



## The combined effects of milking frequency and feeding level on milk quality and metabolic parameters from late lactation dairy goats

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### Abstract

The higher milk fat and protein content are some component that improve the price of the milk bought from dairy farmer in some countries. Also the reduction of the feed cost can reduce the cost of the milk production from dairy farm. Thus this study was conducted to investigate the combined effects of milking management (once-daily milking: ODM or twice-daily milking: TDM) and feeding level (*ad libitum* or adjusted) on milk quality and metabolic parameters from dairy goats in late lactation. This experiment was divided in two successive periods: period of milking frequency reduction (P1) and period of feed adjustment (P2). Milk yield and composition, plasma concentration of glucose, urea,  $\beta$ -hydroxybutyrate (BHB) and Non Esterified Fatty Acids (NEFA), milk fatty acids composition of the 40 dairy goats were determined. During P1, milk fat content decreased (-2.7 g/kg) while protein and lactose content were increased (+1.1 g/kg and +0.7 g/kg, respectively) by ODM compared to TDM. Also Plasma glucose concentration was increased (+0.03 g/kg) by ODM compared to TDM. During P2, only milk protein content was increased by ODM compared to TDM. Conversely, milk fat content and the sum of C18 were increased by feed adjustment. Glucose and BHB concentrations were reduced while NEFA concentration was higher in goats during P2. In conclusion this study demonstrated a lack of increase in milk fat content under ODM management but an increase in milk protein content under this milking management without major impairment of metabolic parameters from dairy goats.

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## Introduction

The results on milk fat are inconsistent in goats under once-daily milking (ODM) by comparison to twice-daily milking (TDM) (Salama *et al.*, 2003; Pulina *et al.*, 2005; Lefrileux *et al.*, 2008). Indeed, an increase in milk fat content was reported in Murciano Granadina dairy goats under ODM management (Salama *et al.*, 2003) while a lack of increase was demonstrated in Alpine dairy goats (Marnet *et al.*, 2005; Lefrileux *et al.*, 2008; Marnet and Komara, 2008) or in Damascus (Papachristoforou *et al.*, 1982) and in Tenerfeña goats (Capote *et al.*, 1999).

In goats, fat content has been shown to be a component of milk mostly reduced under milking interval lengthening up to 24 hr (Pulina *et al.*, 2005; Hammadi *et al.*, 2009). In cows, feed restriction and ODM increased milk fat content (Lacy-Hulbert *et al.*, 1999). Switching from TDM to ODM increased protein content in goats (Salama *et al.*, 2003; Marnet *et al.*, 2005; Lefrileux *et al.*, 2008). However, no data were found on ODM and feeding level combined effects on milk composition in goats.

In dairy ruminants, it is well known that milk fatty acids (FA) are derived, on one hand from *de novo* synthesis and, on the other hand, from FA taken up by the mammary gland from the blood. In this respect, the milk storage in the udder up to 24-h under ODM management could induce disturbances in FA uptake or synthesis by the mammary gland. Although in cows, the mammary efficiency for conversion from acetate and  $\beta$ -hydroxybutyrate taken up from the plasma to short- and medium- chain FA in milk was not impaired by ODM (Guinard-Flament *et al.*, 2007); the combined effect of this management strategy and feeding level on milk FA remains to be determined in goat.

The decrease in plasma concentration of non-esterified fatty acids (NEFA),  $\beta$ -hydroxybutyrate (BHB) and urea, and an increase in glucose has been reported in dairy cows under ODM management (Auldism and Prosser, 1998; Rémond *et al.*, 1999). Moreover, the decrease in NEFA and urea was greater

in restricted cows than in cows grazing *ad libitum* (Auldism and Prosser, 1998). However, no data were found about ODM and feeding level combined effects on these metabolic parameters in dairy goats.

The objectives of this study were to investigate, in late lactation, the effects of ODM vs TDM managements in individually fed goats (*ad libitum* or adjusted) on dairy goats' milk quality and their metabolic parameters.

