS is the

single major factor affecting flock milk yield (Gonzalo et al., 2002; Leitner et al., 2008; Giadinis et al., 2012) and cause negative effects on milk cheese-making suitability (Leitner et al., 2004; Martí-De Olives et al. 2011). In contrast to clinical mastitis, subclinical one is imperceptible and therefore affected udders are milked into the bulk milk tank. Frequently the glands are not treated and the milk yield loss remains during the subsequent lactation (Gonzalo et al., 2004). Infection severity, type of bacteria and unilateral or bilateral character (one or two infected glands, respectively) determine the consequences of subclinical IMI on ewe milk yield (Gonzalo et al., 2002). A relationship between lactation stage and subclinical mastitis has also been reported, in such a way that milk yield is more correlated to the SCC at the end of lactation than at the beginning (Arias et al., 2012).

Milk production losses as affected by subclinical IMI in sheep has been demonstrated by means of different approaches, as much at individual (Saratsis et al., 1999; Gonzalo et al., 2002; Leitner et al., 2003) as at half-udder level (Leitner et al., 2004; Cuccuru et al., 2011). However, quantifying this effect with precision is difficult. The conventional whole-udder approach requires a data set of a numerous samples to take account for the large and significant individual variations (Gonzalo et al., 1994, 2002). Moreover, when only one gland is infected, the effect of mastitis can be underestimated because of a possible compensatory milk production from uninfected half (Leitner et al., 2004).

Subclinical IMI also leads to a modification of main milk components. In ovine milk the content of lactose decreased with IMI (Díaz et al., 1996; Burriel, 1997; Bianchi et al., 2004) mainly because of the reduced synthesis capacity of damaged tissue (Burriel, 1997), but also because of a lesser availability of its precursor, the glucose, due to a competition for energy between secretor cells and those with phagocyte function (Rulquin, 1997). Fat and casein are modified in some way or other depending on the magnitude of milk yield reduction. Frequently, the reduction of milk volume could be greater than the decrease of fat and casein

synthesis as a result of IMI, resulting in a concentration of those components in milk (Schultz, 1977; Burriel, 1997). In adition, lactose can amplify this concentration effect because it is an osmotic regulator of milk, its decrease involving itself a reduction of milk volume (Munro et al., 1984; Burriel, 1997). On the other hand, the components that come from blood, like whey proteins, normally increase because of the higher blood-milk barrier permeability during an IMI (Díaz et al., 1996; Leitner et al., 2003; Bianchi et al., 2004).

With the aim of quantifying the changes in ewe milk yield and composition because of unilateral subclinical mastitis within the current lactation, two studies were developed on two groups of ewes according to the moment of first IMI diagnosis: the first study was done on ewes infected along lactation, in which there were preinfection values that corrected the postinfection ones and improved the precision (Rajala-Schultz et al., 1999). The second study was done on ewes infected from post-partum week, and its objective was to state the curves of lactation and the lactational milk yield and composition changes. To obtain a high reliable estimation, the experiment was carried out by means of both half-udder and whole-udder approaches.

MATERIALS AND METHODS

Animals and Experimental Design

This work was carried out during two annual lactations on the experimental farm flock of Manchega ewes of the Institute for Animal Science and Technology of the Polytechnic University of Valencia (Spain). The trial was accomplished with an initial number of 145 ewes without sign of clinical mastitis (76 and 69 ewes for the first and second year, respectively). Ewe parities were: first, 33; second and plus, 112. The animals were stabled throughout the lactation period and were machine milked twice daily at 08:30 and 17:30 h from third day after lambing.

In each annual lactation period the trial was developed during 16 weeks. All births occurred along 4 weeks, so all animals were checked at least 12 lactation weeks. The presence of IMI in each gland was tested by bacteriological and SCC measures twice a week during the first 15 d after lambing and then once a week for the next 14 wk. Milk yield and main components were checked once a week, both on complete udders at morning and evening machine milking and on half-udders by emptying the gland by hand after an oxytocin injection (productive potential of milk, PPM). Checking on complete udder milk was made from the first week post-partum and checking on half-udder milk was made from the second lactation week, because of the difficulty on emptying completely the glands during the first days of lactation.

To increase the incidence of IMI, and consequently the number of cases to study, 2 management practices were applied. The first was the immersion of all teats in a bacterial suspension of *Staphylococcus simulans* (5×10^7 cfu/mL) between wk 4 and 8 after lambing. The immersions were practised on 4 alternating days, at the morning and evening milking (8 milkings) and immediately before application of the milking unit. The second practice was no dipping of teats after milking throughout the trial.

Bacterial Challenge

The bacterial suspension was made from a *Staph. simulans* strain obtained from a gland with subclinical mastitis from a commercial flock. *Staphylococcus simulans* forms part of the group of CNS that are considered opportunist microorganisms normally found on healthy teat skin as well as on the hands of milkers. Consequently, *Staph. simulans* may easily colonize the teat canal and infect the mammary gland. The bacterial suspension used to dip the teats was prepared according to Hogan et al. (1990). Stock cultures of *Staph. simulans* were stored at −20°C in 50% glycerine. A 6-mL tube of trypticase soy broth (TSB) was inoculated from a vial of stored stock culture and incubated at 37°C for 7 h. One milliliter of this starter culture was used to inoculate

500 mL of TSB, which was then incubated for 16 to 18 h at 37°C on a gyratory shaker. Cells were pelleted by centrifugation, washed twice with a 0.1% water solution of proteose-peptone (no. 3, Difco Laboratories, Detroit, MI), and resuspended in proteose-peptone. A standard plate count was conducted on the stock suspension before it was stored at 5°C. The plate count was used to determine the dilution required to prepare daily challenge suspensions containing 5×10^7 cfu/mL in TSB. The challenge suspension was prepared immediately before use.

