ANTI-SKID RUBBER MATS FLOORING EFFECTS ON THE BEHAVIOURAL ACTIVITY, STRESS, AND MILK YIELD OF DAIRY COWS

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ABSTRACT. Data regarding the effects of anti-skid rubber mat-enriched flooring (RM) on dairy cattle production in Malaysia is limited. Hence, this study evaluates the behavioural activity,and milk yield of 40 dairy cows from 2 concrete-floor (CF) (n=10) and 2 anti-skid rubber mat-enriched floor (RM) (n=10) free-stall design farms with intensive management. Cows housed on RM farms showed a significantly ($p < 0.05$) higher frequency and duration (seconds) of lying down behaviour compared to cows housed on CF, (RM: 4.96 ± 1.33 and 3950.75 ± 744.48 vs. CF: 2.26 ± 1.29 and 1959.6 ± 762.84; mean ± SD). Also, cows housed on RM demonstrated a significantly higher average milk yield (litre/cow/day) than cows housed on CF ($p < 0.05$). A glucose tolerance test (GTT), adrenocorticotropic hormone (ACTH) challenge, and leukocyte count were conducted (n = 5/farm), to further explore the animals' energy metabolism and level of stress, respectively. No significant ($p > 0.05$) differences in the GTT, ACTH, or leukocyte count were observed between cows in CF and RM farms. Thus, the use of RM in dairy farms may improve the milk yield of dairy cattle by promoting the expression of natural activities such as lying down which is important for rumination, without effecting the stress levels or glucose metabolism.

*Keywords***:** dairy cows, floor, stress, production

INTRODUCTION

Numerous studies have reported the detrimental effects of a zero-grazing system on pre-lameness hoof health. Lameness is often observed when cattle are confined to a free-stall barn with a concrete floor (CF). The use of poorly installed concrete surfaces may increase the risk of hoof problems through excessive hoof wear and tear (Dirksen,1997) or animal slippage (Schlichtung,1987), necessitating the implementation of alternative types of flooring such as rubber mats, which may help reduce these problems (Hultgren, 2001).

The benefits of anti-skid rubber mat flooring (RM) are widely acknowledged in Western countries. On dairy farms, the compressibility and improved friction provided by rubber mats were found to reduce the risk of slipping of both the cows and farm workers (Rushen & de Passillé, 2006). Furthermore, the soft texture of this material encourages cows to spend less time standing and more time lying down (Rushen *et al.,* 2007), consequently improving the health of legs and hooves (Vanegas *et al.,* 2006), which is the primary animal welfare concern in the dairy industry.

It is known that the behaviour of animals also corresponds to their level of stress (Koolhaas & Van Reenen, 2016). For instance, cows that are frequently deprived of lying display greater adrenocorticotropic hormone (ACTH) concentrations at the start of and at the end of a 14-h deprivation period (Munksgaard & Simonsen, 1996) suggesting that the situation is stressful causing inability of cows to adapt during this period. The release of cortisol in such situation can be an indicator of poor welfare (Candiani *et al.,* 2008) and has been used as one of the possible markers for the evaluation of chronic stress (Trevisi & Bertoni, 2009).

Chronic stress has been postulated to result in hyperreactivity of the adrenal cortex (Broom, 1988) and may lead to adrenal fatigue (Wilcox *et al.,* 2013). During chronic stress, the disruption of immune system may occur resulting from the prolonged ACTH production and elevation of plasma cortisol concentration (Wilcox *et al.,* 2013) which increases production of neutrophils (Bilandžić *et al.,* 2006).

In contrast, von Borell (2001) suggested that a progressive reduction in blood cortisol might occur if the animals had adapted to long-existing housing conditions assumed to be stressful. However, repeated exposure to the same stressor (homotypic stress) may lead to habituation in glucocorticoid responses (Simpkiss & Devine, 2003).

Glucocorticoids, including cortisol, influence physiological processes such as energy metabolism specifically when animals try to cope and adapt to the stress. Dairy cows under stress display elevated glucocorticoids and blood neutrophilic leukocytosis. In addition, excessive glucocorticoid production has been correlated with insulin resistance (Andrews & Walker, 1999). Hence, the glucose tolerance test (GTT) is often used to determine the energy metabolism of animals potentially overcoming stress.

