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Pre-Stimulation Effects on Teat Anatomy and Milk Flow Curves in Mediterranean Italian Buffalo Cows

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Abstract: Eitgheen Mediterranean Italian buffalo cows were used to demonstrate a relationship between teat anatomy and milk ejection after three different pre-milking treatments (manual pre-stimulation or exogenous oxytocin administration). Teat wall thickness, teat diameter, cisternal diameter and teat canal length were evaluated before and after each treatment. Teat cisternal diameter and teat diameter incresead their lengths while teat wall thickness and teat canal length decreased. The reduction in the teat canal length was observed with all the three treatments with reductions ranging from a minimum of 23.1% (2 min) to a maximum of 29.4% (oxytocin administration). Although, main milking phase was similar among treatments, plateau phase increased ($p = 0.01$) the duration passing from manual stimulation to the administration of exogenous oxytocin (2.84 min). Lag time decreased significantly with the administration of exogenous oxytocin: 0.74 vs. 2.55 min of 2 min pre-stimulation. Even if exogenous oxytocin showed some advantages on some productive parameters, the study showed that 2 min of manual prestimulation are enough for the removal of the alveolar milk fraction.

Key words: Mediterranean Italian buffalo, pre-stimulation, teat anatomy, milk flow curve, treatments

INTRODUCTION

The buffalo (*Bubalus bubalis*) population in the world is about 200 million head (Borghese, 2013) and they contribute considerably to the milk production in many countries of Asia and other parts of the world. The milk produced by buffaloes is an important source of energy: in fact it is cacactherized by fat and protein content both higher than in cattle. Besides, buffaloes have been kept in Italy for centuries to process milk and produce the original buffalo mozzarella cheese Mozzarella di Bufala Campana DOP (Denomination Origin Controlled) that was recognized with the Italian Ministerial Decree on May 10, 1993, published on the G.U. n.219 on 17/9/1993, the trademark was granted by European Union (1996) as Mozzarella di Bufala Campana DOP with Regulation EC n.1107/96.

Although, mechanized milking is today largely diffused in buffalo farms (it's used in Italy for >50 years) because it is the principal way of increasing research productivity and improving milk quality, milk let down is influenced by anatomical, physiological, sanitary and environmental factors (Ambord *et al*., 2009, 2010; Caria *et al*., 2011; Thomas, 2004).

The udder anatomy and the internal arrangement of the mammary tissue, cisternal fraction of milk and teat canal length are quite different in buffaloes compared to dairy cattle (Thomas *et al*., 2004). In buffalo species the milk stored in the cisternal cavity and directly avalaible before milking ranges between 5.0 and 7.6% of total milk (Thomas *et al*., 2004; Ambord *et al*., 2009) and it is lower than recorded one in dairy cattle of about 20%.

Like in cattle, milk ejection in buffaloes occurs in response to elevated oxytocin concentrations (Bruckmaier and Blum, 1998; Thomas *et al*., 2004). However, it has been shown that successful machine milking is much more difficult in buffaloes than in cattle therefore it is necessary a careful udder preparation including pre-milking stimulation for several minutes before the start of milking (Thomas *et al*., 2005) and often exogenous oxytocin is a need to obtain a complete and rapid milk ejection. The importance of oxytocin release for milk ejection are well documented for cow, goat and sheep but less for buffalo (Bruckmaier and Blum, 1998).

The aim of this study was to investigate the effect of the subsequent three treatments (at quarter level) on teat anatomy and milk ejection (milk yield and milkability): two pre-stimulation treatments of 2 and 3 min (2, 3MP) without administration of oxytocin and one exogenous Oxytocin Treatment (OT) without pre-stimulation were used.

MATERIALS AND METHODS

Animals and housing: Eighteen Mediterranean Italian buffaloes, clinically healthy from the farm Caporossa located in Pontinia (Latina) Italy (41°24'25.1"N

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13°08'34.7"E) were used for this experiment. The animals were milked in a herringbone milking parlor (2×5) (DeLaval, Tumba, Sweden). The pulsator rate was 60 cycles min^{-1} and the pulsator ratio was 60% during the experiment milking vacuum level was set at 44 kPa.