Bacteriological Analysis

To obtain milk samples for bacteriological analysis, teats were carefully cleaned with 70% ethanol and the first three streams of foremilk were discarded. Approximately 10 mL of milk were collected aseptically from each gland before the morning milking. Samples were kept at 4ºC for a maximum of 12 h until bacteriological analysis. Twenty microliters of each sample were plated on blood agar plates (5% washed sheep eritrocytes; Biomerieux, Lyon, France). The plates were incubated aerobically at 37ºC and examined at 24 h, 48 h, and 7 d. Cultures with five or more identical colonies were considered positive for IMI. A new IMI in a half udder was diagnosed when the same bacterial species was isolated from two consecutive positive for IMI samples ($\geq$ 250 cfu/mL). A gland diagnosed with IMI was considered infected from the first sampling in which the culture was positive for IMI. Bacteria were identified according to the National Mastitis Council recommendations (Harmon et al., 1990). Identification of staphylococci was carried out using commercial micromethods (API STAPH; BioMèrieux, Lyon, France). SCC was determined with a Fossomatic 90 (A/S N Foss Electric, Hillerød, Denmark) in all samples taken for the bacteriological analysis. Samples remained under refrigeration for 24 to 48 h before being analyzed (IDF, 1995).

Sampling and Analysis

Whole-udder milk yield was determined by volume measurers during morning and evening milking. Half-udder milk yield was estimated by using the productive potential method such

that a first intravenous injection of 3 I.U. of oxytocin was administered to animals after the milking and the glands were completely emptied by handling milking. After a period of 4 hours a second injection of oxytocin (3 I.U.) was administered and glands were completely emptied again, and milk of each gland was collected separately. The obtained milk by this method was measured using graduated test tubes. To estimate the quantity of milk that each gland could synthesize par day (PPM), the milk quantity obtained within 4 hours was multiplied by 6.

From each milk sample, both obtained from complete udders and from half udders, a 50 mL aliquot was transferred into a plastic storage jar and was moved into portable refrigerator and storaged at 4ºC until analysis. Milk composition (fat, protein, true protein, casein, whey protein, lactose and dry matter) was determined by midrange infrared spectroscopy using a MilkoScan FT120 (Foss Electric, Hillerød, Denmark). The mean percentage of components in milk samples from whole-udder were determined by meaning percentages obtained from morning and evening milking weighted by volume of milk.

Grouping of Ewes for Data Analysis

Depending on the moment of the first IMI diagnosis, the infected ewes were divided into two groups to be analysed within two separate studies. The first group included ewes that were free of IMI at first post-partum days and acquired unilateral subclinical mastitis along lactation. The second group comprised ewes that were diagnosed with unilateral subclinical mastitis at first checking, at post-partum period. In both cases a set of healthy ewes were selected to be blocked into trios together with infected ewes as control animals. In each trio, one ewe was infected and the other two ones were healthy throughout the trial and similar in parity, milk production and lactation state at the moment of selection.

Study of ewes infected along lactation. 20 unilaterally infected ewes and the 40 free of IMI ones with which they were blocked were included in this analysis. Ewes with an

inappropriate for the experiment udder health status (clinical mastitis, bilateral infection) and too late infected sheep (with less than 5 weeks postinfection) were excluded. Parities of the 20 ewes were: first, 10; second and plus, 10. The ewes acquired IMI between the 3th and 10th lactation week and kept infected during the rest of the trial. The averaged infection period during which they were permanently infected was 6.5 weeks. The variable "Infection Week" (IW) was defined as the time (in weeks) that passed from the moment of first IMI diagnosis. So, IW = -1 corresponded to the week before the first diagnosis, IW = 0 was the week of first diagnosis, week IW = 1 the week just after the first diagnosis, and so on until IW = 6 (from there on we had not enough animals to be analysed). Therefore two experimental periods were established: a preinfection period of two weeks (IW = -1 and -2), and a postinfection period of 7 weeks (IW = 0, 1, 2, 3, 4, 5, and 6).

Study of ewes infected from post-partum week. 20 ewes with unilateral subclinical mastitis at post-partum period and 40 free of IMI ewes with which they were blocked were included. Therefore, there was not a preinfection period in this case. Ewes with an inappropriate for the experiment udder health status (clinical mastitis, bilateral infection) were excluded. Parities of the 20 ewes were: first, 9; second and plus, 11.

Statistical Analysis

Statistical analyses were performed using the SAS program (SAS, 2011). Several mixed-effects models (by Mixed procedure) were used to study the effects of different factors on Log_{10} SCC, individual milk yield, PPM of half udders and milk composition parameters. At half-udder level the following mixed model was used to analyse data of ewes infected along lactation in the preinfection period:

$$Y_{ijklmn} = \mu + YEAR_i + EIS_j + E_k\ (YEAR \times EIS_{ij}) + GIS_l + GLA_m\ (E \times GIS_{kl}) + IW_n + YEAR \times EIS_{ij} + E \times GIS_{kl} + EIS \times GIS_{jl} + EIS \times GIS \times IW_{jln} + EIS \times GIS \times YEAR_{jli} + e_{ijklmn} \quad [1]$$

Where Y_{ijklmn} = records of each variable; μ = general mean; $YEAR_i$ = year effect (i = 1, first

year; i = 2, second year); EIS_j = fixed effect of ewe infectious status (j = 1, healthy ewes; j = 2, infected ewes); E_k $(YEAR\times EIS_{ij})$ = random effect of the ewe nested in $YEAR\times EIS_{ij}$ interaction (k = 1, 2, 3, …, 60); GIS_l = fixed effect of gland infectious status [l = 1, infected glands from infected ewes (type A glands) or healthy glands from healthy ewes (type C glands); l = 2, healthy contralateral glands to A glands (type B glands) or healthy contralateral glands to C glands (type D glands); letters C and D were randomly assigned to each gland of healthy ewes; GLA_m $(E\times GIS_{kll})$ = random effect of gland nested in $E\times GIS_{kll}$ interaction (m = 1, 2, 3, …, 120); IW_n = fixed effect of infection week (n=0, 1, 2, 3,….6); $YEAR\times EIS_{ij}$ = year × ewe infectious status interaction; $E\times GIS_{kl}$ = ewe × gland infectious status interaction; $EIS\times GIS_{jl}$ = ewe infectious status × gland infectious status interaction; $EIS\times GIS\times IW_{jln}$ = ewe infectious status × gland infectious status x infection week interaction; $EIS\times GIS\times YEAR_{jli}$ = ewe infectious status × gland infectious status x year interaction ; and e_{ijklmn} = residual effect.

