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SPACE ALLOWANCE OF BUFFALO CALVES AND ITS EFFECT ON WELFARE AS INDICATED BY THEIR BEHAVIOR, ADRENAL RESPONSE AND ENVIRONMENTAL POLLUTION

(With 6 Tables)

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المساحة المتاحة من الأرضية ومدى تأثيرها على إرياحية عجلات الجاموس وذلك بالاشارة إلى الدلالات السلوكية والإستجابات الهرمونية والملوثات البيئية الدقيقة

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أجريت هذه التجربة على عدد ٤٥ من عجلات الجاموس التى تم فطامها والتى كانت فى نفس العمر ومتوسط الوزن عند بداية التجربة. قسمت العجول بطريقة عشوائية إلى ٣ مجموعات متساوية وسكنت حيوانات كل مجموعة داخل ٣ مساكن خاص بها تحت الظروف البيئية السائدة(كل ٥ عجلات داخل مسكن مستقل). كانت المساحة المتاحة من الأرضية لحيوانات المجموعات الثلاث تعادل ٥٠, ٧٠, ٩٠% على التوالى من مساحة سطح جسم الحيوانات مع تغيير قياسات كل مسكن شهريا لتوفير المساحة المطلوبة. تم دراسة سلوكيات حيوانات كل مجموعة مع أخذ عينات دم من هذه الحيوانات لتحديد مستوى هرمون الكورتيزول ونسبة الجلوكوز بها وكذلك تم تحديد مستوى التلوث الميكروبى لهواء وارضية كل مسكن من المساكن. أثبتت النتائج التى تم الحصول عليها أن المساحة المتاحة من الأرضية بنسبة ٥٠% من مساحة سطح الجسم غير كافية على الاطلاق لعجلات الجاموس التى فطمت لما لها من تأثير معنوى واضح على حالتها السلوكية مع وجود ارتفاع معنوى لمستوى هرمون الكورتيزول وكذلك نسبة الجلوكوز فى دم هذه الحيوانات وارتفاع معنوى لمستوى التلوث الميكروبى لهواء وارضية المساكن. وقد أوصت الدراسة بإتاحة مساحة من الارضية لا تقل عن ٧٠% من مساحة سطح الجسم فى حالة التسكين المكثف الاقتصادى لعجلات الجاموس المفطومة وذلك لحمايتها من حالات الاجهاد والتلوث الميكروبى داخل المساكن.

SUMMARY

Forty five weaned female buffalo calves were used in this investigation. Animals were 5 months old with a mean live weight of 100 kg at the start of the study. Animals were randomly assigned to 3 groups, 15 calves each. Animals of each group were divided into 3 subgroups, 5 calves each and each subgroup was housed under the prevalent environmental conditions in a separate straw bedded floor pen. Groups were randomly assigned to three treatments differing in the space allowance of each calf which equals 50, 70 and 90% of its body surface. Body surface area was calculated as 0.12 body weight (kg) $^{0.60}$. Pen size was adjusted at monthly intervals to meet the experimented space allowance. Behavioral observations, isolation test, adrenal response and microbial examination of air and bedding materials were carried out during this investigation. The obtained results indicated that 50% of body surface area is inadequate space allowance for weaned buffalo calves compared to 70 or 90%. Space restriction to 50% of body surface area of weaned buffalo calves affected calf welfare by increasing their stress, modifying their behavior and increasing microbial contamination of air and bedding materials in their environment. To fulfill the recent intensification and economic techniques of rearing weaned buffalo calves and, at the same time protect them from facing housing stress, it is recommended to house the weaned buffalo calves in their pens with a space allowance equaling at least 70% of its body surface area.

Key words: *Buffalo, welfare, space allowance, behavior, adrenal, microbial pollution.*

INTRODUCTION

Housing protects animals from adverse weather conditions and provides structured management (feeding, drinking, health check, etc.) under controlled conditions. Recent intensification of rearing techniques has, on one hand, led to renewed economic interest 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). An insufficient space allowance has been shown to directly affect the level of gaseous pollutants and air borne particles in animal dwellings (Sevi *et al.,* 1999). Pollutants may pose a danger to health and life of animal (Novák *et al.,* 2005) and affect the general performance of animals (Zucker *et al.,* 2000). Insufficient space allowances induce a repeated state of stress with a reduction of the productivity (Ingvartsen and Andersen, 1993). It also alters the activity of the pituitary–adrenal axis, immune function, behavior and growth rate (Ingvartsen and Andersen, 1993, Fisher *et al.,* 1997a and Fisher *et al.,* 1997b).

