Influence of Free-Stall Flooring on Comfort and Hygiene of Dairy Cows During Warm Climatic Conditions

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ABSTRACT

An evaluation of behavioral and hygienic conditions was carried out with 4 materials used as free-stall flooring for dairy cows: polyethylene vinyl acetate (EVA) and polypropylene vinyl acetate (PVA) mats, wood shavings, and solid manure. The free-stall type selected by cows was evaluated in response to changes in environmental temperature and humidity. Two tests were used: 1) a preference test, in which 8 cows were housed in a pen with 32 free stalls and 4 types of flooring; and 2) an aversion test, in which 32 cows were placed in 4 pens, each with 8 free stalls. The free stalls in each pen had a single type of bedding material. These tests showed that the comfort of dairy cows was predominantly influenced by environmental conditions. The preference test for lying showed that cows preferred free-stall floors with EVA mats over those with PVA mats, wood shavings, and solid manure (332.4 ± 24.0 vs. 130.8 ± 6.2 , 160.9 ± 23.7 , and 102.6 ± 23.2 min/d, respectively), but under conditions of heat stress, with a temperature–humidity index >80 , they chose wood shavings and solid manure lying areas. These results were confirmed by the aversion test. In all experimental and environmental conditions, the PVA mats were the least suitable. The mats contaminated with organic manure and the free stalls bedded with wood shavings and organic solids did not differ in either the coliform load on the lying surfaces (EVA mats: 290 ± 25 ; PVA mats: 306 ± 33 ; wood shavings: 290 ± 39 ; and solid manure: 305 ± 23 log₁₀ cfu/mL) or the total bacterial count in the raw milk (EVA mats: 232 ± 22 ; PVA mats: 233 ± 24 ; wood shavings: 221 ± 24 ; and solid manure: 220 ± 25 log₁₀ cfu/mL). These results demonstrate that the comfort of dairy cows housed in barns with free stalls as resting areas does not depend only on the material used, but also on the value of the material in microenvironmental conditions.

Key words: dairy cow, free-stall flooring material, behavior, temperature–humidity index

INTRODUCTION

Prolonged exposure to temperatures above the threshold of thermal neutrality induces modifications in the endocrine and metabolic state of the animal to favor adaptation to adverse environmental conditions (Sevi et al., 2001; West, 2003). In conditions of thermal stress caused by high environmental temperatures, significant variations are noted in the energetic, protein, and mineral metabolism. Most of these insights have been derived from research conducted on dairy cows and goats with the aim of evaluating productive measures (Olsson and Hydbring, 1996; St-Pierre et al., 2003) and reproductive traits (Oseni et al., 2003). One of the behavioral traits that varies under conditions of high environmental temperature and humidity is the time cows spend lying down (Shultz, 1984; Overton et al., 2003). This aspect undermines the health of cattle and favors the emergence of lameness (Cook et al., 2004), caused by an increased amount of time that claws are subjected to load and a reduction in their relief (Leonard et al., 1996). Increased standing times play an important role in the productivity and welfare of the animals, such that lying deprivation is associated with a reduction in plasma growth hormone concentrations (Munksgaard and Simonsen, 1996) and increases in cortisol (Fisher et al., 2002).

During heat stress, the comfort provided by the free-stall flooring is critical. Numerous mats and mattresses of different synthetic materials have been marketed with the aim of improving the comfort of dairy cows, reducing the microbial loads that cause mastitis in their environment, and lowering labor costs (McFarland, 2003). The use of mats and mattresses is widespread in the continental and northern regions of Europe, and they are now increasingly being used in areas of the Mediterranean basin, which are characterized by a totally different environmental climate. It has been established that the microbial load on synthetic mats and organic materials differs under the same environmental conditions (Hogan et al., 1999; Reneau et al., 2003). Because the microbial load of lying area surfaces is directly related to the bacterial count of the teats, the material used as free-stall flooring plays a key role in

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Table 1. Composition of the TMR

Item	Amount
Feed, %	
Maize silage	40.00
Water	12.77
Alfalfa hay	9.00
Maize ground	8.50
Beet pulp	6.38
Soybean meal	5.50
Oat hay	4.25
Wheat bran	5.00
Cottonseed	3.84
Soyhulls	2.04
Barley ground	0.90
Rolled soybean	0.77
Calcium carbonate	0.33
Sodium bicarbonate	0.33
Bicalcic phosphate	0.13
Urea	0.10
Sodium chloride	0.08
Magnesium oxide	0.08
Chemical composition, % $\pm$ SD	
DM	55.0 $\pm$ 5.6
CP	16.1 $\pm$ 1.6
Ether extract	4.2 $\pm$ 0.9
Crude fiber	20.0 $\pm$ 2.0
Ash	7.1 $\pm$ 1.3
Nonnitrogen extracts	52.5 $\pm$ 5.0

preventing environmental mastitis (Zdanowicz et al., 2004).

To improve the well-being, hygiene, and, consequently, the health and productivity of dairy cows, we evaluated the materials used as flooring in free-stall resting areas and assessed the climatic characteristics of the microhabitat where cows were housed.

MATERIALS AND METHODS

Experiment 1: Preference Test

Animals. The test was conducted with 8 primiparous Italian-Friesian cows with similar BW, similar body dimensions, and the same sire. Preceding calving, all animals (775.1 ± 15.8 d of age) were maintained on straw bedding. After calving (April 2004), cows were allocated to experimental pens, where they spent 3 mo adapting to the experimental conditions (pre-experimental period). The 3-mo trial began in July 2004. The cows were fed the same TMR (Table 1). Chemical analyses of the TMR (AOAC, 1990) were conducted every 2 wk from July 2004 to September 2004 (Table 1).

Structures and Management. The pen in which the animals were housed provided 32 free stalls arranged in 2 rows placed head to head. The free-stall base surface was concrete. The 2 rows of free stalls were separated by 2 steel tubes, allowing the presence of a shared lunging space. The types of materials used for the free-stall floors were polyethylene vinyl acetate

mats (**EVA**; Cow Mat CM20, DeLaval Inc., Kansas City, MO), coated polypropylene vinyl acetate mats (**PVA**; Arienti Mat, Arienti snc, Cologno Monzese, Italy), wood shavings, and solid manure obtained from a separator. The bedding using the organic materials was approximately 9 cm deep. The 2 mats were made of a single material layer (122×180 cm, with a thickness of 2 cm). Because the base surface was 7 cm below the top of the upper board in the rear of the free stall, a 5-cm layer of fine sand was placed under the mats. The softest material was wood shavings, followed by EVA mats, PVA mats, and solid manure. The 4 types of free stalls were alternated (Figure 1) because, as a species, cows have a hierarchical organization that can involve particular social patterns (Tucker et al., 2004). Nielsen et al. (1997) showed that some animals always lie down close to a particular animal and never close to others. The pen (20×20 m) had an insulated roof, was open on 4 sides, and was equipped with a drinking trough. The free-stall resting area was separated from the feeding area by a 4-m-wide passage. The cows were milked twice daily at 0600 and 1800 h. Wood shavings lost from the free-stall surfaces were replaced daily, whereas the manure lost was replaced every 3 d. Excrement was removed from the mats (both EVA and PVA) daily.

