Application of the Welfare Quality protocol to dairy buffalo farms: Prevalence and reliability of selected measures

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ABSTRACT

Within the general aim of developing a Welfare Quality system for monitoring dairy buffalo welfare, this study focused on prevalence and interobserver reliability of the animal-related variables to be included in the scheme. As most of the measures were developed for cattle, the study also aimed to verify their prevalence for buffaloes. Thirty animal-based measures (22 clinical and 8 behavioral measurements) and 20 terms used for qualitative behavior assessment were assessed in 42 loose-housed buffalo farms. All farms were located in central-southern Italy. Two assessors were used (1 male and 1 female). The time needed to record all measures (animal-, resource-, and management-based) was 5.47 ± 0.48 h (mean $\pm$ SD). Interobserver reliability of animal-based measures was evaluated using Spearman rank correlation coefficient test (r_s). If 0.7 is considered as threshold for high interobserver reliability, all animal-based measures were above this level. In particular, most of the coefficients were above 0.85, with higher values observed for prevalence of animals that can be touched ($r_s = 0.99$) and prevalence of animals with iatrogenic abscess ($r_s = 0.97$), whereas lower coefficients were found for the prevalence of vulvar discharge ($r_s = 0.74$) and dewlap edema ($r_s = 0.73$). Twelve out of the 20 terms used for the qualitative behavior assessment reached a satisfactory interobserver reliability ($r_s = 0.65$). Principal component analysis of qualitative behavior assessment scores was conducted for each assessor. Both principal component 1 and principal component 2 showed high interobserver reliability ($r_s = 0.80$ and 0.79 , respectively). In addition, relevant proportions of animals were affected by welfare issues specific to buffaloes, such as overgrown claws (median = 34.1%), withers hygroma (median = 13.3%), and vulvar or uterine prolapse (median = 9.3%). We concluded that most of the investigated measures could be

reliably included in the final scheme, which can be used as such to monitor buffalo welfare. However, to inform consumers about the welfare status of the animals, the data should be integrated into a single overall assessment of animal welfare, as already performed in the Welfare Quality project for dairy cattle.

Key words: dairy buffalo, Welfare Quality, welfare monitoring

INTRODUCTION

The increased interest by the public in farm animal welfare has resulted in the development of several tools to monitor welfare on farm (Bartussek et al., 2000; Capdeville and Veissier, 2001; Main et al., 2007). These schemes rely on different measures; some of them (resource-based measures) are related to the physical environment and resources available to the animal (e.g., space allowance, housing facilities, flooring, and climatic conditions), others (management-based measures) concern the conduction of the farm (e.g., breeding strategies, milking routine, and health plan). However, more recently, schemes have shifted their emphasis from resource-based and management measures to animal-based measures dealing with behavior (e.g., agonistic behavior, grooming, and fear), health (e.g., body condition, injuries, and udder health), and physiology (e.g., heart rate and respiration rate) of the animals. This shift reflects the perception that many of the welfare outcomes that vary between farms may be due to the interaction between the animals (breed, age, and temperament), the standard of housing and husbandry, and the attitudes of stockers and farm owners (Blokhuys et al., 2003). In addition, animals may experience the same environment differently. Therefore, it is now agreed that animal-based measures are direct indicators of animal welfare and allow the assessment of variations in housing design and management systems, whereas resource and management indicators can only provide indirect measures of animal welfare as they are not able to give information on how the animals are coping with the environment (EFSA, 2012).

Received January 16, 2015.

Accepted June 14, 2015.

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To fill this gap, the European Commission cofinanced the 5-yr (2004–2009) Welfare Quality project (www.welfarequality.net), aimed at developing a European standard for on-farm welfare assessment and product information systems as well as practical strategies for improving animal welfare on farm. Species selected included cattle (dairy cows, fattening bulls, and veal calves), pigs (sows and piglets, fattening pigs), and chickens (laying hens and broilers). In 2007, dairy buffaloes were also included in the project.

Currently, no Welfare Quality protocol exists for dairy buffalo. However, there are some similarities between dairy cattle and buffalo production. Therefore, the protocol developed for dairy cattle was used as starting point. The Welfare Quality protocol for dairy cattle comprises about 30 measures (Welfare Quality, 2009). The measures are aggregated into 12 criteria that are grouped into 4 principles. Then, the principles are integrated into one final score indicating the level of animal welfare in a given farm (Figure 1). The protocol is primarily based on measures taken on animals (e.g., integument alterations, body cleanliness). Resource (e.g., cleanliness of water points, access to pasture) and management (e.g., tail docking and dehorning standard operating procedures) measures are also included in the monitoring system because they may help identify causes of poor welfare and advice farmers on possible improvements. Most of the animal-based measures were evaluated with regards to their validity (meaningful with respect to animal welfare), reliability (reflecting the tendency to give the same results on repeated measurements), and their feasibility (Winckler et al., 2007; Windschnurer et al., 2008; Plesch et al., 2010).

