

## Effects of Machine Milking on Milk Yield and Cortisol Hormone Changes of Dairy Cows with or without Calves

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### ABSTRACT

This study was conducted to investigate the effect of weaning of calves during machine milking on milking adaptation and cortisol concentration in plasma of Shami cattle. 12 heifers at their first lactation were utilized. The animals were randomly classified into two groups of equal numbers; the first group was milked with calf at foot, whereas the second group was milked without calf. Milk yield were recorded weekly for both groups, as well as the collection of blood samples for the determination of cortisol concentration was done in the morning and evening during milking using the ELISA test. Results revealed that, a hormone range of 50-86 ng/ml in the first group and 97-113 ng/ml in the second group. The differences between both groups were not significant. However, the difference of cortisol level in the second group was significant ( $p < 0.05$ ) for measurements before and after morning and evening milking sessions. Variations in periods of before milking were not significant. There was slight elevation in the hormone levels after milking in the first group. The result also indicated a negative correlation between milk yield and concentration of cortisol during morning and evening milking in both groups.

**Keywords:** Dairy heifers, Cortisol hormone, Milk yield, Machine milking, ELISA

### INTRODUCTION

It has been stated that the population of Shami cattle has declined tremendously to 3338 in accordance with the ministry of agriculture census. One of the main problems in this breed is milking in absence of calf, since the presence of calf stimulate the release of oxytocin [1,2]. Some studies found that in cows milked without the presence of their calves, the milk ejection reflex was not stimulated and the cortisol hormone level in the blood plasma was not elevated, although the udder was massaged for a minute before the start of milking process [3]. Machine milking is known to stimulate the release of oxytocin in improved breeds but it may also lead to stress and low milk yield in non-genetically improved breeds [4].

Moreover, when animals are stressed during machine milking, especially in animals that are not accustomed to machine milking, the concentration of cortisol and noradrenalin is elevated [5]. Some studies [6,7] revealed that suckling and machine milking lead to increased dripping in milk and elevated level of oxytocin, prolactin and growth hormones, compared to machine milking without suckling. They attributed that to good stimulation of the teats and the

reaction cow's to its calf. This can explain the increase in milk yield in the beginning of lactation in local breed where milking with suckling is traditionally practiced [8]. Studies on South African's and Latin American's cattle breeds reported that these breeds have not adapted to machine milking in absence of their calves [5,9].

On the other hand, Tancin et al. [10] claimed an elevated blood cortisol level during suckling or machine milking; although the hormone is considered as a standard measure of stress in animals. Moreover, the closer relationship between the mother and the kid leads to stimulate the hypothalamus-pituitary-adrenal cortex axis as being observed in most

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mammals [11]. Some researchers also reported that injecting large doses of cortisol not affect milk ejection reflex [12]. However, stress can clearly affect the milk ejection reflex and leads to elevate the cortisol hormone [13] or when injecting adrenocorticotrophic hormone stimulating factor [14] or adrenocorticotrophic hormone releasing factor [15]. It may also be noticed that the secretion of cortisol hormone during milking is not clearly indicated, it might be a physiolactating hormone, plays a role in the lactating gland itself [16].

This study aims to investigate the effect of machine milking in Shami cows weaning of their calves and to measure the level of cortisol hormone in the blood plasma during machine milking in presence and in absence of the calf.

## MATERIALS AND METHOD

### Research site and animals

The research was conducted at Dair El-Hagar research station, Syria. Twelve Shami heifers of the same age range (28-30 months) and at the trimester were selected for the study. Animals were randomly classified into two groups of equal numbers. Each group was housed in a separate open pen. After delivery, calves were separated from their mothers and reared in individual pens until weaning age (90 days). The first group of heifers was milked in the presence of their calves, whereas the second group individuals were milked without the presence of their calves. During the study period, animals offered their nutritional requirements which were consisted of roughage (wheat straw), Alfa alfa (*Medicago sativa*) and sorghum (*Sorghum bicolor*), concentrate feed (maize, cotton seed cake and corn) in addition to vitamins, minerals, salt and calcium bi-phosphate (concentrate ration offered at 8:00 a.m.). Animals have free access to clean water.

### The milking process

Machine milking was practiced for both groups of cows, two times daily at 6:00 a.m. and at 6:00 p.m. Only the first group was milked in the presence of their calves. Calves were allowed to suckle their mothers' teats for 5-10 s before the commencement of machine milking and then each calf was tied beside his mother and udder massage was then continued for one minute. Machine milking was done for three teats and the fourth left for calf to suckle for 5-10 min, until milking was finished. On the other hand, the second group was hand massaged for 1 min before machine milking.

