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Housing system and welfare of buffalo (*Bubalus bubalis*) cows

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Abstract

Twenty-eight buffalo cows were used to evaluate the effect of housing system on a range of behavioural and physiological variables. Fourteen cows were group-housed in a loose open-sided barn with a concrete floor and 10 m² per head as space allowance (group IS). Fourteen others were group-housed in a similar barn but they could also benefit from an outdoor yard with 500 m² per head as space allowance, free access to potholes for wallowing and spontaneous vegetation (group TS). Animals were subjected to six sessions of instantaneous scan sampling at 10-day intervals. Behavioural variables were expressed as proportions of subjects observed in each category of posture and activity. Phytohaemagglutinin (PHA) was used to perform a skin test based on non-specific delayed type hypersensitivity, whereas 20 mg of ovalbumin were injected subcutaneously to evaluate humoral immune response. Blood samples for evaluation of cortisol concentration were collected immediately prior to exogenous porcine ACTH injection and 1, 2 and 4 h after. The metabolic status of the animals and milk production were also monitored. The proportion of idling animals was higher in group IS than in group TS (P < 0·001). More IS buffalo cows were observed eating at the manger than TS animals (P < 0·001). A higher proportion of TS animals were observed in the sun (P < 0·001). Grazing and bathing activities were recorded only for TS animals. Our findings suggest that buffalo cows kept in intensive conditions and having no access to ample yards and potholes may extend their periods of idling with negative effects on the state of welfare. Immune responses, metabolite concentrations and milk production were not affected by treatment, whereas cortisol levels were higher in IS animals (P < 0·05). The provision of a housing system similar to natural conditions was able to improve the welfare of buffalo cows as indicated by the expression of some species-specific natural behaviours. Such conditions were also associated with lower adrenal cortex response to ACTH injection, possibly as a consequence of the higher degree of initiative allowed to TS cows.

Keywords: *animal welfare, behaviour, buffaloes, housing, immune response.*

Introduction

The definition of animal welfare is a difficult issue. The 'five freedoms' proposed by the Farm Animal Welfare Council (1993) may represent a good starting point. The fact that the natural behaviour of livestock is explicitly mentioned in the objectives of the Report implies that the interpretation of welfare is not simply limited to the animal's functioning or performance. Animals should also be able to develop normally and express natural adaptations in relation to their innate natures. The provision of barren housing systems irrespective of animal's natural behaviours and needs may reduce the welfare of livestock, whereas it has been suggested that the well-being of an animal may be improved through valuable experiences that makes its life richer (Vaarst *et al.*, 2001).

Dairy water buffalo (*Bubalus bubalis*) farming is a traditional Italian enterprise which has been conducted for centuries with extensive rearing systems in low-lying swampy areas of central-southern Italy. Recent intensification of rearing techniques has exposed these animals to a rapidly changing environment that imposes physical and psychological stressors so far unknown to this species. Space restriction presents both physical and psychological components which may result in a marked reduction of animal welfare (Maton and Daelemans, 1989). Lack of space resulted in evidence of stress in cattle (Fisher *et al.*, 1997) and unweaned female buffalo calves (Grasso *et al.*, 1999). These latter animals showed alterations in a number of behavioural and physiological responses as a consequence of space restriction.

In the present study we investigated the effect of two housing systems (with and without an ample outdoor yard including spontaneous vegetation and potholes) on the behavioural, endocrine and immune responses of buffalo cows. These indicators were previously used and adjusted for buffalo calves (Grasso *et al.*, 1999) and heifers (Grasso *et al.*, 2003).

Material and methods

All procedures involving animal handling and testing were approved by the Care and Use Committee of the Istituto Sperimentale per la Zootecnia in Monterotondo (Rome), Italy.

