Qualitative behaviour assessment of dairy buffaloes (*Bubalus bubalis*)

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ARTICLE INFO

ABSTRACT

This study applies qualitative behaviour assessment (QBA) for the first time to dairy buffaloes, using three groups of observers with different cultural backgrounds and different levels of experience in animal behaviour observation and buffalo farming. Eight buffalo heifers aged 16–18 months were subjected to two isolation tests, one performed in the indoor part of their home environment, and one in a novel outdoor paddock. Animals were filmed individually for 2.5 min, and the resulting 16 video clips were shown to three observer panels, consisting of 11 applied animal behaviour scientists from 6 European countries, 11 Italian animal scientists with a background in buffalo farming but no experience in behavioural observation, and 14 Italian undergraduate animal science students with no particular experience. A free choice profiling method was used to instruct observers in QBA, and data for the three panels were analysed separately using Generalised Procrustes Analysis. All three panels showed significant inter-observer agreement ($p < 0.001$) and generated two main consensus dimensions characterised as 'calm-agitated' and 'curious-shy'. There were significant correlations between buffalo scores provided by each of the three observer panels on both these dimensions (*dim1*: Kendall $W = 0.96$, $n = 3$, $\chi^2 = 43.28$, $p < 0.001$; *dim2*: $W = 0.68$, $n = 3$, $\chi^2 = 30.73$, $p < 0.01$). Buffaloes viewed in the familiar indoor pen were assessed by all three panels as more calm and less agitated (dimension 1) than animals viewed in the novel outdoor pen (Wilcoxon $z = -2.52$, $p < 0.01$, $z = -2.52$, $p < 0.01$, $z = -2.38$, $p < 0.01$ for Panels 1, 2, and 3, respectively). Scores on dimension 1 for the same animals viewed in either indoor or outdoor pen were correlated at $r = 0.60$ ($p < 0.10$), 0.74 ($p < 0.05$) and 0.71 ($p < 0.05$) for Panels 1, 2, and 3, respectively. Quantitatively, buffalo in the outdoor pen displayed longer bouts of running and higher frequencies of sniffing (both $p < 0.05$) than those in the indoor pen. Principal component analysis showed meaningful associations between qualitative and quantitative assessments, allowing qualitative dimensions to play a valuable role in interpreting the animals' state. The main outcomes of this study are that QBA can be usefully applied to scientific studies of dairy buffalo, and that substantial differences in observer background do not appear to diminish the reliability of QBA.

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1. Introduction

The qualitative assessment of animal behaviour (QBA) is an integrative, 'whole-animal' methodology based on the qualitative interpretation of the dynamic style in which animals interact with their environment. In other words, it describes not 'what' the animals do, but 'how' they do what they do (Stevenson-Hinde, 1983). This method relies on the ability of human observers to integrate perceived details of behaviour and their context into judgements of animal 'body language', using descriptors such as 'calm', 'tense', anxious' or 'content'. Such terms have an

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expressive, emotional connotation, and provide information that appears relevant to animal welfare and could be a useful addition to information obtained from quantitative indicators (Wemelsfelder, 1997; Wemelsfelder et al., 2001; Rutherford et al., 2012). QBA has so far been applied to farm animal species such as pigs (Wemelsfelder et al., 2001, 2009a) and cattle (Rousing and Wemelsfelder, 2006), and companion animals such as horses (Napolitano et al., 2008) and kennelled dogs (Walker et al., 2010). This study reports the first application of QBA to Mediterranean buffaloes, a dairy animal recently moved from traditional farming techniques based on the extensive use of humid environments to intensive systems similar to those applied to dairy cattle (De Rosa et al., 2009). Intensification of farming techniques has subjected these animals to environmental challenges so far unknown to this species and potentially impairing their welfare, so there is a need to develop welfare assessment tools suited to address these problems in buffalo.