## Materials and methods

### *Animals and housing*

Forty pregnant Alpine and Saanen dairy goats (23 Multiparous and 17 Primiparous) in late lactation ( $37 \pm 3$  days in gestation and  $239 \pm 17$  days in milk and  $66.8 \pm 12.4$  kg of body weight at the start of the experiment) were housed in 2 m x 1 m individual pens with free access to water. Goats with no clinical mastitis during the preceding lactation and before the beginning of the experiment were chosen.

### *Experimental design*

The goats had 1 week of adaptation (AP) to individual pens, followed by 1 week considered as a control period (CP). Goats were all managed under TDM and fed *ad libitum* throughout these two weeks (pre-experimental period). At the end of CP, the goats were allocated to ten groups of four goats according to the average dry matter afternoon feed intake (measured three times during CP), average milk yield (measured during 5 days in AP and 4 days in CP), and average SCC (measured on two milkings during AP). Among each group of 4 goats, the goats were randomly assigned to one of four experimental groups, to obtain ten goats per group, as follows: 1L (ODM and *ad libitum* feed intake), 2L (TDM and *ad libitum* feed intake), 1J (ODM and adjusted feed intake) and 2J (TDM and adjusted feed intake). The experiment was divided into two successive periods: firstly during one week (P1), the two milking frequencies (ODM vs TDM) were tested while the feed was given *ad libitum* and secondly during 3 weeks (P2), the feed was either given *ad libitum* or adjusted to milk yield and body weight (detailed below).

ODM started at the end of CP by suppressing the afternoon milking in twenty goats until the end of the experiment. The TDM goats were milked at 0700 h and 1530 h while the ODM goats were milked at 0700 h only.

Feed adjustment started at the end of P1 until the end of the experiment. During P2, goats from 1J and 2J received a quantity of feed adjusted each week to their body weight (BW) and their milk yield (MY) recorded the previous week with the following formula, according to the requirements proposed in the INRA tables by Sauvant *et al.* (2007):

Quantity of feed (kg DM/d) =  $1 + 0.120 * (BW - 60)/10 + 0.5 * MY$ ; for a diet with a net energy value of 0.84 UFL/kgDM.

The goats from 1L and 2L received their feed *ad libitum* with a weekly adjustment to ensure 10 – 15 % refusals. All the goats were fed with a total mixed ration which was offered individually twice daily in the proportion of two thirds at 1600 h and one third at 0800 h. Two mineral blocks were placed in the waiting area to milking.

#### *Milk composition*

Individual milk samples were taken two days per week at morning and evening milking for milk composition (fat, protein, lactose and urea) analysis by URIANE (inter-professional regional union for analyses north eastern, Capelle-59213, France).

During P2, individual milk samples (5 mL) were taken once a week at morning and evening milking, and stored at -20°C until analysis. The two daily samples from TDM goats were thawed and pooled in the proportion of two thirds for morning sampling and one third for evening sampling. Fatty acid composition of ODM goat samples and of TDM goat pooled samples was determined by the method described by Couvreur *et al.* (2007).

#### *Metabolic parameters*

Jugular blood samples were taken twice a week, 40 min before milking in the morning.

The samples collected by venipuncture into heparinized tubes were immediately centrifuged at 3000g for 10 min at 4°C. Plasma was stored at -20°C until analysis. Plasma glucose was determined by the hexo-kinase-glucose-6-phosphatedehydrogenase method (Cooper, 1973), urea by the Talker and Schubert (1965) method,  $\beta$ -hydroxybutyrate (BHB) by the procedure of Barnouin *et al.* (1986) and Non Esterified Fatty Acids (NEFA) by an adaptation of the enzymatic method described by Bas(1984).

#### *Statistical analysis*

Data were analyzed with the mixed procedure of SAS (2000) with animal as a random effect. The effects of milking frequency (TDM or ODM) were tested during P1. The effects of milking frequency (TDM or ODM) or feeding (*ad libitum* or adjusted) and their interactions were tested during P2. Analysis included the average values obtained for each goat during CP as a co-variable in the model.

