Fernández Martínez, N.; Balasch Parisi, S.; Pérez Baena, I.; Rodríguez Garcia, M.; Peris Ribera, CJ. (2013). Milk yield estimation during suckling using the double oxytocin injection-milking and the double weighing-suckling methods in dairy goats. Small Ruminant Research. 112(1-3):181-185. doi:10.1016/j.smallrumres.2012.12.023.

Milk yield estimation during suckling using the double oxytocin injection-milking and the double weighing-suckling methods in dairy goats

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ABSTRACT: The aim was to verify the validity of the double oxytocin-milking (**DOT**) method as milk yield estimator during the suckling period of lactating dairy goats. To this end, it was necessary to determine whether the weighing-suckling-weighing (**WSW**) and **DOT** methods of milk yield estimation satisfied the criteria to be considered valuable, the accuracy between both methods and the suitability of DOT to evaluate actual milk. At parturition, sixty lactating Murciano-Granadina breed goats were separated into 2 groups, in mixed (**MS**; n = 24) and artificial rearing (**ARS**; n = 36) management systems. Until the sixth wk of lactation (weaning), MS goats suckled one kid while kids from ARS goats were artificially reared; moreover, goats in both systems were submitted to once-a-day milking. Once per wk, actual milk yield for ARS goats and potential milk yield were recorded using DOT method for all goats, except for 12 goats in ARS which remained as a control. Twelve goats from each management system were used to evaluate diurnal variation in milk production (**DVM**) by DOT method for 6 consecutive days in wk 4 of lactation. No difference in DVM was found by DOT method in 4-h milk production of goats in MS ($P = 0.099$) or ARS ($P = 0.220$), which allowed sixfold multiplication of milked milk volume to obtain potential milk per day. ARS goats subjected to a weekly DOT and control group goats showed a similar ($P = 0.379$) lactation curve for the first 6 wks of lactation. The DOT method slightly overestimated (3.4%, $P = 0.005$) the milk yield evaluated by WSW method for goats under an MS, but fitted the actual milk obtained by common milk records for the group of goats in an ARS submitted to the DVM test ($P = 0.357$) and the group in ARS alone ($P = 0.163$). The DOT method applied for 8 consecutive days led to a drop of 6 to 12% in milk yield during the following week for both production systems. In conclusion, DOT was an accurate method to estimate milk yield during the first weeks of lactation both in MS and ARS under the conditions of this experiment.

Key Words: double oxytocin, goats, milk yield estimation.

INTRODUCTION

Milking methods are not valid for estimating milk yield in suckling small ruminants (Boyazoglu, 1963; Linzell, 1972), so weighing-suckling-weighing (**WSW**) and double oxytocin injection (**DOT**) methods (McCance, 1959; Doney et al., 1979) are usually employed to this end in goats (Peris et al., 1997; Delgado-Pertíñez et al., 2009a, b). The DOT method is less labour-intensive, but usually gives an overestimation of milk yield compared to the WSW method in ewes (Coombe et al., 1960; Moore, 1962; Doney et al., 1979). The latter provides a value closer to the real milk production with the exception of the first wk of lactation (Doney et al., 1979), when the newborns are unable to consume much of the milk produced by their mothers. On the other hand, McCance (1959) and Doney et al. (1979) described three criteria that the milk yield evaluation methods adopted must satisfy: 1) over the measuring period, the udder must be emptied to the same extent both at the start and at the end; 2) during the measuring period, the milk secretion rate must not differ significantly from that in other periods from which the estimate is to be extrapolated; and 3) the rate of secretion must not be significantly affected either in the short or long term by the method adopted. No studies on the performance of these criteria have been carried out in dairy goats, which usually produce more milk than ewes and may extend the milk evaluation problem of the first wk of lactation to the full pre-weaning period.

The present study tests different methods for milk yield estimation (WSW and DOT) in different breeding systems (mixed and artificial rearing) to verify the validity of the DOT method as a milk yield estimator in the first weeks of lactation in goats, which involved 1) evaluating whether WSW and DOT satisfied the stated criteria, 2)

measuring the difference between WSW and DOT and 3) assessing the suitability of DOT to evaluate actual milk yield.

MATERIALS AND METHODS

Housing and handling of the experimental animals followed the mandatory principles for care and use of experimental animals in Spain (Real Decreto 1201/2005, Boletín Oficial Estado 252:34367-34291).