Qualitative methodologies have in the past been criticised for being based on subjective and unreliable evaluations, however recently their validity has gained renewed interest and discussion (Meagher, 2009; Whitham and Wielebnowski, 2009). Previous QBA studies have shown good intra- and inter-observer reliability (e.g. Wemelsfelder et al., 2001, 2009a,b; Rousing and Wemelsfelder, 2006; Walker et al., 2010), and have supported the validity of QBA in terms of its correlation with ethogram-based behaviour assessments (Napolitano et al., 2008; Minero et al., 2009) and indicators of physiological stress (Stockman et al., 2011). Most recently Rutherford et al. (2012) demonstrated QBA to be highly sensitive (in a blind trial) to whether growing pigs observed in different test situations had been treated either with anti-anxiety drug azaperone or with neutral saline solution. Generally these studies support that the assessment of animal demeanour through QBA can add a valuable layer of expressive information to animal studies, identifying differences in emotional valence that can be difficult to capture quantitatively. Questions that are still to be investigated, however, are for example whether and how different cultural backgrounds in observers, and different levels of experience with animal behaviour observation and animal farming, affect the reliability of QBA. No information is available as yet on these aspects in buffalo or other animal species.

Thus the aim of this study was to compare the qualitative behaviour assessments of buffalo provided by three groups of observers with different nationalities and different levels of experience in animal behaviour observation and buffalo farming. As in most previous QBA studies, a free-choice profiling (FCP) methodology was used to this end. FCP was originally developed in food science (Arnold and Williams, 1985; Oreskovich et al., 1991), and adjusted for use in animal science by Wemelsfelder et al. (2001). It is characteristic of FCP that it asks observers to develop their own descriptors based on direct observation of animals, a feature we consider essential for being able to test whether or not observers perceive animal body language expressions in similar ways. If pre-determined lists of descriptors were given to observers, the actual process of qualitatively interpreting the animals' expressions would

remain untested, and any found agreement would only concern the quantitative use of terms. Thus we consider FCP to be a more powerful tool for testing the reliability of QBA. The association of QBA data with a range of quantitative behaviour variables was also investigated using principal component analysis.

2. Materials and methods

2.1. Experimental procedures

Animal subjects were 8 Mediterranean buffalo heifers aged 16–18 months. They were group housed in a 5.0 m × 4.6 m indoor slatted floor pen with free access to an outdoor earth floor 5 m × 4.6 m paddock. These animals had previously been kept in intensive farming conditions, where they were subjected to artificial rearing, early weaning and close confinement. These conditions made the animals accustomed to farming practices involving human presence and handling.

In order to test observer agreement in judging buffalo expressions, it was necessary to show observers a variety of different buffalo expressions. To achieve this, buffaloes were subjected to two different isolation tests. One consisted in isolating individual animals from the rest of the group in the indoor part of the home pen, and one in leading animals individually through a single-file chute to an outdoor paddock (5.0 m × 4.6 m, with earth floor and open metal fencing), which was novel to the animals and located approx. 20 m away from the home pen. Four animals were tested first in the home indoor pen and subsequently in the novel outdoor paddock; the other four were tested in the opposite order. During tests subjects were isolated from tactile and visual contact with conspecifics, but could receive auditory and olfactory stimuli from other animals; they could not receive any stimuli from humans. All testing procedures were performed by a stockperson well-known to the animals.

Each animal was confined individually for 2.5 min in each testing condition, and her behaviour during this time was video-recorded using a DVL-157 JVC video camera equipped with a wide-angle lens, located at a corner of the test area at a distance from the fence of 6 m and operated by remote control. From this material a video tape was created containing 16 clips (8 animals in two conditions) of 2.5 min duration each, showing indoor and outdoor tested buffaloes in random order. To give observers time for recording their assessments, each video clip was followed by a blank frame lasting 1.5 min, which was then followed by the next video clip. Thus the total duration of the video recording presented to observers was 64 min.

2.2. Behaviour assessment

2.2.1. Quantitative assessment

The behaviour shown by buffalo heifers in the 16 video clips was analysed quantitatively by means of continuous recording technique (accurate to 1 s). Observations were performed by one trained observer. Training consisted in the observation of 3 outdoor clips and 3 indoor clips with the aim to instruct the observer in recognizing the