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# The effect of extended milking intervals (24, 48 and 72 h) on milk yield, milk composition, mammary physiology and welfare traits in dairy ewes

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The majority of the work today regarding the effects of extended milking intervals has focused on dairy cattle and only to a limited extent on dairy goats and sheep. The aim of this study was to investigate the effect of different non-milking intervals on milk yield and composition, mammary physiology and welfare indices in dairy ewes. Thirty-six multiparous ewes in late lactation were allocated to one of four groups of nine and subjected to 24, 48 or 72 h of non-milking or normal milking interval (12 h) (group A, B, C and D, respectively). Data showed that there were no significant differences in milk yield among the experimental groups during the third day after re-milking. Furthermore, no significant differences in milk lactose, protein and fat concentration among the experimental groups were observed after 7, 14 and 21 d of re-milking, respectively. Non-milking for 72 h resulted in a temporary increase of sodium concentration, Na + /K + ratio and enzymatic activities of plasmin (PL), plasminogen (PG) and plasminogen activator (PA) in milk. However, these parameters had similar values among the experimental groups on day 5 after re-milking. The concentration of lactose in blood was also significantly increased as a result of the 72 h non-milking interval and returned to its initial levels the second day after re-milking. These data, taken together, suggest that early involution events that occurred as an effect of non-milking were fully reversible within a short period of time. Finally, no significant signs of welfare impairment were observed in ewes due to extended milking intervals. In conclusion, non-milking up to 72 h had no negative effects on milk yield and composition, mammary physiology and welfare parameters in dairy ewes.

**Keywords:** Ewe, extended milking intervals, milk yield and composition, plasminogen–plasmin system, welfare.

Extended milking intervals due to bad weather conditions or other unpredictable factors, e.g. power shortage or illness of stockman, occur in the mountainous regions of Greece and other Mediterranean countries. The existing literature on the effects of non-milking is focused on dairy cattle rather than dairy goats or sheep. In dairy cows, the recovery of milk yield is full after 7 d of non-milking interval (Farr et al. 1998; Dalley & Davis, 2006; Singh et al. 2015) but is only partially recovered if non-milking period lasts longer than 11 d (Singh et al. 2015). In goats, a decrease in the quantity of milk produced after 36 h of milk stasis has been observed (Stelwagen et al. 1994b), with changes in apoptotic events being more predominant than those in cell proliferation (Ben Chedly et al. 2009). Whether these adjustments are reversible following re-initiation of milking in goats is not known. Manchega and Lacaune dairy sheep could maintain high rates of milk secretion during up to 24 h of milk accumulation, with no effects on udder health and minor negative effects on milk yield (Castillo et al. 2008a). They suggested that a 24 h milking interval, which means that animals are milked once daily, could be implemented only for large-cisterned dairy breeds of sheep (Castillo et al. 2008b). There is a need for more studies on small ruminants using more extended milking intervals and putting the emphasis on the changes after re-initiation of milking.

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Different results between cows and goats have been observed in milk composition after re-milking; fat yield remained unaffected and protein content increased in dairy cows after 7, 14 and 28 d of non-milking (Singh et al. 2015) but the levels of both milk components were decreased after 36-h of milk stasis in dairy goats (Ben Chedly et al. 2009). In dairy ewes, milk fat content was decreased and that of protein remained unaffected after 24 h of non-milking (Castillo et al. 2008a). Milk lactose declined in all species as a result of the disruption of mammary tight junction and its passage in blood where its levels increased (Castillo et al. 2008a; Ben Chedly et al. 2009; Singh et al. 2015). It is noteworthy that no data exist concerning the effects of longer non-milking intervals (more than 36 h) on milk yield and composition in small ruminants.

Two systems are generally affected by extended periods of not milking. On the one hand, Na + and K + cross through disrupted tight junctions as a result of the mammary involution and their levels increase in milk and blood, respectively (Stelwagen et al. 1994b; Ben Chedly et al. 2009). At the same time, the enzymatic activity of plasmin (PL) and other components of the plasminogen/plasmin system (plasminogen, PG; plasminogen activator, PA) also increase in milk as a consequence of the integrity loss of tight junctions (O'Brien et al. 2002; Stelwagen et al. 2008).

