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Preliminary study on the effect of size of individual stall on the behavioural and immune reactions of dairy calves*

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ABSTRACT

Ten Italian Friesian female calves were housed in individual stalls of two different dimensions (0.73 m x 1.21 m vs 1.00 m x 1.50 m) during the 1st month of life in order to evaluate the effect of space on the welfare of dairy calves. Behavioural observations were made on d 2 to 3 (period 1) and on d 28 to 29 (period 2) during daylight using a time-lapse video recording system. At 10, 20, and 30 d, blood samples were taken and analysed with phytohemagglutinin stimulation to determine lymphocyte proliferation. During the second period, the calves in large stall spent more time lying ($P<0.05$) and grooming ($P<0.001$), and the calves in small stalls exhibited more time searching for feed ($P<0.001$). Lymphocyte proliferation was statistically lower in calves housed in smaller stalls ($P<0.01$). Moreover, lymphocyte proliferation was negatively correlated with time spent searching for feed ($P<0.01$).

KEY WORDS: dairy calves, behaviour, immune reaction, stalls

INTRODUCTION

Individual housing is used not only for veal calves, but also for dairy calves during the 1st month of life because of hygienic and health advantages (Smits and de Wilt, 1991). Much research on veal calves has been undertaken to determine the

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effect of the type of housing on various behavioural, physiological, and productive characteristics (Dellmeier et al., 1985; de Wilt, 1985; Fisher et al., 1985; Friend et al., 1985; Terosky et al., 1997). Some researchers (Broom, 1991) claim that stall size has an impact on animal welfare. Individual calves reared in unsuitable housing facilities may have a modified ethogram, such as alterations of standing and lying postures (de Wilt, 1985). Also, according to Le Neindre (1993), a stall size that is too small prevents the veal calf from lying in a normal position. The results of the most recent and relevant studies on this issue have been widely reviewed by Rushen (1994) and by the Scientific Veterinary Committee (1995). Behavioural studies on bovines that also use immunological parameters to determine stress are relatively few, especially in relation to different housing conditions, and are based on leukocyte counting, the ratio of lymphocytes to leukocytes, and the evaluation of antibody titers (Fisher et al., 1985; Friend et al., 1987; Cummins and Brunner, 1991; Stull and McDonough, 1994). Nevertheless, the immune response is considered a useful indicator for the evaluation of stress in various animal species (Blecha, 1988; Kelley, 1980; Sacerdote et al., 1994), including cattle.

This work aimed to evaluated the possibility of reducing stress in calves and improving the comfort of living conditions by enlarging the size of individual stalls during the 1st month of life. For this purpose, the following indicators of welfare were considered: 1. quantitative and qualitative modifications of behavioural patterns (including abnormal behaviour), and 2. modification of the immune response depending on stall size and its relationship with behavioural patterns.

MATERIAL AND METHODS

This preliminary study was conducted on 10 Italian Friesian female calves (weighing about 40 kg at birth). All animals were healthy and no medical treatment was necessary throughout the study. Five calves were housed from birth to the end of the 1st month of life in individual stalls, 0.73 m x 1.21 m (small stall), and 5 were housed in individual stalls, 1.00 m x 1.50 m (large stall). All stalls were made of fibreglass, had complete walls and roof, solid concrete floor bedded with straw and were located in open air. Contact between calves was possible only through an anterior aperture by which feed and milk were delivered. The average environmental temperature at the time of the presented study (May-June 1994) was of 16.9°C and the relative humidity was of 78.8%. The calves were fed a milk substitute twice daily at 5.00 and 17.00 h; hay and concentrate were also available *ad libitum* from d 3 of life. The study was carried out in a commercial dairy farm. The animals were subjected to the normal management practices which are common on farms where female calves remain in individual stalls until the end of the 1st month of life and are then moved to group pens.

During daylight (from 7.00 to 21.00 h), the behaviour of calves was continually recorded with a camera connected to a time-lapse video recorder (Gavinelli et al., 1994). Recordings were taken for 2 consecutive days following birth (period 1) and for 2 consecutive days at the end of the 1st month of life (period 2). Readings were made using a time-sampling method (Martin and Bateson, 1993) for 1 min in every 10 min (a total of = 168 min of observation per calf per period). The reliability of time sampling was checked against continuous observations. The following activities were considered: standing still (inactivity), lying, exploration, grooming (body care) and searching for feed. The latter was a complex behaviour consisting of a notable repetitive sequence of one or a few of the following single behavioural patterns: licking, sniffing the empty bucket, or licking the feed trough. A similar behaviour, called feed searching, has been described by Redbo (1990) in tied-up heifers fed twice a day and was attributed to the restriction in movements and feeding. The behavioural data (expressed in seconds) were analysed by nonparametric analysis of variance (Kruskal-Wallis test; Siegel and Castellan, 1992).