Based on complexity of interaction between the aforementioned factors, the evaluation of physiological changes corresponding to stress in these animals should be integrated with the behavioural observations to provide a more comprehensive understanding. To date, most research on the effects of RM–enrichment on farm cattle had been conducted in temperate climates.

There is a lack of literature focusing on its effect on temperate cattle raised in tropical regions, including Malaysia. The marked differences in climate, temperature, and humidity between temperate countries and the tropics might affect cattle performance as the European breeds of cattle (*Bos taurus*) had been reported to display poorer reproductive performance, higher mortality rate, and reduction in milk yield after introduction to tropical regions (Huertas *et al.,* 2009). This might be due to the cattle's inability to adapt to the extremely hot and humid climates, feed changes, and diseases present in the tropics. Hence, the effect of RM on the performance of these cattle might be different due to these factors. This study is therefore, conducted to evaluate the effect of RM on the physiology and production of locally-raised dairy cattle in Malaysia.

MATERIALS AND METHODS

Farms and Animals

Two CF farms and two RM-enriched farms are selected for study based on their management similarities. Both farms use semi-intensive practices with the cut–and–carry system and both are free-stall design farms with a stocking density of 1 cow/stall. Animals are fed at 8.30 am and 2.30 pm daily with fresh Napier grass and concentrate *ad libitum*. Water is freely available from the water trough. Similar type of rubber mats made from natural rubber (dimension: 4ft. x 6 ft. x 17 mm) are used in both of the selected RM farms. The rubber mats are introduced into the farms for the period of at least one year and placed as stall bed in the resting area. Three farms (2 CF, 1 RM) are located in the state of Selangor, Malaysia, and one RM-enriched farm is located in the state of Negeri Sembilan, Malaysia. The two adjacent states are located in the mid-west region of peninsular Malaysia. All farms selected in this study are within 20 km radius from each other (Table 1). The sheds and floor in these farms are cleaned twice daily using high water pressure.

Regular health checks of animals in these farms are conducted by veterinarians from the Department of Veterinary Services. Animals selected for this study are considered healthy as there are no history of clinical disease reported within three months prior to and during the period of study. All animals under study have good temperament and do not have any reported issues with administration of any drugs or anthelmintic prior to the study. The dairy cows in these farms are handled by experienced and trained personnel at all times.

A total of 40 (n=10) Friesian-cross lactating cows are selected for the behavioural study. From these, a total of 20 dairy cows (n=5 per farm) are selected for the glucose tolerance test (GTT) and adrenocorticotropic hormone (ACTH) challenge. Both tests were conducted in each animal in all farms, whereby the ACTH challenge was performed 28 days after the GTT. All lactating cows under study are between 4 and 6 years old (mean ± S.D of body weight = 366.6 ± 54.3 kg, body condition score ranging from 3.0 to 3.5), have a parity range between 2 and 4 and are at the mid-lactation stage. Cows were milked twice daily, at 0600h and 1700h. All methods used in this study are approved by the Institutional Animal Care and Use Committee (IACUC), Universiti Putra Malaysia (R054/2017).

Table 1. Distance (km) between concrete floor (CF) farms and anti-skid rubber mat (RM)-enriched farms. Source: Google Map of Hulu Langat District (2020).

Distance (km)	Farm A (CF)	Farm B (CF)	Farm C (RM)	Farm D (RM)
Farm A (CF)	-	14.5	0.4	16
Farm B (CF)	14.5	-	14.7	17.7
Farm C (RM)	0.4	14.7	-	15.8
Farm D (RM)	16	17.7	15.8	-

Average Temperature and Relative Humidity

The study commenced between February 2017 and August 2018. All farms under study are located within the district of Hulu Langat. In year 2017 and 2018, a lower range of average monthly temperature (25 °C to 26 °C) were recorded from January to March for Hulu Langat and its surrounding areas (Figure 1). In 2017, an average monthly temperature of between 26 °C and 27 °C were recorded from April to December. However, in 2018, the highest average monthly temperature of 29 °C was recorded from October until December while a temperature of below than 28 °C was recorded for the rest of the year. The average percentage of relative humidity for Hulu Langat and its surrounding areas for year 2017 and 2018 ranged between 69 % and 84 %, respectively (Figure 2). Collectively, data from Table 1 and, Figure 1 and 2 indicate that farms which are located at a similar distance to each other in the same district share the same average temperature and relative humidity, thus eliminating the possibility of variation in observations due to these factors.