During the last 4 decades, due to the economic interest in mozzarella cheese, the number of buffaloes in Italy has increased from 103,000 in 1980 to approximately 378,000 head in 2015 distributed in about 2,455 farms, which are mainly located in the Campania, Lazio, Apulia, and Molise regions (Italian Ministry of Health, 2015). This geographical area is designated to produce the cheese Mozzarella di Bufala Campana registered in the European Union's list of Protected Designation Origin products, which can be exclusively made with milk from Italian Mediterranean buffalo. The average production in 2013 was 2,222 kg of milk per 270-d lactation (AIA, 2013). As a consequence, buffalo farming has moved from traditional techniques based on the extensive use of marshland environments to intensive systems with no access to grazing areas and water for wallowing. Such changes have negatively affected buffalo behavior (e.g., impaired expression of species-specific behaviors; De Rosa et al., 2009a) and welfare (e.g., increased incidence of uterine prolapses;

Napolitano et al., 2013), and little research has been conducted in objectively assessing and improving dairy buffalo welfare on farm (Napolitano et al., 2005; Saltalamacchia et al., 2007; De Rosa et al., 2009b). Thus, development of a monitoring system for assessing buffalo welfare is critical.

Utilizing the dairy cattle Welfare Quality protocol, the objective of our study was to determine the interobserver reliability of animal-based measures applied to dairy buffaloes for on-farm welfare assessment. We also aimed to provide baseline information on prevalence of selected animal-based measures in water buffalo farms and to identify critical issues that could result in poor welfare.

MATERIALS AND METHODS

Development of the Monitoring System

Animal-based measures used during this project are described in Table 1 and are adapted from the cattle

Figure 1. Welfare Quality integration process followed to aggregate ~30 measures into 12 criteria, 4 principles, and a final score.

Table 1. Measures collected during the farm visits for the different welfare criteria as defined in Welfare Quality

Principle	Welfare criteria	Measure
Good feeding	Absence of prolonged hunger	Very lean, normal, or very fat animals
	Absence of prolonged thirst	Water provision, cleanliness of water points, water flow, functioning of water points
Good housing	Comfort around resting	Udder, flank or upper legs, and lower legs Time needed to lie down, animals colliding with housing equipment during lying down, animals lying partly or completely outside the lying area
	Thermal comfort	Provision of systems to facilitate thermoregulation (potholes, pools, showers, and so on) ¹
Good health	Ease of movement	Presence of tethering, access to outdoor loafing area or pasture
	Absence of injuries	Lameness prevalence (not lame, lame, or severely lame), overgrown claws ¹
		Animals with integument alterations (hairless patches, lesions or swellings, withers hygroma, ¹ dewlap edema, ¹ iatrogenic abscesses ¹)
	Absence of diseases	Coughing, nasal discharge, ocular discharge, hampered respiration
		Diarrhea
Appropriate behavior	Absence of pain induced by management procedures	Vulvar discharge, vulvar or uterine prolapse, ¹ milk SCC, mortality, dystocia, downer cows
		Tail docking—use of anesthetics or analgesics
	Expression of social behavior	Disbudding/dehorning—use of anesthetics or analgesics—animals with nose ring ¹
		Social licking, ¹ social horning ¹
	Expression of other behaviors	Head butts, displacements, fighting, chasing
		Tongue playing, ¹ tongue rolling, ¹ manipulating substrates, ¹ sucking the udder ¹
	Good human-animal relationship	Access to pasture
	Positive emotional state	Avoidance distance at the feeding place Qualitative behavior assessment

¹Measures not included in the Welfare Quality protocol for dairy cattle.

Welfare Quality protocol (Welfare Quality, 2009) with slight adjustments made to accommodate specific dairy buffalo characteristics. Modifications (e.g., inclusion of vulvar or uterine prolapse, presence of systems to facilitate heat dissipation) were developed via direct consultation with a panel of experts (academics, veterinarians, and breeder association representatives).

Farm Visits and Assessors

Fifty farmers were contacted through collaboration with the National Association of Buffalo Farmers by phone and asked whether they were willing to participate in on farm animal welfare assessments. Forty-two farmers agreed and their loose-housed buffalo farms were visited once between September 2007 and March 2008. The farms were distributed throughout the geographical area of central-southern Italy (Campania, Lazio, Apulia, and Molise regions).

Two assessors were used for data collection. Before starting the farm visits, the assessors were trained (5 d; from January 8 to 12, 2007, in Padua, Italy) by a group of cattle experts selected within the Welfare Quality project. During the morning sessions, measures were described and how to record them was explained. Afternoons were dedicated to practice scoring on dairy cattle farms. Training of the 2 assessors was completed in 2 further sessions conducted on buffaloes to practice the collection of the measures on these animals.

Data Collection and Description of Measures

Only lactating buffaloes were assessed. On-farm assessment started approximately 15 min after feed distribution, which took place once in the morning. The measures were collected in the following order: (1) avoidance distance at the feeding rack (**ADFR**); (2) qualitative behavior assessment (**QBA**); (3) behavioral observations (resting, social, and abnormal behavior); (4) clinical scoring; (5) resources checklist; and (6) management questionnaire.

Apart from resources checklist and management questionnaire, the 2 assessors independently and simultaneously collected all the measures. For each farm, the sample size for ADFR and clinical scoring was chosen according to the recommendation of Welfare Quality (2009) protocol for cattle, where the sample size was dependent on herd size. The mean number of assessed animal was 41 (range = 25–64). A brief description of the measures highlighting the specificity for buffalo is given below. For major details of the protocol and assessment, see Welfare Quality (2009) protocol for cattle.