Behavioral and Environmental Measures. The pen was equipped with closed-circuit television cameras with night vision and linked to video recorders (Figure 1). The recordings were made in August 2004 for 144 h. The first 72 h of uninterrupted recording started at 0800 h on August 4 and was repeated 20 d later. Data were obtained from continuous recordings by recording the time when a cow entered and left a free stall. The following data were obtained from an examination of the recordings:

1. bed occupation time of the free stalls per day (**BO**);
2. average duration of each lying event per day (**L**);
3. duration of periods spent standing in the free stall, with 2 or 4 feet per day (respectively, **S2F** and **S4F**);
4. number of interrupted attempts at lying down per hour (**NIL**);
5. mean duration of a single lying bout in minutes (**DLB**); and
6. time spent ruminating in the free stall, during the lying down time per day (**R/L**).

From the data obtained, it was possible to calculate, at 5-min intervals, 2 indices that were useful for the evaluation of well-being (Overton et al., 2003):

7. proportion of eligible lying (**PEL**: number cows lying: number cow in the pen not eating); and

Figure 1. Organization scheme of the preference test: 8 cows housed in a pen provided with 32 free stalls, 8 for each free-stall flooring material: polyethylene vinyl acetate (EVA) mats (Cow Mat CM20; DeLaval Inc., Kansas City, MO), polypropylene vinyl acetate (PVA) mats (Arienti Mat; Arienti snc, Cologno Monzese, Italy), wood shavings, or solid manure.

8. cow comfort index (CCI: number lying bouts: number touching a free-stall surface: lying + 4 feet + 2 feet).

The accuracy in time was in minutes. In addition, data loggers were arranged in the pen as shown in Figure 1. These recorded the temperature and relative humidity of the environment. The data acquired were used to calculate the temperature–humidity index (THI) over each 5-min interval, according to the equation suggested by Srikandakumar and Johnson (2004):

$$\begin{aligned} \text{THI} = & \text{dry bulb temperature} - 0.55 \\ & \times (1 - \text{relative humidity}) \\ & - (\text{dry bulb temperature} - 14.4), \end{aligned}$$

with relative humidity expressed as a decimal and temperature in degrees Celsius. With the timer displayed by the television cameras, we were able to associate every behavioral observation with its temporal context. Every behavioral event was associated with an average THI value in the period in which the event occurred.

Statistical Analysis. In advance, the Shapiro–Wilk test (Shapiro and Wilk, 1965) was conducted, and showed that the variables analyzed had a normal distribution ($P = 99.3\%$). The THI values were divided in 8

classes: A: $\text{THI} < 72$; B: $72 \geq \text{THI} < 74$; C: $74 \geq \text{THI} < 76$; D: $76 \geq \text{THI} < 78$; E: $78 \geq \text{THI} < 80$; F: $80 \geq \text{THI} < 82$; G: $82 \geq \text{THI} < 84$; H: $\text{THI} \geq 84$. This subdivision was used because 90.2% of the THI values recorded were within the range of 72 and 84.

To verify whether any significant distortion was linked to individual cows, we analyzed the preliminary variance using the GLM procedure of SAS (SAS Institute, 1999), setting the individual cows as independent variables. No differences were found. The data were then submitted to ANOVA using the GLM procedure of SAS (SAS Institute, 1999) with the following model:

$$y_{ijk} = \mu + \text{MAT}_i + \text{THI}_j + (\text{MAT} \times \text{THI})_{ij} + \varepsilon_{ijk},$$

where y_{ijk} is the behavioral variable; μ is the mean; MAT_i is the effect of the i th type of material used as free-stall flooring ($i = 1, \dots, 4$); THI_j is the effect of the j th THI class ($j = 1, \dots, 8$); $(\text{MAT} \times \text{THI})_{ij}$ is the effect of the interaction between the i th type of material and the j th THI class; and ε_{ijk} is the error term.

Experiment 2: Aversion Test

Animals and Feeding. The test was conducted on 32 Italian-Friesian primiparous cows with similar BW,

Figure 2. Organization scheme of the aversion test: 4 groups of 8 cows were placed into 4 pens. Each pen was provided with 8 free stalls made with the same flooring material: polyethylene vinyl acetate (EVA) mats (Cow Mat CM20; DeLaval Inc., Kansas City, MO), polypropylene vinyl acetate (PVA) mats (Arienti Mat; Arienti snc, Cologno Monzese, Italy), wood shavings, or solid manure.

similar body dimensions, and the same sire. Feeding was the same as in the preference test (Table 1).

Four groups of 8 cows were formed and were placed after calving (April 2004) into 4 pens. This test was performed contemporaneously with the preference test: starting in July 2004 (after 3 mo of adaptation) and lasting 3 mo (September 2004). The BW and the BCS were recorded when the animals were introduced to the experimental pens (April 2004) and at the end of the trial (September 2004). For the BCS evaluation, photos were taken from the rear and lateral points of view. Scoring of BCS was completed at the same time (at the end of the trial) and by the same person.

Structures and Management. In each of these 4 pens, formed by 8 free stalls, a single material was used: either EVA mats, PVA mats, wood shavings, or solid manure obtained from a separator (Figure 2). All the pens were located within the same shed and were managed as described in Experiment 1. The dimensions and characteristics of the free stalls and the 4 flooring materials were the same as in Experiment 1.

Behavioral and Environmental Variables. The pens were equipped with closed-circuit television cameras with night vision linked to video recorders (Figure 2). The recordings were made at the times described in Experiment 1. In addition to the 8 variables already considered in Experiment 1, we recorded

9. agonistic interactions between animals per hour (AI);

10. duration of the feeding and rumination periods per day (respectively, **F** and **R**); and
11. duration of time spent standing in the passage per day (SP).

Quantitative and Qualitative Milk Production.

Daily milk production was recorded at weekly intervals between July 2004 and September 2004. The milk samples were analyzed by mixing volumes proportional to the production of the 2 daily milkings and placing them in a 200-mL sterile plastic container. Total protein, total fats, and lactose were determined with an infrared spectrophotometer (MilkoScan 133B; Foss Electric, Hillerød, Denmark). The SCC was carried out with a Coulter Counter Z1 (Beckmann Coulter, Fullerton, CA). All determinations were performed in duplicate.