Goats and General Procedures

Sixty multiparous (3 ± 0.2) Murciano-Granadina breed goats (45 ± 2 kg BW) were used at the experimental farm of the Universitat Politècnica de València (Spain). Mating was synchronised by intravaginal sponges (30 mg fluorogestone acetate and 450 IU PMSG; Chrono-gest, CEVA Salud Animal, Intervet, Salamanca, Spain) and all births took place over a 14-d period. At parturition, goats were randomly assigned to a mixed system (**MS**, n = 24 goats) similar to that of Gargoury et al. (1993), or to an artificial rearing system (**ARS**, n = 36 goats) similar to McKusick et al. (2001), for 6 wks. Weekly records (on Tuesday) of actual milk were taken in the 60 goats and readings of potential milk yield were taken in all goats but the control group. In addition, 12 MS goats were used to evaluate WSW and diurnal variation of milk production (**DVM**), 12 ARS goats were used to evaluate DVM and another 12 ARS goats were kept as a control. In the MS, each doe suckled one kid freely and was subjected to once-a-day milking (0800). Kids from the ARS goats were reared in straw-bedded pens (size = 0.3 m^2/kid; 2 bowl water troughs) by using a commercial milk replacer until weaning (wk 6 of age) and does were machine milked once daily (0800).

Both groups of does were kept in separate pens (size = 1.5 m^2/goat; feeder = 0.5 m/goat; 3 bowl water troughs per pen) and received the same total mixed ration twice

daily (at 0900 and 1800 h) throughout the experimental period. The ration was formulated according to Sauvant et al. (2007) and consisted of: 1) a basal diet to meet recommendations for maintenance plus 1.0 L milk/d (2.08 Mcal NE; 99 g MP; 8.7 g Ca; 4.9 g P), which included alfalfa hay (30% as DM), barley straw (26%), beetroot pulp (18%), orange pulp (26%) and 2) a commercial concentrate for dairy goats (1.62 Mcal NE, 135 g MP, 9 g Ca and 4 g P per kg of DM) to meet a total average milk yield of 3.3 L milk per goat per day. This average milk yield value was obtained from previous lactation of the same goats. Rations were offered to the does in an amount 10% higher than the calculated voluntary feed intake.

A high line Casse type milking parlour (2 platforms, 12 does per platform and 6 milking units) was used; machine milking parameters were set to: vacuum = 40 kPa, pulsation rate = 90 ppmin and pulsation ratio = 66%. Does were machine-milked without any udder preparation and using the following routine: machine milking, machine stripping and post-milking teat-dipping (Proactive Plus. 0.15% iodine, 4% glycerin, and 4% sorbitol-based emollient, DeLaval, Drongen, Belgium). Machine stripping involved a vigorous udder massage for 15-20 s just before the teatcups were removed.

Weekly records of actual and potential milk yield were taken at Tuesday milking. Potential milk yield was assessed by the DOT method. To do so, after milk recording (actual milk) goats were injected with 3 IU of oxytocin (**OT**; Hormonipra; Laboratorios Hipra, S. A., Girona, Spain) into the jugular vein, and their udders were emptied again using the milking machine. This milk was discarded and goats returned to the pens, where they remained near but separated from the kids for a 4-h period to prevent suckling. Following this separation period, goats were again injected with 3 IU of oxytocin and machine milked. The milk obtained was measured (potential for 4-h

period) and multiplied by 6 to obtain potential daily milk yield. Samples (50 mL) of actual and potential milk were collected and immediately analysed for milk fat composition and milk density. Milk fat content (ARS: actual = 5.41%, potential = 7.65%; MS: actual = 5.29%, potential = 7.23%) was analysed with an infrared analyser (Milkoscan FT120; Foss Electric, Hillerød, Denmark). Julie C3 Automatic (Scope Electric, Regensburg, Germany) was used to determine milk density. Milk yield was expressed as fat corrected milk (FCM) at 3.5% fat milk using the equation proposed by Sauvant et al. (2007) for goats [FCM yield = milk yield x (1 + (0.0075 x (g/L fat – 35) / 0.4))].