In the postinfection period, the mixed model used to analyse data of ewes infected along lactation at half udder level was the same as [1] with adding the mean value of preinfection period as a covariate (COV). At individual level the model used to analyse data of ewes infected along lactation in the preinfection period was:

$$Y_{ijkl} = \mu + YEAR_i + EIS_j + E_k\,(YEAR\times EIS_{ij}) + IW_l + YEAR\times EIS_{ij} + EIS\times IW_{jl} + e_{ijkl} \qquad [2]$$

The model used in the postinfection period to analyse data of ewes infected along lactation at individual level was the same as [2] with adding the mean value of preinfection period as a covariate (COV). The models used to analyse data of ewes infected from post-partum week were the same that above ([1] and [2]) except that the covariate was not included in any case because of the absence of preinfection period.

To evaluate the influence of infection severity and milk production level on the magnitude of milk losses, both in the study of ewes infected along lactation and that of ewes infected from post-partum wk, several regression analyses were done with the Reg procedure. A file

with mean values of the infected ewes was used to make the regressions. There were two dependent variables: the first was, for each infected ewe, the mean value of the differences between the PPM of healthy and infected gland along the postinfection period (PPMDm); the second was, for each infected ewe, the PPMDm expressed in relative terms, calculated by dividing PPMDm by the postinfection mean PPM of healthy gland (PPMDm%). The independent variables were calculated by different manner depending on the group of ewes considered. In the case of ewes infected along lactation the independent variables were, for each ewe, the mean value of PPM of both glands in the preinfection period (PPMm), and the mean value of Log_{10}SCC of the infected gland during the postinfection period (Log_{10}SCCm). In the case of ewes infected from post-partum wk, the independent variable Log_{10}SCCm was calculated by the same way but the PPMm was calculated, for each ewe, as the mean value of PPM of healthy gland during the 3 first checking. A total of 12 regression equations were obtained, in the way that for each dependent variable (PPMDMm and PPMDMm%) three regression equations were obtained for each study: two regressions with one variable (PPMm or Log_{10}SCCm) and one regression with the two variables all together.

RESULTS

Characteristics of Ewes

In the study of ewes infected along lactation, the infections occurred between weeks 3 and 10 from lambing, although the majority (14 ewes out of 20) were infected between weeks 7th and 9th. Most of the infections, 13 out of 20 isolates, were caused by the same bacteria utilized to carry out the immersions (*Staph. simulans*), whereas the other infections were caused by *Staph. epidermidis* (in three isolates), and *Streptococcus* spp., *Staph. xylosus*, *Micrococcus spp*., and *Str. Bovis* in one isolate each one.

In the group of ewes infected from post-partum week, the most isolated microorganisms were *Staph. simulans* (10 infections), followed by *Streptococcus* spp. (2 isolates); and finally

the germs *Staph. epidermidis, Klebsiella pneumoniae, Staph. caprae, Staph. capitis, Serratia marcencens, Staph. aureus, Staph. xylosus* and *Staphylococcus* spp. caused one mastitis each one.

Effect of Subclinical IMI at Half-Udder Level

In the preinfection period, in the study of ewes infected along lactation year was statistically significant upon all variables, but the YEAR x EIS x GIS interaction effect was not significant. The effect of EIS x GIS interaction was not significant either, so that the average values of all variables were not significant different because of gland's type (A, B, C, and D).

In the postinfection period, both in the study of ewes infected along lactation and that of ewes infected from post-partum week, year significantly affected several variables but the YEAR x EIS x GIS interaction effect was not significant. However, EIS x GIS interaction was significant upon Log_{10} SCC□□, PPM, protein, true protein, casein/protein, whey protein and lactose.

In Table 1 it may be observed the LS means (±SEM) of the considered parameters and their significance levels as affected by gland health status during the postinfection period. In both studies, Log_{10} SCC presented a significant higher mean value in infected glands than in healthy ones, and in milk of B healthy glands (contralateral to infected ones) Log_{10} SCC was significantly higher than in milk from C and D healthy glands. Nevertheless, the three values were very low and typical of free of IMI glands. In both studies, the average (± SEM) PPM in infected glands (A glands) was smaller than that expressed by all healthy ones (B, C and D glands). During the 7 weeks postinfection, in ewes infected along lactation the difference of PPM between the infected glands and their contralateral healthy ones was of 38%. A significant 6.6% average PPM difference was established between B healthy glands (572 mL/d) and C and D glands (average of 534 mL/d), indicating that healthy glands produced

more milk when their contralateral glands were infected than when their contralateral ones were healthy.

When the analysis was made on the ewes infected from post-partum week the mean PPM of infected glands along 11 lactating weeks was 61% smaller than that obtained in the healthy glands of the same animals (A glands, 311 mL/d *vs*. B glands, 798 mL/d). Moreover, the PPM of healthy B glands was higher than that of healthy glands from control ewes (B glands, 798 mL/d *vs*. C glands, 648 mL/d and D glands, 649 mL/d).