In cows, abrupt cessation of milking or reduction of milking frequency results in udder distention and can potentially cause pain and discomfort due to increased intramammary hydrostatic pressure (O'Driscoll et al. 2011), a fact that is associated with welfare impairment. The effect of extended milking intervals on small ruminants' welfare status has not yet been investigated.

The objective of the present study was, therefore, to investigate the effect of different non-milking intervals (24 h, 48 h or 72 h) on milk yield, milk composition (fat, protein, lactose, Na and K), various components of the plasminogen–plasmin system and several welfare traits after re-initiation of milking in dairy sheep.

## Materials and methods

### *Animals and experimental design*

Thirty-six multiparous dairy ewes in late lactation (210 ± 7 d), with an average milk yield of 1·0 ± 0·1 l d were used in this study; 20 from the Chios and 16 from the Karagouniko breed. The animals were housed in the premises of the experimental farm of the Agricultural University of Athens and were milked twice per day (6:00 a.m. and 18:00 p.m). They were randomly allocated to one of four groups, each of 9 ewes, balanced according to their milk yield and breed. Each group pen had the same direction and orientation, the same covered area (2 $m^2$/ewe) and was equipped with similar troughs for feeding. The conditions and facilities in each pen were checked twice per day during the experiment in order to assure homogeneity among the treatments and avoid possible pen effects. The first three groups; A, B and C were subjected to non-milking intervals of 24, 48 and 72 h, respectively, while normal milking interval (12 h) was applied in the 4th group (D) (control group). Milk samples were collected both from the morning and afternoon milking and mixed to obtain the milk sample of each experimental day. Milk yield (ml) was recorded on day 1 prior to the beginning of non-milking period and on days 1, 2, and 3 after re-milking. Milk flow rate (ml/s) was also calculated during the same period as the milk yield divided by the milk flow time. Milk flow time (s) was considered as the time between attachment and detachment of the teat cup. For each experimental day, the average of morning and afternoon milk flow rate is presented.

Individual milk samples were also collected from both udder halves 1 d before the beginning of non-milking period (−1), immediately after (1) and 2, 5, 7, 14 and 21 d after re-milking. Samples derived from the mixing of morning and afternoon milk on days −1, 1, 7, 14 and 21 were immediately analyzed for major milk components (fat, protein and lactose), while additional samples from the morning milking at −1, 1, 2 and 5 d were frozen and stored at −20 °C for the determination of various components of the plasmin – plasminogen system (PL, PG, PA), along with Na and K concentration analysis. In addition, blood samples were collected from the ewes that were subjected to 72 h of non-milking 1 d before the beginning of non-milking period, each day during 3 d of non-milking and the second day after re-milking for the determination of plasma lactose concentration. The guidelines of the Research Ethics Committee of the Agricultural University of Athens, regarding the protection and welfare of animals used for experimental and other scientific purposes were followed throughout the experiment.

### *Determination of milk composition and lactose concentration in blood plasma*

Milk samples were analyzed for fat, protein, lactose, and total solids by using a Milkoscan 133 (Foss Electric, Hillerød, Denmark) calibrated for sheep milk according to the Mojonnier method for fat, Kjeldahl method for protein, and the polarimetric method for lactose (AOAC, 1980). Concentrations of Na and K in the ash fraction of milk were determined according to IDF 119 (IDF, 2007) method using a Shimadzu AA-6800 Atomic Absorption Spectrophotometer equipped with the Shimadzu ASC-6100 autosampler and the software WizAArd v. 2.30.

Blood samples collected in heparinized tubes were centrifuged for 15 min at 1725 × $\boldsymbol{g}$ (Biofuge 17RS, HERAEUS, Sepatech). The plasma (supernatant) was stored at −20° C until analysis for lactose as described by Stelwagen et al. (1994a). The method is described in detail in online Supplementary Materials.

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### *Determination of plasmin (PL), plasminogen (PG), and plasminogen activator (PA) activities in milk*

Activities of PL and PG in milk were determined as described by Politis et al. (1989*a*, *b*). A colorimetric assay was used to measure PA activity in the casein nitrogen (CN) fraction. The principle of this methodology is that PA in the CN fraction converts exogenously supplied PG to PL (Gilmore et al. 1995). Methods are described in detail in online Supplementary Materials.