### Milk yield

Milk yield for each heifer was recorded from the evening production starting from the 7<sup>th</sup> day after delivery to end of lactation. For estimation of daily yield per individual, morning and evening yield was summed up and multiplied by 1.33 because the yield from only three teats [17].

## Blood sampling and analysis

Blood samples were collected using a catheter (Certofix-Mono s430, Brown, 34212 Melsungen, Germany), from the jugular vein to avoid the stress of the animal. Sampling was done before and during milking, two times daily (morning and evening), started from the 2<sup>nd</sup> month of lactation. Sampling periods were designed as follows: 10, 5 and 1 min before milking (BM); 0, 0.5, 1, 1.5, 2, 2.5, 3, 3.5 and 4 min during milking (DM); 5, 10 and 15 min after milking (AM), blood were collected in 10 ml test tubes with anti-coagulant, mixed well and put in cold water at 4°C. Samples were then transferred to the laboratory where centrifuged at 3500 rpm for 15 min and plasma were harvested and kept frozen at -20°C in plastic test tubes, until the begin of the measure of cortisol hormones. ELISA test was used to estimate the concentration of the cortisol hormone using special ELISA kit (IBL-Hamburg, Germany).

## STATISTICAL ANALYSIS

Data collected was subject to statistical analysis after testing its normal distribution with the aid of SAS [18] in accordance with the following model:

$$Y_{ijklm} = \mu + G_i + S_j + P_k + C_1(T_m) + e_{ijklm}$$

Where,

$Y_{ijklm}$  = concentration of cortisol hormone in blood plasma (ng/ml),

$\mu$  = Overall mean,

$G_i$  = Fixed effect of the animal group ( $i=1, 2$ ),

$S_j$  = Fixed effect of blood sampling period ( $j=-10, \dots, +15$ ),

$P_k$  = Fixed effect of milking time ( $k= 6$  o'clock morning, 6 o'clock evening),

$C_1(T_m)$  = Animal variable factor\*group ( $i=1, \dots, 12$ ), ( $m=1, 2$ ),

$e_{ijklm}$  = Residual error.

Least Square Means (LSM) were obtained and the simple Pearson correlation coefficient was used to test the relationship between milk yield and concentration of cortisol hormone.

## RESULTS

### Concentration of cortisol hormone in blood plasma

Figure 1 indicate that the mean cortisol concentration was 50.68 ng/ml 10 min before milking in the first group; as for the second group, it was 97.12 ng/ml with the difference being insignificant ( $p>0.05$ ). The level of the hormone starts to increase gradually with the start of milking, to reach a maximum level (75.98 ng/ml) in the 1<sup>st</sup> group at the end of milking process. In the 2<sup>nd</sup> group, the level of the hormone reached 115.72 ng/ml in the middle of the milking process. Despite the clear variation in cortisol hormone concentration between the study groups, the difference is not significant

( $p > 0.05$ ). The concentration of cortisol hormone continued to be high 10 min after milking and reached 86.33 ng/ml in the 1<sup>st</sup> and 111.1 ng/ml in the 2<sup>nd</sup> group. Generally, the

average concentration of cortisol hormone in the 1<sup>st</sup> group was lower than that in the 2<sup>nd</sup> group during all measurements periods.

