



The relationship between water buffalo cow temperament and milk yield and quality traits



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ABSTRACT

The aims of this study were to assess the relationship between reactivity during milking and daily distances traveled by lactating water buffalo cows and to assess the association of these behavioral traits with milk yield and quality traits. Reactivity during milking was measured as a 4-point milking reactivity score (MRS, N=114 cows) where 1=stands quietly, 2=slight hind leg movements, 3=vigorous hind leg movements, and 4=continual vigorous hind leg movements or the stockperson ties the animal. Cows were classified according to their consistency for MRS within each month as 1) inconsistent (same MRS in fewer than 50% of observations), 2) moderately consistent (same MRS in 50–74.9% of observations), or 3) consistent (same MRS in at least 75% of observations). GPS collars were used to calculate the average daily distance traveled (DDT) by each cow (N=36). Milk yield and quality traits (fat, protein and lactose content, as well as somatic cell count) were measured once a month during the three months of the study. Results showed that DDT was not related to MRS ($F=0.78$, $P=0.47$). Furthermore, MRS was negatively associated with milk yield ($F_{2,66}=3.75$; $P=0.02$), fat content ($F_{2,71}=3.34$; $P=0.04$), and linear somatic cell score (LSCS) ($F_{2,69}=4.06$; $P=0.02$). Reactive cows (MRS 3+4) had lower daily milk yield than MRS 1 cows, with lower fat content and higher LSCL than MRS 2 cows. On the other hand, DDT was not associated with milk yield or any quality traits ($P > 0.05$) other than LSCS ($R^2=0.60$; $P < 0.05$). Since MRS and DDT were not correlated, we conclude that these traits reflect distinct aspects of water buffalo cows' temperament. Milk yield and quality were not related to DDT, indicating that only MRS was associated with the productive traits in lactating water buffalo cows.

1. Introduction

As the animal with the greatest milk production in several countries, domestic water buffaloes (*Bubalus bubalis*) significantly contribute to the world's milk production (Borghese and Mazzi, 2005). Therefore, it is important to understand how handling routines can influence the welfare and productivity of these animals. A useful way to address this issue is to study individual cows' behavioral and physiological responses to handling, as well as how these responses are associated with milk yield and quality traits (Prasad et al., 2011).

These variations are known as animal temperament, which is defined by the expression of individual behavioral differences that are

consistent over time and/or situations (Réale et al., 2007). Animal temperament involves the expression of several behaviors, which makes its measurement difficult. To overcome this challenge, researchers use the operational definition of temperament, which takes into account animals' behavioral responses to handling by humans (Burrow, 1997). Temperament is frequently assessed by visual scores that consider the frequency and intensity of movements and other behaviors reflecting levels of animal fear and reactivity (Breuer et al., 2000; Wenzel et al., 2003; Rousing et al., 2004; Hedlund and Løvlie, 2015).

Some authors have reported an association between dairy cows' reactivity during milking and their degree of susceptibility to stress, which in turn affects milk yield and quality traits (Rousing et al., 2004;

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Bertenshaw et al., 2008; Hedlund and Løvlie, 2015). By contrast, other authors have failed to find a significant relationship between cow behaviors (i.e. flight distance and step and kick responses during milking) and milk yield (Purcell et al., 1988; Uetake et al., 2002; Waiblinger et al., 2002). Yet another set of studies found effects of reactivity on milk yield only for specific behavioral indicators of reactivity, making it even more difficult to construct a general understanding of this phenomenon (Dodzi and Muchenje, 2011; Hedlund and Løvlie, 2015). Although lactating water buffalo cows are known to be more sensitive to handling during milking than dairy cows, few studies have addressed their reactivity to handling, and have focused mostly on cows' stress physiology and milk letdown (Pathak, 1992; Thomas et al., 2005; Bidarimath and Aggarwal, 2007).

In order to get a broader view of cows' individuality, other aspects of temperament should also be considered, such as cows' overall activity levels, which are considered an important aspect of animal temperament (Réale et al., 2007). One study reported that long-term activity levels are linked to short-term reactivity in cattle (Mackay et al., 2013), thus characterizing one aspect of their individuality (Wesley et al., 2012). Moreover, in dairy cattle, some have suggested that daily activity levels can affect milk production (Bewley et al., 2010; Norring et al., 2012), as well as animal behavior (Telezhenko et al., 2012) and health (Gustafson, 1993; Davidson and Beede, 2003).

It is currently unknown to what extent water buffaloes' short-term reactivity is associated with their long-term activity levels. However, a reasonable hypothesis is that animals that are more reactive during milking should also be more active in the pastures, reflecting a temperament dimension that is associated with overall activity. In order to test these ideas, the aims of the current study were: 1) to assess the relationship between water buffalo cows' reactivity during milking and the daily distance traveled (taken as an indicator of general activity); and 2) to evaluate the association of these temperament traits with milk yield and quality traits (protein and fat contents, and somatic cell count).

2. Materials and methods

This study was approved by the commission for the ethical use of animals of FCAV - UNESP (Jaboticabal-SP, Brazil), protocol number 011587/13. Data collection was conducted on a private farm that produces organic milk for mozzarella cheese, located in the municipality of Brodowski, São Paulo State, Brazil.

