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M. Doreau, Sylviane Boulot, W. Martin-Rosset, H. Dubroeuq. Milking lactating mares using oxytocin : milk volume and composition. *Reproduction Nutrition Développement*, 1986, 26 (1A), pp.1-11. hal-00898368

HAL Id: hal-00898368

<https://hal.science/hal-00898368>

Submitted on 11 May 2020

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Milking lactating mares using oxytocin : milk volume and composition

M. DOREAU, Sylviane BOULOT, W. MARTIN-ROSSET (*), H. DUBROEUCQ (*)

*Station des Productions bovines et chevalines,
Laboratoire de la Lactation,*

() Unité Elevage et Alimentation du Cheval,
I.N.R.A., Theix, 63122 Ceyrat, France.*

Summary. Ten lactating mares were milked using oxytocin 5 times during the first two months of lactation. The two sides of the udder were milked alternatively and 60-ml fractions (from each side) were sampled for the analysis of fat, protein and lactose :

- the total amount of milk depended on the time elapsed since the milk was last drawn from the udder by sucking. When that time was 90 min (at 2 months of lactation), a mean amount of 1 200 ml was reached ; the maximal value was 2 185 ml ;
 - the amount of milk collected before oxytocin injection was on the average 40 % of the total but varied between 2 and 79 % ; there was no individual effect ;
 - the accuracy of milking as a method of estimating mare's milk was low when the deuterium oxide dilution technique was used as a reference ;
 - the fat content of the milk increased during milking with a specific effect of oxytocin injection (fig. 3). Variations in protein and lactose were not significant.
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Introduction.

Measurements of milk production and composition can be used to gain knowledge of the nutritional requirements of mares and the growth of their nursing foals. Oxytocin injection promotes udder emptying by stimulating milk ejection.

The estimation of milk production by milking with oxytocin injection has been extensively studied in cows and ewes (Ricordeau and Denamur, 1962 ; Doney *et al.*, 1979 ; Le Du *et al.*, 1979 ; Oftedal, 1984). The quantity sucked by offspring is generally overestimated, especially during early lactation, since it includes residual milk. Because the composition of milk (especially fat) varies during udder emptying of ewes and cows, it is necessary to carry out analysis after complete milking (Gilmore and Gaunt, 1963 ; Labussière, 1969).

The effect of oxytocin on milk release in lactating mares is not known. However, mares milked without oxytocin deliver less than 500 ml at each milking (Bilek *et al.*, 1955 ; Ullrey *et al.*, 1966 ; Johnston *et al.*, 1970), whereas those milked with oxytocin give a mean 1 000 ml (Oftedal *et al.*, 1983) and a maximum of 2 000 ml (Neuhaus, 1961). The above authors did not determine daily milk production. Ashcraft and Tyznik (1976) made an estimate on one side by milking

without oxytocin while the foal sucked the other. This milk yield, lower than all other estimates in the literature, was probably underestimated.

Mare's milk fat increases during milking (Prowosnik, 1931 ; Dittrich, 1938 ; Dyusembin, 1972a ; Cherepanova and Belokobylenko, 1974) but since there is a lack of details in these data, we undertook a more complete study.

After standardizing a milking technique using oxytocin, milk quantities and fat, protein and lactose concentrations were measured on each side of the udder, before and after oxytocin injection and at five stages of lactation. This estimate of milk yield was compared to an accurate one, using deuterium oxide dilution in the foal body, as proposed in mares in a previous paper (Doreau and Dussap, 1980).

Material and methods.

Experimental design. — Ten mares of two French heavy breeds (Bretonne and Comtoise) were maintained each in a box with their foal during the first 8 weeks of lactation. The mares were 5 to 15 years old, multiparous and weighed about 800 kg after foaling. In normal management conditions, the foals suck freely. They may eat hay but intake is known to be low up to 2 months (Martin-Rosset, Doreau and Cloix, 1978). The foals in our experiment were not allowed to drink water while milk production was measured by deuterium oxide labelling. So water intake was obtained almost exclusively from the milk.

The mothers were fed hay-concentrate diets *ad libitum* at a 50 : 50 (5 mares) or 95 : 5 (5 mares) ratio. Hay was a second cut of tall fescue ; a soybean meal concentrate (diet 95 : 5) or a cereal and soybean meal (diet : 50 : 50) was given so that the nitrogen requirements were met. The mares were milked on days 2, 8, 15, 29 and 57 after foaling. Milk yield measurements using the deuterium oxide dilution technique were centered around days 6, 27 and 55.

