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Original article

Effect of calf suckling on oxytocin, prolactin, growth hormone and milk yield in crossbred Gir × Holstein cows during milking

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Abstract — This study evaluated the effect of different milking managements on oxytocin, prolactin and growth hormone release in Gir × Holstein cows. Six cows were suckled by their calves, before and after milking (SM group); another six cows were submitted to exclusive milking (M group). High OT levels were observed during suckling of the SM group, however both groups had similar levels of OT during milking. The SM group presented PRL levels significantly higher than the M group, however only during suckling. For GH levels, the SM group showed higher levels than the M group, however this difference was significant only during the first suckling, cleaning of the teats and at the beginning of machine milking. Despite these results, SM cows produced more milk (milking plus suckling) than M cows, however milk obtained by milking was similar for both groups.

cows / crossbred / milking / suckling / hormone

1. INTRODUCTION

Unspecialized cattle farmers in Brazil have used crossbred Gir (*Bos indicus*) × Holstein (*Bos taurus*) cows to produce both milk and calves during the spring and summer in extensive systems [1] because these crossbred animals are well adapted to tropical conditions [39, 40]. In many cases, this system is economically viable and farmers maintain Gir × Holstein cows [19].

However, some studies with *Bos indicus* × *Bos taurus* cows have demonstrated that these animals are susceptible to lactation failure [1, 26] and retain high levels of residual milk after machine milking [26]. In Brazil, Gir cows have been continuously

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selected for milk yield [22, 38] and at present Gir × Holstein cows exhibit a normal lactation [20]. For these reasons, this crossbreeding program is considered a success [19, 21] and some farmers are now specializing in producing crossbred cows for replacements on other dairy farms [21].

In general, Gir × Holstein cows are reputed not to be well-adapted to machine milking, and many crossbred cows are milked in the presence of their calves, like other breeds considered not well-adapted to exclusive machine milking [4, 31]. Thus crossbred cows are often suckled by their calves immediately before and after milking [34, 35]. Other works have observed that Holstein cows [5] and Lacaune ewes [23] submitted to a combined milking and suckling system produced more milk than during exclusive milking. This management of combined milking and suckling increases the labour of milkers and has been shown to inhibit OT release and decrease milk production in some cases following weaning [23, 41].

Some previous works have studied the influence of a combined milking and suckling system on milk production in crossbred cows [4, 34, 35]. However, these experiments did not analyse hormone release around milking and milking adaptation of crossbred *Bos indicus* × *Bos taurus* cows to exclusive machine milking has not been described in the literature. Thus, the objective of the present study was to evaluate the effect of different milking managements on oxytocin (OT), prolactin (PRL) and growth hormone (GH) release and on milk yield in crossbred Gir × Holstein cows.

2. MATERIALS AND METHODS

The experiment was carried out at the beginning of the summer (the humid season), using 12 crossbred cows (Gir, *Bos taurus* × Holstein, *Bos indicus*) with a proportion of 3⁄4 Holstein blood (F2). All experimental cows had free access to water, pasture (*Panicum maximum*) and a vitamin/mineral mixture. Before each milking, each cow received a mixture of grain in an individual tie-stall, according to their production. Periodic control of the total diet was effected and when necessary, additional concentrate was furnished to provide adequate nutrients for milk production [27].

Experimental cows were divided into two homogeneous groups, with similar lactation number, lactation stage and level of milk production. To avoid the influence of previous milking management in the present experiment, we used cows that had been submitted to the same milking management as in their previous lactation (exclusive milking or suckling and milking combination). All 12 experimental cows were milked twice/day (at 6 and 18 h). Six cows were suckled by their calves, immediately before and after milking thus forming the SM Group; the 6 other cows were separated from their calves and submitted to exclusive milking thus forming the M group.

2.1. Suckling and milking

Suckling lasted 2 min before milking (from –4 to –2 min) and 2 min after milking (from 12 to 40 min). Each calf suckled its own mother, and during milking calves remained tied beside their mother and tactile, visual and vocal contact were possible. Milk consumed by the calves during suckling was measured by weighing the calves before the first suckling and after the last experimental suckling.

Milking machine parameters were constant throughout the experiment. The settings for the low line milking machine (Westfalia Elk Grove Village, IL) were a vacuum of 45 kPa, a pulsation ratio of 70:30% and a pulsation rate of 60 cycles·min^{-1}. Milking routines were constant and the same person performed all experimental milkings. Prestimulation was carried out before milking, during cleaning of the teats and the mastitis

test for both groups (from –1 to 0 min). However, hand milking was not applied. The teatcups were attached at time 0 and detached after interruption of the milk flow (machine milking lasted on average for 8 ± 1.2 min). Milk yield was recorded at each milking throughout lactation.