### *Animal welfare traits*

Udder firmness that could be associated with pain due to the engorgement of mammary tissue was evaluated by the same person throughout the experimental period in the milking parlour by palpating the udder between the hind legs and scoring on a scale from 1 to 3 (1: soft, 2: firm, 3: hard) as previously described by Gleeson et al. (2007) in cows. Number of kick responses (steps or kicks in which the hoof was raised at least to the height of the udder) displayed by the ewes during milking was recorded, since it can serve as a sign of agitation and animal distress (Rushen et al. 1999). Data on these welfare parameters were recorded 1 d before the beginning of non-milking period, immediately after (1) and during the second day after re-milking. Lying behavior of ewes was also recorded using a video camera with infrared lighting (TX-1430OA, Turbo-X) for 1 d before the beginning of non-milking period, during the period of non-milking, immediately after (1) and at the second day after re-milking. The obtained data were stored in a digital video recorder equipped with a hard disk (TX168, Telexper Inc, USA). The recording was performed by using time-lapse photography, every five minutes of an hour.

### *Statistical analysis*

Data for milk yield, milk chemical composition, enzymatic activities of plasmin–plasminogen system and welfare parameters were subjected to analysis of variance with extended milking intervals (24, 48 and 72 h) and breed (Chios, Karagouniko) as fixed effects. Bonferroni correction was used for multiple comparisons among least-square means and significance level was defined at $P < 0{\cdot}05$. All analyses were performed with the SAS software (SAS/STAT, 2011).

## Results

### *Milk yield*

Daily milk yield before the beginning of non-milking period and after re-milking is presented in Table 1. Milk yield (ml) was significantly increased at the first day of re-milking in groups B and C ($P < 0{\cdot}05$). During the 2nd day after re-milking, a significant decline in milk yield (ml) was observed in these groups ($P < 0{\cdot}05$). No significant differences in milk yield among the experimental groups were observed during the third day after re-milking, a fact that indicates full recovery of milk yield within 3 d. Milk flow rate (ml/s) was not significantly different among the experimental groups immediately after re-milking. However, a significant reduction was observed for groups B and C on day 2 after re-milking. Flow rate did not vary among the experimental groups and returned to its initial levels at the third day after re-milking (Table 1).

**Table 1.** Effect of extended milking intervals (24, 48 and 72 h) on milk yield (ml) and milk flow rate (ml/s) of dairy ewes

| Day before and after non-milking† | Group‡ | | | | SEM |
|---|---|---|---|---|---|
| | A | B | C | D | |
| Milk yield | | | | | |
| −1 | 1023 | 1011 | 1036 | 1078 | 95 |
| 1 | 1490$^{ab}$ | 1569$^{a}$ | 1538$^{a}$ | 964$^{b}$ | 143 |
| 2 | 811$^{ab}$ | 579$^{a}$ | 515$^{a}$ | 938$^{b}$ | 83 |
| 3 | 891 | 890 | 808 | 895 | 93 |
| Milk flow rate | | | | | |
| −1 | 7·70 | 8·72 | 9·64 | 10·45 | 0·80 |
| 1 | 10·49 | 8·71 | 8·54 | 8·75 | 1·01 |
| 2 | 6·81$^{ab}$ | 4·62$^{a}$ | 4·79$^{a}$ | 9·06$^{b}$ | 0·95 |
| 3 | 7·44 | 6·10 | 8·53 | 9·01 | 0·87 |

†Day −1: 1 d before the beginning of non-milking period and Days 1, 2 and 3: 1st, 2nd and 3rd day after re-milking
‡Group A, B, C and D subjected to 24, 48, 72 h of non-milking or normal milking interval (12-h), respectively
$^{a,b}$Means within a row with different superscripts are significantly different ($P < 0{\cdot}05$)

### *Milk composition*

Means for the levels of the main milk constituents are presented in Table 2. Protein concentration values were significantly increased ($P < 0{\cdot}05$) in all groups subjected to non-milking, while lactose concentration values were significantly decreased in groups B and C ($P < 0{\cdot}05$) on day 1 after re-milking (Table 2). Milk fat content was not significantly different at the first day after re-milking, however a significant increase in its levels was observed 7 days later for groups B and C ($P < 0{\cdot}05$). No significant differences in milk protein, lactose and fat concentration among the experimental groups were observed after 14, 7 and 21 d of re-milking, respectively.

The concentration of sodium and the Na + /K + ratio were significantly increased in groups B and C ($P < 0{\cdot}05$) (Table 3), while the concentration of potassium was not significantly different among the experimental groups on day 1 after re-milking. Values for all parameters returned to their initial levels at the 5th day after re-milking.