Unweaned female buffalo calves showed evidence of stress with lack of space allowance (Grasso *et al.,* 1999). These latter animals showed alterations in a number of behavioral and physiological responses as a consequence of space restriction. Therefore, it is clearly obvious that it is necessary to extend the study to consider older buffalo calves.

Till now, there is no legislation to determine the space allowances needed for buffaloes. One 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 three fundamental static postures (standing, sternal recumbence and lateral recumbence). However, additional space is needed to express behaviors essential to the animals, including feeding, locomotion, etc., and related to the species (i.e., wallowing) or the age of the animal (i.e., playing).

The present study was to investigate the effect of different floor space allowances on the welfare of weaned buffalo calves through studying their behavior, endocrine response and microenvironment.

MATERIALS and METHODS

I- Experimental design

Fourty five weaned female buffalo calves were used in this investigation. Animals were 5 months old with a mean live weight of 100 kg at the start of the study. Animals were randomly assigned to 3 groups, 15 calves each. Animals of each group were divided into 3 subgroups, 5 calves each and each subgroup was housed under the prevalent environmental conditions in a separate straw bedded floor pen. Groups were randomly assigned to three treatments differing in the space allowance of each calf in relation to its body surface area as the following: -

I. Treatment one (Group 50): Calves were group-housed at 50% of their body surface as space allowance which adopted by Hurnik and Lewis (1991) as a 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.
II. Treatment two (Group 70): Calves were group-housed at 70% of their body surface as an intermediate space allowance.

III. Treatment three (Group 90): Calves were group-housed at 90% of their body surface as a comfortable space allowance adopted by Napolitano *et al.* (2004).

Body surface area was calculated from body weight using the formula stated by Hurnik and Lewis (1991) as follows: -

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

Pen size was adjusted at monthly intervals to meet the experimented space allowance. All animals were weighted when the groups were constituted and, subsequently, at monthly intervals. As the experiment was extended for three months, the monthly starting experimented space allowance was calculated and stated as shown in Table 1.

Table 1: Experimented space allowance of the investigated calves based on their body surface areas.

Experimental period	Starting weight (kg)	Body surface area (m^2)	Space allowance (m^2)		
			group 50	group 70	group 90
1st month	100	1.9	0.95	1.33	1.71
2nd month	130	2.2	1.10	1.54	1.98
3rd month	170	2.6	1.30	1.82	2.34

Calves were ad libitum fed a commercial concentrate mixture for growing calves in addition to barseem. Space at manger was 50 cm / calf. Drinking water was freely available during all the experimental period. For each group, two drinking bowls were presented inside each pen.

II- Behavioral observations

Behavioral observations were carried out after grouping of calves with two weeks which were used as a preliminary period. Behavior of the experimented animals was recorded according to Marten and Bateson (1988) and Fordham *et al.* (1991) using a direct observation method while the observers can observe the experimented calves without being seen by them. Behavior of the experimented calves was recorded for 6 hours / day for 6 days / week (two days for each group) of the last two consecutive weeks of each month as follows: -

- Two hours in the morning after supplying the animals with their morning feeding (8:00 to 10:00 a.m.).
- Two hours in the afternoon (12:00 to 2:00 p.m.).
- Two hours in the evening after supplying the animals with their evening feeding (4:00 to 6:00 p.m.).

Recorded behavior of the experimented calves was analyzed according to Napolitano *et al.* (2004) as follows:-

I- Standing time / calf / recorded hour
II- Lying time / calf / recorded hour
III- % of outstretched legs / lied calves / recorded hour
IV- % of calves showing feeding activities / recorded hour (selection, prehension and mastication)
V- % of calves showing ruminating activities / recorded hour
VI- No. of non-agonistic interactions / calf / recorded hour (licking, sniffing or nuzzling each other)
VII- No. of agonistic interactions / calf / recorded hour (pushing, butting or threating each other)

III- Isolation test

Isolation test was carried out according to Napolitano *et al.* (2004) to examine the behavioral responses of calves housed under the different experimented space allowances. On the last day of each month of the experimental period, calves were subjected to an isolation test. Each animal was exposed to a novel yard 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 the first movement and number of galloping, vocalization, sniffing and buck-kicking were recorded.