With respect to chemical composition of milk (Table 1), it was observed a similar trend in the study of ewes infected from post-partum week and in that of ewes infected along lactation, showing a significantly higher protein, true protein and whey protein in infected glands (A) than in healthy ones (B,C and D). Lactose and the casein/protein ratio were lower in infected glands, while fat, casein and dry matter did not present significant differences between glands.

Factors Influencing Effect of IMI. In Table 2 it is shown the regression equations that correlate the productive level of animals and the infection severity (SCC level) with the PPM losses as affected by IMI, both in the study of ewes infected from post-partum week and in that of those infected along lactation. In equations [1] and [1'] of Table 2 it can be verified a significant relationship between the productive level (PPMm) and the mean PPM differences between glands of infected animals during the postinfection period (PPMDm). Nevertheless, when the PPMDm was expressed in percentage terms (PPMDm%) the regression analysis did not give a significant result (equations [4] and [4']).

In regression equation between PPMDm and Log_{10}SCCm (equation [2]) on the ewes infected along lactation, the determination coefficient was significant but low (R^2=0.25) and the same parameter for PPMDm% (equation [5]) was not significant. In the analysis of ewes infected from post-partum week (equations [2'] and [5']), both of the determination coefficients for PPMDm and PPMDm% (R^2=0.63 and R^2=0.40, respectively) were significant.

All the same, the determination coefficients were better when the regressions included both variables, PPM and Log_{10} SCC.

In the Figure 1 it is shown the evolution of PPM in the four groups of glands (A, B, C and D) of ewes infected along lactation, during the next 7 weeks from the onset of infections. In IW = 0, when infections were diagnosed in glands A, an abrupt drop in the PPM of these glands was observed, and B glands presented a higher PPM than C and D ones. Because of the effect of triple interaction EIS x GIS x IW was not significant upon PPM, the differences between all glands did not varied during the postinfection period.

In the group of ewes infected from post-partum week, the evolution of PPM in the four groups of glands (A, B, C and D) during lactation period is shown in the Figure 2. The effect of IMI was evident from the first checking week (second week from lambing) appearing a difference of approximately 500 mL/d between infected A glands and healthy B ones. From lactation week 2 to 3 all healthy glands (B, C and D) had a light rise in its PPM or held at the same level, and after experimented a decreasing trend until the end of the lactating period. However, in the infected A glands PPM decreased all time from the 2nd lactation week. In spite of these differences at the beginning of the lactating period, the EIO x EIG x SI interaction was not statistically significant. Nevertheless, when the differences of PPM between infected A glands and healthy B glands were expressed in percentage terms with respect to the healthy B glands, it was evidenced a significant rise of PPMDm% ($P < 0.05$) as lactation week advanced, from 51% in week 2 to 66% in week 12.

In the case of the Log_{10} SCC and the milk components affected by IMI it was also observed a difference between glands from the week in which the infection was diagnosed, both in the ewes infected from first week post-partum and those infected along lactation. The interaction EIS x GIS x IW was not significant upon chemical parameters, so that the differences between glands kept constant from the week of first IMI diagnosis until the end of studied periods.

Only in the study of ewes infected along lactation was significant this interaction for Log_{10} SCC (Figure 3). A decreasing trend was observed in A glands from the week of infection to the end of lactating period, while a typical rising trend in the B, C and D glands was observed. Nevertheless, in the ewes infected from post-partum week the effect of EIS x GIS x IW interaction was not statistically significant for Log_{10} SCC (Figure 4).

Effect of Subclinical IMI at Individual Level

During preinfection period at individual level, in the study of ewes infected along lactation year effect was statistically significant for fat and true protein, but the YEAR x EIS interaction effect was not significant for any variable. The effect of EIS was not significant either, so that the average values of all variables were not significant different because of ewe infection status.

In the postinfection period, both in the study of ewes infected along lactation and that of ewes infected from post-partum week, year significantly affected several variables but the YEAR x EIS interaction effect was not significant for any of them. On the other hand, EIS effect was significant upon Log_{10} SCC□□, milk yield, casein/protein and lactose. In Table 3 it can be observed the mean values of parameters determined during postinfection period in individual milk of infected and healthy control ewes, both in the study of ewes infected along lactation and in that of infected from post-partum week. Log_{10} SCC presented a significant higher mean value in infected ewes than in healthy ones that was smaller than at half-udder level in the two studies, because of the milk came from the two glands and the content of cells in infected gland was diluted. The difference of individual milk production between infected and healthy ewes was of 15% in the study of ewes infected along lactation and 17% in that of ewes infected from post-partum week. All milk components followed the same pattern as at half-udder level, but the differences at whole-udder milk were moderated with respect to those

at half-udder one because of a dilution effect. Only the ratio casein/protein and lactose presented significant differences due to IMI at individual level (Table 3).

The results from the analysis at individual level confirmed, in the two studies, the trend throughout time found at half-udder level, showing an abrupt drop of milk production in infected ewes as compared with healthy at the infection diagnosis week. During the postinfection period, milk yield in both studies decreased in a parallel way because of the EIS x IW interaction was not statistically significant. Also on casein/protein ratio and lactose content the effect of IMI was evident from infection diagnosis week in the two studies, and the differences between infected and healthy ewes kept constant during all the postinfection period, the EIS x IW interaction not being significant. Only in the study of ewes infected along lactation was significant this interaction for Log_{10} SCC. A decreasing trend was observed on infected ewes from the week of infection to the end of lactating period, while a typical rising trend in the B, C and D glands was observed. Nevertheless, in the ewes infected from post-partum week the effect of EIS x IW interaction was not statistically significant for Log_{10} SCC.