ADFR. The ADFR is used to assess previous positive and negative human animal interactions. The assessor, starting from a distance of 2 m in front of the animal to be tested, walked slowly (1 step/s) with 1 hand held slightly forward with an angle of approximately 45° in front of the body until signs of withdrawal or until touching of the nose or muzzle. The avoidance distance

was defined as the distance between the hand and the muzzle at the moment of withdrawal or touching and was estimated in steps of 10 cm (from 200 cm to 0 cm). Then, the distance from the hand to the muzzle of the buffalo was placed into 1 of 4 categories: touched, approached to a distance up to 50 cm, approached to a distance of 50 to 100 cm, and approached to a distance higher than 100 cm.

QBA. The QBA is a method developed by Wemelsfelder et al. (2001), which relies on behavioral descriptors spontaneously generated by the assessors following a free-choice profiling approach. However, for feasibility reasons, the method was modified and a fixed list of descriptors was used for welfare monitoring purposes (Wemelsfelder et al., 2009). The list of 20 descriptors (e.g., relaxed, sociable, tense, bored) used for beef cattle (Welfare Quality, 2009) has been chosen for QBA of buffalo after discussion with buffalo experts who deemed this list more appropriate for the description of buffalo behavior. In each farm, the animals were observed as a group for a maximum of 20 min. The minimum amount of time per observation point was 2.5 min. (i.e., a maximum of 6 observation points per QBA). After the observation the assessors scored all descriptors. Next to each descriptor there was a continuous line 125 mm long. Scores for each descriptor were measured as the distance in millimeters from the 0-point (0 mm = descriptor absent; 125 mm = descriptor very strong).

Behavioral Observations (Resting, Social, and Abnormal Behavior). In each farm, before starting the behavioral observations, barns were segmented according to their layout (the number of segments did not exceed 6) and the number of animals per segment, which did not exceed 25. After barn segmentation, continuous recording with a total net observation time of 120 min was performed. The duration of continuous observation per segment was at least 10 min. At the start and at the end of each segment observation the number of feeding, lying within resting area, lying partly or completely outside lying area, and standing animals

in the observed segment was counted. Observations during continuous recording included social interactions and abnormal behaviors. Social interactions were grouped in affiliative and agonistic (see Table 2 for definitions), whereas abnormal behaviors included tongue playing or rolling (the animal is repeatedly twisting, twirling, swinging its tongue in an abnormal way inside or outside the open mouth, or stretches out the tongue for longer than 5 s), object manipulation (the animal chews or licks any equipment), and sucking the udder of a pen mate (the actor gets the udder of a pen mate with its mouth and pulls at it with the muscles of its cheeks and tongue to get milk out of it, for longer than 5 s). Furthermore, time to lying down was recorded along with the incidences of collisions with housing equipment during the recorded lying down movements. Time recording of a lying down sequence started when one carpal joint of the animal was bent and lowered (before touching the ground). The whole lying down movement ended when the hind quarter of the animal fell down and the animal pulled the front leg out from underneath the body. The interactions between bulls and cows were not registered. Although coughing is a clinical variable, it was counted during the behavioral observations.

Clinical Scoring. Clinical examinations were performed from the feeding alley; due to the fact that animals were not dehorned, the feeding racks were not provided with self-locking stanchions and the bull might be present. The assessors stood motionless for 3 to 5 min by the feeding rack, waiting for approaching animals. The approaching of the animals to the feeding alley was encouraged both by the natural curiosity of the species (Napolitano et al., 2005) toward unknown people and by the delivery of feeding 10 min before clinical examinations.

Body condition scoring was assessed following the scheme used for dual-purpose breeds (Welfare Quality, 2009). For lameness, the assessors checked its presence using the system suggested for dairy cattle (Welfare Quality, 2009).

Table 2. Description and classification of social interactions recorded during the behavioral observations

Social interaction	Description
Agonistic behavior	
Head butts	Butting, thrusting or pushing the receiver
Displacements	Physical contact where the actor is butting, thrusting, pushing or penetrating the receiver with forehead, horns, horn base or any other part of the body with a forceful movement and as a result the receiver gives up its position
Chasing	Animal making another animal flee by following fast or running behind it
Chasing up	Use of forceful physical contact against a lying animal, which is forced to stand up
Fighting	Two animals vigorously pushing their heads against each other while stemming their feet into the ground
Affiliative behavior	
Social licking	Animal touching with its tongue any part of the body of a conspecific, except for the anal region or the prepuce
Social horning	Two animals rubbing their foreheads, horn bases, or horns without obvious agonistic intention

For cleanliness and integument alterations, the systems suggested for dairy cattle (Welfare Quality, 2009) were used. However, in the first case, the type of body covering was taken into account, thus buffaloes covered in mud were evaluated as clean animals, whereas for integument alterations 3 further aspects were specified: withers hygroma, defined as an hairless area with skin hyperkeratosis located in the withers region (swelling is also possible); dewlap edema, defined as swelling located at dewlap level; and iatrogenic abscess, mainly located in the hind quarters. The first 2 aspects are indicative of an inappropriate design of feeding rack, whereas the last one reflects the use of oxytocin injection to facilitate milk ejection and may be related to poor handling during milking.