Experimental design
The experiment was conducted from February to July 2000 in a farm of the Istituto Sperimentale per la Zootecnia in Monterotondo (Rome) at latitude 42ºN. Twenty-eight lactating buffalo cows were used. Animals aged about 68 months at the start of the study, with a parity of 2·5 and a mean live weight of 667·5 kg, were equally allocated to two treatments. Fourteen cows were group-housed in a loose open-sided barn with a concrete floor and equipped with self-locking stanchions, where they received 10 m^2 per head of space allowance, as in intensive systems (group IS). The straw bedded resting area and the feeding area were covered by a roof, whereas the exercise area (35% of the total surface of the barn), located between them, was uncovered. Fourteen others were group-housed in a similar barn but they could also benefit from an outdoor yard with 500 m^2 per head as space allowance including spontaneous vegetation and potholes for bathing and wallowing, as in traditional systems (group TS). After parturition experimental animals were separated from calves and allocated to either group where other lactating non-experimental subjects were already present. Non-experimental animals were balanced for parity, body weight and stage of lactation. Therefore, when all the animals (experimental + non-experimental) had been included, there were 40 buffaloes in each group. The two experimental groups were constituted over a 2-week period.

Every day at 08:00 h subjects were offered a complete mixed diet for *ad libitum* consumption (Table 1). For each group an ample drinking trough was available.

Behavioural recordings
Observations were performed in June and July. Animals were subjected to six sessions of instantaneous scan sampling at 10-day intervals. Observations were made every 15 min over an 8-h period (07:30 to 15:15 h), giving a total of 32 sets of observations per session. On observation days, an observer for each group of animals walked slowly past the fence from a distance of 4 m and recorded (using binocular glasses when necessary) posture (standing or lying), location (in the sun or in the shade) and activity such as feeding from the manger (selection, prehension and mastication), ruminating, drinking, locomotion, self-grooming, idling (opened or closed eyes, but no other overt activity). In addition, for TS animals, grazing (searching and

Table 1 *Composition and chemical analysis* (*g/kg dry matter* (*DM*)) *of the complete mixed diet*

	Composition (g/kg DM)	Milk FU per kg DM	Chemical analysis	
			Crude protein	Crude fibre
Lucerne hay	159	0·58	15·2	34·1
Meadow hay	215	0·62	11·0	28·6
Barley meal	110	1·11	12·5	5·4
Maize meal	110	1·27	10·1	2·5
Concentrate	83	1·07	38·0	9·4
Maize silage	313	0·89	8·1	18·9
Mineral mix	9·2	-	-	-
Total	1000	0·88	13·0	19·1

ingesting herbage) and bathing activities were recorded. Due to distance and wallowing any attempt to recognize individual animals failed. Therefore, non-experimental animals were also scored and behavioural variables were expressed as the proportion of subjects observed in each category of posture and activity calculated as : number of animals displaying each posture and activity /40 (total number of animals per group). Rapid behaviours such as agonistic (pushing, butting or threatening) and non-agonistic (licking, sniffing or nuzzling conspecifics) interactions were recorded using the more sensitive technique of continuous recording : during each session these behavioural categories were recorded continuously.

Immune responses

Phytohaemagglutinin (PHA) was used to perform a skin test based on non-specific, delayed type hypersensitivity. At weeks 6 and 22 after grouping, 2 mg PHA (Sigma Chemical Co.) dissolved in 1 ml of sterile saline solution was injected intradermally into the middle of a 2-cm wide circle marked on shaved skin on the upperside of the left shoulder. The skinfold thickness was determined before PHA injection, and 24 h after with a calliper. For each animal, a mean increase in skinfold thickness (24-h thickness – pre-injection thickness) was calculated using two perpendicular measurements performed on the same shoulder.