### *Plasma lactose levels*

An increase of lactose levels (μmol/l) in blood plasma collected from group C was observed as a result of non-milking. In detail, blood lactose concentration increased

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**Table 2.** Effect of extended milking intervals (24, 48 and 72 h) on fat, lactose and protein levels (%) in milk of dairy ewes

| Day before and after non-milking† | Group‡ | | | | SEM |
|---|---|---|---|---|---|
| | A | B | C | D | |
| Protein | | | | | |
| −1 | 5·15 | 5·20 | 5·07 | 5·02 | 0·11 |
| 1 | 5·62$^{a}$ | 6·27$^{b}$ | 6·41$^{b}$ | 5·16$^{c}$ | 0·16 |
| 7 | 5·24$^{a}$ | 5·52$^{b}$ | 5·48$^{ab}$ | 5·20$^{a}$ | 0·09 |
| 14 | 5·16 | 5·35 | 5·14 | 5·24 | 0·10 |
| 21 | 5·16 | 5·31 | 5·11 | 5·11 | 0·10 |
| Lactose | | | | | |
| −1 | 5·71 | 5·58 | 5·43 | 5·65 | 0·09 |
| 1 | 5·33$^{a}$ | 4·63$^{b}$ | 3·90$^{c}$ | 5·49$^{a}$ | 0·19 |
| 7 | 5·54 | 5·44 | 5·47 | 5·53 | 0·06 |
| 14 | 5·57 | 5·44 | 5·37 | 5·41 | 0·09 |
| 21 | 5·41 | 5·40 | 5·25 | 5·33 | 0·09 |
| Fat | | | | | |
| −1 | 4·98 | 5·58 | 5·30 | 5·21 | 0·25 |
| 1 | 4·91 | 5·24 | 5·10 | 4·97 | 0·32 |
| 7 | 5·39$^{a}$ | 6·77$^{b}$ | 6·75$^{b}$ | 5·27$^{a}$ | 0·29 |
| 14 | 5·02$^{a}$ | 6·05$^{b}$ | 6·14$^{b}$ | 5·53$^{ab}$ | 0·22 |
| 21 | 5·32 | 5·55 | 5·75 | 5·41 | 0·22 |

†Day −1: 1 d before the beginning of non-milking period and Days 1, 7, 14 and 21: 1st, 7th, 14th and 21st day after re-milking
‡Group A, B, C and D subjected to 24, 48, 72 h of non-milking or normal milking interval (12-h), respectively
$^{a,b,c}$Means within a row with different superscripts are significantly different ($P < 0·01$)

**Table 3.** Effect of extended milking intervals (24, 48 and 72 h) on indicators of tight junctions' permeability (sodium and potassium concentration and Na + /K + ratio) in dairy ewes

| Day before and after non-milking† | Group‡ | | | | SEM |
|---|---|---|---|---|---|
| | A | B | C | D | |
| Sodium (mg/100 g) | | | | | |
| −1 | 41·67 | 53·28 | 43·68 | 33·97 | 10·57 |
| 1 | 50·40$^{a}$ | 127·55$^{b}$ | 187·55$^{c}$ | 44·03$^{a}$ | 10·63 |
| 2 | 46·59$^{ab}$ | 61·85$^{b}$ | 92·52$^{c}$ | 34·23$^{a}$ | 10·58 |
| 5 | 37·22 | 39·68 | 49·30 | 36·59 | 10·63 |
| Potassium (mg/100 g) | | | | | |
| −1 | 77·53 | 77·59 | 74·87 | 61·48 | 8·48 |
| 1 | 63·66 | 54·73 | 61·88 | 67·58 | 8·69 |
| 2 | 64·05 | 74·07 | 78·96 | 66·29 | 8·54 |
| 5 | 61·62 | 71·73 | 80·72 | 62·19 | 8·67 |
| Na + /K + ratio | | | | | |
| −1 | 0·60 | 0·64 | 0·67 | 0·66 | 0·18 |
| 1 | 0·80$^{a}$ | 2·62$^{b}$ | 3·32$^{c}$ | 0·58$^{a}$ | 0·20 |
| 2 | 0·76$^{a}$ | 0·97$^{a}$ | 1·37$^{b}$ | 0·76$^{a}$ | 0·19 |
| 5 | 0·64 | 0·57 | 0·65 | 0·65 | 0·20 |