Nutritional data concerning the effect of diet on milk yield and composition and the relationship to foal growth will be published elsewhere (Doreau *et al.*, 1986 and unpublished).

Milking procedure. — The mares were hand-milked at about 3 p.m. when the sucking frequency of the foals was close to the mean daily frequency (Schoen, Banks and Curtis, 1976). This single milking was taken as representing the mean quantity of milk consumed by the foal in one suckling. The time between the last sucking and milking was equal to the mean time between two sucklings, estimated from behavioural data on mares of the same breeds (Martin-Rosset, Doreau and Cloix, 1978) : 30, 40, 50, 60 and 90 min for days 2, 8, 15, 29 and 57 after foaling. The mares were always milked by the same milker. The following sequence was used :

- a muzzle was put on the foal for longer than the mean interval between two sucklings ;
- the muzzle was removed and the foal allowed to suck. The hungry foal emptied the udder almost completely ;
- the muzzle was put back on the foal during the mean interval between two sucklings ;

- the mare was milked without any special udder preparation. When milk ejection stopped, 40 IU of oxytocin were injected intravenously and the mare was milked. The foal was left in the box with its mother to minimize the mare's stress. The whole procedure lasted between 5 and 12 min ;
- the muzzle was removed, and since the efforts of the foal to suck were unsuccessful, the udder was assumed to be well emptied.

Milk sampling. — At each milking, the milk, totally collected in 60-ml fractions in flasks, was taken alternatively from the right or left side before and after oxytocin injection. The first fraction milked and the first one collected after injection were taken from the right side. When one side was emptied, the other was milked until empty. This sampling procedure allowed us to calculate the quantity of milk obtained from each side before and after oxytocin injection. The corresponding fat, protein and lactose concentrations were also measured.

Daily milk yield was estimated by the formula $Y = M \times \frac{24}{I}$, where M was the quantity of milk collected by milking and I the interval between the last suckling and milking (in hours).

All 60-ml fractions of milk were analysed using the Gerber volumetric method for fat content and infra-red spectrophotometry for lactose and protein contents. This method, calibrated with cow's milk, led to a slight overestimation of the protein content, but the relative variations during milking were probably independent of the method (Doreau *et al.*, 1985).

Estimation of milk yield by the deuterium oxide dilution technique. — This method has been described by Doreau and Dussap (1980). In this trial, deuterium oxide was injected intravenously (0.6 g/kg live weight) at 9 a.m. Blood samples were taken at 3 p.m. four times during 5 days after injection. The hypothesis for calculating milk dry matter content and the production of metabolic water, as estimated in the previous paper, was maintained. No correction was made for hay intake ; a maximal intake of 1 kg of hay at 2 months leads to a calculated water intake (including metabolic water) of only 0.3 kg.

Results and discussion.

Amount of milk collected (table 1). — Total amounts varied significantly with physiological stage and diet. The effect of the first was chiefly the consequence of the increasing intervals between the last suckling and milking (30 to 90 min according to the stage). The amounts per unit of time were less variable. They decreased slightly, but not significantly ; standard deviations were high during the colostral stage and week 1. This seemed to show a maximal rate of secretion as soon as foaling occurred.

The coefficient of variation in milk quantity was high during the colostral stage : 70 % ; it decreased to 33 % at week 2 and to 18 % at week 8. The mean difference between the two diets, not detailed in this paper, was 273 ml. The

TABLE 1
Volume of milk collected from mares (means, standard deviation, minimum, maximum)

	Mean results	Results for each stage of lactation					Effect of stage of diet
		colostrum	1 week	2 weeks	4 weeks	8 weeks	
Total volume collected (ml)	964 ± 439 (265 - 2 185)	556 ± 393 (265 - 1 420)	767 ± 388 (450 - 1 760)	889 ± 293 (405 - 1 405)	1 075 ± 308 (445 - 1 690)	1 573 ± 297 (925 - 2 185)	**
Volume collected per min of secretion (ml)	18.3 ± 7.6 (8.1 - 49.3)	20.5 ± 11.6 (8.2 - 49.3)	19.1 ± 9.7 (11.2 - 44.0)	17.8 ± 5.9 (8.1 - 28.1)	17.9 ± 5.2 (12.4 - 27.5)	16.4 ± 3.7 (10.3 - 24.3)	**
Volume collected before injection of oxytocin (ml)	386 ± 290 (15 - 1 380)	300 ± 242 (75 - 895)	317 ± 376 (125 - 1 380)	315 ± 199 (15 - 630)	355 ± 213 (20 - 170)	642 ± 284 (310 - 1 280)	NS
Difference of volume collected from the two sides (ml)	63 ± 104 (0 - 555)	65 ± 107 (0 - 235)	52 ± 61 (0 - 160)	47 ± 75 (5 - 170)	61 ± 91 (10 - 220)	98 ± 166 (5 - 555)	NS