Five weeks after grouping, cows were injected subcutaneously with 20 mg ovalbumin (OVA, Sigma Chemical) dissolved in 4 ml sterile saline solution and emulsified in an equal volume of incomplete Freund's adjuvant. Another injection without adjuvant was repeated 8 weeks after grouping. Antibody titre was evaluated before the first antigen administration (pre-immunization) and at 10-day intervals after the first immunization (eight samples) on serum collected from a jugular vein using vacuum tubes. An enzyme-linked immuno-sorbant assay (ELISA) was performed in 96-well, U-bottomed microtitre plates. Wells were coated with 100 µl of antigen (10 mg of OVA per ml of phosphate buffer) at 4ºC for 12 h, washed and incubated with 1% milk powder (200 µl) at 37ºC for 1 h to reduce non specific binding. After washing, the serum (1 : 100 dilution in phosphate buffered saline (PBS); 100 µl per well) was added and incubated at 37ºC for 1 h. Buffer alone was used as negative control. The extent of antibody binding was detected using a horseradish peroxidase-conjugated anti-bovine IgG (Sigma Chemical). Plates were again incubated for 1 h at 37ºC after washing and adding 100 µl per well (1 : 2000 in PBS) of conjugate. Buffer alone provided blank wells. Following a further washing, 100 µl of substrate (1 mg of tetra methyl benzidine free base tablets, 1 ml dimethyl sulphoxide, 9 ml phosphate-citrate buffer, 2 µl H_2O_2) were added to each well. After 30 min, 50 µl of 2 mol/l H_2SO_4 was added to terminate reactions. Optical density was measured at a wavelength of 450 nm (OD_{450})using an ELISA reader. The intra- and inter-assay CVs were 0·04 and 0·05, respectively. The assay was optimized in our laboratory for concentrations of coating antigen, serum and detector antibody.

Adrenal response test

At week 23 after grouping, animals were injected with 1·98 IU per kg $M^{0.75}$ (Fisher *et al.*, 1997) of porcine ACTH (Sigma Chemical) into the jugular vein. Blood samples for evaluation of cortisol concentration were collected in vacuum tubes immediately prior to injection and 1, 2 and 4 h after injection. Heparinized blood was centrifuged and the resultant plasma stored at –20ºC until assayed. Hormone concentration was determined using a bovine radio-immuno-assay kit (Immunotech, Marseille, France). The sensitivity of the assay was 20 nmol/l. The inter- and intra-assay coefficients of variation were 0·089 and 0·039, respectively.

Body condition score, milk production and blood metabolite analyses

The buffalo cows were scored for body condition (BCS) at the beginning and at the end of experimental period (scale 0 to 5 units; Edmonson *et al.*, 1989).

Animals were milked twice daily at 05·30 and 17·30 in a herring-bone parlour using pipeline milking machines (Tecnozoo, Zelo Buon Persico, Italy). Daily milk yield, and milk fat, protein and somatic cell content were determined 4 weeks after grouping and then at monthly intervals (five recordings). Milk yield was recorded by means of graduated measuring cylinders attached to individual milking units. Subsequently, individual milk samples were withdrawn from cylinders and placed in 40-ml plastic containers. Samples were analysed for fat and protein content (International Dairy Federation (IDF), 1990) using an infrared spectrophotometer (Milko Scan 605; Foss Electric, Hillerød, Denmark) and somatic cell count (IDF, 1995) using a Somacount 300 (Bentley Instruments, Chaska, USA).

Blood samples were collected from the jugular vein in vacuum tubes 10 days after grouping and then at 10-day intervals. After centrifugation plasma aliquots were frozen stored until metabolite determinations. Glucose, cholesterol, triglicerides, NEFA, urea, creatinine, albumins, total proteins, calcium, phosphorus, bilirubin, aspartate aminotransferase, alanine aminotransferace, alkaline phosphatase, lactate dehydrogenase and gamma glutamyl transferase were determined using a Monarch 1000 Chemistry System, (International laboratory, Lexington, MA, USA).

Statistical analysis
Data were analysed with the Statistical Analysis Systems Institute package (SAS, 1990). Due to the lack of individual recordings, behavioural data were analysed using the day of observation as the experimental unit and treatment and period of observation (07:30 to 09:00, 09:15 to 12:00 and 12:15 to 15:15 h) as main factors. For all other dependent variables the cow was used as the experimental unit. During the behavioural observations, drinking, locomotion, self-grooming, agonistic and non agonistic interactions were rare. Thus, no statistical analysis was executed for these variables. Behavioural data were analysed using an analysis of variance with two factors (treatment and period of observation). An angular transformation was used to homogenize variance of feeding activity. Since grazing and bathing activities were recorded only for TS animals, they were analysed using an ANOVA with one factor (period of observation). Immunological, cortisol, blood metabolites and milk data were analysed with analyses of variance for repeated measures with treatment (IS and TS) as a non-repeated factor and time and time × treatment as repeated factors. Square root (cortisol, NEFA and phosphorous data) and logarithmic (somatic cell count) transformations were used to normalize skewness. Initial and final BCS data were separately analysed using an ANOVA with one factor (treatment). Where appropriate, the t test was used to identify differences between least-square means.