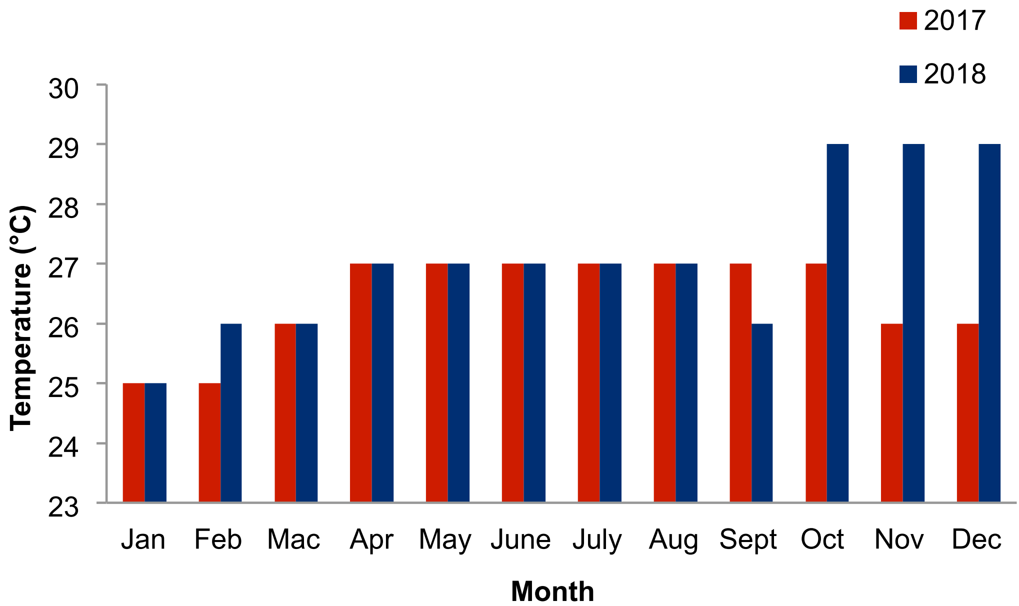

Figure 1. Average of monthly temperature recorded for Hulu Langat district and surrounding areas in year 2017 and 2018. Retrieved from WorldWeatherOnline.com (2020).

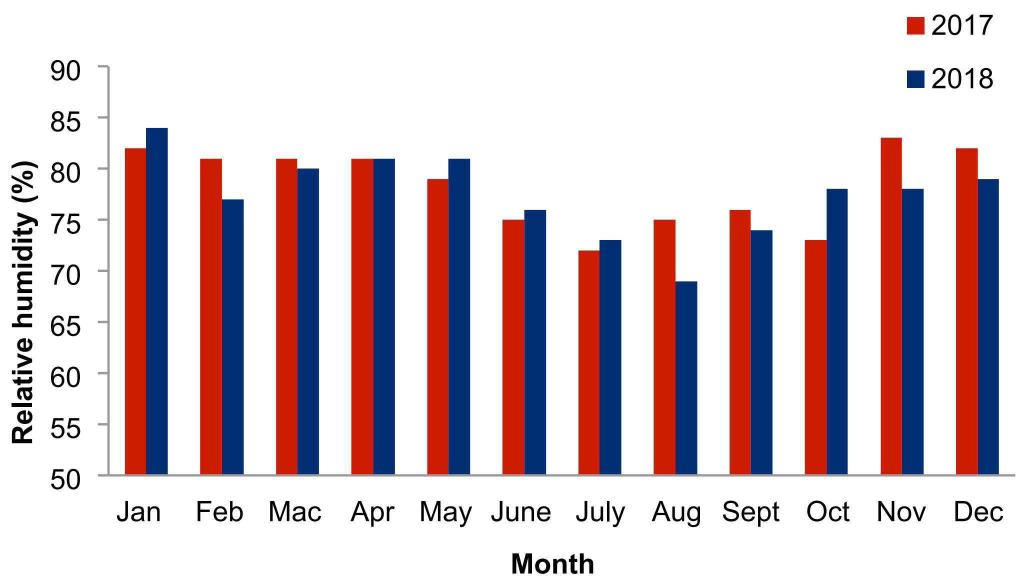

Figure 2. Average percentage of relative humidity recorded for Hulu Langat and surrounding areas in year 2017 and 2018. Retrieved from WorldWeatherOnline.com (2020).

