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Influence of space allowance on the welfare of weaned buffalo (*Bubalus bubalis*) calves

F. Napolitano[a,*], G. De Rosa[b], F. Grasso[b], C. Pacelli[a], A. Bordi[b]

[a] *Dipartimento di Scienze delle Produzioni Animali, Università degli Studi della Basilicata, Via N. Sauro 85, 85100 Potenza, Italy*

[b] *Dipartimento di Scienze Zootecniche e Ispezione degli Alimenti, Università degli Studi di Napoli 'Federico II', Via Università 133, 80055 Portici (Napoli), Italy*

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Abstract

Twenty weaned female buffalo calves were used to evaluate the effect of space allowance in relation to their body surface area on a range of behavioural and physiological parameters. Body surface area in m^2 was calculated as 0.12 body weight$^{0.60}$. Ten calves received 50% of body surface as space allocation (Group 50), 10 others received 90% of body surface area (Group 90). Animals in Group 50 lay with a lower number of outstretched legs than calves in Group 90. Buffaloes from Group 50 were observed standing more frequently than animals from Group 90 ($P<0.001$). The proportions of idling ($P<0.01$) and lying idle observations ($P<0.001$) were higher for Group 90 than for Group 50. Group 90 performed a higher number of non-agonistic interactions than Group 50 ($P<0.01$), whereas the opposite was observed for the number of agonistic interactions ($P<0.01$). When exposed to open field testing, Group 50 animals displayed an increased duration of movement, number of galloping events and more vocalisation. Neither immune responses to phytohemagglutinin and ovalbumin nor the cortisol response to exogenous ACTH were affected by treatment. It was concluded that 50% of body surface area may be an inadequate space allowance for weaned calves.

Keywords: Animal welfare; Behaviour; Buffalo; Immune response; Open field test; Space allowance

1. Introduction

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, on one hand, led to renewed economic interest in this species whose milk is used to make 'mozzarella cheese' and, on the other, imposed a unique and extreme environmental stress. Space restriction presents both physical and psychological conditions which may result in a dramatic 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. Following this it was deemed necessary to extend the study to consider older animals.

At present, no legislation on buffalo space allowances exist either at Italian or at European level. One

* Corresponding author. Tel.: +39-0971-202-294; fax: +39-0971-470-719.

E-mail address: napolitano@unibas.it (F. Napolitano).

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way to set minimum space allowance is based on body surface area, as suggested by Hurnik and Lewis (1991) for pigs and cattle.

Minimum space allocation should allow at least the three fundamental static postures (standing, sternal recumbence and lateral recumbence). However, animals have space needs that are well beyond ground occupation, because additional space is needed to express behaviours essential to the animals, e.g., feeding, locomotion, etc., and related to the species (i.e., wallowing) or the age (i.e., playing). The present study investigated the effect of floor-space allowance on calf welfare through the behavioural, endocrine and immune responses.

2. Materials and methods

2.1. Experimental design

Twenty weaned female buffalo calves were used. Animals were 18 weeks old with a mean live weight of 120 kg at the start of the study. Animals were randomly allocated to two treatments differing in pen sizes in relation to their body surface. Ten calves were group-housed at 50% of body surface as space allowance (Group 50) and 10 others were group-housed at 90% of body surface area (Group 90). Body surface area was computed from body weight using the following formula:

$$\text{Body surface area } (\text{m}^2) = 0.12 \text{ body weight } (\text{kg})^{0.60}$$

as indicated by Hurnik and Lewis (1991). Those authors adopted 50% of body surface area as minimum space to be assigned to each animal based on the consideration that three-dimensional objects would always occupy less than 50% of their surface.

Pen size was determined using the mean calf weight for each group to account for increase in body weight, hence, body surface area. Buffalo calves were housed in slatted floor pens and pen sizes adjusted at monthly intervals. All animals were weighed when the groups were constituted and, subsequently, at monthly intervals. For group 50, the corresponding space allowances per calf were 1.1 and 1.9 m^2 at the first and last month of the experiment, respectively, whereas Group 90 calves received 1.9 and 3.4 m^2/animal. The corresponding initial and final dimensions were 5.0×2.2 m and 5.0×3.8 m for Group 50 and 5.0×3.8 m and 5.0×6.8 m for Group 90. For both groups space at manger was 50 cm/calf. The experiment lasted for 30 weeks.