Results

Behavioural recordings
Table 2 shows relevant results obtained from behavioural recordings. Lying down was not affected by treatment. There was a significant period of observation effect ($P < 0{\cdot}001$). More animals lay down at periods 2 (09:15 to 12:00 h; least-square mean±s.e. : 0·59±0·035) and 3 (12:15 to 15:15 h; 0·54±0·035) than period 1 (07:30 to 09:00 h; 0·26±0·038).

The proportion of animals idling was higher in group IS than in group TS ($P < 0{\cdot}001$), whereas there was no influence of period of observation on this behavioural activity.

More IS buffalo cows were observed eating at the manger than TS animals ($P < 0{\cdot}001$). Feeding was not affected by period of observation, whereas ruminating was higher at period 2 (0·17 ± 0·014) and 3 (0·16 ± 0·014) than period 1 (0·08 ± 0·015; $P < 0{\cdot}001$). In addition, more IS animals were observed eating from 07:30 to 09:00 h (0·29±0·023) than from 09:15 to 12:00 h (0·17±0·021; $P < 0{\cdot}01$). Conversely, no significant differences among periods of observation were found for TS animals, thus explaining the significance of the interaction space allowance × period of observation ($P < 0{\cdot}01$).

Table 2 *Effect of housing system on behavioural categories (least-square mean and s.e.) observed over 8-h periods in six sessions (data are expressed as proportion of animals observed in each category)*

	Housing system†			
	IS	TS	s.e.	Significance
Lying down	0·47	0·46	0·03	
Location in the sun	0·21	0·74	0·02	***
Idling	0·45	0·08	0·03	***
Feeding	0·23	0·13	0·01	***
Ruminating	0·15	0·13	0·01	
Grazing	NR	0·21	0·11	
Wallowing	NR	0·31	0·08	

† IS = intensive system; TS = traditional system; NR = not recordable.

A higher proportion of TS animals were observed in the sun ($P < 0{\cdot}001$). Irrespective of the treatment the proportion of animals recorded in the sun was highest in period 1 (0·58±0·026), intermediate in period 2 (0·47±0·021) and lowest in period 3 (0·39±0·021). The mean ambient temperatures of periods 1, 2 and 3 were 19·9, 24·5 and 29·8ºC, respectively.

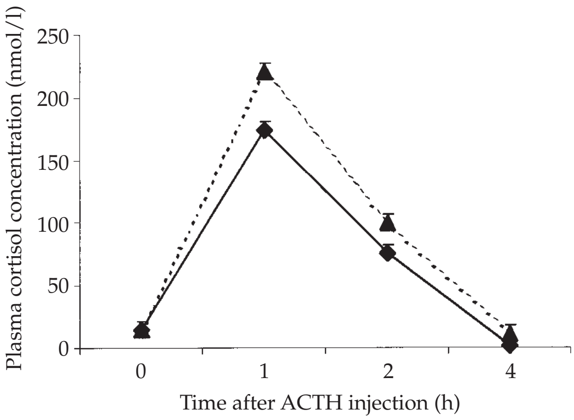

Figure 1 Plasma cortisol concentration (least-square mean ± s.e.) after intravenous injection of exogenous ACTH in buffalo cows kept in intensive (IS ····▲····) and traditional (TS —◆—) systems.

Table 3 *Milk yield, protein, fat and somatic cell count (least-square mean ± s.e.) during the lactation of buffalo cows*

	Month after parturition					
	1	2	3	4	5	s.e.
Milk yield (kg/day)	9·90	9·87	8·04	7·28	6·74	0·253
Protein concentration (g/kg)	45·4	43·6	45·2	46·8	43·6	1·56
Fat concentration (g/kg)	75·8	74·3	80·9	82·7	81·6	0·58
Somatic cell count (cells per ml)	228 172	229 975	284 354	†	†	34 683

† Not recorded.