2.2. Blood samples

Blood samples were taken at the morning milkings on days 40, 41, 42 and 43 of lactation, before and after udder stimulation. At each experimental milking, samples were taken at: –5, –3, –1, 1, 2, 3, 5, 7, 10, 13 and 15 min. For both groups, the samples at –5 min were defined as basal levels, and the sample at 0.5 min was taken during the cleaning of the teats and the mastitis test. For the SM group, samples at –3 and at 13 min were taken at the middle of suckling, and each suckling duration was fixed at 2 min (calves suckled from –4 min to –2 min and from 12 to 14 min respectively).

Samples were collected in chilled heparinized tubes and immediately centrifuged at 4 °C and 3000 *g* for 15 min. Plasma was preserved at –20 °C until analysis. Plasma concentrations of OT were measured by the EIA method [24] and plasma concentrations of PRL and GH were determined using the RIA method [15].

2.3. Statistical analysis

SAS software [36] was used for evaluating data by means of analysis of variance, Student t-tests and Newman-Keuls tests. The probability level was $P < 0.05$, and all values presented in this work are given as the mean ± standard error of the mean (sem). The model used to evaluate the effect of type of milking management took the group, cow identity, time of sample, day of milking and all possible interactions into account. The relationships between milk yield and hormone levels were evaluated by Pearson correlations.

3. RESULTS

The hormone profiles are shown in Figure 1. OT levels measured during the cleaning of the teats were higher than the basal levels for both groups of cows. Highest OT levels were observed during suckling, however there was no significant difference between peaks measured during suckling and milking in the SM group. At the same time, both groups had similar levels of OT during machine milking. However, the hormone profiles observed during milking were different because OT increased more rapidly in the SM group (2 min after the beginning of milking) than in the M group (5 min).

Baseline levels of PRL were similar for both groups. In general, the SM group presented higher PRL levels than the M group but only during suckling were PRL levels significantly higher. However, PRL levels varied between sucklings and the PRL level observed during the suckling following milking was higher than that measured during the suckling before milking. It is important to observe that at the end of milking, PRL release increased for both groups (Fig. 1).

Although GH levels were significantly higher for the SM group than for the M group, there were significant differences between groups only during the first suckling, cleaning of the teats and at the beginning of machine milking. As shown in Figure 1, both groups presented similar levels of GH from 7 min to 15 min, because the M group showed an increase in GH levels after the end of milking, while the GH levels of the SM group remained stable.

In contrast to the hormone results, milk production recorded during milking was similar for both groups (SM = 14.68 ± 0.26 L·day^{-1} and M = 15.10 ± 0.40 L·day^{-1}). However, calves suckled 3.75 ± 0.45 L·day^{-1}, consequently SM cows produced more milk (milking plus suckling = 18.43 ± 1.22 L·day^{-1}) than the M cows (15.10 ± 0.40 L·day^{-1}). There were no significant correlations between the levels of hormones studied and milk yield.