Behavioural Study

The behavioural study was conducted according to Yanar *et al.* (2010) with slight modifications. Ten lactating cows from each farm were filmed using an action camera (4K Sports Ultra HDDV, Model SLDV4K; Serene Life™, China) for two hours per day, from 1000h until 1200h. Due to the time constraint and limitations imposed by the owner of the farm, the duration of observation for this activity was permitted for only two hours. Therefore, it is assumed that the selected time period should be used effectively for resting and the cows should be spending more time lying down instead of standing. In addition, none of the dairy cattle operational activities like milking or treatment was conducted during the recording. Observation and video recording were conducted for five days within the month of April 2017. The behavioural activities (i.e., standing, lying, feeding, drinking, and walking) of the cows are classified as follows: standing is defined as a body in an upright position and supported by four legs; lying is described as direct contact of the left or right side of the cow's body with the ground; drinking is described as the head over or in the water trough; feeding is described as the head over or in the bunk and lastly, walking is defined as moving at least three legs forward in sequence. Based on the recorded video, the frequency and duration of each behaviour displayed were analysed off-line with a behavioural coding software (Solomon Coder© version beta 17.03.22).

GTT and ACTH Challenge

The GTT and ACTH challenge were carried out as described by Huzzey *et al.* (2012) with a slight modification of ACTH challenge dosage (Schwinn *et al.,* 2018). All cows under study underwent the GTT before the ACTH challenge. However, these tests were conducted independently of each other in the same cow, at least 28 days apart. Both of these tests were conducted on 20 out of the total 40 Friesian-cross lactating dairy cows (n=5) from two CF and two RM–enriched farms. Each study was performed daily in the morning, from 0900h until 1200h. The feed was removed two hours before the start of experiment. Each cow was restrained and fitted with an indwelling catheter (2.1 x 133 mm, 5.25 IN, 14 G, BD Angiocath) at either the right or left jugular vein. The catheter was secured and wrapped with elastic or adhesive bandages to prevent the cows from dislodging the catheter during the test. The bodyweight of all animals was measured pre-test to determine the dosages for both analyses. During GTT, animals were administered (0.25 g/kg of body weight) a glucose solution (50 % wt./vol, B. Braun Medical Industries, Penang, MY) intravenously and blood samples were collected at -15, 0, 15-, 30-, 60-, and 120- minutes relative to glucose administration. Blood samples were taken at 0 min or in order to establish the concentration of glucose m these animals at the point of glucose administration.

Cows were subjected to the ACTH challenge at 28 days post GTT. As for the ACTH challenge, animals were administered (0.16 µg/kg of body weight) ACTH intravenously (Anaspec, San Jose, CA), and blood samples were collected at 0, 30, 60, 90, and 120 min after the administration of ACTH. An elevation of the plasma corticosteroid concentration above the basal concentration was previously reported to occur 30 minutes after an ACTH administration (Kolver & Matthews, 2014). Therefore, a measurement of the value was made at 0 min to determine the basal concentration of plasma corticosteroid in the animals prior to the challenge. At the end of each test, the catheters were then removed, and the cows were returned to their respective group pens. All blood samples were collected using EDTA tubes and were stored in a cooler maintained at 2–8 °C by ice pack during transportation from the farm to the laboratory. Upon arrival at the laboratory, blood samples were centrifuged immediately for 15 minutes at 2800 G. Plasma samples were collected and stored at -20 °C for subsequent analysis of glucose and cortisol concentration. Samples of plasma from the GTT underwent a routine laboratory analysis. Samples of plasma collected from the ACTH challenge were evaluated for cortisol concentration using radioimmunoassay (RIA) (Cortisol RIA kit, Beckman Coulter, Czech Republic). The intra- and inter-assay coefficient of variation (CV) for the cortisol assays were 6.2 % and 6.7 %, respectively.