Every day at 08:30 h, subjects were offered unified feed ad libitum. For each group two drinking bowls were available all the time.

2.2. Behavioural recordings

Observations were started 2 weeks after grouping. Animals were subjected to seven sessions of instantaneous scan sampling at 4-week intervals. With this method on the instant of each sample point the observer records whether or not the behaviour pattern is occurring. Observations were made every 10 min over a 6-h period (10:00 to 16:00 h), giving a total of 36 sets of observations per session. On observation days, an observer for each treatment walked slowly past the front of each pen from a distance of 4 m and recorded posture (standing or, when lying, number of outstretched legs) and activity such as feeding (selection, prehension and mastication), ruminating, drinking, locomotion, idling (opened or closed eyes, but no other overt activity). Subsequently, the proportions of standing idle and lying idle activities were calculated scoring the animals that were simultaneously standing and idling or lying and idling, respectively. Behavioural variables were expressed as proportion of observations calculated as number of observations in which the activity was performed/36 (number of scan samplings). In addition, the average number of outstretched legs was determined in relation to the number of observations during which the animal was lying down. 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, where during each session these behavioural categories were recorded continuously.

2.3. Immune responses

Phytoemagglutinin (PHA) was used to perform a skin test based on non-specific delayed type hypersensitivity. At weeks 7 and 30 PHA (1 mg, Sigma) dissolved in 1 ml of sterile saline solution was injected intradermally into the middle of 2-cm wide circles marked on shaved skin on the upperside of each 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−preinjection thickness) was calculated using the two measurements gathered from shoulders.

Calves were injected subcutaneously with 10 mg (5 mg per shoulder) of ovalbumin (OVA, Sigma) dissolved in 2 ml of sterile saline solution and emulsified in an equal volume of incomplete Freund's adjuvant 3 weeks after grouping. Two other injections without adjuvant were repeated 7 and 22 weeks after grouping. Antibody titer was evaluated before the first antigen administration (preimmunization), at weekly intervals after the first immunization (four samples) and fortnightly after the second (four samples) and third injection (three samples) on serum collected from jugular vein using vacuum tubes. An enzyme-linked immunosorbent assay (ELISA) was performed in 96-well, U-bottomed microtiter plates. Wells were coated with 100 μl of antigen (10 mg of OVA/ml of phosphate buffer) at 4 °C for 12 h washed and incubated with 10% milk powder (200 μl) at 37 °C for 1 h to reduce non-specific binding. After washing, the serum (1:100 dilution in PBS; 100 μl per well) was added and incubated at 37 °C for 1 h. Buffer alone provided negative control wells. The extent of antibody binding was detected using a horseradish peroxidase-conjugated anti-bovine IgG (Sigma). Plates were again incubated for 1 h at 37 °C after washing and adding 100 μl per well (1:10 000 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 sulfoxide, 9 ml phosphate−citrate buffer, 2 μl H_2O_2) was added to each well. After 30 min 50 μl of 2 M H_2SO_4 was added to terminate reactions. Optical density was measured at a wavelength of 450 nm using an ELISA reader. The intra- and inter-assay CV were 3.5 and 6.5%, respectively. The assay was optimized in our laboratory for concentrations of coating antigen, serum and detector antibody.

2.4. Isolation test

At weeks 16 and 30 calves were subjected to an isolation test. Each animal was exposed to a novel environment (an 18×5-m outdoor paddock) and isolated from tactile and visual contact with other animals for 5 min. However, they could receive auditory and olfactory stimuli from conspecifics. Latency time to the first movement, duration of movement and number of vocalisation, galloping, flight attempt, buck-kicking and sniffing were recorded.

2.5. Adrenal response test

At week 28 animals were injected with 1.98 i.u. per kg L $W^{0.75}$ (Fisher et al., 1997) of porcine ACTH (Sigma) 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 −20 °C until assayed. Hormone concentration was determined using a bovine RIA kit (Immunotech, Marseille, France). The sensitivity of the assay was 20 nmol/l. The inter- and intra-ssay coefficients of variation were 8.9 and 3.9%, respectively.