The assessors, in addition to the measures for absence of disease proposed for dairy cattle (Table 1), checked the animals for a common pathology in buffaloes, vaginal or uterine prolapse. For this purpose, the assessors recorded the animals with evident extroversion of vagina or uterus, indicating actual prolapse, the animals presenting stitching at vaginal level, indicating a recent prolapse, and the animals with edema at vaginal region, expressing a remote prolapse. During the clinical scoring, animals wearing nose ring or other craft devices were also recorded. Their presence reflects the tendency of that animal to suck the udder of companions and it represents an indirect measure of cross-sucking, as it may be rarely expressed during the 2-h behavioral observations.

At the end of the farm visits, a resources checklist and a management questionnaire were filled. Management practices inducing pain, such as dehorning and tail docking, are not common in buffalo farming and none of the visited farms adopted these practices. In addition, the farmer declared no downer cows, whereas only few herd records were available for milk SCC, as most of the farms were not enrolled in the national milk-recording scheme. Therefore, only data concerning the incidence of dystocia (mostly gathered through direct farmer interview) and mortality (mostly taken from farm records) are shown.

Statistical Analysis

Data were analyzed using the software SAS (1990). Farm was used as experimental unit. For each farm and each assessor, clinical scores and the 4 categories of ADFR were expressed as prevalence (i.e., percentage of animals with signs of each condition), whereas behavioral data, including coughing, were expressed as number of events per animal per hour and QBA scores as millimeters (distance from the 0-point of the scale for each descriptor). In addition, for each farm

and each assessor the means of avoidance distance and duration of lying down behavior were calculated. Then, for all of them the interobserver reliability was computed using the Spearman correlation coefficient (r_s), expressing the degree of agreement between the 2 assessors. Limits of agreement were also calculated to test whether bias existed between observers (Bland and Altman, 1986). Apart from QBA descriptors, if a variable was reliable, for each farm the mean of the 2 values gathered by the 2 assessors was calculated. The means of the measures concerning dirtiness were also used to calculate Spearman correlation coefficients among 3 body regions (legs, flanks, and udders). For each measure medians were calculated and grouped into 5 quintiles so that the range of the results from 20% of the farms fell within each quintile. Finally, for QBA data, a principal component analysis (PCA; covariance matrix, no rotation) of scores was conducted for each assessor. The scores attributed to the observed farms on the first 2 main components of the PCA were then tested for interobserver reliability using Spearman correlation coefficient.

RESULTS AND DISCUSSION

Farm Characteristics

The main features of the farms are reported in Table 3. The time needed to record all measures (animal-based, resource, and management) was 5.47 ± 0.48 h (mean $\pm$ SD). Thirty-six percent of farms had an outdoor paddock, but none used pasture. Of the 42 farms, 3 used cubicles in the resting area, 15 deep-bedded straw, and the remaining concrete floor without bedding (20 farms) or with dried manure solids as bedding material (4 farms). Fifteen (7 using showers in the feeding area, 5 presenting concrete pools and 3 with potholes in the outdoor paddock) out of 42 farms used facilities for thermoregulation. In hot conditions, heat dissipation in buffaloes relied on wallowing and the lack of water may have impaired effective thermoregulation with negative effects on buffalo welfare and milk production (Tripaldi et al., 2004; De Rosa et al., 2009b). For the manger frontage, although the average (0.7 m) was close to the suggested value for buffalo (0.75 to 0.80 m; i.e., 0.05 to 0.10 m higher than cattle due to the fact that buffalo were not dehorned), 17 farms showed a value below 0.60 m. The average of drinking frontages (8.26 cm) was within the range of the recommended values for buffalo (7 to 8.5 cm; i.e., 1 to 1.5 cm higher than cattle). However, 14 farms out of 42 showed a value below 6 cm. Mean culling rate was relatively low (9.3%) compared with those reported for dairy cattle. In fact, in buffalo farms is it common to maintain buffalo of

Table 3. General features of the farms (n = 42 farms)

Variable	Mean $\pm$ SD	Median	Minimum	Maximum
Head, no.	355 $\pm$ 209	300	89	900
Lactating animals, no.	118 $\pm$ 64	97	30	306
Space allowance, m ² /head	16.94 $\pm$ 22.19	10.95	3.30	119.90
Space allowance in the resting area, m ² /head	7.68 $\pm$ 17.88	3.35	1.00	117.50
Manger frontage, m/head	0.70 $\pm$ 0.28	0.66	0.24	1.48
Drinking frontage, cm/head	8.26 $\pm$ 5.77	8.00	1.00	26.00
Feed curb height, cm	42.88 $\pm$ 8.38	40.00	30.00	64.00
Feed neck rail height, cm	105.79 $\pm$ 15.86	105.00	73.00	150.00
Milk production, kg/head per year	1,914 $\pm$ 290	1,885	1,119	2,615
Culling rate, %	9.3 $\pm$ 5.0	9	1	20

the sixth parity and above in production. However, the increasing trend in milk production and the intensification of the farming system suggest that this variable should be constantly monitored. Mean milk production was in line with the values reported on the official statistics of milk recording activity (AIA, 2008). Five farms showed values above 2,300 kg/head per year. It is worth noting that the standard lactation length in buffalo is only 270 d long.