Significance level of statistical tests : * : 5 % (two-way analysis of variance)
 ** : 1 %

In the 5 successive stages, the total volumes correspond to milk secretion with 30, 40, 50, 60 and 90 min intervals between sucklings.

analysis of variance did not show any significant individual effect. Some mares gave very different amounts of milk from one week to another and sometimes more during the colostral stage than in week 4 of lactation.

The maximal amount of milk collected was 2 185 ml for a 90-min interval between the last suckling and milking. This value was close to the maximal values obtained by Neuhaus (1961) and to data from the Soviet Union, with dairy mares weighing about 500 kg (see review of Doreau and Boulot, 1986). Only a few authors obtained greater amounts. However, Fedotov (1965) and Fedotov and Duisembaev (1974) respectively obtained 2 600 and 2 700 ml of milk with one milking. The results are not easy to compare because in the literature the duration of secretion between the last emptying of the udder and milking is not indicated. Moreover, in our trial, maximal udder capacity may not have been reached with a 90-min secretion.

The amount of milk collected before oxytocin injection (fig. 1) depended on the physiological stage but not on the diet ; on the average, it was 40 % of the total amount. This ratio varied between 2 and 79 % but no effect of physiological stage, diet or animal was found. One mare spontaneously (*i.e.* before injection) gave 220 ml of milk in the colostral stage and 10 ml in week 4 of lactation. The lack of individual effect is surprising ; other workers indicate great individual variations in milk retention (Cherepanova and Belokobylenko, 1974). Moreover, we did not find any relationship between the apparent calm or stress of the mare and the amount of milk collected without oxytocin injection. Perhaps the muzzled foal caused a release of oxytocin during an attempt to suck, inhibiting further release during milking.

The volume collected after injection (V_2) was poorly related with the volume collected before injection (V_1) : $V_2 = 0.32 V_1 + 453$ ($r = 0.40$, $n = 50$,

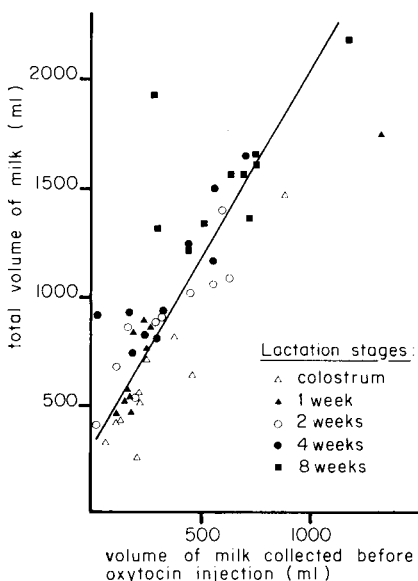


FIG. 1. — Relation between total volume of milk and volume collected before injection of oxytocin.

$P < 0.01$, V_1 and V_2 in ml). However, the total volume (V_T) could be roughly predicted with V_1 (fig. 1) : $V_T = 1.72 V_1 + 295$ ($r = 0.87$, $n = 50$, $P < 0.01$, V_1 and V_T in ml).

In 2 milkings out of 50, oxytocin was injected a second time because only 15 and 20 ml of milk were collected before the first injection. The amount collected after the second injection was 8 % of the total. The dose of oxytocin per kg of live weight was slightly higher than in the trials of Neuhaus (1961) or Oftedal *et al.* (1983). When related to blood volume, it probably led to maximal plasma concentrations measured during suckling (Sharma, 1974) or during dairy mare milking (Kuanyshebekova, 1975). However, the dose we used was very high in comparison with those generally injected in cows. The very small volume sometimes collected without injection could indicate that the cistern of the udder had a very limited capacity or was not full when the ducts were. Tumanova (1966) indicates a cistern volume of 200 to 300 ml. Masloboev (1961) has estimated it as 10 % of total udder capacity, and Cherepanova and Belokobylenko (1974) as between 40 and 100 % ; however, anatomical observations (Barone and Monnet, 1955) show the total capacity of udder cisterns to be about 60 ml in dry mares.