IV- Adrenal response

During the last two days of each month of the experimental period, two blood samples, 5 ml each were drawn from the jugular vein of each animal of randomly selected three calves of each pen. Blood samples were taken from selected calves of each pen at the same time with the aid of three technicians to prevent any expected activity stress on the animals. The first blood sample was drawn into centrifuge tubes and centrifuged for 30 minutes at 3000 r.p.m and the obtained sera were assayed within three hours for their glucose concentration according to Tinder (1969). The second blood sample was drawn into centrifuge tubes and centrifuged for 30 minutes at 3000 r.p.m and the obtained sera were frozen at –80 ºC and kept for further analysis to determine their cortisol level using TDx FLx system according to Dandliker and Sassure (1973).

V- Air and bedding materials sampling

Air samples were collected once weekly from the examined animal enclosures by means of liquid impinger as described by Cown *et al.* (1956). Twenty ml of sterile nutrient broth was used for collecting airborne-dust particles. The liquid impinger was adjusted at a rate of 5 liter / minute. The air samples were collected at a mid-day following the routine work and during the ordinary activity of individuals, which care-after the animals. During air sampling, liquid impinger was moved inside the animal house in order to trap all the suspended dust particles to get a representative air samples. The collected air samples were carried with minimum delay for microbiological examination.

Bedding material samples were collected once weekly during the trial. Samples of bedding were collected from beneath of each 5 buffalo calves of each pen. Straw was chopped to facilitate handling and 10 gm portions were suspended in 90 ml nutrient broth. After thorough shaking, 1: 10 serial dilutions were prepared in sterile nutrient broth (Rendos *et al.,* 1975).

The total colony count, Coliform count, Clostridium perfringens count, Faecal streptococcal count and Total yeast and moulds count were done according to the technique described by Johnson and Curl (1972); Oblinger and Koburger (1975); Beerens *et al.,* (1980); Cruickshank *et al.* (1980) and Carter and Cole (1990).

VI- Statistical analysis

Statistical analysis of the collected data were carried out according to procedures of completely random design SAS (1995). Behavioral, cortisol and microenvironment data were analysed with analyses of variance for repeated measures with space allowance as a non-repeated factor and time and time x space allowance as repeated factors.

RESULTS

The results of this study were illustrated in Tables 2, 3, 4, 5 and 6.

Table 2: Effect of space allowance on behavioral patterns of the experimented calves.

Variables	Space allowance (m^2)		
	Group 50	Group 70	Group 90
Standing time (min. / hour)	38±4 [a]	21±2 [b]	18±2 [b]
Lying time (min. / hour)	22±2 [a]	39±6 [b]	42±3 [b]
Outstretching legs (% of lying calves)	20 [a]	60 [b]	70 [b]
Feeding activities (% of calves / hour)	50 [a]	80 [b]	80 [b]
Ruminating activities (% of calves / hour)	20 [a]	50 [b]	60 [b]
Non-agonistic interactions (No. / hour)	6±1 [a]	17±3 [b]	14±2 [b]
Agonistic interactions (No. / hour)	11±3 [a]	3±1 [b]	3±1 [b]

Figures in the same raw with different superscripts differ significantly ($p<0.01$).

Table 3: Effect of space allowance on behavioral response of the experimented calves to isolation test.

Variables	Space allowance (m^2)		
	Group 50	Group 70	Group 90
Latency time before the first movement (s)	9±2 [a]	11±1 [a]	9±1 [a]
Duration of the first movement (s)	112±6 [a]	64±4 [b]	58±3 [b]
Galloping (No.)	14±2 [a]	7±2 [b]	6±1 [b]
Vocalization (No.)	18±3 [a]	8±2 [b]	9±1 [b]
Sniffing (No.)	12±1 [a]	11±2 [a]	11±2 [a]
Buck kicking (No.)	0 [a]	0 [a]	0 [a]

Figures in the same raw with different superscripts differ significantly ($p<0.01$).

Table 4: Serum cortisol (µg / L) and glucose (Mmol / L) concentrations of the experimented animals.

Variables	Space allowance (m^2)		
	Group 50	Group 70	Group 90
Cortisol	0.97±0.02 [a]	0. 76±0.02 [b]	0.71±0.02 [b]
Glucose	6.91±0.10 [a]	5.22±0.20 [b]	5.31±0.20 [b]

Figures in the same raw with different superscripts differ significantly ($p<0.01$).

Table 5: Microbial pollution of air collected from buffalo calves' pen.