Interobserver Reliability

Tables 4 and 5 show the interobserver reliability of the animal-based and QBA variables, respectively. Limits of agreement mostly confirm the results expressed in terms of r_s . Although no threshold figure of r_s exists above which a variable may be considered reliable, it has been suggested that a value above 0.7 expresses high reliability (Martin and Bateson, 2007). Table 4 indicates that the interobserver reliability of all animal-based measures were well above the threshold limit of 0.7. In particular, most of the coefficients were above 0.8, whereas lower coefficients were found for the prevalence of vulvar discharge (0.74) and dewlap edema (0.73). For the measures used to assess human-animal relationship, the higher coefficient was observed for the prevalence of animals that could be touched (0.99). These results may be attributed to the fact that a touched animal can be easily identified, whereas avoidance distances have to be subjectively estimated. Taken together, these results show that animal-based measures, as also reported for dairy cattle (EFSA, 2012), may be reliably included in a monitoring scheme assessing buffalo welfare at farm level.

Table 5 shows the Spearman coefficients for each of the qualitative terms used for QBA. According to the arbitrary threshold of 0.7, 3 terms out of 20 reached a satisfactory interobserver reliability (≥ 0.7). Another 5 terms reached values ≥ 0.6 and < 0.7 , whereas 4 terms reached a low level of reliability (≥ 0.5 and < 0.6) and the remaining 8 terms were not reliable (< 0.5). The

PCA of scores was conducted for each assessor to gain more information on how they used the various terms to account for the variation between farms. The PCA of assessors' scores showed similar clustering of terms along the 2 principal components (**PC**). The first PC distinguishes between positive and negative mood and the second PC differentiates these moods in low and high level of arousal. Both PC1 and PC2 showed high interobserver reliability ($r_s = 0.80$ and 0.79 , for PC1 and PC2, respectively; $P < 0.001$). These results indicate that QBA can be reliably applied to buffaloes for welfare monitoring purposes.

In our study, the 2 observers used for the calculation of interobserver reliability were intensively trained (5 consecutive days with a group of experts in the same training sessions). This may have caused an overestimation of interobserver reliability. However, any welfare-monitoring schemes should be based on an accurate training of the observers to achieve a reliable application (van Reenen and Engel, 2009).

Scores of Animal-Based Measurements

Table 6 shows the median, minimum, and maximum values of the animal-based measures recorded during the farm visits as well as their distribution in 5 quintiles. The highest median prevalence of affected buffaloes was observed for dirtiness (legs, flanks, and udders). In 40% of farms all the sampled animals showed dirty hindquarters. These results may be at least partly attributed to the fact that the farm visits took place during fall-winter period. However, buffaloes are highly motivated to wallow (De Rosa et al., 2009b) and in absence of appropriate facilities they may be forced to wallow in the dung. Therefore, a dirt-free floor is essential to keep these animals clean. These measures were also highly correlated ($r_s = 0.91$, 0.82 and 0.81 , for legs vs. flanks, legs vs. udders, and flanks vs. udders, respectively; $P < 0.001$). Therefore, we suggest including only one of these measures in the final buffalo welfare-monitoring scheme.

In our study only 20% of farms showed a prevalence of thin animals higher than 10%, with a maximum value of 26.2%, whereas 40% of farms displayed a prevalence of fat animals ranging from 9.4 to 30%. Although Mediterranean buffaloes are exclusively used for milk production, from a morphological and metabolic point of view these animals are more similar to dual-purpose cattle than to dairy animals (Campanile et al., 1998). This different metabolism may explain the higher prevalence of thin animals frequently observed in dairy cattle (Whay et al., 2003).

No data on buffalo lying behavior are available. However, in our study the mean time needed to lie down (4.3 ± 0.7 s) was in the range observed in dairy cattle (e.g., Plesch et al., 2010). In the Welfare Quality protocol for dairy cattle, the threshold for the time taken to lie down was 5.2 s, whereas 6.3 s is the threshold for a serious problem impairing the passage from standing to a lying down position. In our study, 4 farms displayed values higher than 5.2 s, with 2 of them showing values higher than 6.3 s. The prevalence of animals lying partly

or completely outside lying area was high with 40% of farms showing values above 33%. Previous results (De Rosa et al., 2009b; Sabia et al., 2014) showed that when available buffaloes tend to spend a high proportion of time in potholes, pools, or ponds. As only few farms had these facilities, we hypothesized that the buffaloes were induced to rest in more humid areas, such as the feeding alley, rather than in the resting area. The low number of farms with cubicles may explain the low prevalence of animals colliding with housing equipment when lying down.

As integument alterations, lesions were more frequent than hairless patches (Table 6). In addition, the latter may be more difficult to assess due to the sparse and heterogeneous hair coat of adult buffalo and its exclusion from the final protocol may facilitate the assessment. Twenty percent of farms showed a prevalence of withers hygroma higher than 35%. These results indicated that in several farms the height of feeding neck rail was too low. In fact, 60% of farms showed a height less than 110 cm, whereas the suggested value

Table 4. Interobserver reliability of animal-based variables expressed as Spearman correlation coefficient (r_s) and limits of agreement (mean of differences $\pm$ 2 SD) between observers ($n = 42$)