Grazing and bathing activities were recorded only for TS animals. Grazing activity involved more animals at 07:30 to 09:00 h (0·44±0·025) compared with 09:15 to 12:00 h (0·09±0·023, $P < 0{\cdot}001$) and 12:15 to 15:15 h (0·12±0·023, $P < 0{\cdot}001$). The proportion of animals performing bathing behaviour was higher at period 2 (0·46±0·054) than period 1 (0·20±0·058, $P < 0{\cdot}01$) and 3 (0·27±0·054, $P < 0{\cdot}05$).

Immune responses

Delayed type hypersensitivity to a percutaneous injection of PHA was not affected by treatment. Animals showed a higher reactivity to PHA at week 22 (7·73 ± 0·4 mm) after grouping compared with week 6 (6·32 ± 0·4 mm, $P < 0{\cdot}0184$).

Immunoglobulin G (IgG) concentration was not affected by treatment, whereas a significant effect of time of sampling was clearly evident ($P < 0{\cdot}001$). The antibody titre (pre-immunization value = 0·07±0·03 OD_{450}) reached a peak 40 days after the first immunization (1·33±0·03 OD_{450}).

Cortisol response to exogenous ACTH

The changes observed in the plasma levels of cortisol after ACTH challenge are presented in Figure 1. Both treatment and time influenced cortisol concentration ($P < 0{\cdot}05$ and $P < 0{\cdot}001$, respectively), whereas no significant space allowance × time interaction was found. Group TS showed a lower concentration of cortisol (65·99 ± 5·23 nmol/l) than animals of group IS (86·02 ± 5·23 nmol/l). Peak concentrations occurred in the samples collected 1 h after ACTH injection, whereas the samples taken before hormone challenge presented a higher cortisol concentration (14·16 ± 5·33 nmol/l) than those withdrawn 4 h after (5·94 ± 5·33 nmol/l; $P < 0{\cdot}01$).

BCS, milk production and blood metabolite status

Initial (3·17 and 2·86, s.e. = 0·12) and final (3·87 and 3·55 BCS units; s.e. = 0·11, for IS and TS, respectively) BCS did not differ significantly between groups.

Treatment did not affect daily milk yield, milk fat and milk protein concentrations or somatic cell

Table 4 *Metabolic variables† (least-square mean ± s.e.) of buffalo cows kept in intensive (IS) and traditional (TS) housing systems*

	Housing system			Significance of effects		
	IS	TS	s.e.	Treatment	Time	Treatment × time
Glucose (mmol/l)	3·48	3·44	0·05		***	
Cholesterol (mmol/l)	2·81	2·74	0·12		***	
Triglycerides (mmol/l)	0·10	0·10	0·0			
NEFA (mmol/l)	0·32	0·26	0·03		***	
Urea (mmol/l)	7·96	7·88	0·19		***	
Creatinine (µmol/l)	199·34	135·16	39·52			
Albumins (g/l)	41·80	40·92	0·37		***	
Total proteins (g/l)	86·85	86·50	1·05		***	
Calcium (mmol/l)	2·49	2·50	0·02		***	
Phosphorus (mmol/l)	1·77	1·68	0·06		***	
Bilirubin (µmol/l)	4·34	3·80	0·50		**	
AST (U/l)	146·84	164·68	6·74			
ALT (U/l)	55·35	58·49	2·40		***	
ALP (U/l)	370·11	443·12	54·91		***	
LDH (U/l)	1500·41	1603·17	50·15		***	
GGT (U/l)	26·95	27·43	1·62		*	

† Abbreviations: NEFA = non-esterified fatty acids; AST = aspartate aminotransferase; ALT = alanine aminotransferase; ALP = alkaline phosphatase; LDH = lactate dehydrogenase; GGT = gamma glutamyl transferase.

count. As expected, an effect of time on these variables was detected (Table 3).