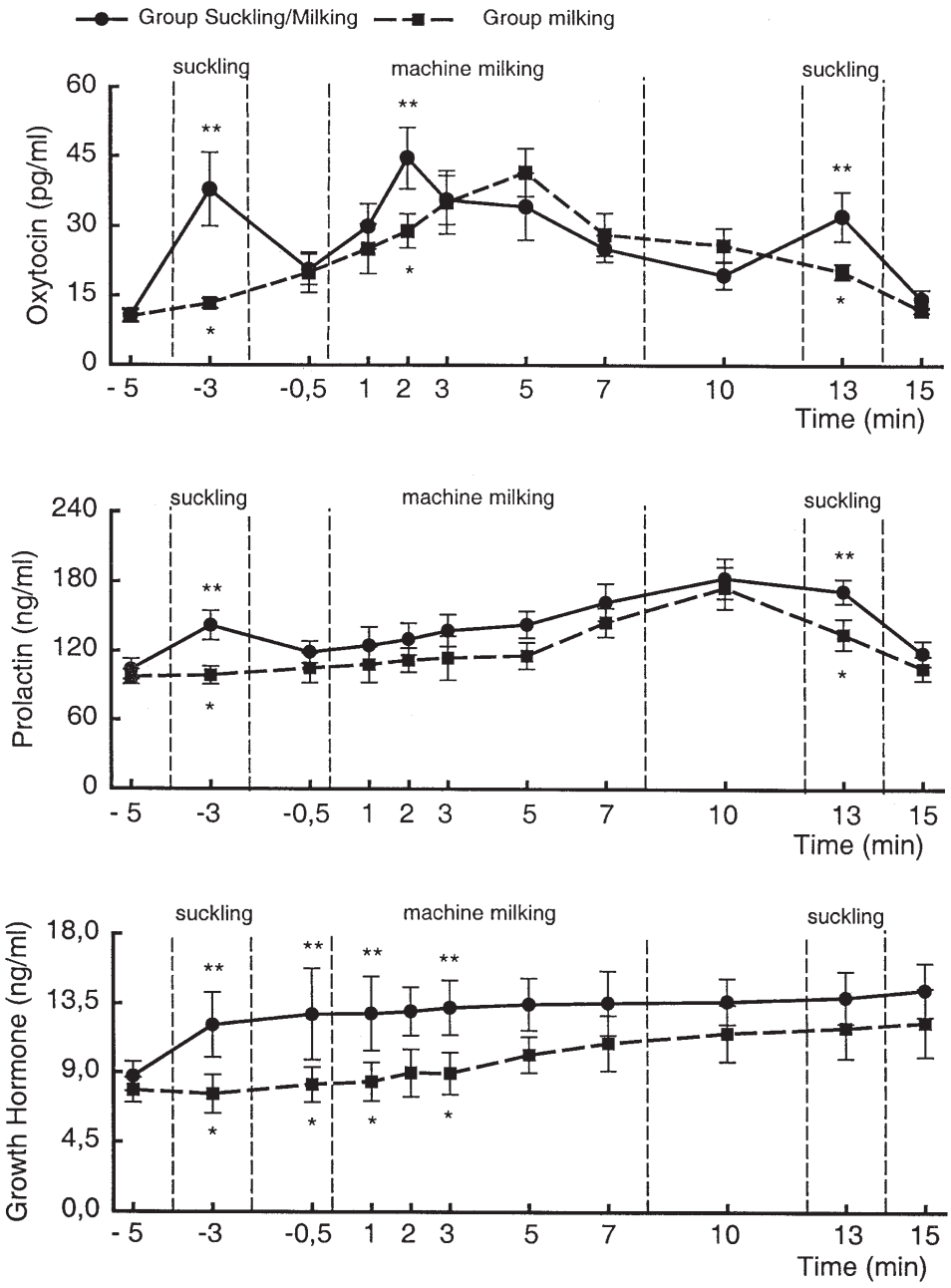

Figure 1. Oxytocin (OT), prolactin (PRL) and growth hormone (GH) release (means ± sem) before and after different milking managements with or without suckling in Gir × Holstein cows. Pre-stimulation was carried-out before milking (from –1 to 0 min). The vertical dotted lines indicate the beginning and the end of suckling (from –4 to – 2 min and from 12 to 14 min, respectively) or machine milking episodes (from 0 to 8 min). Means with different symbols differ $P < 0.05$.

4. DISCUSSION

In this study, the baseline levels of OT, PRL and GH were similar for both groups of cows. Also, only OT levels had a slight but significant increase during the cleaning of the teats for all cows. As described in previous studies, pre-stimulation caused OT release [9, 13, 25]. Although OT levels first peaked during suckling in the SM group, OT peaks measured during suckling did not differ from the OT peaks measured during milking. In contrast, Holstein cows [5] and Lacaune ewes [23] showed the highest OT levels during suckling, however in these experiments suckling and milking were carried out at two distinct times.

In the present experiment, calves suckled immediately before milking. This prior udder stimulation probably influenced OT release and milk ejection during milking of the SM group. Consequently, OT increased more rapidly in the SM group (2 min after the beginning of milking) than in the M group (5 min). Nevertheless, OT levels measured at the first min of milking in the M group were significantly higher than the basal levels. As demonstrated by other authors, small increases in OT levels reaching a threshold level are effective in inducing milk ejection in cows [37]. Furthermore, the variations in OT levels reported in the present experiment could be considered normal after a comparison of our results with those of other studies on Holstein cows submitted to exclusive machine milking [9, 14].

