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Evaluation of different habituation protocols for training dairy jennies to the milking parlor: Effect on milk yield, behavior, heart rate and salivary cortisol

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ABSTRACT

The aim of the trial was to apply different habituation protocols for machine milking to never machine-milked donkeys observing the effect on behavior, heart rate, salivary cortisol concentration and milk yield. Sixty lactating dairy donkeys were studied; the animals were divided into three groups of 20 each to evaluate whether a habituation protocol for dairy donkeys could help ensure good animal welfare and production. None of the animals had been subjected to mechanical milking procedures and had never entered the milking parlor. The trial lasted 15 days (two sessions per day), for a total of 30 experimental sessions: 9 days of training sessions for Groups A and B, and 6 days of milking sessions for all three Groups. Behavioral patterns, heart rate (**HR**) and salivary cortisol were measured during each experimental session. Two groups (i.e. A and B) received two different pre-milking habituation handling protocols; as the control group, Group C received no habituation and was directly milked in the milking parlor. For the first 3 days (phase 1; sessions 1–6), Groups A and B both passed through the milking parlor while the milking machinery was switched off. In the next 6 days (from Days 4–9, sessions 7–18), Group A donkeys received a "less intense" treatment, involving 2 days passing through the milking parlor where the machinery was switched on during phase 2 (sessions 7–10) and, for the next 4 days being stopped in the milking stall (phase 3; sessions 11–18). Meanwhile, the donkeys in Group B, on all 6 days, were confined in the milking stall, and their udders were brought into contact with the milking cluster, which was turned on at the time. Subsequently, all groups involved in the trial were machine-milked in the milking parlor for 6 days (phase 4; sessions 19–30). Donkeys that received the pre-milking habituation handling (Groups A and B) showed less reactive behaviors (kicks and steps) and incoming stops when milking started compared to the control group ($P < 0.01$). Differences in HR were observed particularly between the control and the other groups ($P < 0.05$). Results suggest that a pre-milking habituation protocol providing short contact with the milking cluster (as in Group B) can result in better response from the animals with less reactive behavior, lower heart rate increase and higher milk production.

1. Introduction

Donkeys have been domesticated for approximately 5000 years, and are still used for various different purposes, particularly for work and for milk and meat production (Rossel et al., 2008; Ali et al., 2014). Most donkeys are now concentrated in the tropical regions where they are used principally as working animals (De Palo et al., 2016a). More recently, breeding has been increasing due to donkeys' adaptability to different types of activity, ranging from onotherapy to garbage collection (Minero et al., 2016). Moreover, in some countries, donkey milk and meat is also highly remunerative. Indeed, an interest in equid milk production, such as donkey (Polidori et al., 2011; De Palo et al., 2016a) and horse milk (Centoducati et al., 2012; Salimei and Fantuz 2012; De Palo et al., 2016b) for human consumption, in particular in Italy, France and Belgium, is gaining popularity and economic importance (Veneziano et al., 2011). Milk from dairy donkeys is considered an excellent hypoallergenic substitute for children with cow's milk protein allergies (Gokbulut et al., 2016) or intolerance (Monti et al., 2007). When studying heifers, some authors have reported that a pre-habituation period, as a training, to the sight and sounds of a conventional

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milking parlor is recommended and common practice (Jago and Kerrisk, 2011). Studies on species different from cows, that usually are not habituated to machine milking, as buffaloes, showed a great sensibility to this novelty (Napolitano et al., 2013; Polikarpus et al., 2014). So, we can hypothesize that also donkeys could show great sensibility, and so reactions, to machine milking procedures. Moreover, fear and stress can affect Alveolar milk ejection, because of its relation to oxytocin concentration and to myoepithelial contraction (Bruckmaier and Wellnitz, 2008). Considering this, also milk ejection represent an important parameter that need to be evaluated. In the light of these considerations, we aimed to study how machine milking can impact on donkey welfare through their behavior and their reactions to a novel technology and environment, as well as on milk yield.

2. Materials and methods

2.1. Donkey management

This study was approved by the Ethics Committee for animal testing–CESA (process number 58337-X/10). The research was conducted at a commercial farm (Lamacarvotta s.a.s.) during the month of May in southern Italy (40°71′ N, 16°78′ E). Sixty Martina Franca multiparous dairy donkeys, which had previously only ever been hand-milked, were selected for the trial. None had ever been subjected to mechanical milking or entered a milking parlor. Animals were randomly subdivided into three homogeneous groups (20 heads per group), according to age, body weight and days in milk (DIM). All donkeys involved in the trial were housed together, in the same stall. The stall was equipped by mobile fences useful to temporary divide the three experimental groups, each day, before starting the training procedures. Each day, all foals were separated by their dams and, subsequently, the three jennies groups were subdivided in order to facilitate all experimental procedures. The three temporary box in the same stall guaranteed a minimum space allowance of $8m^2$ per head, feeding stations and water stations. All animals were individually marked with a nontoxic marking spray (Raidex; Dettingen an der Erms, Germany), on the croup and on both sides, with an acronym made up of the Group ID (A, B or C) and a progressive number (from 1 to 20), in order to identify each animal involved in the trial, also in the milking parlor and during video recordings.