#### **Results and discussion**

Milk yield was reduced by 18% in goats under ODM management compared to TDM during both periods: P1 or P2 (Table 1 and 3). But ODM management, increased milk protein content during these both periods (Table 1 and 3). Indeed, the reduction in milk yield caused by ODM induced an increase in protein content in accordance with previous studies conducted in goats (Salama *et al.*, 2003; Marnet *et al.*, 2005; Lefrileux *et al.*, 2008) or cows (Holmes *et al.*, 1992; Pomiès and Rémond, 2002). Such an increase in protein content could be explained by soluble serum protein leakages in the milk. However, we don't agree with this explanation because the decreases in lactose content previously reported in cows at different stage of lactation (Holmès *et al.*, 1992; Carruthers *et al.*, 1993; Stelwagen *et al.*, 1994) was not observed in our experiment suggesting the lack of mammary tight junction opening (lactose leakage from milk to blood) in goats under ODM management. Because we observed an increase in lactose content by 1% (Table 1).

**Table 1.** Milk yield and composition after the first week of milking frequency switching from TDM to ODM in late lactation goats.

Milk yield and composition	MF <sup>1</sup>		SEM	Effects
	TDM (n = 20)	ODM (n = 20)		
Milk yield (kg/d)	2.20	1.80	± 0.02	***
Fat content (g/kg)	37.1	34.4	± 0.68	*
Protein content (g/kg)	37.9	39.0	± 0.26	**
Lactose content (g/kg)	44.2	44.9	± 0.14	**
Urea content (g/kg)	0.53	0.52	± 0.01	NS
Fat yield (g/d)	80.7	62.1	± 1.76	***
Protein yield (g/d)	81.6	69.8	± 1.18	***

NS = not significant ( $P \geq 0.1$ ); \*  $P < 0.05$ ; \*\*  $P < 0.01$ ; \*\*\*  $P < 0.001$ .

<sup>1</sup>MF: milking frequency, TDM: twice-daily milking, ODM: once-daily milking.

However, ODM, compared to TDM, reduced fat content by 7% during P1 (Table 1) but milk fat, was not influenced by milking frequency during P2 (Table 3). Thus, the reduction in milk yield did not induce an increase in fat content. Indeed, the fat content was decreased by ODM on the contrary to the increase demonstrated in previous studies conducted in goats (Salama *et al.*, 2003) or in cows and ewes (Holmes *et*

*al.*, 1992; Nudda *et al.*, 2002; Pomiès and Rémond, 2002). Fat content increased in goats receiving adjusted feed compared to *ad libitum* feed that agrees with what was reported in late lactation restricted cows (Lacy-Hulbert *et al.*, 1999). Nevertheless, milk fat content was not different between milking treatment during the period of feed adjustment.

**Table 2.** Plasma metabolic parameters after the first week of milking frequency switching from TDM to ODM in late lactation goats.

Metabolic parameters	MF <sup>1</sup>		SEM	Effects
	TDM (n=20)	ODM (n=20)		
Glucose (g/L)	0.57	0.59		**
Urea (g/L)	0.37	0.37		NS
BHB <sup>2</sup> (mg/L)	34.7	33.4		NS
NEFA <sup>3</sup> (µeq/L)	128	109		NS

NS = not significant ( $P \geq 0.1$ ); \*\*  $P < 0.01$ .

<sup>1</sup>MF: milking frequency, TDM: twice daily milking, ODM: once daily milking

<sup>2</sup>BHB: β-hydroxybutyrate;

<sup>3</sup>NEFA: Non Esterified Fatty Acids.