2.6. Statistical analysis

Data were analysed with the Statistical Analysis System package (SAS, 1990). Behavioural, immunological and cortisol data were analysed with analyses of variance for repeated measures with space allowance as a non-repeated factor and time and time×space allowance as repeated factors. A $\log_{10}(1+\text{value})$ transformation was used to normalize skewness in the number of outstretched legs, non-agonistic and agonistic interactions. Where appropriate, *t*-test was used to identify differences between least squares means.

Average daily weight gain was analysed using ANOVA with one factor (space allowance).

3. Results

3.1. Behavioural recordings

Table 1 shows relevant results obtained from behavioural recordings. Space allowance markedly affected the number of outstretched legs ($P<0.001$).

Table 1
Effect of space allowance on behavioural categories (least squares mean±S.E.M.) observed over 6-h of observations in seven sessions

	Space allowance		S.E.M.	*P* value
	50% BS	90% BS		
No. of outstretched legs/animal	0.71	1.00	0.04	0.001
Standing[a]	0.65	0.49	0.02	0.001
Idling[a]	0.18	0.25	0.02	0.01
Standing idle[a]	0.09	0.06	0.01	ns
Lying idle[a]	0.09	0.19	0.02	0.001
Feeding[a]	0.41	0.30	0.02	0.001
Ruminating[a]	0.26	0.31	0.02	0.05
No. of non-agonistic interactions/animal	5.23	7.47	0.54	0.01
No. of agonistic interactions/animal	7.77	2.06	0.52	0.01

BS, body surface area.
[a] Number of observations in which the activity was performed/total number of scan samplings.

Animals in a restricted space lay with a lower number of outstretched legs than calves provided with more free space.

Buffaloes from Group 50 were observed in the standing posture more frequently than animals from Group 90 ($P < 0.001$).

The proportions of idling ($P < 0.01$) and lying idle observations ($P < 0.001$) were higher for Group 90 than for Group 50, whereas the number of times animals were observed standing idle tended to be lower for calves with more space allowance ($P = 0.12$).

Although Group 90 ate less frequently than Group 50 ($P < 0.001$), the proportion of ruminating was lower for buffaloes kept in a restricted space ($P < 0.05$). Both behavioural categories increased over weeks ($P < 0.001$).

Mean daily weight gain was similar for the two experimental groups (0.85 and 0.88±0.02 kg for Groups 50 and 90, respectively).

Non agonistic and agonistic behaviours showed opposite patterns. Group 90 performed a higher number of non-agonistic interactions than Group 50 ($P < 0.01$), whereas number of agonistic interactions was higher between animals receiving 50% of body surface as space allowance than between subjects having 90% of body surface ($P < 0.001$).

3.2. Behavioural response to isolation

There was no effect of space allowance on latency time to the first movement or on number of sniffings, flight attempts and buck-kickings. Conversely, duration of movement ($P < 0.05$), number of gallopings

Table 2
Effect of space allowance on behavioural responses (least squares mean±S.E.M.) during two 5-min isolation tests

	Space allowance		S.E.M.	*P* value
	50% BS	90% BS		
Latency time to first movement (s)	5.80	7.20	0.80	ns
Duration of movement (s)	106.30	87.70	5.60	0.05
Gallop, no.	17.10	14.00	0.80	0.01
Vocalisation, no.	62.10	40.50	6.40	0.05
Sniffing, no.	13.80	15.10	1.40	ns
Flight attempts, no.	0.50	0.30	0.20	ns
Buck-kicking, no.	1.40	1.70	0.40	ns

BS, body surface area.

($P<0.01$) and vocalisations ($P<0.05$) were affected by space allowance (Table 2).

Calves performed a higher number of gallopings at week 30 after grouping than at week 16 (10.7±1.08 vs. 20.4±1.08; $P<0.001$).

3.3. Cortisol response to exogenous ACTH

On overall the concentration of plasma cortisol after ACTH injection tended to be higher in buffaloes from Group 50 (74.5±7.6 nmol/l) compared with Group 90 (59.4±7.6 nmol/l), but these differences were not statistically significant. Conversely, time of sampling had a significant effect on hormone concentration ($P<0.001$). Peak concentrations occurred in the samples taken 1 h after ACTH injection, whereas cortisol concentrations decreased to pre-injection concentrations 4 h after the treatment with exogenous ACTH (Fig. 1). No significant space allowance×time interaction was found.