2.2. Experimental design

The trials lasted 15 days, with two experimental sessions per day, one in the morning (11:00 to 14:00) and one in the afternoon (16:00 to 19:00), for a total of 30 experimental sessions. Groups A and B received habituation handling for 9 days (training sessions) and were subsequently machine-milked for 6 days (milking sessions). Group C (control) donkeys did not receive any pre-milking habituation and were directly machine-milked for 6 days. Four hours before milking, all foals were separated from their dams. The experimental design is reported in Fig. 1. All groups were tested the same days in a random order.

The 9-day adaptation period differed from Group A to B. On the first 3 days (phase 1, sessions 1–6), both groups passed through the switched-off milking parlor. From days 4–9 (sessions 7–18), they received two different habituation protocols. Group A received a less intense treatment regime, consisting in 2 days (sessions 7–10) passing through the switched-on milking parlor, and 4 days (sessions 11–18) passing through and being confined in a milking stall for 120 s with the milking parlor switched on. By contrast, Group B received a more intense treatment, by which they were confined in the milking stall for 120 s on all 6 days (sessions 7–18), receiving an udder massage and bringing the switched-on milking cluster into contact with the udder for a few seconds, without being attached. All animals were trained one by one. The less intense habituation period (Group A) aimed to emphasize the new environment effect (milking parlor) excluding any cluster effect, while the more intense treatment (Group B) aimed to evaluate the effect of udder stimulation by the cluster in the milking stall.

Before the experimental sessions, the animals in each group were led into the holding area by the same three farmers (who had hand-milked the donkeys, and were therefore known to the animals). All the donkeys stayed in the holding area for 20 min before entering the milking parlor. When the milking parlor door was open, two farmers led the donkeys into the milking parlor while the other was in the milker pit. Donkeys enter in one line. Only on the first occasion was necessary to led the donkeys into the milking parlor, as subsequently they entered alone once the door was opened. Donkeys were able to choose the entrance order. The milker pit contained the milker and one person to supervise the heart rate (**HR**) monitoring systems (Fig. 2).

2.3. Milking parlor

The herringbone milking parlor was made up of three stalls for donkeys. Before the trial, the noise was measured by a phonometer (Delta ohm 2110L, ZetaLab s.r.l., Padua, Italy). This was placed in the parlor with the milking cluster switched on and recorded noise levels of 72 dB.

2.4. Behavior recordings

All sessions were video-recorded in order to capture all of the donkeys' behavioral patterns. The cameras (ADJ mod. OWC5, San Cesareo, RM, Italy) were located: i) outside the milking parlor, to record all donkeys in the holding area; ii) in the milking parlor, to record the animals' behavioral patterns and the time taken from entering to leaving the milking parlor. This parameter was recorded only during training sessions in which the animals were simply passing through the milking parlor (phase 1). The behavioral patterns recorded in the holding area were the incoming stops, in the milking parlor were incoming stops, exit stops leaving the parlor, kicks, stepping, defecation, urination and vocalization (Table 1). All individual behavioral parameters were assessed by watching the videos by the same trained observers. Trained observers watched videos from the incoming to the moment of leaving parlor, registering, for each animal, the number (frequency per head) of each behavior.

2.5. Heart rate

A HR monitoring system (Polar Equine RS800 CX, Polar Electro Oy, Helsinki, Finland) was applied on the shaved skin of the left side, at the third intercostals space, in the heart projection area. Before the beginning of the trial, for three days, the HR monitoring system was attached to each animal for 60 min/day and 30 min before starting the experiment to habituate donkeys to it. The HR was recorded throughout the milking phases described in Table 1: mean HR was calculated for each phase over 5 s (holding area, being confined in the milking stall, milking). All data were downloaded by "ProTrainer 5 Equine" free software on a personal computer and subsequently analyzed.

2.6. Milk yield

Milk yield for each donkey was measured at each milking session. During milking, the milk produced by each jenny was collected in a glass jar and weighed to determine milk yield. All donkeys involved in the trial, excluding primiparous, were just hand milked by farmers but no information about their milk yield in previous lactations was available. Farmers milked donkeys only when milk was requested by buyer.

2.7. Salivary cortisol

Saliva was collected using Salivettes (Salivette ® Cortisol, Code Blue,