Furthermore, *de novo* fatty acids synthesis in the mammary gland or fatty acids taken up from the blood did not seem to be affected by ODM management since our experiment demonstrated the lack of effect of ODM on the percentage of short chain fatty acids or the sum of C18 (indicator of body fat mobilization) in accordance with previous studies in cows and goats (Chilliard *et al.*, 2006; Lefrileux *et al.*, 2008; respectively).

Only an increase in plasma glucose concentration (by 3% in goats under ODM management (Table2) was observed after milking frequency switching from TDM to ODM in accordance with a previous study in cows (Auldism and Prosser; 1998; Rémond *et al.*, 1999; Grala *et al.*, 2016) probably due to a depressed uptake of glucose by the mammary gland as reported in cows under ODM management (Guinard-Flament *et al.*, 2007).

However, in our experiment, this effect of ODM disappeared throughout the trial suggesting a metabolic adaptation to this milking management strategy. Furthermore, plasma urea, BHB or NEFA concentrations were not influenced by milking frequency (Table 2).

Milk urea content was not influenced by milking frequency in our study. Also, there was neither an effect of parity, nor an interaction between milking frequency and parity during P1.

**Table 3.** Milk yield and composition, and percentage of short-chain fatty acids (FA) and sum of C18 for 3 weeks in late lactation goats under TDM or ODM management and receiving a diet either given *ad libitum* or adjusted to their body weight and milk production (adjusted feeding).

Milk yield and composition	MF <sup>1</sup>			FL <sup>2</sup>			Group <sup>3</sup>				Effects <sup>4</sup>	
	TDM	ODM	SEM	L	J	SEM	2L	1L2J	1J	MF	FL	
Milk yield (kg/d)	1.93	1.51	0.55	1.74	1.70	0.55	1.99 <sup>a</sup>	1.49 <sup>b</sup>	1.86 <sup>a</sup>	1.54 <sup>b</sup>	***	NS
Fat content (g/kg)	39.9	39.0	0.85	37.3	41.6	0.85	38.7 <sup>b</sup>	35.8 <sup>b</sup>	41.0 <sup>a</sup>	42.2 <sup>a</sup>	NS	**
Protein content (g/kg)	38.7	41.2	0.52	39.8	40.0	0.52	38.5 <sup>b</sup>	41.2 <sup>a</sup>	38.9 <sup>b</sup>	41.2 <sup>a</sup>	***	NS
Lactose content (g/kg)	43.3	43.8	0.20	43.6	43.5	0.20	43.5 <sup>a</sup>	43.7 <sup>a</sup>	43.2 <sup>a</sup>	43.8 <sup>a</sup>	NS	NS
Urea content (g/kg)	0.68	0.70	0.01	0.68	0.70	0.01	0.68 <sup>a</sup>	0.69 <sup>a</sup>	0.69 <sup>a</sup>	0.70 <sup>a</sup>	NS	NS
Fat yield (g/d)	73.2	56.2	2.10	65.5	64.0	2.11	74.7 <sup>a</sup>	56.3 <sup>b</sup>	71.8 <sup>a</sup>	56.2 <sup>b</sup>	***	NS
Protein yield (g/kg)	70.8	60.3	1.88	69.4	61.8	1.88	73.8 <sup>a</sup>	65.0 <sup>b</sup>	68.0 <sup>ab</sup>	55.6 <sup>c</sup>	***	**
Σ short chain FA (%)	25.7	24.6	0.17	25.6	24.8	0.17	26.1 <sup>a</sup>	25.0 <sup>a</sup>	25.3 <sup>a</sup>	24.2 <sup>a</sup>	NS	NS
Σ C18 (%)	26.5	26.5	0.31	25.6	27.4	0.32	25.9 <sup>b</sup>	25.2 <sup>b</sup>	27.1 <sup>a</sup>	27.7 <sup>a</sup>	NS	***

NS = not significant ( $P \geq 0.1$ ); \*\*  $P < 0.01$ ; \*\*\*  $P < 0.001$ .