Hygienic Traits. Digital photographs were taken of the mammary gland (from the rear and from the 2 sides) and of the distal parts of the front and rear limbs (from the side and the rear) simultaneously with the weekly milk sampling. These photos were used to determine manure-covered areas to assess a dirtiness score of the mammary gland and the distal portion of the rear limbs (Reneau et al., 2003). Every 4 wk during milking, the following measurements of rear hooves were taken: hoof angle, dorsal toe length, and heel depth.

Every week a locomotion score was determined using a 4-point scale (Cook et al., 2004). The different free-stall lying area surfaces were subjected to coliform counts every week using cotton swabs in a saline solu-

Table 2. Least squares means $\pm$ SE for free-stall surface on duration of lying (L), bed occupation (BO), standing with 4 (S4F) and 2 (S2F) feet, number of interrupted lying down periods (NIL), duration of lying bouts (DLB), duration of ruminating while lying (R/L), proportion of eligible lying (PEL), and cow comfort index (CCI) in cows housed in a pen for the preference test

Item	EVA ¹	PVA ²	Wood shavings	Solid manure
BO, min/d	417.2 $\pm$ 68.3 ^A	222.3 $\pm$ 28.3 ^B	194.7 $\pm$ 34.6 ^B	159.9 $\pm$ 27.1 ^B
L, min/d	332.4 $\pm$ 24.0 ^A	130.8 $\pm$ 6.2 ^B	160.9 $\pm$ 23.7 ^B	102.6 $\pm$ 23.2 ^C
S4F, min/d	49.2 $\pm$ 4.0 ^A	46.2 $\pm$ 6.2 ^a	26.8 $\pm$ 3.7 ^{B,b}	45.7 $\pm$ 3.2 ^a
S2F, min/d	35.7 $\pm$ 4.6 ^A	45.3 $\pm$ 3.7 ^A	7.0 $\pm$ 2.8 ^B	11.7 $\pm$ 1.1 ^B
NIL, n/h	0.4 $\pm$ 0.0 ^B	1.1 $\pm$ 0.1 ^A	0.5 $\pm$ 0.0 ^B	0.6 $\pm$ 0.1 ^B
DLB, min	71.9 $\pm$ 5.7 ^b	95.4 $\pm$ 15.8 ^a	72.5 $\pm$ 7.4 ^b	73.3 $\pm$ 8.7 ^{ab}
R/L, min/d	42.0 $\pm$ 4.6	35.8 $\pm$ 4.4	40.7 $\pm$ 4.6	40.4 $\pm$ 4.4
PEL ³	0.7 $\pm$ 0.0 ^A	0.2 $\pm$ 0.0 ^B	0.8 $\pm$ 0.1 ^A	0.8 $\pm$ 0.1 ^A
CCI ⁴	0.8 $\pm$ 0.1 ^A	0.3 $\pm$ 0.1 ^B	0.8 $\pm$ 0.1 ^A	0.6 $\pm$ 0.1 ^A

^{a,b}Different letters within a row indicate a difference between the means of the floors ($P < 0.05$).

^{A-C}Different letters within a row indicate a difference between the means of the floors ($P < 0.01$).

¹Polyethylene vinyl acetate mats (Cow Mat CM20; DeLaval Inc., Kansas City, MO).

²Polypropylene vinyl acetate mats (Arienti Mat; Arienti snc, Cologno Monzese, Italy).

³Number lying:number in pen not eating.

⁴Number lying:number touching a stall surface: lying + 4 feet + 2 feet.

tion during the early morning before cleaning. Each free-stall lying area surface was divided into 9 squares (3×3), and the center of each square was swabbed. All samples were kept at 4°C, transferred within an hour to the laboratory, and processed during the day. The coliform counts were performed using the method of the most probable number (Harrigan, 1998). The most probable number technique is a quantitative method for routinely examining coliform sample counts. The swabs were inoculated using the following method: Undiluted material was inoculated into 3 tubes of lactose broth (Oxoid, Milan, Italy) at double strength with Durham tubes, and 2 serial dilutions (10^{-1} and 10^{-2} dilution) were made for inoculating 3 tubes at normal concentrations for each dilution. After 48 h of incubation at 37°C, the tubes were analyzed for the coliform count (Harrigan, 1998). The coliform count of each lying area surface was expressed as the logarithm to base ten of the arithmetic mean values of the 9 squares sampled.

Milk bacterial counts were conducted weekly. Ten milliliters of milk were collected aseptically after cleaning the teats with a 70% ethanol solution and discarding the first 3 streams of milk. The milk bacterial count was estimated using an electronic system that measures the hygienic quality of milk by counting the individual bacterial cells in raw milk (Bactoscan; Foss Electric). All hygienic traits were recorded from July 2004 to September 2004.

Statistical Analysis. The statistical analysis of behavioral measures was identical to that described in Experiment 1. Somatic cell counts and bacterial counts were normalized by calculating the common logarithm. The following model was used for the productive and hygienic variables:

$$y_{ijk} = \mu + \text{MAT}_i + \varepsilon_{ijk},$$

where y_{ijk} is the productive and hygienic traits; μ is the mean; MAT_i is the effect of the i th type of material as free-stall flooring ($i = 1, \dots, 4$); and ε_{ijk} is the error term.

RESULTS

Behavioral Aspects in the Preference Test

Free-Stall Flooring Material. Table 2 shows variations in some of the behavioral aspects of cows in relation to the 4 different flooring materials used on the free-stall lying surfaces. The cows included in the preference test had a greater ($P < 0.01$) occupation (BO) and duration of lying time in free stalls with EVA mats than in stalls with the other materials. Wood shavings and PVA mats were preferred more ($P < 0.01$) than solid manure for lying. Cows stood with 4 feet (S4F) least in free stalls with wood shavings compared with EVA mats ($P < 0.01$) or the other materials ($P < 0.05$), whereas the least-used flooring materials for standing with 2 feet (S2F) were wood shavings and solid manure ($P < 0.01$). The greatest number of interrupted attempts at lying down per hour (NIL) was on PVA mats ($P < 0.01$). The duration of single lying bouts (DLB) was greater in the free stalls with PVA mats compared with those with EVA mats and wood shavings ($P < 0.05$). The PEL index showed that the cows preferred the flooring materials of EVA mats, wood shavings, and solid manure ($P < 0.01$) to PVA mats. The CCI was lowest for PVA mats ($P < 0.01$).