Animals and treatments: Three different treatments were investigated. The animals were randomly assigned to each individual treatment at every experimental milking. Milking was performed after 2 min (2MP) or 3 min pre-stimulation (3MP) or intramuscolarly with 20 IU of exogenous Oxytocin (OT) without prestimulation, respectively.

Pre-stimulation treatment included cleaning of the teats and the udder with a wet cotton towel before manual prestimulation or esogenus Oxytocin (OT). The anatomical measurements were effected before and after the treatments.

Teat ultrasound cross section and teat length measurement: Longitudinal cross section b-mode ultrasound images (Honda Electrics HS101V) of the right front and right rear teat were performed before milking in all experimental animals. The following parameters were measured: teat canal length; teat cistern diameter; teat wall thickness and teat diameter, measured by ultrasound imaging on the right front and right rear before and after the treatment. The image was recorded with a 5 MHz -probe and the teat in a cup of hand-warm water. The ultrasound probe was applied to the outside of the cup by using ultrasound gel (Thomas *et al*., 2004). Teat diameter (Ø) teat wall thickness (average of both walls of the cross section was used for data evaluation) cisternal diameter, 4 cm above the teat tip and teat canal length were evaluated. Tirthy six quarters were analyzed (18 front and 18 rear quarters) before and after each different treatment.

Milk flow curve detection: Milk flow curves were recorded during evening milking from each animal in lactation using 2 electronic mobile milk flow meters (LactoCorder®, WMB, Balgach, Switzerland). Each Lactocorder were installed between the milking cluster and the milking tube before each evening milking session. For each animal the milk flow curve was recorded after single treatment. In the study, the following parameters were measured and evaluated:

- Milk yield (kg)
- Maximum milk flow rate (kg min^{-1}) (maximum milk flow in the main milking process)
- Average milk flow rate (kg min^{-1})
- Lag time (lag time from the beginning of measurement until a 0.25 kg min^{-1} threshold in the milk flow was reached)
- Main milking time (min) (time of main milking process by machine: beginning of the milk flow = 0.25 kg min^{-1} until a milk flow of 0.10 kg min^{-1} at the decline is reached)
- Incline phase (min) (time part of the main milking process from the milk flow of 0.25 kg min^{-1} to the top of the plateau phase)
- Duration of plateau phase (min) (time from the top of the incline to the top of the decline)
- Decreasing phase (min) (Time from the top of decline until the milk flow falls below 0.10 kg min^{-1})
- Total milking time (time from attachment the cluster and detachment of the cluster at the end of quarter milking included stripping)

Statistical analysis: A total of 108 quarter milk flow curves were recorded. All data are presented as arithmetic mean values and Standard Error of the Mean (SEM). Parameters measured were compared by ANOVA using SPSS Software (Ver. 13.0, SPSS, Inc., Chicago, IL, USA). Differences in means were localized by Bonferroni's t-test in order to classify the effect of the different treatments (2MP, 3MP or OT administracion).

RESULTS

Teat anatomy of all teats (front and rear quarters) before and after each single treatment are shown in Table 1. Teat canal length after treatment showed significant differences compared to the measure pre treatment: in fact, teat canal length significantly ($p<0.001$) reduced its length with a decrement ranging from a minimum of 23.1% (2MP) to a maximum of 29.4% (OT). The other measured anatomical teat parameters have showed different variations after each treatment: teat cisternal diameter significantly ($p<0.01$) increased with OT treatment, comprised between a minimum of 21.37% (2MP) and a maximum of 49.62% (OT); teat wall thickness showed a significant ($p<0.001$) reduction ranging from 9.28% (2MP) to 18.56% (3MP). Teat diameter increased up to a maximum of 11.62% (OT) with significant difference. Therefore, the effect of each prestimulation is the reduction of teat canal length on the contrary the teat diameter was increased significantly. Among the treatments, the exogenous oxytocin administration