†Day −1: 1 d before the beginning of non-milking period and Days 1, 2 and 5: 1st, 2nd and 5th day after re-milking
‡Group A, B, C and D subjected to 24, 48, 72 h of non-milking or normal milking interval (12-h), respectively
$^{a,b,c}$Means within a row with different superscripts are significantly different ($P < 0·05$)

from 58·66 ± 3·83 (1 d before the beginning of non-milking period) to 193·77 ± 7·08 (on day 1 after the beginning of non-milking period) and 292·12 ± 9·38 (on day 2 after the beginning of non-milking period). It remained constant at the 3rd day of the beginning of non-milking period (293·31 ± 8·19) and finally returned to its initial levels at the 2nd day after re-milking (58·56 ± 3·81) ($P < 0·001$).

### *PL–PG system*

An increase of the PL, PG, PA and PL + PG enzymatic activities (U/ml) in milk from C group occurred on day 1 after re-milking (Table 4). Values remained high at the 2nd day after re-milking and returned to their initial levels at the 5th day after re-milking. Finally, the ratio PG/PL, which can be used as an indicator of the activation of plasminogen to plasmin, was not significantly different among the experimental groups during the whole period ($P > 0·05$).

### *Welfare traits*

Values for kicks responses were not significantly different among the experimental groups ($P > 0·05$; Table 5). On the other hand, increased udder firmness scores were observed immediately after re-milking for groups A, B and C ($P < 0·05$). Extended intervals of non-milking did not influence the daily lying time of ewes ($P > 0·05$; Table 5).

Both breeds responded similarly to the extended milking intervals. Milk yield, flow rate and composition and plasma lactose for both breeds are presented in online Supplementary Figures S1–S4.

## Discussion

The first finding emerging from the present study was that milk yield returned to its initial levels during the 3rd day after re-milking. Firstly, a significant increase in milk yield in all experimental groups compared to the controls was observed on day 1 after re-milking due to the accumulation of milk in the udder followed by a great decline only in groups B and C on day 2 after re-milking (Table 1). A possible explanation for the decline in milk yield is that it might be a result of early involution events that are related with an increase in apoptosis and a decrease in proliferation of secretory cells. These results are in agreement with previous studies that reported a decrease of milk yield after 24-h of re-milking and return to the pre-treatment milk levels within a period ranged from 30 h to a week after 40 h, 2, 4 and 7 d milk stasis in dairy cows (Farr et al. 1998; Dalley & Davis, 2006; Stelwagen et al. 2008; Singh et al. 2015). In goats, a decrease of milk yield was reported after 36 h of non-milking (Stelwagen et al. 1994b; Ben Chedly et al. 2009).

In the present study, a decrease in milk flow rate was observed in groups B and C on day 2 after re-milking (Table 1), a fact that is possibly related with the reduction

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**Table 4.** Effect of extended milking intervals (24, 48 and 72 h) on PL-PG system activity (Plasmin-PL, Plasminogen-PG, Plasminogen activator-PA, PL + PG, PG:PL ratio) in dairy ewes

| | Group‡ | | | | |
|---|---|---|---|---|---|
| Day before and after non-milking† | A | B | C | D | SEM |
| Plasmin (U/ml) | | | | | |
| −1 | 56·24 | 54·43 | 55·33 | 55·77 | 2·80 |
| 1 | 56·54[a] | 54·26[a] | 68·32[b] | 56·85[a] | 2·81 |
| 2 | 56·61[a] | 55·17[a] | 62·68[b] | 56·12[a] | 2·80 |
| 5 | 55·16 | 54·27 | 54·98 | 55·85 | 2·81 |
| Plasminogen (U/ml) | | | | | |
| −1 | 62·11 | 61·34 | 61·36 | 61·17 | 2·92 |
| 1 | 61·94[a] | 61·93[a] | 75·15[b] | 61·17[a] | 3·04 |
| 2 | 61·85[a] | 60·26[a] | 67·42[b] | 60·71[a] | 2·94 |
| 5 | 60·48 | 60·27 | 61·28 | 60·58 | 3·04 |
| Plasminogen activator (U/ml) | | | | | |
| −1 | 128·23 | 127·42 | 123·74 | 126·33 | 3·85 |
| 1 | 130·40[a] | 127·57[a] | 146·08[b] | 127·30[a] | 3·85 |
| 2 | 128·28[a] | 127·71[a] | 136·02[b] | 124·66[a] | 3·85 |
| 5 | 128·37 | 127·16 | 124·61 | 125·71 | 3·85 |
| PL + PG (U/ml) | | | | | |
| −1 | 118·40 | 115·76 | 116·68 | 116·96 | 5·67 |
| 1 | 118·53[a] | 116·18[a] | 143·46[b] | 118·04[a] | 5·80 |
| 2 | 118·51[ab] | 115·42[a] | 129·10[b] | 116·86[a] | 5·69 |
| 5 | 115·69 | 114·54 | 116·25 | 116·45 | 5·80 |
| PG:PL ratio | | | | | |
| −1 | 1·12 | 1·13 | 1·12 | 1·10 | 0·02 |
| 1 | 1·10 | 1·14 | 1·11 | 1·08 | 0·02 |
| 2 | 1·11 | 1·09 | 1·10 | 1·09 | 0·02 |
| 5 | 1·10 | 1·11 | 1·12 | 1·09 | 0·02 |