THI. Table 3 illustrates the behavioral patterns in relation to variations in the THI. With THI < 74 the cows spent more time lying down (L) compared with

Table 3. Least squares means $\pm$ SE for temperature–humidity index (THI) classes¹ on duration of lying (L), bed occupation (BO), standing with 4 (S4F) and 2 (S2F) feet, number of interrupted lying down periods (NIL), duration of lying bouts (DLB), duration of ruminating while lying (R/L), proportion of eligible lying (PEL), and cow comfort index (CCI) in cows housed in a pen for the preference test

Item	A	B	C	D	E	F	G	H
BO, min/d	159.3 $\pm$ 26.3	146.4 $\pm$ 17.1	124.2 $\pm$ 17.0	128.6 $\pm$ 15.8	134.9 $\pm$ 25.7	146.6 $\pm$ 24.0	159.4 $\pm$ 30.3	162.3 $\pm$ 31.2
L, min/d	123.0 $\pm$ 21.2 ^a	102.6 $\pm$ 13.1 ^a	75.4 $\pm$ 11.5 ^{ab}	72.1 $\pm$ 9.5 ^{ab}	63.6 $\pm$ 17.1 ^{ab}	63.2 $\pm$ 14.8 ^b	63.5 $\pm$ 18.8 ^b	63.3 $\pm$ 23.3 ^b
S4F, min/d	25.5 $\pm$ 2.1 ^{b,c}	30.2 $\pm$ 2.1 ^b	32.7 $\pm$ 3.3 ^b	37.1 $\pm$ 3.9 ^b	45.8 $\pm$ 5.6 ^a	49.6 $\pm$ 5.8 ^{a,a}	54.8 $\pm$ 7.2 ^{a,a}	60.0 $\pm$ 4.1 ^{a,a}
S2F, min/d	10.8 $\pm$ 3.0 ^{c,b}	13.7 $\pm$ 2.0 ^c	16.1 $\pm$ 2.4 ^c	19.4 $\pm$ 2.4 ^{BC,a}	25.5 $\pm$ 3.1 ^B	33.7 $\pm$ 3.5 ^B	41.2 $\pm$ 4.2 ^A	39.0 $\pm$ 3.8 ^A
NIL, no./h	0.5 $\pm$ 0.0 ^{CD,b}	0.5 $\pm$ 0.0 ^C	0.4 $\pm$ 0.0 ^C	0.5 $\pm$ 0.1 ^{BC}	0.7 $\pm$ 0.1 ^{BD,a}	0.7 $\pm$ 0.1 ^B	0.8 $\pm$ 0.1 ^A	1.0 $\pm$ 0.1 ^A
DLB, min	85.8 $\pm$ 8.2	80.2 $\pm$ 8.6	78.8 $\pm$ 6.6	79.4 $\pm$ 9.2	78.3 $\pm$ 8.4	74.6 $\pm$ 8.0	74.6 $\pm$ 13.9	74.5 $\pm$ 12.4
R/L, min/d	43.3 $\pm$ 4.4	42.6 $\pm$ 4.4	42.90 $\pm$ 4.6	39.4 $\pm$ 4.4	39.9 $\pm$ 4.3	38.7 $\pm$ 4.4	37.4 $\pm$ 4.6	34.4 $\pm$ 4.9
PEL ²	0.8 $\pm$ 0.0 ^A	0.7 $\pm$ 0.0 ^A	0.6 $\pm$ 0.0 ^{B,a}	0.5 $\pm$ 0.0 ^{B,b}	0.4 $\pm$ 0.0 ^C	0.4 $\pm$ 0.0 ^C	0.4 $\pm$ 0.0 ^C	0.3 $\pm$ 0.0 ^C
CCI ³	0.8 $\pm$ 0.1 ^{A,a}	0.7 $\pm$ 0.1 ^A	0.6 $\pm$ 0.1 ^b	0.6 $\pm$ 0.1 ^b	0.5 $\pm$ 0.1 ^{BC}	0.4 $\pm$ 0.1 ^{BC,c}	0.4 $\pm$ 0.1 ^{C,c}	0.4 $\pm$ 0.1 ^{C,c}

^{a–d}Different letters within a row indicate a difference between the means of the floors ($P < 0.05$).

^{A–D}Different letters within a row indicate a difference between the means of the floors ($P < 0.01$).

¹A: THI < 72; B: 72 $\geq$ THI < 74; C: 74 $\geq$ THI < 76; D: 76 $\geq$ THI < 78; E: 78 $\geq$ THI < 80; F: 80 $\geq$ THI < 82; G: 82 $\geq$ THI < 84; H: THI $\geq$ 84.

²Number lying/number in pen not eating.

³Number lying/number touching a stall surface: lying + 4 feet + 2 feet.

THI > 80 ($P < 0.05$). The lying down time was 123.0 min/d for THI below 72, whereas at THI > 84 the time was 63.3 min/d. Standing with 2 (S2F) or 4 feet (S4F) in the free stall was influenced ($P < 0.01$) by the THI: This behavioral pattern increased steadily with an increasing in the THI. Time spent standing with 4 feet (S4F) for THI < 76 were lower compared with that for THI > 78 ($P < 0.01$). For THI values ranging between 76 and 78, S4F was lower than for THI > 80 ($P < 0.05$). Time spent standing with 2 feet (S2F) was greater for THI > 76 than for THI < 76 ($P < 0.01$). Interrupted lying events per hour (NIL) were more frequent at THI values > 78 ($P < 0.01$) than at THI < 78. The PEL index revealed a resting discomfort for cows with an increase in the THI. Differences ($P < 0.01$) were recorded between THI < 78 and the other THI, and between THI < 74 and higher THI. The CCI was similar to the PEL index: The values fell with an increase in the THI, and the greatest differences ($P < 0.01$) were found between THI < 74 and THI > 76, and between THI < 78 and THI > 82.

Interaction Between THI and Free-Stall Flooring Material. Figure 3 shows the results between the materials used for free-stall flooring and the THI classes for behavioral aspects of the interaction. Bed occupation (BO) in the various THI classes was always highest for the EVA mats, followed by PVA (for THI classes up to 80), solid manure, and wood shavings ($P < 0.01$). In free stalls bedded with wood shavings, there was a difference ($P < 0.05$) between THI < 76 and THI > 80. With an increase in the THI, the differences were greatest for PVA and solid manure ($P < 0.01$). The values for L in THI < 76 were greater ($P < 0.01$) for EVA mats. For wood shavings and solid manure, the values increased with greater THI ($P < 0.01$). In general, cows lay down less on the PVA mats ($P < 0.01$) than on the other materials. The free stalls used least for standing with 4 feet (S4F) were those with wood shavings ($P < 0.01$). The mats were used more for standing on with only the front feet (S2F) than were the other 2 materials in the free-stall areas ($P < 0.01$). In addition, the S2F values for THI < 76 were lower ($P < 0.001$) compared with the S2F values for THI > 78. For the THI interval between 76 and 78, the cows spent less time ($P < 0.01$) with their front feet in the free stalls (S2F) compared with THI > 80. The use of free stalls with EVA mats remained constant, whereas the use of free stalls with PVA mats increased ($P < 0.01$). The R/L values for all types of free-stall materials fell gradually with an increase in the THI ($P < 0.05$). The CCI fell for the free stalls with EVA and PVA mats ($P < 0.01$) with an increase in the THI. The free stalls bedded with wood shavings and solid manure showed an increase in the CCI with an increase in THI values ($P < 0.05$).