Table 4 shows that blood metabolite levels were not affected by treatment and there were no treatment × time interactions, whereas, as expected, time from calving influenced most of these variables ($P < 0{\cdot}05$ to $P < 0{\cdot}001$).

Discussion

Confinement of domestic animals radically changed their life by affecting the habitat, activity and capability of individuals to choose whether to stay or leave. Under confinement, animals have less space for movement, and environmental stimuli facilitating the expression of proper species-specific behaviours are often lacking. The concept of boredom has been used to explain the state of animals in these conditions (Wemelsfelder, 1993). Other authors have stated that a high level of idling can be considered an abnormal behavioural expression (Barnett *et al.,* 1992; Hanlon *et al.,* 1994; Chaplin *et al*., 2000). A number of farm animals are able to display long periods of inactivity that may reflect the inadequacy of their surrounding environment (Fraser and Broom, 1990). In our study buffalo cows kept in intensive conditions and having no access to ample yards and potholes extended their periods of idling. Conversely, TS animals had the opportunity to perform other basic natural behaviours such as looking for food and bathing which were denied to IS cows. In fact, in the former group a high proportion of animals was observed bathing in the potholes and grazing. As a consequence, a lower proportion of these animals ate at the manger, irrespective of the period of observation. Although the data do not describe time budgets it was expected that the wider range of activities available to the TS animals would have implied trade-offs for the set of activities that were common to both groups. Therefore, it was not surprising that idling was greater in the IS group and perhaps this represents substitution activity for time that would otherwise have been spent grazing and wallowing.

A higher proportion of IS cows was observed eating early in the day when the environmental temperature was lower than later when it was hotter. In the same period of the day (07:30 to 09:30 h) more TS buffaloes grazed in the yard; these observations are consistent with the well known phenomenon that high temperatures depress ingestive activities. However, the presence of potholes facilitated thermoregulation of the TS animals; they were observed in the sun in higher proportions than were IS cows.

Basal cortisol level is not considered a very effective means by which to study the functional state of the adrenal cortex (Rushen, 1991; Pearce and Paterson, 1993). In chronic stress after an initial onset, plasma glucocorticoid concentration tends to fall back towards control levels (Harbuz and Lightman, 1992), thus the stressful condition can be overlooked. Conversely, the ACTH test is more sensitive and alteration of the adrenal response to ACTH stimulation has been observed even without any variation in basal plasma corticosteroids (Friend *et al*., 1979). This is likely to be due to the fact that exposure of an animal to a chronic stress alters the responsiveness of the adrenal gland to subsequent acute stressors. A number of authors have observed an effect of housing conditions on cortisol response to exogenous ACTH challenge in cattle (Friend *et al*., 1979 and 1985; Dantzer *et al.,* 1983) and pigs (Pearce and Paterson, 1993). It has been stated that the pituitary-adrenal axis can be affected by the degree of control an animal is able to exert over the surrounding environment (Dantzer *et al*., 1983). The present results suggest that the lack of space, lower degree of initiative and inability to perform some species-specific behaviours induced a higher reactivity of the adrenal gland as a possible consequence of chronic stress.

Neither cellular nor humoral immunity were affected by the treatment. A previous study has reported reduced IgG production and decreased skinfold thickening in buffalo calves kept at restricted space allowance (Grasso *et al.,* 1999). These different results may be due to the age of the experimental subjects : young animals may be more sensitive to environmental stressors. The reduced response to PHA percutaneous injection observed at week 6 compared with week 22 may be attributed to an impairment of immune functions in the peripartum period (Mallard *et al*., 1998).

Housing system did not markedly affect the metabolic status of buffalo cows which instead was clearly influenced by time from calving.

In conclusion, although no effect on immune response and metabolic profile could be detected, the provision of an housing system close to natural conditions was able to improve the welfare of buffalo cows as indicated by the expression of some species-specific natural behaviours. Such conditions also determined a lower adrenal cortex response to ACTH injection possibly as a consequence of the higher degree of initiative allowed to TS cows.

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