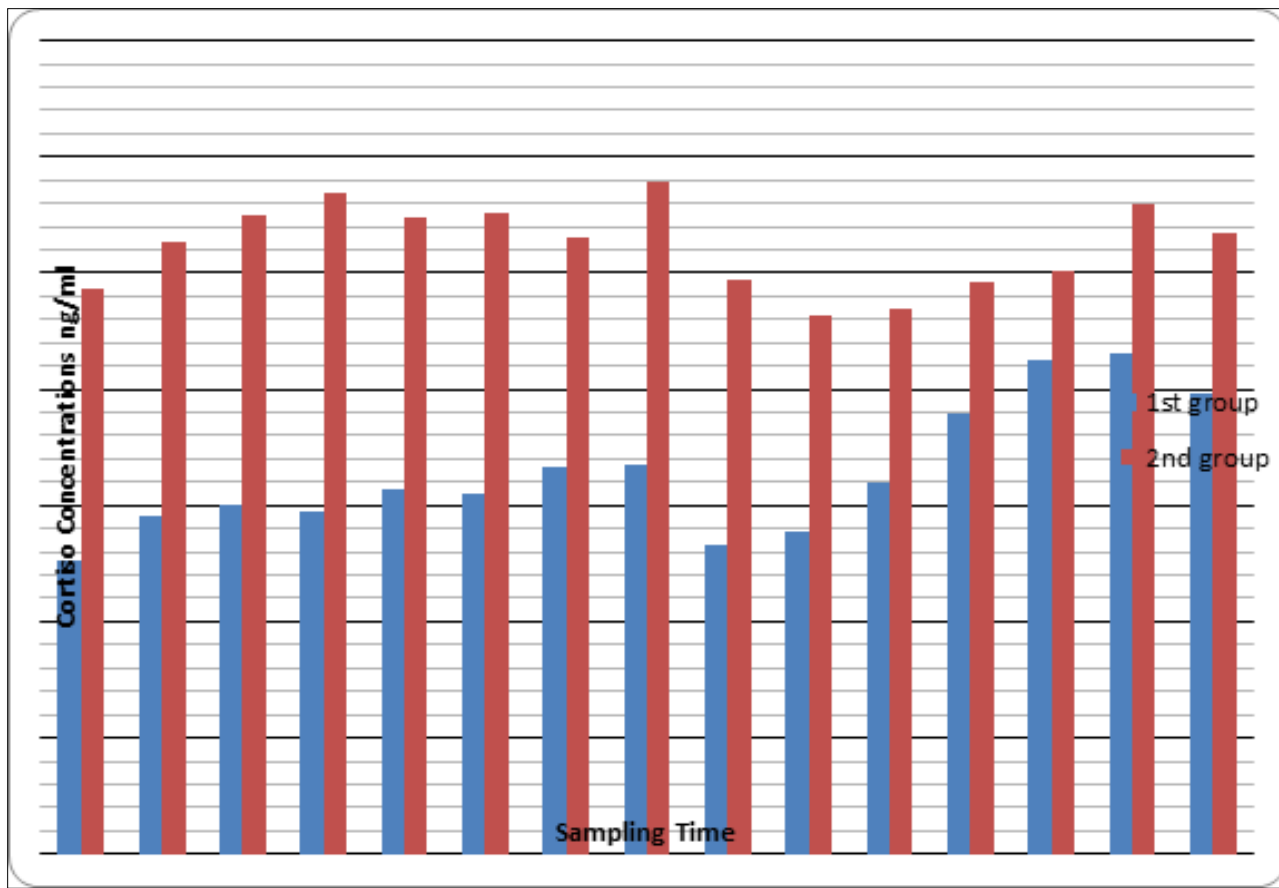


Figure 1. Mean concentration of cortisol hormone (ng/ml) in different milking periods.

**Concentration of cortisol hormone in blood plasma during morning milking**

Results reveal that there is no statistical difference in mean cortisol concentration between the two groups before morning milking. During milking the hormone continued to elevate in the 1<sup>st</sup> group compared to the 2<sup>nd</sup> group but the difference was not significant. However, after milking the difference in cortisol concentration in the two groups was significant ( $p < 0.05$ ) as shown in **Figure 1**.

**Concentration of cortisol hormone in blood plasma during evening milking**

The same trend was shown regarding the variation in cortisol concentration during evening milking (**Table 1**). Significant difference in the hormone concentration was shown in the 1<sup>st</sup> group after milking, whereas in the 2<sup>nd</sup> group no statistical differences were exposed before, during and after milking. By comparing the morning and evening milking with regard to the concentration of the cortisol hormone in different measurement periods, the difference was found to be significant ( $p < 0.05$ ) as shown in **Table 2**.

**Table 1.** Correlation coefficient of daily milk yield and concentration of cortisol in blood plasma of Shami heifers in presence (1<sup>st</sup> group) and withdrawal (2<sup>nd</sup> group) of their calves during machine milking.

Animals groups	Number of records	Correlation coefficient
All animals	335	-0.320***
1 <sup>st</sup> group	166	-0.160*
2 <sup>nd</sup> group	169	-0.320***
1 <sup>st</sup> group (morning milking)	85	-0.28*
1 <sup>st</sup> group (evening milking)	81	-0.41***
2 <sup>nd</sup> group (morning milking)	85	-0.32*
2 <sup>nd</sup> group (evening milking)	84	-0.31*

\*=significant at  $p < 0.05$

\*\*=significant at  $p < 0.01$

\*\*\*=significant at  $p < 0.001$

**Table 2.** LS-means  $\pm$  SE of daily milk yield, lactation milk yield and lactation length in Shami heifers in presence (1<sup>st</sup> group) and absence (2<sup>nd</sup> group) of their calves.