Figure 3. Effect of temperature–humidity index (THI) classes (A: THI < 72; B: 72 ≤ THI < 74; C: 74 ≤ THI < 76; D: 76 ≤ THI < 78; E: 78 ≤ THI < 80; F: 80 ≤ THI < 82; G: 82 ≤ THI < 84; H: THI ≥ 84) and free-stall flooring on bed occupation (BO), duration of lying (L), standing with 4 (S4F) and 2 (S2F) feet, number of interrupted lying down periods (NIL), duration of lying bouts (DLB), duration of ruminating while lying (R/L), and cow comfort index (CCI) in cows housed in a single pen with different free-stall flooring materials (preference test).

Table 4. Least squares means $\pm$ SE by free-stall surface on duration of lying (L), bed occupation (BO), standing with 4 (S4F) and 2 (S2F) feet, standing on passage (SP), number of interrupted lying down periods (NIL), duration of lying bouts (DLB), feeding (F), ruminating (R), duration of ruminating while lying (R/L), proportion of eligible lying (PEL), cow comfort index (CCI), and agonistic interactions (AI) in cows housed in different pens with different free-stall flooring materials

Item	EVA ¹	PVA ²	Wood shavings	Solid manure
BO, min/d	961.6 $\pm$ 153.5	1,011.3 $\pm$ 113.2	894.0 $\pm$ 191.4	915.6 $\pm$ 160.3
L, min/d	660.3 $\pm$ 115.6	599.7 $\pm$ 86.0	749.0 $\pm$ 161.8	677.7 $\pm$ 140.7
S4F, min/d	174.9 $\pm$ 21.9 ^{BC,a}	249.1 $\pm$ 14.1 ^A	112.8 $\pm$ 16.8 ^{C,b}	184.5 $\pm$ 14.6 ^B
S2F, min/d	126.5 $\pm$ 165.0 ^A	162.5 $\pm$ 13.1 ^A	32.2 $\pm$ 12.8 ^B	53.4 $\pm$ 5.2 ^B
SP, min/d	62.4 $\pm$ 3.5 ^B	96.4 $\pm$ 2.4 ^A	34.5 $\pm$ 5.4 ^C	45.3 $\pm$ 4.5 ^C
NIL, no./h	3.6 $\pm$ 0.1 ^C	9.5 $\pm$ 1.0 ^A	4.5 $\pm$ 0.3 ^B	5.5 $\pm$ 0.5 ^B
DLB, min	67.8 $\pm$ 4.1 ^B	84.7 $\pm$ 4.1 ^A	67.6 $\pm$ 4.1 ^B	67.2 $\pm$ 3.0 ^B
F, min/d	322.6 $\pm$ 10.0 ^A	269.2 $\pm$ 9.4 ^B	321.4 $\pm$ 11.2 ^A	319.3 $\pm$ 10.0 ^A
R, min/d	507.4 $\pm$ 12.3 ^{A,b}	453.5 $\pm$ 12.6 ^B	542.3 $\pm$ 11.4 ^{A,a}	497.1 $\pm$ 12.0 ^A
R/L, min/d	229.9 $\pm$ 31.6	195.4 $\pm$ 30.2	222.8 $\pm$ 31.5	221.1 $\pm$ 30.3
PEL ³	0.6 $\pm$ 0.1 ^b	0.4 $\pm$ 0.1 ^{B,c}	0.8 $\pm$ 0.1 ^{A,a}	0.7 $\pm$ 0.1 ^{ab}
CCI ⁴	0.6 $\pm$ 0.0 ^B	0.5 $\pm$ 0.1 ^{B,b}	0.8 $\pm$ 0.1 ^A	0.8 $\pm$ 0.1 ^a
AI, no./h	0.5 $\pm$ 0.0	0.6 $\pm$ 0.1	0.5 $\pm$ 0.0	0.6 $\pm$ 0.1

^{a-c}Different letters within a row indicate a difference between the means of the floors ($P < 0.05$).

^{A-C}Different letters within a row indicate a difference between the means of the floors ($P < 0.01$).

¹Polyethylene vinyl acetate mats (Cow Mat CM20; DeLaval Inc., Kansas City, MO).

²Polypropylene vinyl acetate mats (Arienti Mat; Arienti snc, Cologno Monzese, Italy).

³Number lying:number in pen not eating.

⁴Number lying:number touching a stall surface: lying + 4 feet + 2 feet.

Behavioral Aspects in the Aversion Test

Free-Stall Flooring Material. Table 4 shows variations in the behavioral traits with the 4 different materials used for the free-stall lying areas in the aversion test. There were no differences in bed occupation and lying down times among EVA mats, PVA mats, wood shavings, and solid manure. Standing with 4 feet (S4F) was most frequent for cows stabled with PVA mats ($P < 0.01$). Wood shavings and manure were used less for S4F than were EVA mats ($P < 0.05$). The cows kept in pens with free stalls floored with mats spent more time standing with 2 feet in the free stall (S2F) compared with those standing in free stalls with wood shavings and solid manure ($P < 0.01$). The time cows spent standing in the passage (SP) was greatest for those in the pen with free stalls bedded with PVA mats ($P < 0.01$). The greatest number of interrupted lying periods (NIL) was with PVA mats ($P < 0.01$), whereas the cows housed in free stalls with EVA mats ($P < 0.01$) had the lowest NIL. The greatest duration of lying bouts (DLB) was found for the cows with PVA mats ($P < 0.01$). These animals spent less time ($P < 0.01$) feeding (F) and ruminating (R). Greater ($P < 0.05$) R was noted in the free stalls with wood shavings than in those with EVA mats. Cows' proportion of eligible lying (PEL) on PVA mats was lower than for the free stalls with wood shavings ($P < 0.01$), solid manure ($P < 0.05$), and EVA mats ($P < 0.05$). The same index was lower ($P < 0.05$) for mats than for the organic bedding materials. The CCI differed according to the materials used for the free-stall

lying area. In particular, the highest CCI was with wood shavings compared with the mats ($P < 0.01$). The CCI in the pens with solid manure was greater than that in the pens with PVA mats ($P < 0.05$).