Variable	Spearman statistics		Limits of agreement		
	r_s	P -value	Mean	Mean + 2 SD	Mean - 2 SD
Prevalence of thin animals, %	0.90	<0.001	0.29	4.59	-4.00
Prevalence of fat animals, %	0.99	<0.001	-0.12	4.63	-4.88
Prevalence of animals with dirty udders, %	0.83	<0.001	-1.14	8.23	-10.51
Prevalence of animals with dirty flanks, %	0.93	<0.001	-0.44	5.98	-6.86
Prevalence of animals with dirty legs, %	0.84	<0.001	-0.52	7.44	-8.48
Duration of lying down behavior, s	0.79	<0.001	-0.03	0.52	-0.57
Prevalence of animals lying partly or completely outside lying area, %	0.97	<0.001	0.03	2.87	-2.81
Prevalence of collision with equipment during lying down behavior, %	0.87	<0.001	-0.40	8.60	-9.40
Prevalence of animals with hairless patches <20 cm, %	0.95	<0.001	-0.48	2.60	-3.55
Prevalence of animals with hairless patches >20 cm, %	0.98	<0.001	0.00	5.44	-5.46
Prevalence of animals with lesions <20 cm, %	0.92	<0.001	-0.56	5.42	-6.53
Prevalence of animals with lesions >20 cm, %	0.99	<0.001	0.21	5.27	-4.84
Prevalence of animals with withers hygroma, %	0.96	<0.001	0.33	6.49	5.82
Prevalence of animals with dewlap edema, %	0.73	<0.001	-0.68	5.26	-6.62
Prevalence of animals with iatrogenic abscesses, %	0.97	<0.001	-0.41	4.07	-4.88
Prevalence of animals with overgrown claws, %	0.95	<0.001	0.64	7.26	-5.97
Prevalence of animals with nasal discharge, %	0.91	<0.001	1.06	6.07	-3.94
Prevalence of animals with ocular discharge, %	0.88	<0.001	0.62	3.01	-1.77
Prevalence of animals with vulvar discharge, %	0.74	<0.001	0.24	2.06	-1.58
Prevalence of animals with vulvar or uterine prolapse, %	0.97	<0.001	0.68	4.23	-2.86
Prevalence of animals with nose ring, %	0.94	<0.001	-0.27	3.75	-4.29
Agonistic behavior (chasing, fighting, displacement, butting), no. of events/animal per hour	0.87	<0.001	0.00	0.32	-0.31
Affiliative behavior (social licking, social horning), no. of events/animal per hour	0.90	<0.001	-0.00	0.07	-0.07
Abnormal behavior (bar biting or licking, tongue playing, and so on), no. of events/animal per hour	0.95	<0.001	-0.00	0.04	-0.04
Avoidance distance at manger, m	0.92	<0.001	-0.01	0.10	-0.12
Prevalence of animals that can be touched, %	0.99	<0.001	0.30	3.84	-3.24
Prevalence of animals approached at <0.5 m, %	0.88	<0.001	1.17	13.91	-11.56
Prevalence of animals approached at ≤ 1 and ≥ 0.5 m, %	0.83	<0.001	-3.98	10.40	-18.36
Prevalence of animals approached at >1 m, %	0.80	<0.001	2.51	11.68	-6.66

Table 5. Interobserver reliability of each term used for Qualitative Behavior Assessment expressed as Spearman correlation coefficient (r_s) and limits of agreement (mean of differences $\pm$ 2 SD) between observers ($n = 42$)

Term	Spearman statistics		Limits of agreement		
	r_s	P -value	Mean	Mean + 2 SD	Mean - 2 SD
Active	0.67	<0.001	6.29	40.07	-27.50
Relaxed	0.68	<0.001	-6.52	28.33	-41.37
Uncomfortable	0.82	<0.001	1.71	37.33	-33.91
Calm	0.49	<0.001	-3.55	43.86	-50.96
Content	0.63	<0.001	-3.64	35.56	-42.84
Tense	0.45	<0.01	1.83	45.13	-41.46
Enjoying	0.59	<0.001	12.02	51.30	-27.25
Indifferent	0.56	<0.001	7.74	60.62	-45.15
Frustrated	0.62	<0.001	2.12	42.39	-38.15
Friendly	0.18	NS	-1.71	52.21	-55.64
Bored	0.63	<0.001	0.64	40.33	-39.04
Positively occupied	0.54	<0.001	0.76	35.83	-34.31
Inquisitive	0.36	<0.05	-6.05	51.55	-63.65
Irritable	0.44	<0.01	-2.00	37.30	-41.30
Nervous	0.53	<0.001	-5.00	35.21	-45.21
Boisterous	0.48	<0.001	2.29	42.45	-37.88
Uneasy	0.48	<0.001	4.57	38.23	-29.08
Sociable	0.16	NS	5.41	49.57	38.76
Happy	0.76	<0.001	4.88	37.00	-27.24
Distressed	0.78	<0.001	8.55	38.29	-21.20

is approximately 130 to 150 cm. Dewlap edema was much less frequent, possibly because the height of feed curb was closer to the buffalo needs (35 to 45 cm). In our study, the percentage of animals with iatrogenic abscesses was lower to that reported by Saltalamacchia et al. (2007), although animals comprised 21.7 to 36.7% of the population in the 20% of farms affected. The buffalo udder cistern contains only 5% of the milk, whereas the remaining is stored in the alveoli and small ducts (Thomas et al., 2004). The alveolar milk ejection relies on the action of endogen oxytocin on myoepithelial cells (Bruckmaier and Blum, 1998). As a consequence, buffaloes tend to release the milk incompletely in response to various negative factors, including unkind interactions with handlers during milking, and oxytocin injections are often used to gather the milk. Such injections can cause iatrogenic abscesses, which in turn represent a clear sign of mismanagement of the milking routine.