Weighing-suckling-weighing milk yield estimation

Daily milk yield by the WSW method was measured during wk 3 of lactation in 12 MS does at 4-h interval on two consecutive days. The experimental period started after machine milking (0800) on the first experimental day (Sunday) and finished after milking was recorded in wk 3 (0800; Tuesday). Milk obtained in the first milking was discarded and the does returned to the pens, where they remained nearby but separated from the kids for a 4-h period to prevent suckling. Following this separation period, the kids were weighed to the nearest 10 g and allowed to suckle from their mothers for 5 min and weighed again to evaluate the milk yield produced by the dams. This process was repeated for each 4-h period of the two days experimental period. At 0800 daily machine milkings, actual milk obtained was recorded and milk sampled for composition. Daily milk production (mL) was estimated by the sum of milk yield obtained by the WSW method (g) plus actual milk (mL), after transformation of weighed milk to volume by milk density (1,030 ± 0.3 g/L). Final daily milk yield evaluation was the average value of both experimental days.

Diurnal variation in milk production

The experimental setup to evaluate diurnal variation in milk production (**DVM**) by the DOT method was a 6 x 6 Latin square design (Montgomery, 1997), conducted for six consecutive days and immediately after finishing the experiment with the WSW method, starting on Wednesday of the 4th wk. So, goats were injected on a total of eight consecutive days, from Tuesday 3rd wk to Tuesday 4th wk records. The 24 h in a day were divided into the six 4-h milk production measurement periods already mentioned, starting after the daily milking at 0800 h. The same twelve goats used for the WSW experiment that were rearing single kids (MS) and 12 goats from ARS, which were between d 22 and d 29 of lactation, were randomly assigned to six different day x time period combinations (4 goats per combination) within the restrictions of a Latin square design. Goats were machine-milked at 0800 h and milk was discarded. Potential milk for each day x time period combination was calculated as described above.

Statistical Analysis

Daily variation in milk yield by DOT in the ARS and MS does was analysed using the GLM procedure (SAS Inst. Inc., Cary, NC). The model included the fixed effects of period of the day and day of experiment, the random effect of the animal and residual error.

To study the accuracy of the WSW and DOT methods for evaluating milk yield in the MS does, a paired data analysis, blocking by goat, was used. Average values for the two consecutive experimental days (WSW) and for the two following days (DOT) were used in a T test from SAS.

For ARS, the fit between actual (control vs one DOT per wk groups) and between actual and potential milk yields was analysed with a repeated measures model that included the fixed effects of method and wk of record, the random effect of animal, the corresponding interactions and residual error. When an interaction was non significant ($P > 0.05$), the corresponding interaction term was pooled with the error. MIXED procedure (SAS) was used. Separation of the means, if appropriate, for the determination of a significant ($P < 0.05$) main effect was done using pairwise contrasts (PDIFF option from SAS).

RESULTS AND DISCUSSION

The first criterion that milk yield evaluation methods must satisfy to be considered as valuable states (McCance, 1959; Doney et al., 1979) that over the measuring period, the udder must be emptied to the same extent both at the start and at the end. Both oxytocin injections and both machine milkings done at the beginning and at the end of each milk yield evaluation period by DOT and by WSW methods, respectively, established the same conditions for the udder milk content at these times, as the first criterion required.

No DVM for the 4-h milking intervals was found in the MS and ARS goats when measured by the DOT method, as shown in Table 1. Milk yield did not differ among time periods, suggesting a consistent milk secretion throughout the 24 h of the day, which satisfied the second criterion and allowed us to multiply sixfold the volume obtained after the second injection of the DOT method to evaluate milk yield, under these experimental conditions. These results agree with those of McCance (1959) and Cardellino and Benson (2002) in ewes.

Lactation curves for the ARS goats that were not subjected to the DVM evaluation and for control group did not differ (Figure 1; P = 0.379). So, it seems that a weekly application of the DOT method did not affect permanently the rate of milk secretion, which would satisfy the third criterion stated in the introduction section.