<sup>1</sup>MF: milking frequency, TDM: twice daily milking, ODM: once daily milking

<sup>2</sup>FL: feeding level, L: *ad libitum*, J: adjusted;

<sup>3</sup>Groups: 2L (TDM, *ad libitum*), 1L (ODM, *ad libitum*), 2J (TDM, adjusted), 1J (ODM, adjusted) ; within row none of the values differed ( $P > 0.10$ )

<sup>4</sup>No interaction was found between milking frequency and feeding level for all the variables.

In the present study, Non-Esterified Fatty Acids (NEFA),  $\beta$ -hydroxybutyrate (BHB) or urea concentrations were not modified under ODM management on the contrary to the decrease observed for these metabolic parameters in cows (Auldist and Prosser, 1998; Rémond *et al.*, 1999).

This discrepancy could be explained by the different lactation stage (late lactation in our study vs early lactation from studies of Auldist and Prosser, 1998 or Rémond *et al.*, 1999) or

to the species (goats in our study vs cows from studies of Auldist and Prosser, 1998 or Rémond *et al.*, 1999).

The plasmatic NEFA concentration, and the milk fat content and sum of C18 increases observed in goats under feed adjustment during P2 (Table 3 and 4) suggested a body fat mobilization as a consequence of feed adjustment, even if the total fat yield remained unchanged by feeding treatments in our experiment (Table 3).

**Table 4.** Plasma metabolic parameters for 3 weeks in late lactation goats under TDM or. ODM management and receiving a diet either given *ad libitum* or adjusted to their body weight and milk production (adjusted feeding).

Metabolic parameters	MF <sup>1</sup>			FL <sup>2</sup>			Group <sup>3</sup>				Effects <sup>4</sup>	
	TDM	ODM	SEM	L	J	SEM	2L	1L	2J	1J	MF	FL
Glucose (g/L)	0.54	0.55	0.004	0.56	0.53	0.004	0.55 <sup>a</sup>	0.57 <sup>a</sup>	0.52 <sup>b</sup>	0.53 <sup>b</sup>	NS	***
Urea (g/L)	0.34	0.35	0.01	0.34	0.36	0.01	0.32 <sup>a</sup>	0.35 <sup>a</sup>	0.36 <sup>a</sup>	0.36 <sup>a</sup>	NS	†
BHB <sup>5</sup> (mg/L)	34.5	33.6	1.20	36.1	32.0	1.20	36.4 <sup>a</sup>	35.8 <sup>ab</sup>	32.7 <sup>ab</sup>	31.3 <sup>b</sup>	NS	*
NEFA <sup>6</sup> (µeq/L)	140	134	6.92	112	163	6.94	119 <sup>b</sup>	105 <sup>b</sup>	162 <sup>a</sup>	164 <sup>a</sup>	NS	***

NS = not significant ( $P \geq 0.1$ ); †  $0.05 < P < 0.1$  (tendency); \*\*\*  $P < 0.001$ . <sup>1</sup>MF: milking frequency, TDM: twice daily milking, ODM: once daily milking <sup>2</sup>FL: feeding level, L: *ad libitum*, J: adjusted

<sup>3</sup>Group: 2L (TDM, *ad libitum*), 1L (ODM, *ad libitum*), 2J (TDM, adjusted), 1J (ODM, adjusted); within row none of the values differed ( $P > 0.10$ )

<sup>4</sup>No interaction was found between milking frequency and feeding level for all the variables

<sup>5</sup>BHB: β-hydroxybutyrate

<sup>6</sup>NEFA: Non Esterified Fatty Acid.

### Conclusion

This study demonstrated a lack of increase in milk fat content under ODM management without major impairment of metabolic parameters. The milk fat content was increased when the goats were under feed adjustment but this feeding management involved an increase in NEFA as an indicator of fat mobilization. However, the milk protein content was increased by ODM management. Nevertheless, the milk urea content and the sum of short chain of fatty acids were not modified by ODM management or feed adjustment.