†Day −1: 1 d before the beginning of non-milking period and Days 1, 2 and 5: 1st, 2nd and 5th day after re-milking
‡Group A, B, C and D subjected to 24, 48, 72 h of non-milking or normal milking interval (12-h), respectively
[a,b]Means within a row with different superscripts are significantly different ($P < 0·05$)

of lactose concentration in milk and the disrupted tight junctions as a result of the beginning of mammary involution. Fleet & Peaker (1978) in goats and Farr et al. (1998) in dairy cows reached the same conclusions, since after the first 24 h after the beginning of non-milking period, secretory rate and mammary blood flow decreased markedly over the next days.

Although milk yield returned to its initial levels within 3 d after re-milking, milk composition was only fully restored on day 21 after re-milking. It is also noteworthy that milk lactose and protein returned to their initial levels faster than milk fat. The reason for these differences is not known. Extended milking intervals resulted in an increase of protein levels on days 1 and 7 and of fat concentration on days 7 and 14 in groups B and C. On the other hand, a decrease of lactose levels at the 1st day after re-milking was observed in groups B and C (Table 2). There are no published data that could explain the increment of milk fat concentration that is reported 7 d after re-milking, but this increase may be related with different regulatory mechanisms for milk fat secretion relative to the aqueous phase of milk (Salama et al. 2003; Hervas et al. 2006; Rémond & Pomiès, 2007). On the other hand, the increase of protein levels and the decrease of lactose concentration in milk are possibly attributable to the disruption of mammary tight junctions that allow the passage of blood protein, e.g. serum albumin, into milk and of milk lactose into blood, respectively (Stelwagen et al. 2008; Ben Chedly et al. 2009; Singh et al. 2015). Contradictory results concerning the effects of non-milking on fat content exist for dairy cows and small ruminants. In dairy cows, milk fat yield remains unaffected as a result of different extended milking intervals, with the exception of 28 d milk stasis, when milk fat appeared to decrease after re-milking possibly due to the reduced activity of enzymes associated with milk fat composition during involution (Stelwagen et al. 2008; Singh et al. 2015). On the other hand, studies implemented in goats (Ben Chedly et al. 2009) and ewes (Castillo et al. 2008a) showed a significant reduction of milk fat levels after 36 and 24 h of non-milking, respectively.

The second finding of the present study is that Na + /K + ratio and PL-PG system were affected by extended milking intervals. However, these early involution events were fully reversible within 5 d (Tables 3 and 4). PL, PG and PL + PG activities increased in the milk of ewes that were subjected to 72 h of non-milking, suggesting that milk stasis can lead to increased overall proteolytic activity in milk. However, the key event is the increase in PG-