Variables	Space allowance (m^2)

	Group 50	Group 70	Group 90
Total bacterial count (cfc/m^3)	$39.1 \times 10^{3} \pm 2.42^{a}$	$16.5 \times 10^{3} \pm 1.32^{b}$	$14.3 \times 10^{3} \pm 1.22^{b}$
Total coliforms count (cfc/m^3)	$2.51 \times 10^{2} \pm 0.30^{a}$	$0.9 \times 10^{2} \pm 0.10^{b}$	$0.81 \times 10^{2} \pm 0.11^{b}$
Faecal streptococcal count (cfc/m^3)	$1.6 \times 10^{2} \pm 2.1^{a}$	$0.3 \times 10^{2} \pm 1.3^{b}$	$0.1 \times 10^{2} \pm 1.5^{b}$
Clostridium perfringens count (cfu/m^3)	0.0	0.0	0.0
Total yeast and moulds count (cfc/m^3)	$1.39 \times 10^{3} \pm 0.21^{a}$	$0.83 \times 10^{3} \pm 0.15^{b}$	$0.73 \times 10^{3} \pm 0.11^{b}$

Figures in the same raw with different superscripts differ significantly ($P<0.01$).

Table 6: Microbial pollution of bedding materials of buffalo calves' pen.

Variables	Space allowance (m^2)		
	Group 50	Group 70	Group 90
Total bacterial count (cfc/gm)	$89.1 \times 10^{6} \pm 1.0$	$46.5 \times 10^{5} \pm 1.02^{b}$	$34.3 \times 10^{5} \pm 1.01^{b}$
Total coliforms count (cfc/gm)	$51.1 \times 10^{3} \pm 1.30^{a}$	$23.2 \times 10^{3} \pm 1.1^{b}$	$19.6 \times 10^{3} \pm 1.01^{b}$
Faecal streptococcal count (cfc/gm)	$17.3 \times 10^{3} \pm 2.01^{a}$	$9.5 \times 10^{3} \pm 0.89^{b}$	$7.9 \times 10^{3} \pm 0.79^{b}$
Clostridium perfringens count (cfu/m^3)	0.0	0.0	0.0
Total yeast and moulds count (cfc/gm)	$59.8 \times 10^{5} \pm 2.21^{a}$	$31 \times 10^{4} \pm 1.11^{b}$	$29 \times 10^{4} \pm 1.01^{b}$

Figures in the same raw with different superscripts differ significantly ($P<0.01$).

DISCUSSION

I- Behavioral observations

Studying the animal behavior plays an important role in measuring its welfare. Standing and resting behaviors play a major role in animal comfort. Deprivation of lying and resting may have detrimental effects on animal welfare. Reduced space allowance affects substantially on standing and lying behaviors with an end result of reduced well-being and productivity (Leonard *et al.*, 1994).

Table 2 showed the relevant results obtained from behavioral observations. Space allowance significantly affected standing time ($p<0.01$). Housed calves of group 50 were observed in the standing posture for longer time than calves of group 70 or those of group 90, which were insignificantly different. However, lying time was significantly higher for calves either of group 70 or group 90 than for those of group 50 ($p<0.01$).

Moreover, Space allowance was found to affect significantly on the number of outstretching legs ($p<0.01$). A lower number of calves in a restricted space of 50% of their body surface lied with outstretched legs than calves provided with more free space. However, the percentage of outstretching legs among lying calves were not significantly differed between group 70 and group 90. This finding agreed with that of Napolitano *et al.* (2004) using 50 and 90% of body surface area as a studied space allowance and indicated that, the 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.

The percentages of eating as well as ruminating activities were lower for calves kept in a restricted space ($p<0.01$). Housed calves of group 50 were observed in eating and ruminating activities with a lower percentage than calves of group 70 or those of group 90, which were insignificantly different. This finding was in agreement with that of Napolitano *et al.* (2004) and may be related to the crowdness of the calves in group 50 which forbidden a large number of them from being at the manger in the same time with a prominent effect on their eating, and subsequently, ruminating activities.

In fact, a restricted environment may make the animals more prepared to react to threat and aggression. Non agonistic and agonistic behaviors showed opposite patterns. Calves of groups 70 and 90 performed non-agonistic interactions with a higher number than those of group 50 ($p<0.01$), whereas the number of agonistic interactions was higher between calves receiving 50% of body surface as space allowance than between subjects having either 70% or 90% of body surface, which were insignificantly different ($p<0.01$). This finding agreed with Barnett *et al.* (1992) who 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 behaviors may be determined by forced non-agonistic interactions, which in turn, can induce animals to fight or flee (Hanlon *et al.*, 1994).

II- Behavioral response to isolation test

In bovine calves, De Passillé *et al.* (1995) classified the behaviors recorded during isolation test according to their motivations that might underlie each response. These authors described three main clusters (fear, exploration and locomotion) using factor analysis. Vocalization was included among variables indicating fear, whereas ambulatory behaviors were associated with locomotory motivation.