On the other hand, milk yield evaluated through DOT method (3,329 mL/d) was significantly (P = 0.005) higher than the milk yield obtained through WSW method (3,220 mL/d), which constituted an overestimation of 3.4%. Benson et al. (1999) obtained a similar overestimation percentage in ewes (3.24%) for DOT compared to WSW method, although this was not significant for them. A high experimental error for these authors could explain the lack of significance in this case. A higher milk yield estimation through DOT method could be related to the fact that the first OT injection induced milk letdown of residual milk and milking completely emptied the udder whereas, for the WSW method, a certain quantity of milk always remained in the udder, which may slow down the activity of secreting cells by the negative effect of feedback inhibitor of lactation (Rennison et al., 1993; Peaker and Wilde, 1996). On one hand, Stull et al. (2007), Hernandez et al. (2008), Pai and Horseman (2008) and Marshall et al. (2010) proposed that serotonin (5-hydroxytryptamine, 5-HT) is a feedback inhibitor of lactation. One mechanism responsible for this process is that serotonin alters barrier function and in this way dissipates the transepithelial gradients necessary for milk secretion (Stull et al., 2007). Other authors (Silanikove et al. 2006, 2010) proposed the plasmin-based concept. Thus, mild activation of the plasmin system results in the production of β-CN f(1-28), which is a potent blocker of K^+ channels in the apical membrane of mammary epithelial cells, affecting the osmotic-coupled water flow and so milk volume. It seems that increased milking frequency or the degree of emptying of

the udder dilute the content of β-CN *f*(1-28), which in turn reduces the inhibition on fluid secretion.

In ARS, significant differences (P = 0.005; Figure 1) were found only for wk 4 postpartum when comparing actual milk yield of ARS does used to assess the DVM with those used as a control. The same results were obtained for potential milk yield. This increase in milk yield for goats injected twice daily with oxytocin for eight consecutive days when compared to goats injected twice daily on only one day per wk could be due to the fact that oxytocin might increase membrane permeability, thereby increasing the supply of nutrients to the alveolar cells (Cowie et al., 1980). On the other hand, when milk is removed immediately after oxytocin injections, as happened in this experiment, oxytocin can also accelerate the rate of transit of synthesised milk constituents from the cytoplasm to the alveolar lumen (Cowie et al., 1980).

Estimated milk yield by the DOT method in ARS does submitted to DVM evaluation did not differ (P = 0.357) from the actual milk values obtained by once-daily milking during the rest of the experimental weeks (interaction wk x method, P = 0.945). The same result was obtained (P = 0.922) for goats not subjected to DVM. This result is important because authors (Peris et al., 1997; Delgado-Pertíñez et al., 2009a, b) usually compare potential MS to actual ARS milk yield. The results from this experiment may validate such comparisons.

In Figure 1 (ARS) we can observe that after wk 4, when goats were under DOT method daily for eight days (solid line), a milk production drop between 10-12% happened in wk 5 while the group under one weekly potential milk yield evaluation (broken line) and the control group presented a drop between 2-4%. Similar results occurred in Figure 2, where the drops presented by goats under DOT method daily for eight days (solid line) and the group under one weekly potential milk yield evaluation

(broken line) were of 7% and 2%, respectively. Bruckmaier (2003) observed that within one wk after beginning a chronic oxytocin treatment in cows there was a reduction in milk ejection when oxytocin was withdrawn. This author put forward two possible reasons: a reduced release of oxytocin from the pituitary or a possible down-regulation of an oxytocin receptor that caused reduced sensitivity to oxytocin in the udder. In an experiment with cows, Mačuhova et al. (2004) found that the reduction of spontaneous milk removal after a chronic OT treatment was due to reduced contractibility of myoepithelial cells in the mammary gland at a physiological range of OT concentrations. In the same line, Belo and Bruckmaier (2010) concluded that a desensitisation of the udder toward OT occurs when it is exposed to elevated OT plasma concentrations due to chronic high-dosage treatment. Both results suggested that the reduction in milk ejection after a chronic OT treatment is not due to a reduced OT release from the pituitary.

In summary, DOT method satisfied the three criteria to be taken as a valuable method to evaluate milk yield during the pre-weaning period in does under an MS. Diurnal variation in milk production was not significant, and daily milk production can therefore be reliably estimated from 4-h yield measurements. This information is useful in estimating lactation curves during suckling plus milking period of lactation and thus in defining feeding and management strategies for dairy goats. The DOT method overestimates milk yield measured by WSW by 3.39% under an MS, but fits the actual milk obtained by normal recording under ARS.

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Figure 1. Least squares means (± SEM) for the control group (n = 12), and for the actual and potential daily milk yield from goats (n = 12) under milk yield diurnal evaluation (solid line) or goats (n = 12) without milk yield diurnal evaluation (broken line) for an artificial rearing system. Milk yield diurnal evaluation (indicated with vertical arrows) took place at wk 4 postpartum.