Three blood samples per calf were taken at 10, 20, and 30 d of age from the jugular vein using Vacutainers® (Becton Dickinson Vacutainer Systems, Rutherford, NJ). All calves of both groups were submitted to the same handling procedures for blood sampling. The samples were used to evaluate lymphocyte proliferation induced by phytohemagglutinin. Phytohemagglutinin was chosen on the basis of preliminary studies showing that it elicited a better proliferative response of calf lymphocytes in comparison to other polyclonal mitogens (Sacerdote, personal communication). Peripheral lymphocytes were separated by gradient centrifugation by a specific lymphocyte separation medium (Ficoll-Paque, Pharmacia, Uppsala, Sweden), and microcultures of 1 x 10^6 lymphocytes were set up in RPMI 1640, 10% fetal calf serum in presence or in absence of phytohemagglutinin at three dilutions (2.0, 0.5, and 0.125 mg/ml). After 48 h of incubation at 37°C, 1 mCi of [^{3}H]thymidine (specific activity = 2Ci/mmol; Amersham, Little Chalfont, United Kingdom) was added to all cultures. Eighteen hours later, cells were harvested by an automatic cell harvester, and radioactivity was measured in a liquid scintillation counter. Background values (thymidine incorporation of unstimulated cells) were subtracted from mitogen-induced proliferation (Sacerdote et al., 1994).

Data were analysed using a two-way analysis of variance, followed by a Tukey t test for multiple correlation. Spearman correlation ranks were calculated between lymphocyte proliferation and behavioural data.

RESULTS

Table 1 shows the mean duration of each activity. During the first recording period, time spent exploring, searching for feed, lying, and standing still (inactive)

was similar for calves in both stall sizes. Time spent grooming was significantly higher for calves housed in large stalls ($P < 0.001$).

During the second period, feed searching activity was higher for calves in small stalls ($P < 0.001$), while grooming was higher for those in large stalls ($P < 0.001$). Calves reared in large stalls spent more time lying than calves in small stalls ($P < 0.05$) and had longer exploration times, even though the difference was found not significant.

TABLE 1

Mean time spent on various activity for calves housed in stalls of different size in the two observation periods during the daylight

	Period 1		Period 2	
	large stall	small stall	large stall	small stall
	(s/min)			
	x ± SE	x ± SE	x ± SE	x ± SE
Exploring	8.83 ± 0.70	8.60 ± 0.71	8.12 ± 0.64	6.94 ± 0.62
Grooming	1.32 ± 0.19[a]	0.38 ± 0.14[a]	1.55 ± 0.19[b]	0.78 ± 0.16[b]
Searching for feed	5.15 ± 0.55	6.87 ± 0.65	4.57 ± 0.50[c]	11.09 ± 0.79[c]
Standing still	0.48 ± 0.17	0.18 ± 0.10	0.09 ± 0.05[d]	0.25 ± 0.12
Lying	44.16 ± 0.93	43.98 ± 0.93	45.54 ± 0.84[d]	40.94 ± 0.97[d]

a = $P<0.001$
b = $P<0.001$
c = $P<0.001$
d = $P<0.05$

Table 2 shows the lymphocyte proliferation values between the two groups of calves. At 10 d of age these values were lower for calves in small stalls than for calves in large stalls. Lymphocyte proliferation significantly increased during the 1st month of life, but lymphocyte proliferation of calves reared in small stalls always remained significantly lower than that of calves reared in large stalls. The response of lymphocyte proliferation during the second period, considering the reference dilution (0.5 g/ml of phytohemagglutinin), was negatively correlated with feed searching activity ($r = -0.78$; $P < 0.01$). In the same period, a positive correlation was found between lymphocyte proliferation at the same dilution and time spent lying, which approached statistical significance ($r = 0.63$; $P = 0.06$).