Table 1: Teat measurements before and after three different treatments (2, 3 min of manual prestimulation and exogenus oxytocin administration)

Time point (mm)	N	Teat canal length	Teat cistern diameter	Teat wall thickness	Teat diameter
Before PS	108	25.5±0.4[a]	13.1±0.5[a]	9.7±0.2[a]	32.7±0.3[a]
After 2 min PS	36	19.6±0.6[b]	15.9±0.6[ab]	8.8±0.2[ab]	33.5±0.4[a]
After 3 min PS	36	19.5±0.5[b]	17.1±1.0[ab]	7.9±0.2[b]	32.9±0.6[a]
After Oxy adm	36	18.0±0.4[b]	19.6±0.7[b]	8.4±0.2[ab]	36.5±0.5[b]

Different letters in columns mean significant differences for $p<0.05$

Table 2: Milk Yield and milk flow parameters at different treatments

Parameters	N	2 min of PS	3 min of PS	Oxytocin injection	Overall mean
Milk Yield (kg)	108	1.33±0.12	1.36±0.13	1.61±0.140	1.43±0.070
1 Milk Yield (kg)	108	0.21±0.02^{b}	0.24±0.02^{ab}	0.32±0.020^{a}	0.26±0.010
2 Milk Yield (kg)	108	0.41±0.05^{b}	0.47±0.05^{ab}	0.68±0.060^{a}	0.52±0.030
3 Milk Yield (kg)	108	0.61±0.08^{b}	0.68±0.07^{ab}	0.98±0.080^{a}	0.76±0.050
Maximum MF (kg min^{-1})	108	0.42±0.03	0.41±0.03	0.46±0.030	0.43±0.020
Average MF (kg min^{-1})	108	0.28±0.02	0.30±0.02	0.33±0.030	0.31±0.010
Lag time (min)	108	2.55±0.36^{a}	2.17±0.30^{ab}	0.74± 0.07^{b}	1.82±0.170
Main milking time (min)	108	3.94±0.53	3.80±0.41	3.95±0.320	3.90±.0.25
Plateau phase (min)	108	1.52±0.31^{a}	1.78±0.28^{a}	2.84±0.290^{b}	2.05±0.180
Decline phase (min)	108	2.30±0.54^{a}	1.88±0.38^{a}	0.94±0.260^{b}	1.71±0.240
Incline time (min)	108	0.15±0.04	0.17±0.05	0.16±0.040	0.16±0.030
Blind time (min)	108	1.31±0.23	1.01±0.19	0.95±0.220	1.09±0.120
Total milking time (min)	108	8.12±0.55^{a}	7.22±0.42^{a}	5.71±0.290^{b}	7.02±0.270

Different letters a row without common letter are significantly different ($p<0.05$)

realized the highest effect on canal reduction and on teat diameter and on cisternal diamenter increasing. The treatment of 3 min produced the highest reduction in teat wall thickness ($p<0.05$).

Milk yield and main milk flow parameters of all teats (front and rear quarters) are shown in Table 2. Milk yield extracted by quarters did not show statistically significant differences among treatments, even if they ranged from 1.33±0.71 kg per quarter (2MP) to 1.61±0.87 kg per quarter (OT). The milk yield obtained after 1-3 min of milking was higher significantly ($p<0.05$) in OT group than in 2MP min pre-stimulation group. Maximum and average flow did not show significant differences among treatments. Lag time decreased significantly from 2.55±2.15 min (2MP) to 0.74±0.39 min (OT) that resulted more convenient in terms of time. Similar values were found in different experiences by Iranian researchers on Khuzestani buffalo: Roshanfekr *et al*. (2010) found an overall let down value of 1.57 min in 60 lactating Khuzestani buffalo cows while in a previous trial (Roshanfekr, 2001) the let down was not influenced by the time of milking: 1.50 and 1.60 min, respectively in am or in pm.