THI. Table 5 illustrates cows' behavioral patterns in relation to the THI in the aversion test. The time spent lying down per day (L) was greater for THI <72 than for all the other THI classes ($P < 0.01$). The time spent by animals standing on 4 feet (S4F) increased with the THI. The time spent with the front feet in the free stall (S2F) increased in proportion to the THI index. All the S2F at THI <80 were lower ($P < 0.01$) than the S2F at THI >80. The cows stood in the passage (SP) for more time when the THI increased. The number of interrupted lying down periods (NIL) was lowest in the THI between 72 and 76. There was a considerable increase for THI >82 ($P < 0.01$). The mean duration of every single lying bout (DLB) was inversely proportional to the THI. The duration of lying bouts was higher for THI <72 than for THI >80 ($P < 0.05$). The cows spent more time feeding (F) when the THI <80 ($P < 0.01$). Less time was spent in rumination (R) as the THI increased. There were differences ($P < 0.01$) between THI <78 and the higher classes. The 2 indices of well-being, the PEL index and the CCI, decreased with an increase in the THI.

Interaction Between THI and Free-Stall Flooring Material. Figure 4 describes the interactions among the free-stall flooring materials and the THI. Both the duration of bed occupation of the free stalls

Table 5. Least squares means $\pm$ SE for temperature-humidity index (THI) classes¹ on duration of lying (L), bed occupation (BO), standing with 4 (S4F) and 2 (S2F) feet, standing on the passage (SP), number of interrupted lying down periods (NIL), duration of lying bouts (DLB), feeding (F), ruminating (R), duration of ruminating while lying (R/L), proportion of eligible lying (PEL), cow comfort index (CCI), and agonistic interactions (AI) in cows housed in different pens with different free-stall flooring materials

Item	A	B	C	D	E	F	G	H
BO, min/d	150.2 $\pm$ 36.9	106.6 $\pm$ 22.1	99.3 $\pm$ 19.4	106.5 $\pm$ 16.0	111.4 $\pm$ 28.9	114.9 $\pm$ 25.1	119.8 $\pm$ 32.0	124.4 $\pm$ 39.6
L, min/d	138.8 $\pm$ 12.8 ^A	88.3 $\pm$ 10.6 ^B	75.1 $\pm$ 10.4 ^B	74.2 $\pm$ 15.3 ^B	74.2 $\pm$ 16.3 ^B	69.8 $\pm$ 18.8 ^B	69.0 $\pm$ 21.1 ^B	69.8 $\pm$ 20.9 ^B
S4F, min/d	6.7 $\pm$ 1.0 ^E	11.8 $\pm$ 0.9 ^D	16.5 $\pm$ 1.6 ^C	23.1 $\pm$ 2.8 ^B	25.1 $\pm$ 2.8 ^B	29.1 $\pm$ 2.9 ^{AB,b}	31.7 $\pm$ 3.6 ^{A,c}	36.3 $\pm$ 2.1 ^A
S2F, min/d	4.7 $\pm$ 1.4 ^{B,c}	6.4 $\pm$ 0.9 ^{B,c}	7.7 $\pm$ 1.1 ^{B,b}	9.3 $\pm$ 1.1 ^{B,a}	12.1 $\pm$ 1.5 ^{B,a}	16.0 $\pm$ 1.7 ^A	19.2 $\pm$ 2.2 ^A	18.3 $\pm$ 1.9 ^A
SP, min/d	5.5 $\pm$ 1.0 ^{B,b}	5.4 $\pm$ 1.0 ^{B,b}	5.9 $\pm$ 1.0 ^{B,b}	6.9 $\pm$ 1.1 ^b	7.8 $\pm$ 1.1 ^b	8.8 $\pm$ 1.1 ^a	8.3 $\pm$ 1.1 ^a	10.4 $\pm$ 1.2 ^{A,a}
NIL, no./h	0.7 $\pm$ 0.1 ^{B,b}	0.6 $\pm$ 0.1 ^{C,a}	0.5 $\pm$ 0.1 ^C	0.6 $\pm$ 0.1 ^{AB}	0.7 $\pm$ 0.1 ^B	0.7 $\pm$ 0.1 ^{B,a}	0.9 $\pm$ 0.1 ^A	1.0 $\pm$ 0.1 ^A
DLB, min	79.8 $\pm$ 3.9 ^a	75.4 $\pm$ 3.8	74.5 $\pm$ 3.1	74.0 $\pm$ 3.5	71.8 $\pm$ 3.5	67.9 $\pm$ 3.2 ^b	66.3 $\pm$ 4.2 ^b	64.9 $\pm$ 5.3 ^b
F, min/d	48.3 $\pm$ 3.5 ^A	56.7 $\pm$ 4.7 ^A	43.0 $\pm$ 3.8 ^A	47.4 $\pm$ 3.5 ^A	48.5 $\pm$ 3.8 ^A	21.2 $\pm$ 3.0 ^B	15.5 $\pm$ 2.6 ^{BC,a}	8.2 $\pm$ 2.5 ^{C,b}
R, min/d	92.2 $\pm$ 5.5 ^A	89.3 $\pm$ 5.4 ^A	94.5 $\pm$ 5.6 ^A	84.7 $\pm$ 5.3 ^A	60.2 $\pm$ 5.0 ^B	68.5 $\pm$ 5.0 ^B	61.4 $\pm$ 5.1 ^B	55.4 $\pm$ 5.0 ^B
R/L, min/d	59.4 $\pm$ 7.6	58.3 $\pm$ 7.5	57.5 $\pm$ 7.8	53.8 $\pm$ 7.6	54.5 $\pm$ 7.5	52.9 $\pm$ 7.6	51.1 $\pm$ 7.9	46.9 $\pm$ 8.3
PEL ²	0.9 $\pm$ 0.1 ^{A,a}	0.8 $\pm$ 0.1 ^{A,b}	0.8 $\pm$ 0.1 ^{A,b}	0.7 $\pm$ 0.1 ^c	0.7 $\pm$ 0.1 ^c	0.6 $\pm$ 0.1 ^d	0.6 $\pm$ 0.1 ^{B,d}	0.5 $\pm$ 0.1 ^{B,d}
CCI ³	0.9 $\pm$ 0.1 ^A	0.8 $\pm$ 0.1 ^A	0.8 $\pm$ 0.1 ^B	0.7 $\pm$ 0.1 ^B	0.7 $\pm$ 0.1 ^C	0.5 $\pm$ 0.1 ^{D,a}	0.5 $\pm$ 0.1 ^D	0.5 $\pm$ 0.1 ^{D,b}
AI, no./h	0.1 $\pm$ 0.0	0.1 $\pm$ 0.0	0.1 $\pm$ 0.0	0.1 $\pm$ 0.0	0.1 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.1 $\pm$ 0.0

^{a-c}Different letters within line indicate a difference between the means of the floors ($P < 0.05$).

^{A-E}Different letters within line indicate a difference between the means of the floors ($P < 0.01$).