Parameters	1 <sup>st</sup> group	2 <sup>nd</sup> group
Morning milking (kg)	7.7 $\pm$ 0.61***/**	3.02 $\pm$ 0.61 N.S
Evening milking (kg)	5.90 $\pm$ 0.61*	2.51 $\pm$ 0.61 N.S
Lactation milk yield (kg)	2507 $\pm$ 551*	703 $\pm$ 226
Lactation length (day)	210 $\pm$ 34*	122 $\pm$ 22

NS: Not Significant ( $p > 0.05$ )

\*=significant at  $p < 0.05$

\*\*=significant at  $p < 0.01$

\*\*\*=significant at  $p < 0.001$

### Milk yield

Statistical analysis of the milk yield data indicated that the difference in mean milk yield between the 1<sup>st</sup> and 2<sup>nd</sup> groups were significant ( $p < 0.01$ ). Milk yield in the morning milking were 7.7 kg compared to 3.02 kg in the 1<sup>st</sup> and 2<sup>nd</sup> groups respectively (**Table 1**). In the evening milking, the same trends were revealed. It has also been demonstrated that the milk yield in the 1<sup>st</sup> group was significantly different ( $p < 0.001$ ) between the morning and evening milking, however, in the 2<sup>nd</sup> group the difference was not significant ( $p > 0.05$ ). These results were reflected in the lactation yield, it was 2507 kg for 210 days lactation length in the 1<sup>st</sup> group and 703 kg for a period extended for 122 days in the 2<sup>nd</sup> group. The difference between lactation yields as well as lactation lengths were significant ( $p < 0.05$ ) as displayed in **Table 1**.

### Correlation between milk yield and concentration of cortisol hormone

**Table 1** reveals that the correlation coefficient between daily milk yield and mean concentration of cortisol hormone in

blood plasma. It can be notice that a significant negative correlation were obtained between the two traits in the morning and evening milking in both groups.

### DISCUSSION AND CONCLUSION

The study found that the concentration of cortisol hormone in blood plasma was lower in the 1<sup>st</sup> than in the 2<sup>nd</sup> group, but this could not be clearly attributed to the presence or absence of the calf, as the difference was not significant. The insignificant high concentration of the hormone in the blood plasma of the 2<sup>nd</sup> group could be attributed to the stress to which the group individuals were subjected by withdrawal of their calves or the reaction to the milking process.

The concentration of the hormone obtained in this study for Shami cows was higher than that obtained for Holstein Friesian [10,13,19]. In Gir breed, the concentration of cortisol hormone was found to be in the range 80-100 ng/ml which is closer to the estimated range in this study [20]. The concentration of the hormone in Gir breed was found to be negatively correlated with milk yield, which is in agreement with results in this study results and different to the case of

Holstein Friesian, which might be due to the adaptation of the latter breed to machine milking without showing stress. Some studies found that the level of cortisol is not affected by the milking method whether hand or machine milking, whereas other studies claimed that machine milking could increase the level of cortisol hormone in the blood compared with hand milking [16,21]. However, a third group of researchers referred to variations in cortisol concentration at individual level in the same breed. For instance, Faltys et al. [22] found an elevation in cortisol concentration during machine milking in some cows than others. It could be concluded that the concentration of cortisol hormone in cow's blood plasma can be affected by many factors including breed, method of milking, individuality and the stress to which the animal subjected.

The results in this study are in close agreement with Peeters et al. [23] who found high milk yield in cows milked with their calves beside, they considered the presence of the calf as stimulant factor for milk ejection reflex. Lupoli et al. [24] found a high level of cortisol hormone in the blood plasma of cows milked without the presence of their calves.

The negative correlation between milk yield and cortisol concentration in this study could be attributed to two key reasons: machine milking and the presence or absence of calves beside their mothers during milking. It may seem that machine milking in Shami cows had a negative effect, whether milked in presence or absence of calves, and this is clear from the elevated level of cortisol in their blood due to the stress during machine milking. Withdrawal of calves in Shami cows were obviously affecting the performance of cows as being indicated with high cortisol level and low lactation yield and this is clear in the 2<sup>nd</sup> group.

Varner et al. [25] found an elevated level of cortisol hormone and decreased milk yield in cows transported from one place to another. These findings support that the decreasing in milk yield might not be always due to machine milking. Other reports Lefcourt et al. [26] suggested that when heifers milked for the first time, they will be subject to stress lead to the release of catecholamine (adrenalin and nor adrenalin); in turn, this will be accompanied with an elevated concentration of cortisol hormone. Blum et al. [27] Explained that the milking process does not lead to release of catecholamine. However, other researchers Lefcourt et al. [26,28] reported that the increase in catecholamine lead to suppress the release of oxytocin and consequently non stimulation of milk ejection reflex resulting in low milk yield.

The results in this study are supported with the results obtained by Miyazawa [29] regarding the negative correlation between milk yield and cortisol concentration in blood plasma and the release of the hormone in response to milking process. Research studies indicated that the decrease in milk yield in stressed animals could be related to the

mechanism of milk release in milk cistern [30] as had been earlier explained by Gorewit and Tucker [31].

The study concluded that machine milking is a stress factor in Shami heifers lead to elevate the cortisol hormone and the stress increased with withdrawal of calves during milking process and that is reflected in none or weak stimulation of milk ejection reflex and consequently low lactation milk yield and short lactation length.

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