DISCUSSION

Infection Characteristics of Ewes

Various CNS bacteria are the most abundantly occurring in isolates associated with subclinical mastitis in sheep flocks (Leitner et al., 2001, 2004; Gonzalo et al., 2002). The CNS bacteria are usually ignored by farmers and veterinarians because they are not considered as major pathogenic bacteria. However, in the present study, CNS infection, mainly that caused by novobiocin-sensitive CNS (NSCNS) induced the inflammatory response, reflected in a high SCC, which is consistent with previous findings in sheep (Ariznabarreta et al., 2002; Gonzalo et al., 2002; Leitner et al., 2003).

Effect of IMI on Milk Yield

In the present work the difference of PPM between the infected glands and their contralateral healthy ones, over the following 7 weeks to the onset of infection (that occurred between the 3th and the 10th lactation week), was of 38%. This difference was much higher (61%) when infection was already present at post-partum week and persists during the whole considered lactation period (from week 2 to week 12), not knowing the onset of infections. It is worth mentioning that the PPM values obtained in the two types of analysis can not be really compared between them, because of in the group of ewes infected from post-partum week there was not a preinfection value to correct the results. In other words, might be there was already a difference between A and B gland's PPM before the first checking that would allow to an overestimation of the true PPM difference between glands. However, from 38% to 60% there is a high distance that allowed us to hypothesize, first, that the reduction in milk yield could be biggest when IMI occurred in early lactation as reported in cows (Lucey and Rowlands, 1984; Rajala-Schultz et al., 1999; and second, that in the study of ewes infected from post-partum, higher value of PPM reduction could be due to a harder effect of a persistent mastitis if it was acquired in one or more previous lactations, as indicated in cow researches (Rajala-Schultz et al., 1999; Sloth et al., 2003).

The results of the study of infections occurred along lactation draw an individual milk loss of 14% if percentage was calculated based on the PPM values from half-udder approach (the addition of halves of infected ewes, 924 mL/d, *vs.* the addition of halves of control ewes, 1,068 mL/d). This percentage was consistent with the 15% of milk loss obtained from the whole-udder approach. So that, it can be said that the actual milk loss as a result of unilateral subclinical IMI in the conditions of the present research, could be quantified in 15% for the following 7 weeks after the onset of infections. This result was consistent with a previous work in which a similar approach was used (Peris et al., 1996), and in general with other authors who reported losses of milk yield by unilateral subclinical mastitis from 3 to 14.4%,

depending on the methods used to estimate it, on the bacteria involved and on the time of infection permanency (Dario et al., 1996; Gonzalo et al., 2002). The individual milk yield lost by ewes infected from post-partum week was of 17%, value that was very similar to the 15% lost by ewes infected along lactation. This find contrast with the great difference obtained at half-udder level between the two types of analysis (38% *vs.* 61%). Might be ewes with mastitis from the first lactation week were higher yielding animals before the infection than were their healthy herdmates, according to what generally occur in cows (Gröhn et al., 1995; Rajala-Schultz et al., 1999; Wilson et al., 2004), that would reduce the estimated individual differences due to IMI.

Comparison of PPM of healthy glands from control ewes with PPM of healthy glands from infected ewes gave the possibility to demonstrate a compensation phenomenon previously suggested in sheep (Peris et al., 1996; Leitner et al., 2003), by which when only one half is infected the other half try to compensate by producing more milk, so that the loss of individual milk is moderated. The importance of knowing the existence and the magnitude of this compensatory phenomenon lie in the underestimation of real importance of subclinical IMI on milk yield if it is ignored, together with the fact that this adaptation could involve an overstrain of the mammary gland. The result of the present research, with a 6.6% more milk obtained in the uninfected gland during the postinfection period, confirm with statistical significance the previous results of Peris et al. (1996), where an increase of 7.4% were obtained. In that research the results had not statistical significance probably because of the limited data, in which only 8 unilateral infected animals were studied. This compensatory effect was estimated in 13% in cows (Woolford, 1985).

In the study of ewes infected from post-partum week it can not be said that the higher PPM of B glands of infected ewes compared with glands of control sheep was due to a compensatory effect, because there were not previous to infection values. Maybe healthy

glands of the infected ewes had already higher milk yield than control ewes before the onset of checking period.

Effect of IMI on Milk Composition and SCC

Several components of milk were affected by subclinical IMI, the effect being established with higher statistical significance at half-udder level than at individual one. The reason of that may be the absence of individual factors because samples own to the same animal and that half-udder samples provide higher variation ranges than the whole-udder ones (Le Roux et al., 1995). The IMI reduced clearly the lactose content and the ratio casein/protein, the values being according to those reported by others authors (Díaz et al., 1996; Burriel, 1997; Bianchi et al., 2004). However, fat and casein did not present a significant variation, not even at the half-udder level. The reduction of secreted milk volume due to IMI could be at the basis of this absence of modification because of a concentration effect on these components synthesized components (Schultz, 1977; Burriel, 1997). In the literature, the content of fat frequently increases (Burriel, 1997) or remains without changes as affected by IMI (Díaz et al., 1996; Leitner et al., 2003). With respect to casein, some authors found a reduction of its content (Leitner et al., 2004), an increase of it (Bianchi et al., 2004) or any variation (Díaz et al., 1996). In any case, when the ratio casein/protein (parameter independent of the milk volume) was established, it was confirmed that it decreased as a result of infection (Bianchi et al., 2004) or elevated SCC (Pellegrini 1997; Pirisi et al., 1999). This is an important find for ewe milk producers because of, according to Klei et al. (1998), the ratio casein/protein is the parameter that justify the variations of cheese yield dues to proteins in milk, more than only casein concentration. In the present work, the increase of whey proteins in half udder milk as a result of the increase of the blood-milk barrier permeability during an IMI is likely that was the responsible of the reduction in the ratio casein/protein, as it was described previously (Díaz et al., 1996; Bianchi et al., 2004), because of casein content did not varied.