¹A: THI < 72; B: 72 $\leq$ THI < 74; C: 74 $\leq$ THI < 76; D: 76 $\leq$ THI < 78; E: 78 $\leq$ THI < 80; F: 80 $\leq$ THI < 82; G: 82 $\leq$ THI < 84; H: THI $\geq$ 84.

²Number lying/number in pen not eating.

³Number lying/number touching a stall surface: lying + 4 feet + 2 feet.

over a 24-h period (BO) and the duration of lying over a 24-h period (L) followed similar patterns, with free stalls floored with mats being occupied less when there was an increase in the THI and the opposite with the organic bedding materials. The cows stood with 4 feet (S4F) or 2 feet (S2F) more when the THI increased; however, only in free stalls with wood shavings did this behavior remain relatively constant for THI > 72. The NIL increased with THI for all the materials, with the exception of wood shavings. In all the THI classes, the NIL was greatest ($P < 0.01$) for the free stalls with PVA mats. The rumination time (R) at all THI was greater in the free stalls with EVA mats, and declined with increased THI. The CCI fell with an increase in the THI ($P < 0.01$) for the free stalls floored with mats, whereas the organic materials had constant values for all THI.

Quantitative and Qualitative Production, BW and BCS, and Hygienic Parameters in the Aversion Test. Table 6 shows milk yields and milk components as well as BW and BCS (starting and final) for the 4 materials used for free-stall flooring. Milk production is displayed for the 32 cows in the aversion test. The total protein content ($N \times 6.25$) of the milk from cows housed on PVA flooring was lower than from cows on wood shavings or on solid manure ($P < 0.05$). The daily production of lactose was lower from cows in PVA-floored free stalls compared with cows in free stalls with wood shavings ($P < 0.05$). Although the starting BCS was not different, the final BCS of the cows in pens with PVA mats was lower than those of cows in the pens with wood shavings ($P < 0.05$).

Table 7 shows the SCC, locomotion scores, cleanliness scores, hoof measurements, and bacterial counts of free-stall surfaces and the milk of cows involved in the aversion test. The SCC and the scores for the dirtiness of the udder and of the distal parts of the rear limbs were not influenced by the free-stall flooring material, nor were the traits for the morphology of the hoof (wall-sole angle, maximum wall length, and heel height). The bacterial counts of milk and coliform counts of the free-stall lying surfaces were not different. The locomotion scores increased for cows resting on PVA compared with the other materials ($P < 0.05$).

DISCUSSION

The comfort provided by free-stall flooring should be evaluated by using the preference test. This type of test has been used since the 1970s to evaluate structures in relation to animal well-being (Manninen et al., 2002; Tucker et al., 2004). The reliability of this test is very high, in that the animals are placed in the position of choosing the best option, from their perspective, on the

Figure 4. Effect of temperature–humidity index (THI) classes (A: THI < 72; B: 72 ≤ THI < 74; C: 74 ≤ THI < 76; D: 76 ≤ THI < 78; E: 78 ≤ THI < 80; F: 80 ≤ THI < 82; G: 82 ≤ THI < 84; H: THI ≥ 84) and free-stall flooring on bed occupation (BO), duration of lying (L), standing with 4 (S4F) and 2 (S2F) feet, number of interrupted lying down periods (NIL), duration of lying bouts (DLB), duration of ruminating while lying (R/L), and cow comfort index (CCI) in cows housed in 4 different pens with different free-stall flooring materials (aversion test).

Table 6. Least squares mean $\pm$ SE for productive traits of dairy cows housed in 4 pens with different free-stall flooring materials

Item	EVA ¹	PVA ²	Wood shavings	Solid manure
Milk yield, kg/d	27.3 $\pm$ 0.2	26.9 $\pm$ 0.2	27.0 $\pm$ 0.3	27.1 $\pm$ 0.3
Milk fat, g/d	36.2 $\pm$ 0.9	35.7 $\pm$ 0.8	37.1 $\pm$ 0.8	36.8 $\pm$ 1.0
Milk protein, g/d	35.4 $\pm$ 0.5	34.6 $\pm$ 0.5 ^b	36.1 $\pm$ 0.5 ^a	36.1 $\pm$ 0.5 ^a
Milk lactose, g/d	48.5 $\pm$ 0.4	47.3 $\pm$ 0.4 ^b	49.2 $\pm$ 0.4 ^a	47.9 $\pm$ 0.4
r, ³ min	19.1 $\pm$ 1.5	19.3 $\pm$ 1.6	19.0 $\pm$ 1.4	19.5 $\pm$ 1.3
k ₂₀ , ⁴ min	9.5 $\pm$ 3.2	10.1 $\pm$ 2.8	9.7 $\pm$ 3.0	10.0 $\pm$ 3.3
a ₃₀ , ⁵ mm	27.8 $\pm$ 4.7	24.7 $\pm$ 5.4	22.7 $\pm$ 4.8	27.6 $\pm$ 5.0
Starting BW, kg	460.2 $\pm$ 9.3	460.0 $\pm$ 10.2	462.3 $\pm$ 9.7	461.2 $\pm$ 9.4
Final BW, kg	522.4 $\pm$ 11.3	524.0 $\pm$ 10.9	523.9 $\pm$ 11.6	524.6 $\pm$ 10.6
Starting BCS	3.0 $\pm$ 0.4	2.9 $\pm$ 0.4	3.1 $\pm$ 0.2	2.9 $\pm$ 0.3
Final BCS	2.8 $\pm$ 0.4	2.4 $\pm$ 0.3 ^b	3.0 $\pm$ 0.3 ^a	2.9 $\pm$ 0.3

^{a,b}Different letters within a row indicate a difference between the means of the floors ($P < 0.05$).

¹Polyethylene vinyl acetate mats (Cow Mat CM20; DeLaval Inc., Kansas City, MO).

²Polypropylene vinyl acetate mats (Arienti Mat; Arienti snc, Cologno Monzese, Italy).

³Clotting time.

⁴Rate of clot formation.