A total of 114 lactating water buffalo cows (Murrah purebred and Murrah×Jaffarabadi crossbred, 99 multiparous and 15 primiparous) were evaluated from March to May 2013. The animals were kept in a single social group. The cows averaged 93.25 ± 59.77 (mean \pm standard deviation) days in lactation and had experienced 3.67 ± 1.92 calvings. They were milked twice a day (at 05:00 A.M. and 02:00 P.M.) in a tandem milking parlor, with calves tied close to their mothers, but without access to the udder during milking. Natural suckling was used just before the pre-dipping procedure to stimulate milk let-down. After suckling for less than one minute, the calves were tied in the milking parlor structure close to the cows' head. Milking was always carried out by the same two stockpersons. After each milking session, the calves stayed with their mothers for approximately one hour, with access to the udder to suckle the residual milk.

Between the morning and afternoon milkings, the animals were kept indoors with free access to drinking water and fed ad libitum with a total mixed ration composed of corn silage, chopped sugarcane, wet brewer's spent grain and mineral supplements. After the afternoon milking, the cows were moved to the indoor cow sheds, where they had free access to one of two paddocks.

Before assessing milking reactivity, preliminary observations were conducted over seven days to habituate the cows to the observer's presence. The reactivity assessments were performed in the milking parlor by only one trained observer. Direct observations with focal

sampling and continuous recording (Martin and Bateson, 1993) were performed at the time of fitting the teat cups, and the milking reactivity scores (MRS) based on leg movement were assigned as follows: 1=cow stands quietly; 2=cow shows some slight movements with one or both hind legs; 3=cow shows vigorous movements with one or both hind legs; and 4=cow shows continual vigorous hind leg movements, or the stockperson ties the hind legs (adapted from Paranhos da Costa and Broom (2001)).

The MRS was assigned during both the morning and afternoon milking sessions, yielding at least 14 measurements per month for each cow. Cows were classified according to the consistency of their MRS within each month, following the criterion used by Paranhos da Costa and Broom (2001), which defines three groups: inconsistent (N=36), for cows that received the same MRS score in fewer than 50% of the observations within each month; moderately consistent (N=38), for those that received the same MRS score in 50–74.9% of the monthly observations; and consistent (N=40), for cows that received the same MRS score in more than 75% of the monthly observations. For cows classified as consistent, the MRS classification for each cow was used to define a single MRS per cow per month.

The distances traveled per day were assessed in 36 water buffalo cows (randomly assigned from the 114 studied cows) over a period of 10 weeks. GPS collars (GPS Plus-2 Vecronic Aerospace GmbH, Berlin) were used to record the distance traveled, which was used as an indicator of individual activity levels. The collars were fitted randomly to groups of three to five cows each time, and each group of cows remained with the collar for four whole days and nights, totaling 96 h of individual monitoring. During this period, cows alternated between two paddocks, as follows: paddock 1 (49.5 ha) – from weeks 1–5 and week 10, and paddock 2 (27.7 ha) – from weeks 6–9. Both paddocks had a complex vegetation cover, with forest fragments combining different species of trees and bushes, as well as some areas of rock outcrop. The predominant grass species in both paddocks were Panicum maximum Jacq cv Colonião and Cynodon nlemfuensis. A paddock effect was included in the statistical analysis of the average daily distance traveled for correction purposes.

Each collar was configured to record animal location (UTM - Universal Transversor Mercator) at 30-s intervals. For each record (location point), the following variables were logged: collar number, date, time, latitude, longitude, altitude and the dilution of precision (DOP, a measure of the predictive accuracy of the obtained location; smaller DOP values indicate more accurate location measurements).

The GPS Plus (VECTRONIC Aerospace) and Microsoft Excel programs were used to calculate the distances traveled by each cow every 30 s, using Pythagoras' theorem in the following equation: Distance traveled= $(T_2 \text{ Latitude} - \text{Latitude } T_1)^2 + (T_2 \text{ Longitude} - \text{Longitude } T_1)^2)^{0.5}$, where: Latitude T_1 : is the first latitude point recorded by the GPS collar; Latitude T_2 : is the second latitude point recorded by the GPS collar after 30 s; Longitude T_1 : is the first longitude point recorded by the GPS collar; Longitude T_2 : is the second longitude point recorded by the GPS collar after 30 s. Subsequently, the values were summed to obtain the distance traveled per cow per day (m). The distances traveled per cow per day were used to estimate the means and then the average of the four days was calculated (DDT).

The daily milk yield per water buffalo cow (kg/day) was measured once a month during the three months of data collection. Simultaneously, individual samples were collected from each animal to evaluate milk quality traits. Samples were sent to the Clínica do Leite Laboratory, located in the Department of Animal Science of the Escola Superior de Agricultura "Luiz de Queiroz", at the University of São Paulo, Piracicaba, São Paulo State, Brazil. The following milk quality traits were assessed: fat (% , mass solute/mass total solution), protein (% , m/m) lactose (% , m/m) and total solids contents (% , m/m), and the somatic cell count (SCC, $\times 1000$ cells/mL). Infrared spectroscopy was used to determine fat, protein and lactose contents, and SCC was assessed by flow cytometry.

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