Influence of Production Level and Severity of Infection on Milk Yield Loss

In the present research regressions were established to test the influence of production level of animales and infection severity on the mean of PPM lost par ewe along the postinfection period. The regressions showed that the more productive animals lost more quantity of milk because of IMI than the less productive ones, but these losses were proportional to the production level in healthy conditions, the predictions being more reliable in the case of ewes infected from post-partum week (higher R^2) than in those infected along lactation. The regressions also indicated that the infection severity affected the mean of PPM loss between glands of infected ewes during the postinfection period, both in absolute (PPMDm) and relative terms (PPMDm%), being more pronounced as Log_{10}SCCm increased. The association between the two variables, PPMDm and Log_{10}SCCm, appeared closer in ewes infected from post-partum week than in those infected along lactation. This difference was probably due to the larger number of observations in the whole lactating period test. In general, those results were consistent with Gonzalo et al., (1994) that obtained a mathematical model to estimate the individual milk yield as a function of Log_{10} SCC in sheep, accounting for 73% of the variation in milk yield. It is worth mentioning that in the research of these authors a total of 8,403 samples from 3,202 ewes from 22 herds were studied, which provide a huge SCC variability corresponding to a great diversity of bacteria species, factor that is proved to affect the SCC and the milk production (Gonzalo et al., 2002).

Influence of Time from Infection on Milk Yield Loss and Composition Changes

The obtained results in the study of ewes infected along lactation, both at half and whole-udder level, demonstrated that the milk yield dropped dramatically from the very week in which the infection was diagnosed. In the same way, the yield reduction in one gland was accompanied by a rapid increase in the yield of the other gland in the very week of infection. All that denoted a quick response of mammary secretor tissue to subclinical IMI, both in the

infected glands and in their collateral uninfected ones, according to Knight and Peaker (1991) in goats. Moreover the milk yield differences in absolute terms remained constant during the 7 weeks following the onset of infections. In sheep, possible fluctuations of milk yield losses within the following weeks from infection have not been studied. In cows, the investigation of that has been focused on clinical mastitis, which is always treated after diagnosis. In these cases, it has been reported that after treatment the level of milk production for mastitic cows does not return completely to the level of that for healthy herdmates (Rajala-Schultz et al., 1999; Wilson et al., 2004).

In the ewes infected from post-partum week, the absolute differences between glands were evident from the first checking week, and remained the same during the rest of lactation. Nevertheless, the interest of this analysis lies in the estimation of lactational losses for milk yield and contents in sheep infected from the first wk postpartum, and also in the elucidation of the evolution of infection consequences along the lactation curve. In this sense, it should be pointed out that whilst there were not variations on the absolute magnitude of milk yield losses along the lactation period, the relative ones increased as lactation advanced because of the typical declining trend of lactation curve, the consequences of IMI getting worse as lactation advanced.

The study of Log_{10}SCC in milk from ewes infected during lactation showed that in the very week of infection, ewes experimented also a rapid and strong inflammatory response to IMI that next is slightly moderated. However, the trend of Log_{10}SCC of ewes infected from post-partum week, represented the typical curve for this parameter in ovine livestock (Fuertes et al., 1998) in all glands, infected and healthy. The absence of the progressive reduction of Log_{10}SCC as lactation advanced that was observed in milk from ewes infected during lactation, reinforces the hypothesis that probably a considerable number of these infections were permanent infections acquired in previous lactations. Finally, in both studies the

differences in the studied milk components between glands infected and uninfected were manifested from the very week of infection diagnosis and also remain constant along the postinfection period.

CONCLUSIONS

The individual milk loss as a result of unilateral subclinical IMI acquired along lactation has been quantified in 15% for the following 7 weeks. This loss of milk yield was smaller than what it could be thanks to a 6.6% more milk produced by the uninfected half to compensate some of the milk lost by infected one. This compensatory adaptation highlights the risk for underestimate subclinical mastitis in sheep. The lactational milk yield loss in ewes infected from lambing was 17%. A rapid response of the mammary secretor tissue to subclinical IMI was noticed from the very week in which IMI were diagnosed and remained constant within the rest of lactation. The milk losses were proportional to the production level of ewes and infection severity affected the milk loss. The present research confirms the previous knowledge about the clear decrease of lactose content and the ratio casein/protein because of subclinical IMI in sheep. Those finds warn us about the negative consequences of subclinical IMI on the yield and quality of ewe milk and suggest the importance of subclinical mastitis control and treatment programs for the improvement of udder health status.

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TABLES AND FIGURES

Table 1. LS means (±SE) of the considered parameters as affected by the gland health status[1] during the postinfection period