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**Table 5.** Effect of extended milking intervals (24, 48 and 72 h) on the major welfare indices in dairy ewes

| Day before and after non-milking† | Group‡ A | B | C | D | SEM |
|---|---|---|---|---|---|
| Kick responses | | | | | |
| −1 | 0·67 | 0·56 | 0·45 | 0·34 | 0·24 |
| 1 | 0·54 | 1·88 | 1·15 | 0·77 | 0·37 |
| 2 | 0·61 | 1·11 | 0·56 | 0·83 | 0·26 |
| Udder firmness | | | | | |
| −1 | 1·00 | 1·00 | 1·05 | 1·11 | 0·05 |
| 1 | 1·77[a] | 1·88[a] | 2·00[a] | 1·00[b] | 0·20 |
| 2 | 1·00 | 1·00 | 1·06 | 1·00 | 0·03 |
| Percentage of ewes lying (%) | | | | | |
| −1 | 37·01 | 37·05 | 37·33 | 36·77 | 1·39 |
| 24-h of non-milking in C group | 37·05 | 37·01 | 37·05 | 36·74 | 1·25 |
| 24-h and 48-h of non-milking in C and B group, respectively | 34·90 | 34·48 | 34·62 | 35·00 | 1·08 |
| 24-h, 48-h and 72-h of non-milking in C, B and A group, respectively | 39·86 | 39·27 | 39·31 | 38·96 | 1·15 |
| 1 | 37·64 | 38·44 | 38·54 | 38·16 | 0·97 |
| 2 | 40·28 | 40·24 | 40·45 | 39·86 | 1·07 |

†Day −1: 1 d before the beginning of non-milking period and Days 1 and 2: 1st and 2nd day after re-milking
‡Group A, B, C and D subjected to 24, 48, 72 h of non-milking or normal milking interval (12-h), respectively
[a,b] Means within a row with different superscripts are significantly different ($P < 0·05$)

derived activity despite that fact that the PL activity also increased. The increase in PG-derived enzymatic activity observed in the present study could be attributed to enhanced entrance of PG from blood to milk. Silanikove (2016) proposed that PG crosses the mammary epithelium barrier by the transcellular route. However, although there was an increase in PL + PG activity, the PG/PL ratio (that is a parameter used for the assessment of the conversion of PG to PL and that is independent of milk) was not affected by extended milking intervals. Thus, there is no activation of the PG-PL system due to non-milking up to 72 h. Our results are similar to those of previous researchers who observed enhanced Na+ levels in milk of goats (Stelwagen et al. 1994b) and increased enzymatic activities of PL and PG in milk of dairy cows (Stelwagen et al. 2008), which were connected with impairment of mammary tight junction integrity after milk stasis. Values for PL activity, Na+ concentration and Na:K ratio in milk increased as the milking stasis was extended from 4 to 24 h in Manchega ewes in an experiment that studied the effects of short-term non-milking intervals (Castillo et al. 2008a).

The third finding emerging from the present study is that no significant effects of extended milking intervals on ewe welfare status were detected (Table 5). The increased score of udder firmness in ewes subjected to non-milking before re-milking was a result of milk accumulation and the resulting udder pressure caused by the omitted milking, as previously shown in dairy cows (Gleeson et al. 2007; O'Driscoll et al. 2011) and ewes (Koutsouli et al. 2017). However, although the greatest score for udder firmness is observed after 2–3 d of non-milking in dairy cows (Tucker et al. 2009) and goats (Fleet & Peaker, 1978), this finding was not confirmed by our data, since the same score for udder firmness was recorded in ewes subjected to 24, 48 and 72 h of non-milking. Number of kick responses and daily lying time of ewes were not significantly affected by extended milking intervals (Table 5). A decreased lying time was observed in lactating cows and was attributed to milk accumulation and the resulting udder pressure caused by omitted milking (O'Driscoll et al. 2011). However, other researchers demonstrated that abrupt cessation of milking had no effect (Tucker et al. 2009) or increased lying time (Chapinal et al. 2014) in dairy cattle. In dairy ewes, 24 h of non-milking also increased their daily lying time (Koutsouli et al. 2017). These discrepancies could be attributed to the different species, the different stage of lactation and the varied milk yield that is accumulated in the mammary gland or to possible differences in the experimental conditions and reflect the necessity for further investigation with the inclusion of additional observation criteria of animal's welfare such as vocalizations/bleating.

## Conclusion

The results of the present study indicate that milk yield returned to pre-treatment levels 3 d after re-milking. At the same time, early involution events that occurred as an effect of extended milking intervals were also fully reversible (on day 5 after re-milking). Finally, no significant signs of welfare impairment were observed in ewes due to the extended periods of not milking.

## Conflict of interest

None declared.

## Supplementary material

The supplementary material for this article can be found at https://doi.org/10.1017/S002202991800047X.

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