Main milk time is similar for all the treatments althougth plateau phase and decreasing phase showed opposite trend. Plateau time was increased significantly by oxytocin administration while this treatment influenced decreasing time with an opposite trend, confirming the favourable effect of oxytocin on productive parameters. Total milking time data revealed a significant difference ($p<0.05$) between pre-stimulation treatments and oxytocin administrations: they show that time decreased from 8.12 min (2MP) to 5.71 min (OT) as oxytocin provoked a more rapid emptying of the udder quarter.

DISCUSSION

In this study, the values of teat anatomy found in pre-treatment are similar to those reported by Ambord *et al*. (2009) (average value 25.9 mm) (2010) (average value 23.6 mm) and Boselli *et al*. (2010) (average value 27.1 mm) before stimulation treatment and beginning of milking while they are lower than ones found by Thomas *et al*. (2004) on Murrah buffaloes (average value 31.0 mm). The lower length of teat canal in the Mediterranean Italian in comparison with Indian Murrah could be explained by the high selection pressure applied on the Mediterranean Italian breed in the last 50 years to adapt it to the mechanic milking.

The teat canal reduction was significant in all the applied treatments, even if the higher reduction was obtained by the treatment OT. If researchers compare the results obtained with treatment 3MP with the values of Ambord *et al*. (2010) these researcher obtained higher reduction: 37.29 vs. 23.53%, 14.8 vs. 19.5 mm because of the reduced number of animals and teat controlled by these researchers.

The lag time (1.82 min) found is similar to one obtained by Roshanfekr *et al*. (2010) on Khuzestani Breed (1.56 min) even if effected in different conditions. The OT treatment effected a higher (+17%) milk production as oxytocin produced a complete emptying of udder quarters.

The milk flow profiles (Fig. 1-3) were influenced by the treatments according the increased oxytocin blood concentration with OT treatment which provoked a higher and more rapid udder emptying (Fig. 3) according Thomas *et al*. (2005, 2008).

The low correlations found between teat canal length (after treatments) versus maximum milk flow ($r = 0.02$, $p = 0.876$) and average milk flow ($r = 0.08$, $p = 0.396$) suggest that milk flow should not be related only to this parameter. Probably, other anatomical factors affect the milk flow as the diameter of teat canal and the muscles that close the apical part of this.