### References

**Auldist MJ, Prosser CG.** 1998. Differential effects of short-term once-daily milking on milk yield, milk composition and concentrations of selected blood metabolites in cows with low or high pasture intake. Proceedings of the New Zealand Society of Animal Production **58**, 41-43.

**Barnouin J, El Idilbi N, Chilliard Y, Chacornac JP, Lefaivre R.** 1986. Micro-dosage automatisé sans déprotéinisation du bêta-hydroxybutyrate plasmatique chez les bovins. (Automated micromethod for determining 3-hydroxybutyrate in bovine plasma without acid precipitation). Annales Recherches Vétérinaires **17**, 129-139.

**Bas P.** 1984. Détermination enzymatique des acides gras non estérifiés dans le plasma de chèvre. (Enzymic determination of non-esterified fatty acids in goat plasma). Annales Recherches Vétérinaires **15**, 7-16.

**Capote J, Lopez JL, Peris S, Suchs X, Argüello A, Darmanin N.** 1999. The effects of milking once or twice daily throughout lactation on milk production of canarian dairy goats. In Milking and Milk Production of Dairy Sheep and Goats. N. Zervas and F. Barillet, eds. EAAP Publication **95**, 267-273 p.

**Carruthers VR, Davis SR, Bryant AM, Henderson HV, Morris CA, Copeman PJA.** 1993. Response of Jersey and Friesian cows to one-a-day milking and prediction of response based on udder characteristics and milk composition. Journal of Dairy Research **60**, 1-11.  
<https://doi.org/10.1017/S0022029900027291>

**Chilliard Y, Pomiès D, Pradel P, Rémond B.** 2006. La monotraite ne modifie pas la composition en acides gras du lait, chez la vache en bilan énergétique équilibré. Rencontres Recherches Ruminants **13**, 328.

**Cooper GR.** 1973. Methods for determining the amount of glucose in blood. CRC crit. Rev. ClinicLaboratory Science **4**, 101-107.



**Couvreur S, Hurtaud C, Marnet PG, Favardin P, Peyraud JL.** 2007. Composition of milk fat from cows selected for milk fat globule size and offered either fresh pasture or a corn silage-based diet. *Journal of Dairy Science* **90**, 392-403.  
[https://doi.org/10.3168/jds.S0022-0302\(07\)72640-1](https://doi.org/10.3168/jds.S0022-0302(07)72640-1)

**Guinard-Flament J, Delamaire E, Lamberton P, Peyraud JL.** 2007. Adaptations of mammary uptake and nutrient use to once-daily milking and feed restriction in dairy cows. *Journal of Dairy Science* **90**, 5062-5072.  
<https://doi.org/10.3168/jds.2007-0259>

**Grala TM, Handley RR, Roche JR, Walker CG, Phyn CV, Kay JK.** 2016. Once-daily milking during late lactation in pasture-fed dairy cows has minor effects on feed intake, body condition score gain, and hepatic gene expression. *Journal of Dairy Science* **99**, 1- 15.  
<https://doi.org/10.3168/jds.2015-1042>

**Hammadi M, Ayadi M, Barmat A, Khorchani T.** 2009. Effects of milking interval on secretion and composition of milk in three dairy goats breeds. In *Proceedings of the 60th Annual Meeting of the European Association for Animal Production*. Wageningen Academic Publishers The Netherlands Eds. Abstract, p. 473.

**Holmès CW, Wilson GF, Mackenzie DDS, Purchas J.** 1992. The effects of milking once daily throughout lactation on performance of dairy cows grazing pasture. *Proceeding of the New Zealand Society of Animal Production* **52**, 13-16.

**Lacy-Hulbert SJ, Woolford MW, Nicholas GD, Prosser CG, Stelwagen K.** 1999. Effect of milking frequency and pasture intake on milk yield and composition of late lactation cows. *Journal of Dairy Science* **82**, 1232-1239.  
[https://doi.org/10.3168/jds.S0022-0302\(99\)75346-4](https://doi.org/10.3168/jds.S0022-0302(99)75346-4)

**Lefrileux Y, Pommaret A, Raynaud S.** 2008. Impacts de la monotraite dans une exploitation caprine fromagère à haut niveau de production. *Rencontres Recherches Ruminants* **15**, 167-170.