Table 3 showed the result of isolation test on the behavioral response of the experimented calves. The data indicated that there was no significant effect of space allowance either on the latency time to the first movement, number of sniffing or number of buck-kicking. Meanwhile, the duration of the first movement and the number of galloping as well as vocalization were significantly longer and higher ($p<0.01$) during isolation test for calves of group 50 rather than those either of group 70 or group 90 which were insignificantly different. This finding agreed with Dellmeier *et al.* (1985); De Passillé *et al.* (1995) and Jensen (1999) who observed that animals housed in less spacious environments have a lowered threshold for release of locomotory behaviors which were somehow suppressed during confinement. They also indicated that chronic suppression of free locomotion resulted in an increased expression of this behavior after release from confinement. The increased levels of locomotory behavior 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.

III- Adrenal response

The data illustrated in Table 4 showed the effect of the studied space allowance on the blood levels of cortisol and glucose of the experimented calves. Cortisol and glucose levels were significantly higher ($p<0.01$) in the serum of the experimented calves of group 50 than in the serum of those of group 70 or group 90, which were insignificantly different. The significant increase in the blood cortisol level of the experimented calves that allowed 50% of its body surface as a space allowance indicating an occurrence of stress due to housing in less spacious environments. This increase in blood cortisol may be related to an outpouring of ACTH which intern causes the adrenal cortex to increase its secretion of glucocorticoids including cortisol (McDonald, 1969; Burchfield *et al.*, 1980 and Stephens, 1981). However, the increase in blood glucose level of the same calves may be related to the fact that glucocorticoids, including cortisol, act mainly on the hepatocytes which induced to produce gluconeogenic enzymes which in turn increase the rate of gluconeogenesis and enhance the conversion of protein to glucose. Moreover, cortisol causes a moderate reduction in the rate of glucose utilization by the body cells, which leads to a rise in blood glucose level (Guyton and Hall, 1996).

IV- Airborne and bedding materials microorganisms

The data illustrated in table 5 showed the effect of the studied space allowance on air-borne microbial contamination inside the pens of the experimented buffalo calves. Microbial concentration of air were significantly higher ($p<0.01$) in the pen of the experimented calves of group 50 than in those of group 70 or group 90, which were insignificantly different.

Moreover, table 6 showed the effect of the studied space allowance on microbial contamination of bedding materials of the pens of the experimented buffalo calves. Microbial concentration of bedding materials were significantly higher ($p<0.01$) in the pen of the experimented calves of group 50 than in those of group 70 or group 90, which were insignificantly different.

The quality of air supplied to animals has significant factors. Dust contributed by animal operations is a serious nuisance problem, specially when loaded at the air. It is responsible for a decline of the general health, productivity and performance of animals. In addition, dust can transport various biological excitants of animal diseases particularly in a high density animal confinement operation (Adams and Moss, 2003). Occurrence and persistence of many diseases producing agents in the soil and bedding material has been over-locked as problem in the disease control (Huss, 1980; Smith & Young, 1980). Due to capability of the organisms to survive for long time, many diseases can be arising from these contaminated floors. Under most farm conditions, eliminating infectious agents from the environment was difficult, but good management procedures could aid in maintaining a level of environmental contamination that was less than critical (Kotb, 2006).

The routine examination of animal environment for a wide range of pathogens is very important as to assessment the hygienic quality of animal environment. These bacteria are termed indicator organisms and they often regarded as being of great significance when assessing the microbial safety of animal environment. The principle bacteria employed as indicators are Coliforms, Faecal streptococcal and Clostridium perfringens (Cruickshank *et al.*, 1980).

The microbial count inside buffalo's pens in air and bedding materials of group 50 was higher than those of other pens and this result may be attributed to accumulation of faecal matter under animals which was constantly damp and soiled with animal manure as a result of low space allowance for each animals inside these pens. The extent of bacterial contamination in livestock habitations naturally depends on many factors including the rate of ventilation, extent of dustiness, sedimentation rate, standard of hygiene and the rate at which bacteria are produced by occupied animals as well as desiccation and maceration of soil (Hartung, 1989; Zuker *et al.*, 2000).

It was concluded that 50% of body surface area is inadequate as space allowance for weaned buffalo calves than 70 or 90%. Space restriction to 50% of body surface area of weaned buffalo calves was severely affected its welfare and resulted in a stressful situation with some modifications of their behavior. To fulfill the recent intensification and economic techniques of rearing weaned buffalo calves and, on the same time protect them from facing housing stress, it is recommended to house the weaned buffalo calves in their pens with a space allowance equals to 70% of its body surface area.

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