The increase in the amount of milk given spontaneously by mares between the colostral stage and week 8 of lactation was perhaps due to familiarization with milking. More probably, it was caused by an increase in the volume of milk in the udder ; intramammary pressure can stimulate the myoepithelial cells, as in other species (Denuccio and Grosvenor, 1971).

The right side of the udder gave a mean of 12 %, or 63 ml more milk than the left side. For 18 % of the milkings, however, the left produced more milk than the right. The difference between the two sides varied within the extreme values of 0 and 555 ml (table 1). It depended neither on physiological stage nor on diet. Two mares gave rise to the difference, which was small in the other 8 animals. The difference between the two sides was not significant for the milk collected before oxytocin injection (mean of 17 ml) but was significant for the milk collected after injection. Neuhaus (1961) has shown that the first side milked produces 35 % more milk than the other. The milking procedure in this trial could partially explain the difference observed. However, the quarters may not be physiologically symmetrical.

Comparison of the two methods for estimating milk production. — The relationship between the individual values obtained by milking or D_2O dilution at the same stage of lactation was not significant ($r = 0.17$). Moreover, one of the data obtained by milking was aberrant (63.4 kg). We considered the D_2O dilution technique to be the reference : it had been verified by Dove and Freer (1979) and Doreau and Dussap (1980) in lambs fed milk from a bottle. In this trial, the correlation coefficients between D_2O concentration and time were between 0.990 and 0.999. The main purpose of milking was to determine variations in milk composition. The natural conditions of nursing seemed to be optimized when the secretion time before milking came after the udder had been emptied as much as possible by suckling. When the estimation of the rate of milk secretion is the most important measurement, the best method is milking after a given secretion time,

once the udder has been emptied by oxytocin injection. So, the method used here might slightly overestimate the milk secretion rate. However, the amount of residual milk is unknown.

Up to week 4 of lactation, the estimation of milk production was higher by milking than by D₂O dilution, but at 4 and 8 weeks of lactation, the two means were similar (fig. 2). However, the relationships between the two methods were different when the mares were classed according to mean milk production : for the 5 lowest producers, the two methods gave similar results between weeks 1 and 8 ; for the 5 highest producers, production was overestimated by milking before 2 weeks and underestimated after 4 weeks. These results must be interpreted with caution owing to wide individual variation. Nevertheless, during the first week of lactation, the difference between the two methods might be explained by the fact that the foal's appetite was not enough to drive it to empty the udder ; this is also true in sheep (Ricordeau and Denamur, 1962) and cattle (Le Neindre *et al.*, 1976). At weeks 4 and 8, underestimation by milking might be due to the frequency of foal sucking which could be higher than expected in the high producers. According to Masloboev (1961), daily milk production drops when the interval between milking increases from 45 min to 2 h. However, sucking frequency has never been related to milk intake.

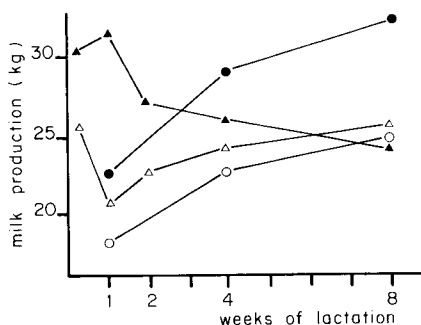


FIG. 2. — Comparison of two methods to estimate mare's milk production : effect of lactation stage and level of milk production. ● : D₂O dilution in foal body, high producers ; ▲ : milking mare, high producers ; ○ : D₂O dilution in foal body, low producers ; △ : milking mare, low producers.

Therefore, the estimation of mare's milk production by milking was not reliable in our experimental conditions. One milking was probably not enough to obtain a good estimate. Several successive milkings might have a galactopoietic effect (Dyusembin, 1972a). Moreover, our knowledge of the lactation physiology and behaviour of the mare is, at present, too limited to replicate nursing by milking.

Milk composition. — The milkings included 4 samples of about 120 ml of milk from both sides of the udder (60 ml from each side) : (1) : first fraction milked ; (2) : last fraction collected before oxytocin injection ; (3) first fraction collected after oxytocin injection ; (4) : last fraction milked.