As also reported by De Rosa et al. (2003), lameness was virtually absent. This may be attributed to a different metabolism and feeding regimen used in buffaloes as compared with cattle (Campanile et al., 1998; Terramoccia et al., 2000). However, due to the changes occurring in modern buffalo enterprises (e.g., adoption of concrete floors, decreased forage-to-concentrate ratio), this measure should be kept in the welfare-monitoring scheme. Although in the final Welfare Quality (2009) protocol the prevalence of overgrown claws was not included, the high prevalence registered in the present study suggests that this aspect should be not neglected

in buffaloes. This high prevalence may be due to housing factors such as the large use of concrete floors impairing the regular growth of claws and to management factors such as the low frequency of hoof trimming. In our study, only 16 out of 42 farms performed foot trimming regularly (i.e., once a year).

No animals were observed with signs of coughing, hampered respiration, or diarrhea, therefore no data on these issues are reported in Table 6. About 80% of farms had a prevalence of animals with ocular discharge below the warning threshold (3%), indicated by the Welfare Quality (2009) protocol with almost 20% of farms exceeding the alarm threshold (6%). Conversely, 60% of farms showed a prevalence of animals with nasal discharge above the warning threshold (5%) with 12 farms exceeding the alarm threshold (10%). Only 4 farms were above the alarm threshold (4.5%) indicated for vulvar discharge. A high prevalence of animals with signs of recent or remote vulvar or uterine prolapse was observed. According to Zicarelli (2000), an incidence above 2% may be considered as warning threshold, whereas the alarm incidence may be set at 7%. Table 6 shows that more than 60 and 40% of the farms were above the warning and alarm threshold, respectively. These results confirm that vulvar and uterine prolapses are one of the major welfare issues in modern dairy buffalo enterprises. This pathology has been associated with unbalanced rations fed to the cows in the dry and transition periods, and inappropriate feeding from birth to weaning leading to reduced pelvis development

Table 6. Median score and quintile distribution of animal-based variables¹

Variable	Median	First group	Second group	Third group	Fourth group	Fifth group
Prevalence of thin animals, %	4.2	0-2.0	2.5-4.0	4-6.2	7.1-8.5	10-26.2
Prevalence of fat animals, %	5.7	0-0	0-2.2	2.5-8.6	9.4-17.6	18.3-30
Prevalence of animals with dirty udders, %	93.4	0-57.0	65-82.1	84-95	97.1-100	100-100
Prevalence of animals with dirty flanks, %	94.1	0.8-34.0	41.2-82.1	82.5-97.1	97.1-100	100-100
Prevalence of animals with dirty legs, %	97.1	1.5-70.0	82-95	95.5-98.5	98.8-100	100-100
Duration of lying down behavior, s	4.0	3.5-3.8	3.9-4	4-4.2	4.2-4.6	4.7-6.8
Prevalence of animals lying partly or completely outside lying area, %	27.7	0-3.6	3.7-15.9	16.9-32.2	33.3-57.8	60.3-94
Prevalence of collision with equipment during lying down behavior, %	0	0-0	0-0	0-8.3	12.5-28.6	30-70
Prevalence of animals with hairless patches <20 cm, %	2.9	0-0	0-2	2-4	4.3-7.5	11-21.4
Prevalence of animals with hairless patches >20 cm, %	14.5	0-5.0	5-10	10-18.6	18.9-45	46-74
Prevalence of animals with lesions <20 cm, %	10	0-2.9	2.9-7.5	8-11	11.4-14.3	17-25
Prevalence of animals with lesions >20 cm, %	23.4	4-9.4	10-17.1	18-26	26.6-35	38.7-62.5
Prevalence of animals with withers hygroma, %	13.3	0-4	4-10	10-15.4	15.7-31.5	35-74.5
Prevalence of animals with dewlap edema, %	2.3	0-0	0-1.4	1.4-3	3.2-7.2	8-25
Prevalence of animals with iatrogenic abscesses, %	4.2	0-0	0-1.4	2.5-8.6	8.6-21.2	21.7-36.7
Prevalence of animals with overgrown claws, %	34.1	4.3-14.3	19.8-28.6	31-35.9	39-54.4	55-82.9
Prevalence of animals with nasal discharge, %	7.6	0-0	1-5.7	5.7-8.3	8.5-12	12-30
Prevalence of animals with ocular discharge, %	0.5	0-0	0-0	0-1	1.4-3.7	3.9-23
Prevalence of animals with vulvar discharge, %	0	0-0	0-0	0-0	0-1.5	1.5-5.7
Prevalence of animals with vulvar or uterine prolapse, %	9.3	0-1.4	1.7-5.5	5.7-12.5	12.7-16.7	17-28
Dystocia: calvings requiring major assistance, %	2.5	0-1	1-2	2-3.5	4-10	10-12
Mortality, animal culled due to diseases or accidents, %	1	0-0.5	0.5-1	1-1.5	1.5-2.5	3-5
Prevalence of animals with nose ring, %	0.7	0-0	0-0	0-1.7	1.7-6	7.1-12
Agonistic behavior (chasing, fighting, displacement, butting), no. of events/animal per hour	1.0	0.1-0.6	0.6-0.8	0.8-1.2	1.2-1.8	1.9-6.6
Affiliative behavior (social licking, social horning), no. of events/animal per hour	0.1	0.7-0.3	0.3-0.2	0.2-0.1	0.1-0.1	0.1-0
Abnormal behavior (bar biting or licking, tongue playing, and so on), no. of events/animal per hour	0	0-0	0-0	0-0	0-0.03	0.03-0.31
Avoidance distance at manger, m	0.37	0.15-0.22	0.23-0.32	0.34-0.39	0.39-0.5	0.53-0.81
Prevalence of animals that can be touched, %	20.0	67.1-32.5	32.0-22.2	21.3-14.3	14.3-9.4	8.0-2.7
Prevalence of animals approached at <0.5 m, %	47.1	75.0-58.8	57.1-51.4	50.0-40.6	40.0-35.7	35.0-18.6
Prevalence of animals approached at ≤1 and ≥0.5 m, %	25.0	4.3-11.4	11.7-20.0	21.9-26.5	27.1-35.0	35.9-47.1
Prevalence of animals approached at >1 m, %	5.4	0-0	1.4-4.1	4.7-7.0	8.0-11.3	11.4-38.6