3.4. Immune responses

Delayed type hypersensitivity to a percutaneous injection of PHA was not affected by space allowance. At 24 h post injection Groups 50 and 90 displayed a skinfold thickening of 3.32 and 3.71 mm (S.E.M.= 0.324), respectively.

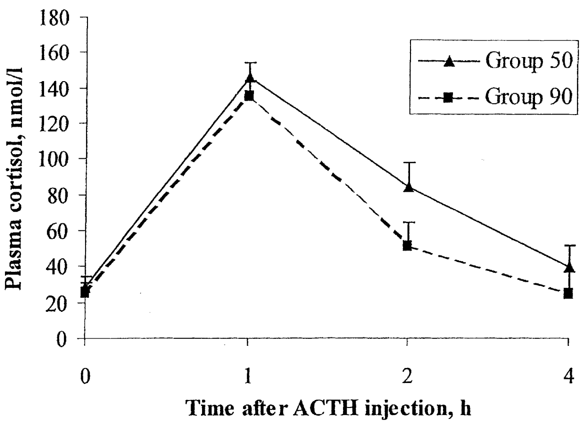

Fig. 1. Least squares mean (±S.E.M.) of plasma cortisol concentrations in buffalo calves after intravenous injection of exogenous ACTH.

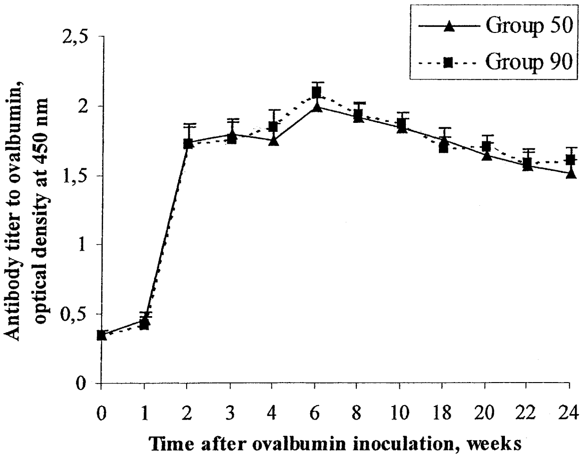

Fig. 2. Effect of space allowance on antibody response to OVA injected subcutaneously at weeks 0, 4 and 18 (least squares mean±S.E.M.).

IgG concentration was not affected by group, whereas a significant effect of the sampling week was obviously evident ($P<0.001$). The antibody titer increased 2 weeks after the first immunisation and reached a plateau until the second injection. The third injection of ovalbumin did not markedly affect serum antibody levels (Fig. 2).

4. Discussion

At the beginning of the experiment the space allowance for Group 50 was slightly lower than that recommended by Directive 91/629/EEC on the laying down minimum standards for the protection of bovine calves (European Union, 1991) as amended by Directive 97/2/EC (European Union, 1997). The Directive recommends 1.5 m^2 for calves of less than 150 kg of live weight (LW), whereas in the present study 1.1 m^2/calf was used. However, there was no cause for concern as our final space allowance (1.9 m^2/head) was higher than that recommended by the Directive for calves of more than 220 kg LW (1.8 m^2/calf). In addition, throughout the experiment each animal received more space than that suggested by Directive 86/609/EEC on the protection of animals used for experimental and other scientific purposes (European Union, 1986).

Lying and resting behaviours play a central role in maximising animal comfort. Uncomfortable housing conditions may prevent animals from lying down reducing well-being and productivity (Leonard et al., 1994). Space restriction had a substantial effect on standing and lying behaviours. The amount of time spent lying by Group 90 (51%) was similar to that previously reported and reviewed by Haley et al. (2000) for loose housed cows, whereas Group 50 displayed a markedly lower amount of lying behaviour (35%). This latter value was even lower than that expressed by cows housed in tie-stalls (Deschamps et al., 1989; Krohn and Munksgaard, 1993). A reduced space allowance may make it more difficult to perform the movements needed to lie down and this may explain the differences observed between the groups. A crowded environment may reduce the ease with which animals change position from standing to lying by increasing the risk of falls. In addition, these differences could be also due to the fact that lying patterns were restricted by other calves. In particular, buffaloes could cause the interruption of pen mate resting by stepping on them. This latter hypothesis is also supported by data on leg positions. According to Le Neindre (1993), group reared calves stretch their legs less often than calves in large stalls. In the present study, a reduction in space allowance resulted in animals assuming postures with a higher number of bent legs, possibly in order to reduce the chance to be trodden on. Accordingly, buffaloes with higher space allowance showed greater levels of idling compared to animals housed in a restricted space possibly because they were not disturbed by other animals' activities, as also stated by Grasso et al. (1999). More importantly, Group 50 displayed decreased levels of lying idle, which is likely to represent a fundamental form of resting. Deprivation of lying and resting may have detrimental effects on animal welfare (Munksgaard and Simonsen, 1996).