Parameter	First diagnosis	Infected ewes A (Infected)	Infected ewes B (Healthy)	Healthy ewes C (Healthy)	Healthy ewes D (Healthy)	Signif.
Number of ewes		20	20	40	40	
Log_{10} SCC	Along Lact.	6.21 ± 0.05[a]	4.85 ± 0.05[b]	4.71 ± 0.03[c]	4.74 ± 0.03[c]	***
SCC, g.m^2, x 10^3 cells/mL		1,622	71	51	55	-
	Post-partum	6.17 ± 0.04[a]	4.77 ± 0.04[b]	4.64 ± 0.02[c]	4.63 ± 0.02[c]	***
		1,479	59	44	43	-
PPM, mL/d	Along Lact.	352±16[a]	572 ± 16[b]	528 ± 11[c]	540 ± 11[c]	***
	Post-partum	311±23[a]	798 ± 23[b]	648 ± 18[c]	649 ± 18[c]	***
Fat, %	Along Lact.	8.65±0.16	8.52 ± 0.16	8.76 ± 0.12	8.74 ± 0.12	NS[3]
	Post-partum	8.11 ± 0.20	8.13 ± 0.17	8.36 ± 0,11	8.24 ± 0,11	NS
Protein, %	Along Lact.	5.81 ± 0.04[a]	5.50 ± 0.04 [b]	5.51 ± 0.03 [b]	5.50 ± 0.03 [b]	***
	Post-partum	5.63 ± 0,04[a]	5.38 ± 0,03 [b]	5.39 ± 0,02 [b]	5.41 ± 0,02 [b]	***
True Protein, %	Along Lact.	5.51 ± 0.06 [a]	5.18 ± 0.06 [b]	5.19 ± 0.04 [b]	5.19 ± 0.04 [b]	***
	Post-partum	5.33 ± 0,06 [a]	5.06 ± 0,03 [b]	5.08 ± 0,03 [b]	5.06 ± 0.03 [b]	**
Casein, %	Along Lact.	4.69 ± 0.11	4.50 ± 0.08	4.52 ± 0.04	4.52 ± 0.04	NS
	Post-partum	4.36 ± 0.03	4.28 ± 0.03	4.35 ± 0.02	4.36 ± 0,02	NS
Casein/Protein	Along Lact.	78.58 ± 0.51[a]	80.29 ± 0.43[b]	80.39 ± 0.33[b]	80.46± 0.30[b]	**
	Post-partum	77.60 ± 0.35[a]	79.32 ± 0.30[b]	80.39 ± 0.22[b]	79.91 ± 0.23[b]	*
Whey Protein, %	Along Lact.	0.97 ± 0.05[a]	0.84 ± 0.05[b]	0.84 ± 0.03[b]	0.86 ± 0.04[b]	*
	Post-partum	0.95 ± 0.04[a]	0.80 ± 0,03[b]	0.76 ± 0.02[b]	0.77 ± 0.02[b]	*
Lactose, %	Along Lact.	4.56 ± 0.06[a]	4.92 ± 0.06[b]	4.92 ± 0.04[b]	4.90 ± 0.04[b]	***
	Post-partum	4.59 ± 0.05[a]	5.01 ± 0.04[b]	5.05 ± 0.03[b]	5.05 ± 0,03[b]	***
Dry Matter, %	Along Lact.	20.10 ± 0.17	19.94 ± 0.18	20.10 ± 0.12	20.11 ± 0.12	NS
	Post-partum	19.01 ± 0.27	19.48 ± 0.23	19.71 ± 0.16	19.60 ± 0.16	NS

[a,b,c]Means within a row with different superscripts differ;
[1]A = infected glands; B = healthy glands contralateral to A glands; C = healthy glands of healthy control ewes; D = healthy glands of healthy control ewes, contralateral to C glands;
[2]g.m.: geometrical mean;
***: $P < 0.001$; **: $P < 0.01$; *: $P < 0.05$;
[3]NS: non statistically significant.

Table 2. Regression equations for the mean value of PPM difference between infected and healthy glands of infected animals

Variable		Regression equations	Pairs. no.	R^2	Sign.
PPMDm[1] (mL/d)	[1] Along lactation diagnosis	PPMDm = 0.5 x PPMm[3] – 107	20	0.58	***
	[1'] Post-partum diagnosis	PPMDm = 0.8 x PPM- 214	20	0.62	***
	[2] Along lactation diagnosis	PPMDm = 170 x Log_{10} SCC[4] – 845	20	0.25	*
	[2'] Post-partum diagnosis	PPMDm = 369 x Log_{10} SCC – 1,794	20	0.63	***
	[3] Along lactation diagnosis	PPMDm = 96 x Log_{10} SCC + 0.4 x PPM – 666	20	0.65	***
	[3'] Post-partum diagnosis	PPMDm = 259 x Log_{10} SCC + 0.6 x PPM – 1,594	20	0.87	***
PPMDm%[2]	[4] Along lactation diagnosis	PPMDm% = 0.02 x PPM + 24	20	0.11	NS[5]
	[4'] Post-partum diagnosis	PPMDm% = 0.03 x PPM + 36	20	0.10	NS
	[5] Along lactation diagnosis	PPMDm% = 10 x Log_{10} SCC – 27	20	0.14	NS
	[5'] Post-partum diagnosis	PPMDm% = 27 x Log_{10} SCC – 105	20	0.40	**
	[6] Along lactation diagnosis	PPMDm% = 8 x Log_{10} SCC + 0.01 x PPM – 22	20	0.19	NS
	[6'] Post-partum diagnosis	PPMDm% = 26 x Log_{10} SCC – 0.01 x PPM - 103	20	0.42	**

[1] PPMDm: mean value of PPM difference between infected and healthy glands of infected animals;
[2] PPMDm%: mean value of PPM difference between infected and healthy glands of infected animals in percentage terms;
[3] PPM: in ewes infected along lactation, the mean value of PPM of in both glands in the preinfection period; in ewes already infected at post-partum period, the mean value of PPM of the healthy gland during the 3 first checking;
[4] Log_{10} SCC: mean value of the infected gland during postinfection period (variation interval: 5.42-7.07);
***: $P < 0.001$; **: $P < 0.01$; *: $P < 0.05$;
[5] NS: non statistically significant.

Table 3. LS means (±SE) of the considered parameters in individual milk as affected by the ewe health status during the postinfection period