⁵Clot firmness after 30 min.

basis of environmental conditions (Dawkins, 1990), although some characteristics of this kind of test make it imperfect. The preference test intrinsically creates a condition of low animal density. This situation contrasts with the reality of a commercial dairy farm, where the tendency is to exploit all the available space and where there is often overcrowding. For all these reasons, the preference test should be accompanied by an aversion test, characterized by a more realistic situation in which there is only one free stall for every cow—but above all, in which only one type of material is used as flooring—to evaluate the behavior under real field conditions in which the cow does not have the possibility of choice. The results showed that the material used for free-stall flooring has a decisive influence on the amount of rest which, together with feeding behavior,

gives us greater indications of the comfort offered to the animals. Our tests revealed a certain discomfort on the part of the cows using the PVA mats. The cows that were given the opportunity to choose between the 4 types of flooring material had a certain aversion toward PVA mats, which was supported in the aversion test by the reduced duration of lying for the cows in free stalls with PVA mats. These results were confirmed by the greater times spent standing with 4 (S4F) or 2 feet (S2F) on the PVA mats, as well as by the greater number of interrupted lying down (NIL) periods per hour. One aspect that apparently contrasted with the thesis that the PVA mats were less comfortable was the average length of each individual lying bout, which was greater than for the other flooring materials considered in both the preference and aversion tests, but this result may

Table 7. Least squares mean $\pm$ SE for SCC, locomotion score, cleanliness scores, hoof measurements, total bacteria counts of raw milk and coliform counts of cows housed in 4 pens with different free-stall flooring materials

Item	EVA ¹	PVA ²	Wood shavings	Solid manure
SCS, log ₁₀ no. cells/mL	4.7 $\pm$ 0.2	4.9 $\pm$ 0.2	4.7 $\pm$ 0.2	4.7 $\pm$ 0.2
Locomotion score ³	1.2 $\pm$ 0.1 ^b	1.8 $\pm$ 0.2 ^a	1.2 $\pm$ 0.2 ^b	1.3 $\pm$ 0.1 ^b
Udder cleanliness score ⁴	1.4 $\pm$ 0.1	1.4 $\pm$ 0.1	1.5 $\pm$ 0.1	1.4 $\pm$ 0.1
Rear distal legs score ⁵	1.2 $\pm$ 0.1	1.3 $\pm$ 0.1	1.3 $\pm$ 0.1	1.2 $\pm$ 0.1
Hoof angle, degrees	42.5 $\pm$ 2.7	42.4 $\pm$ 2.9	43.1 $\pm$ 3.0	42.0 $\pm$ 2.7
Dorsal toe length, cm	8.1 $\pm$ 0.2	8.0 $\pm$ 0.6	8.3 $\pm$ 0.5	8.4 $\pm$ 0.6
Heel depth, cm	4.6 $\pm$ 0.2	4.1 $\pm$ 0.3	4.6 $\pm$ 0.3	4.5 $\pm$ 0.2
Milk bacteria count, log ₁₀ cfu/mL	231.8 $\pm$ 22.5	232.5 $\pm$ 24.0	220.7 $\pm$ 23.7	220.2 $\pm$ 25.3
Lying area surface coliform counts, log ₁₀ cfu/mL	289.6 $\pm$ 25.3	305.9 $\pm$ 33.2	290.3 $\pm$ 39.2	305.0 $\pm$ 22.6

^{a,b}Different letters within a row indicate a difference between the means of the floors ($P < 0.05$).

¹Polyethylene vinyl acetate mats (Cow Mat CM20; DeLaval Inc., Kansas City, MO).

²Polypropylene vinyl acetate mats (Arienti Mat; Arienti snc, Cologno Monzese, Italy).

³Range from 1 (normal progress) to 4 (lying down and absence of standing).

⁴Range from 1 (total absence of dirt) to 5 (more than 90% of udder covered by dirt).

⁵Range from 1 (total absence of dirt) to 5 (more than 90% of rear distal legs covered by dirt).

be linked to the difficulty of getting up, because the surface is slippery. This hypothesis is supported by the frequent observations of cows slipping during a change of position (from lying to standing, and vice versa).

Another fundamental measure that can be garnered from these tests is the absolute influence of environmental temperature and relative humidity on the behavior of dairy cows in relation to the free-stall surface material. This aspect has not been adequately examined in the literature. Many studies have been concerned with differences in the behavior of cows during rest in relation to seasonal variations (Manninen et al., 2002; Wagner-Storch et al., 2003) and in structural aspects of the pens (Fulwider and Palmer, 2004; Tucker et al., 2004; Veissier et al., 2004), but none have considered them in relation to variations in the THI. Our tests showed that heat stress had an absolute influence on well-being and that the free-stall surface material on which the cows rested was of fundamental importance. Therefore, an increase in the THI was accompanied by a reduction in the duration of lying and an increase in the time spent standing with 2 (S2F) and 4 feet (S4F) in the free stall. In addition, there was an increase both in standing in the passage (SP) and in the number of interrupted lying down (NIL) periods. In agreement with the results of Keister et al. (2002), the data collected in our study showed that the time dedicated to feeding and rumination declined as heat stress increased. The preference test showed that the animals willingly used the EVA mats above all other flooring materials for lying down, but in situations of high temperature and humidity (THI >80) they preferred organic materials, which offered a greater degree of comfort.

In the aversion test, the animals used PVA and EVA mats more than organic beddings for lying down at THI <74 and switched to organic beddings at THI >78. The behaviors shown in both the tests are probably due to differences in the transmission of heat from the cow to the free-stall surface material in warm climates. Plastic polymers may create a heat insulation that does not contribute to heat dissipation from the cow to the lying area surface. The overall comfort of dairy cattle, as measured by the time lying down, appears to be influenced more when cattle are not provided with a choice of free-stall flooring surface, as in the aversion test.

For an overall evaluation of the value of the 4 flooring materials in climates with high THI, we found that neither the coliform counts on the stall surfaces nor the total bacterial counts in the milk were different between mats contaminated with organic manure and free stalls bedded with organic solids and wood shavings. These results disagree with Eckes et al. (2001), who found a greater microbial presence on organic beddings. Additional microbiological surveys aimed at isolating envi-

ronmental mastitis pathogens in different climatic conditions and on different free-stall flooring materials are warranted. The materials used as free-stall flooring in warm climatic conditions had a limited influence on the dirtiness score of the mammary glands and the distal parts of the rear limbs and on the morphology of the hooves.

Somatic cell counts and total bacterial counts in raw milk were not statistically different under our conditions. From these observations, it is clear that udder health (evaluated through the SCC) and milk hygiene (measured as the total bacterial count) were not influenced by the material used as the free-stall lying surface.

CONCLUSIONS

The materials used as free-stall flooring cannot be used as absolute standards in relation to the comfort of the dairy cows, because they modify their value according to the microclimatic conditions in which they are used. In particular, in conditions of heat stress, the material on which the cow lies must perform additional functions of extreme importance, such as being able to dissipate body heat as well as sweat and transpiration. In conditions of low THI, the best surface material in a free-stall rest area is that on which the cows spend the most time lying down. With high THI values, however, the cows reduce their lying times (with an increase in time spent standing) independently of the material, although these lying times are reduced less on surfaces with wood shavings and solid manure than on EVA mats. Industries that provide technological aids for improving animals' well-being will therefore have to take into account these new aspects of the comfort of dairy cows during conditions of heat stress. The results of the present work clearly indicate the necessity of evaluating both materials that are already in use and those that are innovative, not only according to their influence on personnel costs, but also on the hygienic value and comfort of rest, while taking into account the different microenvironmental conditions in which the materials are to be used.

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