Milk composition during milking varied whatever the diet, mare or stage of lactation. Figure 3 shows that fat concentration rose considerably as soon as oxytocin was injected. The protein and lactose concentrations fell only between

TABLE 2
Composition of milk fractions milked before and after oxytocin injection (analysis of variance on differences. 50 data pairs)

		Concentration (%)		Global effect of oxytocin (a)	Effect of lactation stage (b)	Individual effect (b)
		mean	standard deviation			
Fat	before oxytocin (1)	1.13	0.59	0.17	2.57	
	after oxytocin (2)	1.58	0.60	0.37	2.91	
	difference (2)-(1)	0.45	0.25	- 0.11	1.02	NS
Protein	before oxytocin (1)	3.38	0.80	2.43	6.20	
	after oxytocin (2)	3.28	0.75	2.35	6.13	
	difference (2)-(1)	- 0.10	0.13	- 0.83	0.04	NS
Lactose	before oxytocin (1)	6.27	0.31	5.38	6.96	
	after oxytocin (2)	6.22	0.31	5.44	6.98	
	difference (2)-(1)	0.04	0.05	0.00	0.31	NS

*** Significant effect at a 1 % level

* Significant effect at a 5 % level

NS non significant effect.

(a) Student's t-test on paired data
 (b) Two-way analysis of variance on differences

fractions 3 and 4. The increase in milk fat content during milking was verified in the mares; there were fewer variations between milking onset and end than previously reported (Cherepanova and Belokobylenko, 1974). This can be explained by a difference in udder emptying. There was no large increase between the last fraction and the preceding one. Fat content at the onset of milking was variable, sometimes being less than 1 ‰. This might explain why Neuhaus (1959) reported some zero milk fat content.

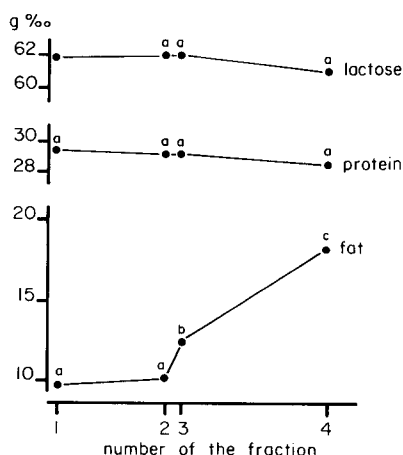


FIG. 3. — Variations in fat, protein and lactose concentrations during milking. Fractions 1 to 4 (60 ml milk from each quarter) are defined in text. Means with different superscripts differ ($P < 0.05$).

The slight decrease in protein and lactose contents at the end of milking, already observed in other species (see review of Labussière, 1985), can be partially (0.2 ‰) explained by a passive variation caused by the increase in fat content.

As a result of these variations, it is necessary to milk mares after oxytocin injection in order to collect a representative sample for milk composition analysis. Table 2 shows a significant difference in fat content between samples collected before and after oxytocin injection, whatever the physiological stage. These differences vary greatly with the mare. The errors committed in milking without oxytocin were always less than 0.4 and 0.9 ‰ for the lactose and protein contents, respectively. The error in fat content could reach 5 ‰ but was less than 2 ‰ in half of the milkings.

There is no significant difference in composition between the two sides when all the samples are considered. During the colostrals phase, however, the left side, which gave less milk, had a 2 % higher fat content than the right side. In the two mares which had a very different milk volume in the two sides, the left side had a higher fat content.

Reçu en mars 1985.

Accepté en octobre 1985.

Résumé. *Traite des juments allaitantes à l'aide d'ocytocine : volume et composition du lait recueilli.*

Dix juments allaitantes ont été traites à l'aide d'ocytocine à 5 reprises durant les deux premiers mois de lactation. Les deux quartiers étaient traits alternativement par fractions de 60 ml recueillies pour analyse des matières grasses, protéines et lactose :

- la quantité de lait recueillie a varié avec la durée de sécrétion de la mamelle depuis la tétée précédente. Quand cette durée était de 90 min (à 2 mois de lactation) elle a atteint en moyenne 1 200 ml ; la valeur maximale a été de 2 185 ml ;
- la quantité de lait collectée avant l'injection d'ocytocine a été en moyenne de 40 % de la quantité totale, mais a varié entre les valeurs extrêmes de 2 et 79 % ; il n'y a pas eu d'effet animal sur cette proportion ;
- l'utilisation de la traite pour estimer la production laitière des juments semble peu fiable, lorsqu'elle est comparée à la méthode de diffusion de l'eau lourde dans l'organisme du poulain, considérée comme référence ;
- la teneur en matières grasses du lait s'est accrue au cours de la traite, avec un effet spécifique de l'injection d'ocytocine (fig. 3). Les variations des teneurs en protéines et en lactose n'ont pas été significatives.

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