**Marnet PG, Gomis B, Guinard-Flament J, Boutinaud M, Lollivier V.** 2005. Effets d'une seule traite par jour (Monotraite) sur les performances zootechniques et les caractéristiques physico-chimiques du lait chez les chèvres Alpine à haut potentiel. *Rencontres Recherches Ruminants* **12**, 225-228.

**Marnet PG, Komara M.** 2008. Management systems with extended milking intervals in ruminants: Regulation of production and quality of milk. *Journal of Animal Science* **86** (Supplement.1), 47- 56.  
<https://doi.org/10.2527/jas.2007-0285>

**Nudda A, Bencini R, Mijatovic S, Pulina G.** 2002. The yield and composition of milk in Sarda, Awassi, and Merino sheep milked unilaterally at different frequencies. *Journal of Dairy Science* **85**, 2879-2884.  
[https://doi.org/10.3168/jds.S0022-0302\(02\)74375-0](https://doi.org/10.3168/jds.S0022-0302(02)74375-0)

**Papachristoforou C, Roushias A, Mavrogenis AP.** 1982. The effect of milking frequency on the milk production of Chios ewes and Damascus goats. *Annales Zootechnie* **31**, 37-45.

**Pomiès D, Rémond B.** 2002. La traite des vaches laitières une fois par jour pendant l'ensemble de la lactation: conséquences sur les performances zootechniques et la qualité du lait. *Rencontres Recherches Ruminants* **9**, 195-198.

**Pulina G, Fancellu S, Battacone G, Nudda A.** 2005. Effects of milking interval on hourly milk secretion rate in goats. *Journal of Animal Science* **83**(1), 363.

**Rémond B, Coulon JB, Nicloux M, Levieux D.** 1999. Effect of temporary once-daily milking in early lactation on milk production and nutritional status of dairy cows. *Annales Zootechnie* **48**, 341-352.  
<https://doi.org/10.1051/animres :19990502>

**Salama AA K, Such X, Caja G, Rovai M, Casals R, Albanell E, Marin MP, Marti A.** 2003. Effects of once versus twice daily milking throughout lactation on milk yield and milk composition in dairy goats. *Journal of Dairy Science* **86**, 1673-1680.  
[https://doi.org/10.3168/jds.S0022-0302\(03\)73753-9](https://doi.org/10.3168/jds.S0022-0302(03)73753-9)

**SAS.** Institute Inc. 2000. SAS/STAT Software: User's Guide, Release 8.0 696 SAS Institute Inc. Cary. NC.

**Sauvant D, Giger-Reverdin S, Meschy F.** 2007. In: Alimentation des caprins Alimentation des bovins, ovins et caprins. Besoins des animaux- Valeurs des aliments. Tables INRA 2007, Institut National de la Recherche Agronomique (INRA), Paris, France, p. 137- 148.

**Stelwagen K, DavisSR, Farr VC, Eichler SJ, Politis I.** 1994. Effect of once-daily milking and concurrent somatotropin on mammary tight junction permeability and yield of cows. *Journal of Dairy Science* **77**, 2994-3001.  
[https://doi.org/10.3168/jds.0022-0302\(94\)77240-4](https://doi.org/10.3168/jds.0022-0302(94)77240-4)

**Talke H, Schubert GE.** 1965. Enzymatic urea determination in the blood and serum in the Warburg optical test. *Klin Wochenschr***43**, 174-175.

**Tucker CB, Lacy-Hulbert SJ, Webster JR.** 2009. Effect of milking frequency and feeding level before and after dry off on dairy cattle behavior and udder characteristics. *Journal of Dairy Science* **92**, 3194-3203.  
<https://doi.org/10.3168/jds.2008-1930>