Parameter	First diagnosis	Infected ewes	Healthy ewes	Signif.
Number of ewes		20	40	
Log_{10} SCC	Along Lact.	5.80 ± 0.04^{a}	4.95 ± 0.03^{b}	***
SCC, $g.m^{1}$, x 10^{3} cells/mL		631	89	-
	Post-partum	5.64 ± 0.04^{a}	4.91 ± 0.03^{b}	***
		427	81	-
Milk Yield, mL/d	Along Lact.	897 ± 50^{a}	$1{,}053 \pm 35^{b}$	**
	Post-partum	982 ± 87^{a}	$1{,}186 \pm 65^{b}$	*
Fat, %	Along Lact.	7.08 ± 0.26	7.35 ± 0.19	NS[2]
	Post-partum	7.09 ± 0.18	$7.25 \pm 0{,}14$	NS
Protein, %	Along Lact.	5.69 ± 0.13	5.61 ± 0.09	NS
	Post-partum	$5.39 \pm 0{,}08$	$5.38 \pm 0{,}06$	NS
True Protein, %	Along Lact.	5.50 ± 0.13	5.39 ± 0.09	NS
	Post-partum	$5.06 \pm 0{,}09$	$5.05 \pm 0{,}07$	NS
Casein, %	Along Lact.	4.50 ± 0.11	4.48 ± 0.08	NS
	Post-partum	4.14 ± 0.07	4.21 ± 0.05	NS
Casein/Protein	Along Lact.	78.03 ± 0.54^{a}	79.70 ± 0.39^{b}	**
	Post-partum	76.78 ± 0.43^{a}	78.44 ± 0.31^{b}	***
Whey Protein, %	Along Lact.	0.98 ± 0.06	0.92 ± 0.04	NS
	Post-partum	0.90 ± 0.04	0.84 ± 0.03	NS
Lactose, %	Along Lact.	4.67 ± 0.07^{a}	4.94 ± 0.05^{b}	**
	Post-partum	4.80 ± 0.06^{a}	5.06 ± 0.04^{b}	***
Dry Matter, %	Along Lact.	18.48 ± 0.39	18.81 ± 0.28	NS
	Post-partum	18.01 ± 0.25	18.50 ± 0.20	NS

[a,b,c]Means within a row with different superscripts differ;
[1]g.m.: geometrical mean;
***: $P < 0.001$; **: $P < 0.01$; *: $P < 0.05$;
[2]NS: non statistically significant.

Figure 1. Productive potential of milk (PPM) of glands of ewes infected along lactation. Values are LS means with SEM indicated by vertical bars of infected A glands (○), B contralateral to A glands (□), C glands of healthy control ewes (▲) and D glands of healthy control ewes (♦) , before the onset of infection (IW < 0) and after the onset of infection (IW ≥ 0).

Figure 2. Productive potential of milk (PPM) of glands of ewes already infected at post-partum week. Values are LS means with SEM indicated by vertical bars of infected A glands (○), B contralateral to A glands (□), C glands of healthy control ewes (▲) and D glands of healthy control ewes (♦).

Figure 3. Log_{10} SCC in milk of glands of ewes infected along lactation. Values are LS means with SEM indicated by vertical bars of A glands (○), B contralateral to A glands (□), C glands of healthy control ewes (▲) and D glands of healthy control ewes (♦) before the onset of infection (IW < 0) and after the onset of infection (IW ≥ 0).

Figure 4. Log_{10} SCC in milk of glands of ewes already infected at post-partum week. Values are LS means with SEM indicated by vertical bars of A glands (○), B contralateral to A glands (□), C glands of healthy control ewes (▲) and D glands of healthy control ewes (♦).

Figure 1

Martí-De Olives

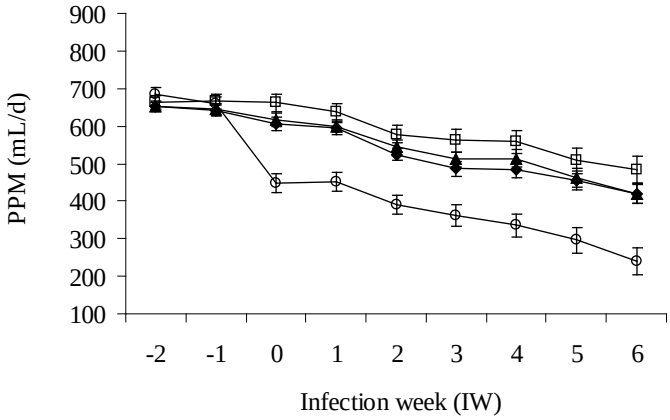

Figure 2

Martí-De Olives

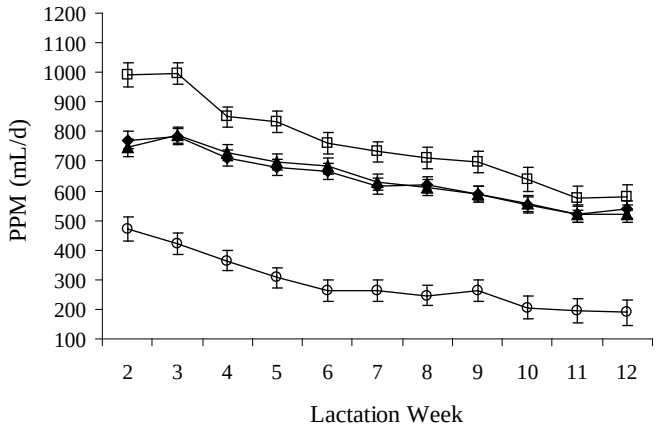

Figure 3

Martí-De Olives

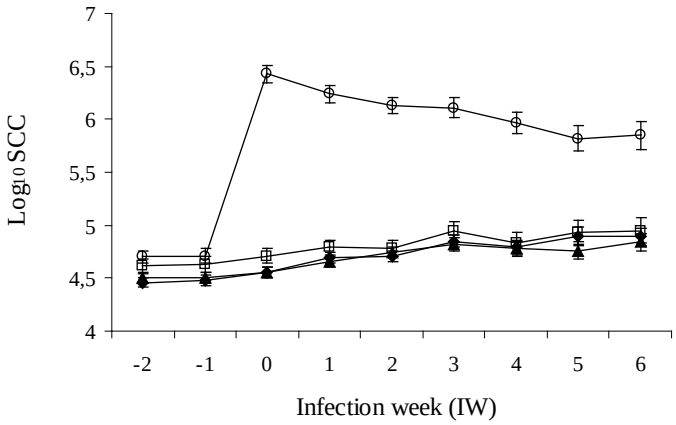

Figura 4
Martí-De Olives