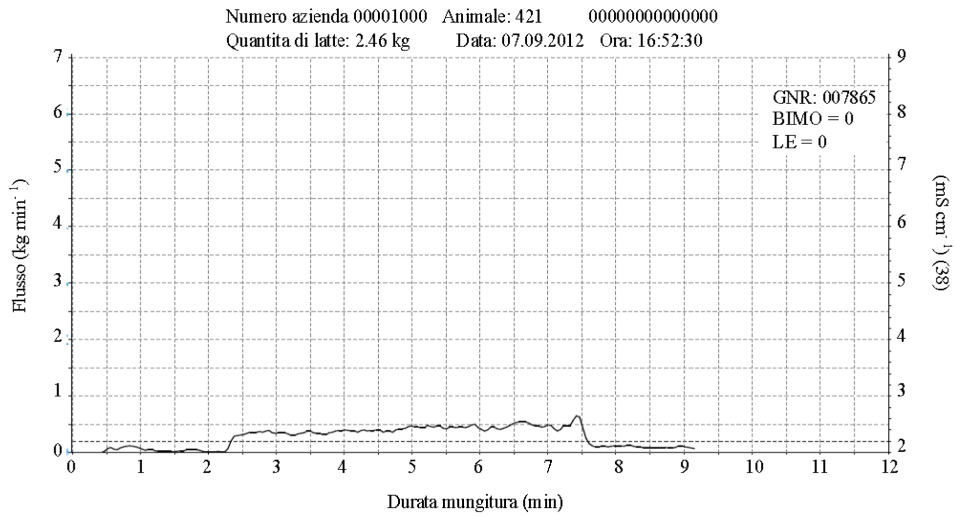

Fig. 1: Milk flow profile with treatment 2MP

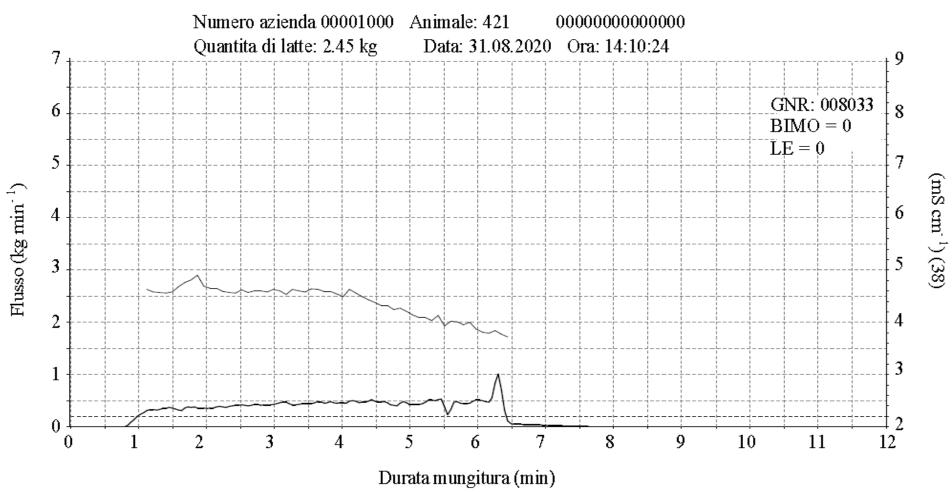

Fig. 2: Milk flow profile with treatment 3MP

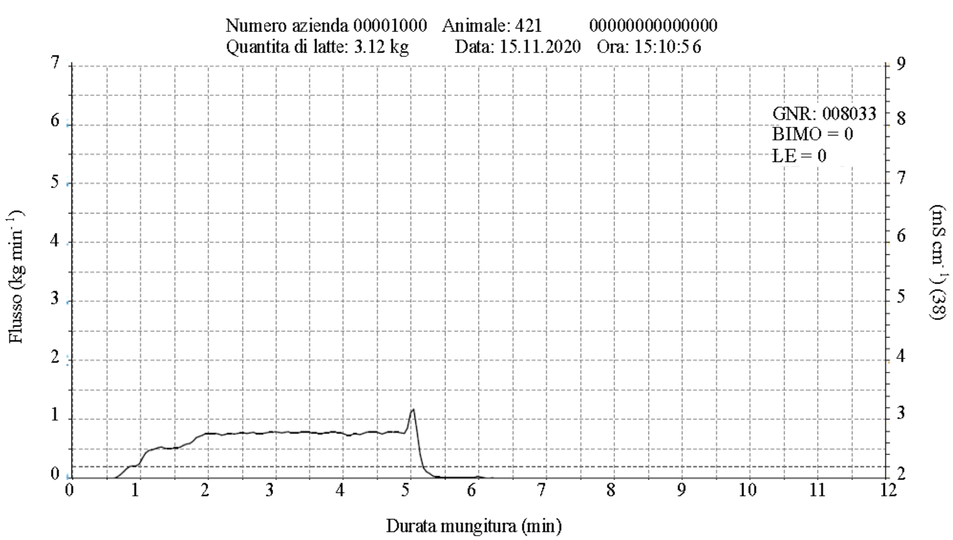

Fig. 3: Milk flow profile with treatment OT

CONCLUSION

The results show that all the treatments cause a reduction of teat canal length, a teat diameter widening, higher milk yields (1-3 min) and reduction of milking time. Even if the oxytocin treatment provoked the highest effects, 2MP is enough for the removal of alveolar milk fraction in Mediterranean Italian buffalo cow and therefore could be considered more favourable than OT because of the collateral effects.

Pre-stimulation must be introduced as milking routine as this practice increases the milk ejection, reduces milking time as it is shown by milk flow profiles with economic advantages for farmers and with welfare advantages for the animals.

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