¹Each group represents the results from 20% of the farms, with the minimum and maximum result in each quintile being indicated; first group shows the top results for each measure and fifth group the bottom.

(Zicarelli, 2000). Therefore, technical advices for the introduction of corrective measures concerning feeding management should be given to the farmers. About 60% of farms declared a percentage of dystocia (i.e., calvings requiring major assistance) below the warning threshold (2.75) indicated by Welfare Quality (2009) protocol, with 26% of farms above the alarm threshold (5.5). These data should be taken with caution, as they mostly derived from farmer estimates. As to median mortality, only 2 farms were above the alarm threshold (4.5%) reported by the Welfare Quality (2009) protocol.

Field data indicate that cross-sucking is a common behavior in buffalo calves (possibly related to early separation from the mothers and early weaning) and it is often shown also by adult animals. To prevent milk loss, farmers apply to the nose of the animals expressing this behavior either weaning rings or other sucking-discouraging craft devices. Our data show that in 60% of farms this problem is virtually absent, whereas in the remaining 40% cross-sucking represents an issue often leading to increased culling rates of the sucking cows.

Previous studies on buffaloes report low levels of aggressive interactions in weaned calves (Napolitano et al., 2004), heifers (Napolitano et al., 2013), and cows (De Rosa et al., 2009b). Similarly, in the present study, only 2 farms exceeded 3 agonistic events per animal per hour. Sociopositive behaviors, including licking and horning, were not frequently expressed. A low level of sociopositive interactions in dairy buffaloes was also recorded by De Rosa et al. (2009b). These authors observed that the availability of a pool increased 1.5-fold the expression of these behaviors. Affiliative behaviors are not included in the Welfare Quality (2009) protocol for dairy cattle.

Abnormal behaviors were rarely observed, as only 20% of farms exhibited a prevalence higher than 0.03 events per animal per hour, whereas it was absent in 60% of the farms. The Welfare Quality (2009) dairy cattle protocol does not include this measure. Due to the low level registered in the present study, this measure may also be excluded from the dairy buffalo protocol without losing much information.

A recent study (De Rosa et al., 2014) reported that positive stockperson interactions negatively correlated with the number of kicks during milking and the percentage of animals treated with oxytocin. Similarly, Saltalamacchia et al. (2007) observed a negative correlation between positive stockperson interactions and kicking and a positive correlation between kicking and prevalence of oxytocin injection. In addition, negative stockperson interactions were positively correlated with avoidance distance (De Rosa et al., 2014). In our study, median avoidance distance and percentage of animals

touched at the feeding rack were 0.37 m and 20%, respectively.

CONCLUSIONS

To protect buffaloes from effects of intensification of farming techniques detrimental to animal welfare, the development of a monitoring scheme of welfare is needed. Its application may help to improve both the consumer perception of the chain production quality and the farm management and housing condition. The results of the current project showed that the interobserver reliability of the measures proposed was very high. Therefore, most of the proposed measures can be reliably included in the final scheme, which can be used to monitor buffalo welfare after appropriate refinement (e.g., exclusion of hairless patches and abnormal behaviors). We observed high proportions of animals affected by welfare problems specific to buffaloes. In particular, further studies should be conducted to tackle issues such as the relationships between feeding management and vulvar or uterine prolapse, weaning management and cross-sucking, concrete flooring and overgrown claws, wallowing behavior and lying outside the resting area or body dirtiness, as well as milking routine and iatrogenic abscess. However, to implement this scheme and obtain an efficient advisory tool, the welfare status of each farm should be benchmarked with that of a reference population with the aim to identify and promote the application of the best farming practices.

ACKNOWLEDGMENTS

This study was supported by the European research project Welfare Quality, co-financed by the European Commission (Brussels, Belgium) within the 6th Framework Programme, Contract No. FOOD-CT-2004-506508. The text represents the authors' views and does not necessarily represent a position of the European Commission, who will not be liable for the use made of such information. Thanks are due to L. Zicarelli (Dipartimento di Medicina Veterinaria e Produzioni Animali, Università degli Studi di Napoli Federico II) for his valuable comments on the early draft of the protocol and G. Migliori (Dipartimento di Agraria, Università degli Studi di Napoli Federico II) for technical assistance.

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