In agreement with Barnett et al. (1992), it was observed that a shortage of free space increased aggression. That effect is probably due to a reduced ability of subordinate animals to withdraw from the presence of a dominant animal when the space allowance was lower.

In addition, increased levels of standing and active behaviours may be determined by forced non-agonistic interactions, which in turn, can induce animals to fight or flee (Hanlon et al., 1994). In fact, these behavioural categories may make them more prepared to react to threats and aggression in a restricted environment. In our study, space reduction resulted in increased aggression and reduced non-agonistic interactions. Forced non-agonistic interactions induced animals to fight, thus reducing motivation in performing passive interactions. Meunier-Salaun et al. (1987) suggested that crowding might determine non-agonistic avoidance behaviour aimed at preventing an increase in aggression under circumstances of space restriction.

The behaviour of animals responding to a stimulus is likely to be the result of a combination of different motivational systems competing for animal behaviour control (Rushen, 2000). In bovine calves, De Passillé et al. (1995) classified the behaviours recorded while animals were tested in an open field according to the motivations that might underlie each response. These authors described three main clusters (fear, exploration and locomotion) using factor analysis. Vocalisation was included among variables indicating fear, whereas ambulatory behaviours were associated with locomotory motivation. Jensen (1999) observed that animals housed in less spacious environments have a lowered threshold for release of locomotory behaviours which were somehow suppressed during confinement.

When exposed to the open field test, animals receiving 50% of body surface as space allocation displayed increased duration of movement, number of galloping and more vocalisation. Space restriction may prevent animals from performing certain types of locomotory behaviour that are regularly expressed in less restrictive conditions. Therefore, the increased levels of locomotory behaviour in these animals may reflect a build-up of internal motivation to perform locomotion and gallop while calves were housed in a more confined environment. Numerous authors have observed that chronic suppression of free locomotion results in an increased expression of this behaviour after release from confinement (Dellmeier et al., 1985; De Passillé et al., 1995; Jensen, 1999). Furthermore, Dellmeier et al. (1985) described in farm animals a phenomenon termed 'damming up' related to the expression of behaviours which are somehow exaggerated compared to the suppressed behavioural categories. They found increased levels of buck-kicking, cantering and trotting with increasing degree of confinement. Accordingly, in the present study, animals housed in a restricted space exhibited a higher number of galloping events which can be correlated to the damming up phenomenon. In addition, novelty may induce increased levels of exploration and locomotion aimed to give the animal information about an unknown environment.

Neither immune responses (skin test and antibody response to ovalbumin) nor the cortisol response to exogenous ACTH were affected by treatment with different space allowances. In particular, skin thickening was low in animals of both groups thus indicating a possible immune suppression induced by both treatments. Little information on the effect of space allowance on buffalo welfare is available. Therefore, the absence of differences between the groups observed in the present study for immune and endocrine variables is not easy to explain.

5. Conclusions

Space restriction to 50% of body surface area resulted in some modifications of buffalo resting and non-agonistic behaviour. Increased levels of locomotory behaviour and vocalisation during open field testing suggested higher levels of motivation to move and be fearful, respectively.

It was concluded that for weaned calves 50% of body surface area may be a less adequate space allowance than 90%. It is likely that the provision of an environment more close to natural conditions than the slatted floor would have determined a further increase of the welfare of buffalo calves. However, in Italy these conditions are not used for growing animals.

Cortisol and immune responses were unaffected by space allowance. Therefore, based on the present results, behavioural measurements seem to be more sensitive for the detection of stressful conditions as compared to other commonly used endocrine or immune indicators of welfare.

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