DISCUSSION

This preliminary research showed that the size of stalls seemed to affect behaviour as well as immunological characteristics of dairy calves during early development. In this study, the difference in time spent lying down was obvious at the end of

TABLE 2

Mean values of lymphocyte proliferation (cpm) for calves at different ages housed in stalls of different sizes

Stall sizes[1] and age[2]	Phytohemagglutinin		
	2 µg/ml	0.5 µg/ml	0.125 µg/ml
		cpm	
Small stalls	x ± SE	x ± SE	x ± SE
10d	13061 ± 3418	24744 ± 5063	11501 ± 5594
20d	20718 ± 3935	37673 ± 7583	22998 ± 9096
30d	30590 ± 5556	54709 ± 6422	26370 ± 5608
Large stalls	x ± SE	x ± SE	x ± SE
10d	20545 ± 2655	51157 ± 15808	26328 ± 4956
20d	26387 ± 7083	71108 ± 20909	25758 ± 2901
30d	54047 ±16651	120324 ± 32156	35070 ± 2420

[1] differences between stall sizes are significant: $F_{1,26} = 8.37$; $P<0.01$

[2] differences among ages within each stall size are significant: $F_{2,26} = 4.14$; $P<0.05$

the 1st month of life. Small stalls did not satisfy the resting requirements of the calf, in agreement with observations for veal calves during the fattening period (Ketelaar de Lauwere and Smits, 1991; Le Neindre, 1993).

Calves in large stalls spent more time grooming than those in small stalls. In 13-week-old calves, Le Neindre (1993) noted a reduction in grooming when calves were housed in small pens. Calves reared for long periods in small individual stalls that do not allow them to turn around cannot perform grooming on the hind part of their bodies, an activity that calves perform many times daily (Fraser and Broom, 1990). Calves reared in both sizes of stalls spent time exploring, but exploration time was longer for calves in large stalls during the second period. In all animals, including cattle, time spent exploring is highly motivated (Kerr and Wood Gush, 1987; Fraser and Broom, 1990) to know and to adapt to the environment.

Another behavioural difference that was observed in the present study, especially during the second period of observation, was a greater amount of time spent searching for feed by calves in small stalls. Many researchers (de Wilt, 1985; Broom, 1991), studying the effects of housing on calves, have observed, for calves in stalls, the presence of oral stereotypies, such as tongue rolling (tongue playing), excessive chewing of the pen, and excessive grooming. Feed searching often adjoins stereotypies, especially after feeding time in heifers (Redbo, 1990), while often adjoins stereotypies before feeding time in sows (Rushen, 1985). In this study the presence of feed searching can be interpreted as a warning sign of the developement of oral stereotypies and therefore an indicator of poor welfare (Broom, 1991).

Lymphocyte proliferation was significantly higher in calves reared in large stalls than in those reared in small stalls; this difference was already apparent at 10 d of age, confirming that the immune response can be considered a further useful indicator of stress (Kelley, 1980) even after a stressful situation of relatively short duration (Canali et al., 1996). The negative correlation found between lymphocyte proliferation and searching for feed clearly demonstrates the role of this behaviour as an indicator of poor welfare and supports the interpretation of searching for feed as a warning signal in the first month of life.

CONCLUSIONS

In this preliminary study stall size seemed to have an effect on both immunological and behavioural traits. In particular, exploration, grooming, lying and searching for feed were affected by stall size. The presence of lower lymphocyte proliferation in calves reared in stalls with smaller dimensions could be regarded as an index of poor welfare caused by a stressful environment.

There is a need for more work in this area to confirm these preliminary observations. Indications of adequate stall size for calves related to the different phases of growth would also be important to prevent either problems connected with the welfare of the calf or negative effects on health and productivity.

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STRESZCZENIE

Wstępne badania nad wpływem wielkości klatek na zachowanie się i reakcje odpornościowe cieląt ras mlecznych

Dziesięć cieląt – jałówek rasy włoski fryz utrzymywano przez pierwszy miesiąc życia w indywidualnych klatkach o dwóch rozmiarach (0,73 x 1,21m vs. 1,00 x 1,50 m) celem określenia wpływu wielkości zajmowanej powierzchni na samopoczucie cieląt. Obserwacje prowadzono od 2 do 3 dnia (okres 1) i od 28 do 29 dnia życia (okres 2) w czasie dnia świetlnego stosując system czasowy zapisu kamerą video. W 10, 20 i 30 dniu pobierano próby krwi do określenia namnażania się limfocytów stosując metodę stymulacji fitohemaglutyninowej. W drugim okresie cielęta trzymane w większych klatkach spędzały więcej czasu leżąc ($P<0,05$) oraz pielęgnując ciało ($P<0,001$), natomiast trzymane w mniejszych klatkach spędzały więcej czasu na poszukiwaniu paszy ($P<0,001$). Namnażanie limfocytów było mniejsze u cieląt trzymanych w mniejszych klatkach ($P<0,05$) i było ujemnie skorelowane z czasem spędzonym na poszukiwaniu paszy ($P<0,01$).