Apparently, in the present study the calf presence facilitated OT release during machine milking of the SM group, because the SM and M groups presented similar OT release during milking. In contrast, other authors have reported that dairy cows show higher OT release during suckling than milking in the calf's presence [18, 42]. However, the difference between these results and our study could be attributed to the fact that the latter authors compared two different groups submitted to suckling or milking in the calf's presence. It is possible that a combined milking and suckling system (consequently, milking in the calf's presence) has a positive effect on OT release and milk ejection for the Gir × Holstein cows of the SM group.

As shown by our results, the SM group presented higher PRL levels than the M group and PRL levels of the SM group were significantly higher during suckling than milking. Previously, other authors had demonstrated that the PRL release by cows during exclusive suckling was higher than during exclusive milking [2, 32], and that the calf's presence positively influenced PRL levels during milking [3]. Although PRL levels appeared to be superior during suckling than milking in a combined system of suckling and milking, these differences were not significant [5, 23].

It is important to observe that at the end of milking, the PRL release increased for both groups of cows. This explains why in the present work the difference between the groups was smaller during the suckling following milking, and indicates that suckling and milking stimulation improved PRL release. In the same way, other studies have demonstrated that machine milking continues influencing PRL levels several minutes after the end of milking [14, 28, 29]. Yet PRL is apparently not essential for galactopoiese in ruminants [33]. Recently, PRL administration in dairy goats has been found to significantly influence milk yield [16]. In dairy ewes, PRL is released during and after milking and milk yield decreases simultaneously from mid- to late-lactation [29].

Comparing GH levels measured in the present study, we observed that there was a significant difference between the groups of cows. This difference existed only at the beginning of udder stimulation (suckling, cleaning and the beginning of milking), because while GH levels remained stable for the SM group, GH levels increased after the end of milking for the M group. Other research has demonstrated that GH release is

higher in a combined system of suckling and milking than in exclusive milking [5]. It is generally accepted that GH positively influences galactopoiese and since many studies have reported that exogenous GH increases milk yield [7, 10], it is interesting to know that it is possible to influence GH levels by udder stimulation.

Apparently, the stimulus induced by machine milking was effective in the present experiment because OT, PRL and GH were released during and after milking. In fact, milk obtained during milking was similar for the SM and M groups. Our experimental crossbred cows of both groups produced more milk than Gir cows [34, 35] and other crossbred Gir × Holstein cows [34, 35] but less milk than Holstein cows [5]. At the same time, our experimental calves suckled more than in previous studies with crossbred calves [34, 35]. Consequently, SM cows produced more milk (milking with more suckling) than M cows.

For the SM group, it is possible that milk obtained by machine milking was influenced by suckling at the previous milking. Other authors have already demonstrated that different management of cows and calves during milking could influence milk yield [5, 34, 35] and calf growth [11, 35]. It is important to note that the time of suckling was limited to 4 min/milking during our experiment, and that normally the calf remains with crossbred cows for several hours after milking [35]. Thus it is possible that crossbred calves (that received a mixture of grain and had access to pasture), normally suck more milk than necessary for their development which would influence the amount of milk obtained during the next milking.

Although *Bos taurus* cows are well-adapted to machine milking, cows managed in a combined system of milking and suckling may not eject milk during subsequent exclusive machine milking [5, 8]. Indeed, milk yield shows a significant decrease when suckling is abruptly ceased in cows [5] and ewes [23]. As previously described, this kind of disturbance could be attributed to inhibition of OT release [23, 41], likely due to psychological stress caused by weaning [23]. However, OT release increases gradually several days after weaning and ewes then present a normal milk ejection [23].

Similarly, other authors have observed that crossbred cows susceptible to lactation failure exhibit high residual milk, and have suggested that inhibition of OT release is a possible explanation for lactation failure [26]. This could explain why cows with poor milk yield depend on frequent udder stimulation, and in many cases present a failure of lactation when submitted to the classical management strategy of 2 exclusive milkings/day. Furthermore, suckling after a period without suckling or milking has been shown to reinitiate milk secretion in beef cows [17].

In conclusion, it is possible that the beneficial effects of frequent milk removal could be partially attributed to the simultaneous release of OT, PRL and GH caused by udder stimulation. Several authors have demonstrated that GH administration [6], OT administration [30] or a combination of GH and PRL administration [12] has a positive effect on milk yield. It then seems possible that hormones released during and after udder stimulation have a concomitant action in milk synthesis, which remains to be elucidated. In this work, both types of milking management were effective in inducing OT, PRL and GH release in Gir × Holstein cows, however crossbred cows submitted to suckling before and after milking were more productive than those submitted to exclusive milking.

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