Figure 1

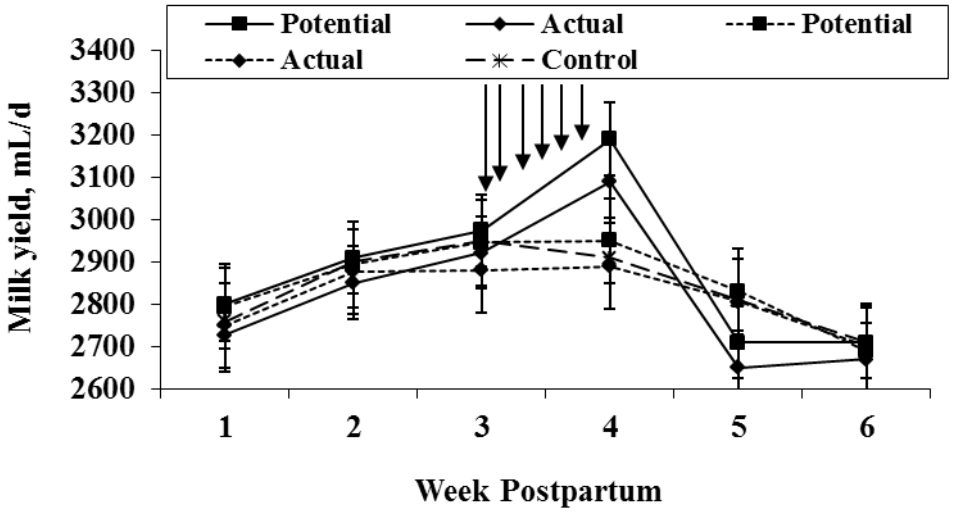
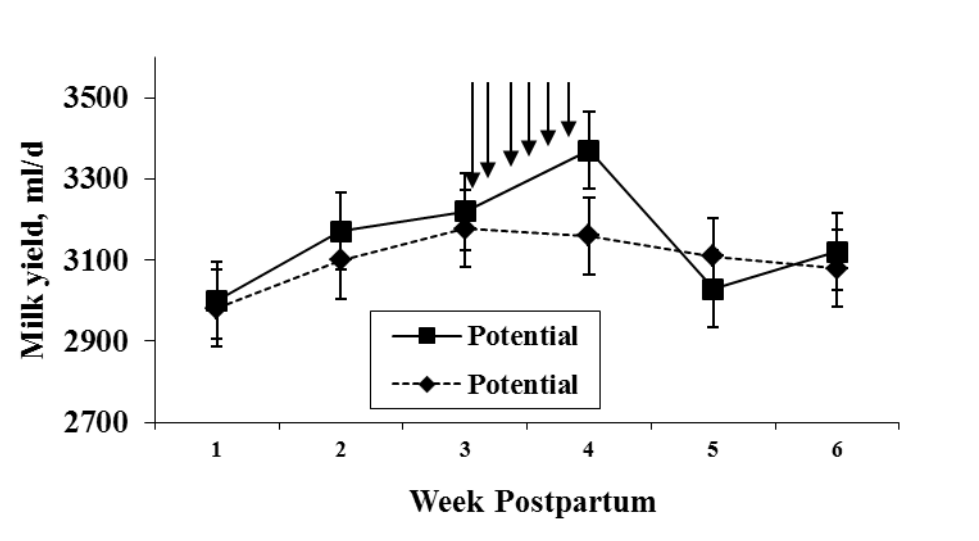

Potential
Actual
Potential
Actual
Control
Milk yield, mL/d
3400
3300
3200
3100
3000
2900
2800
2700
2600
1
2
3
4
5
6
Week Postpartum

Figure 2. Unadjusted means (± SEM) for potential daily milk yield from goats (n = 12) under milk yield diurnal evaluation (solid line) or goats (n = 12) without milk yield diurnal evaluation (broken line) for a mixed system. Milk yield diurnal evaluation (indicated with vertical arrows) took place at wk 4 postpartum.

Figure 2


Milk yield, ml/d
3500
3300
3100
2900
2700
1
2
3
4
5
6
Week Postpartum
Potential
Potential