Radioimmunoassay

All reagents and samples were brought to room temperature before pipetting. Each of the antibody-coated tubes were labelled in duplicate accordingly for total count (TC), Standard 0 (S0), Standard 1 (S1), Standard 2 (S2), Standard 3 (S3), Standard 4 (S4), Standard 5 (S5), quality control (QC) and sample tubes. The cortisol calibrators were pipetted to each tube according to their concentrations (50 µl of 0 nmol/L cortisol calibrator for S0, 50 µl of 20 nmol/L cortisol calibrator for S1, 50 µl of 50 nmol/L cortisol calibrator for S2, 50 µl of 200 nmol/L cortisol calibrator for S3, 50 µl of 720 nmol/L cortisol calibrator for S4 and 50 µl of 2160 nmol/L cortisol calibrator for S5). Then, 50 µl of each sample was added to each sample tube. All of the labelled tubes were added with 500 µl of 125 I-labeled cortisol tracer including tubes for the total count and mixed well. All tubes were then incubated for 1 hour at 18 – 25 °C on orbital shaker (≥ 400 rpm). Contents in each tube were decanted carefully except the tubes for the total count. The gamma-counting procedure was conducted using Packard Cobra II Gamma Counter (USA) to determine the count per minute (CPM) bound and total CPM (T). The concentrations of samples were determined from standard curve analyses using GraphPad Prism 6.

Leukocyte Profiles

The white blood cell (WBC) count was determined for each animal from blood samples collected in EDTA tubes during the glucose tolerance test. All samples were stored in a cooler and were transported to the Clinical Pathology Laboratory, Faculty of Veterinary Medicine, Universiti Putra Malaysia (UPM) for white blood cell differential count (i.e., neutrophils, lymphocytes, eosinophils, and monocytes) using an automated blood analyser. The neutrophils to lymphocytes ratio (N: L ratio) was determined through simple calculation. The analysis of blood samples for WBC was conducted within 24 hours after collection from the respective farms.

Average Milk Yield

The average milk yield (litre/cow/day) from each farm (n=10) was calculated based on a 30-day farm records for the month of January 2017. The daily milk yield for each cow consisted of milk (L) produced in the morning and evening milking. Comparisons of the milk yield between and within the group of flooring types were performed.

Calculations and Statistical Analyses

The data were analysed using IBM® SPSS® Statistics version 20. The Shapiro–Wilk test was performed to check for data normality. In the behavioural study, the average duration and frequency for five days observation of each behavioural parameter were analysed with an independent t-test, with results presented as mean ± standard deviation (S.D.) and with Mann–Whitney test, with results expressed as median (interquartile range). For the GTT and

ACTH challenge, a mixed-design ANOVA or split-plot ANOVA (SPANOVA) was performed by incorporating the fixed terms for flooring types, time, and their interaction in this model. Differences were determined by F-tests, and pairwise comparisons between the group means, as well as the associated *p*–values were acquired. Model effects were deemed statistically significant when the Type I error rate was less than 5 %. The area under the curve (AUC) and clearance rate (CR) of GTT and ACTH challenge were determined to provide additional evidence that flooring type may be associated with changes in energy metabolism and stress level. AUC for the glucose response to the GTT and cortisol response to the ACTH challenge were calculated using the trapezoidal method, and the clearance rate (CR) of glucose and cortisol were calculated as described by Kaneko (2008) by the following formula:

$$\text{CR (\%/min)} = \frac{\ln[t_a] - \ln[t_b]}{t_b - t_a} \times 100$$

where $[t_a]$ and $[t_b]$ are the concentrations of the glucose or cortisol at times a and b, respectively. The average milk yield from each farm was compared between groups using independent t-test results expressed as mean ± S.D. Significance was assigned at $p < 0.05$.

RESULTS

Behavioural Study

The mean duration of lying behaviour of cows housed on RM was significantly higher than cows housed on concrete: t (38) = 8.35; $p < 0.01$ (Table 2). In contrast, the mean duration for standing behaviour of cows housed on CF was significantly higher than cows housed on RM: t (38) = 6.80; $p < 0.01$. As for walking behaviour, the mean duration differed significantly between the two groups of flooring types, t (28.54) = 4.73; $p < 0.01$. A Mann–Whitney U test indicated that the duration of drinking behaviour was greater for cows housed on CF (Median = 142.71) than cows housed on an RM (Median = 6.64), U = 108.0;

Table 2. Duration (sec) and frequency of standing, lying, walking, feeding, and drinking behaviour of cows housed on CF and cows housed on RM–enriched farms. Values are presented as mean ± S.D and median (interquartile range).

Behavioural Parameter		CF n = 20	RM n = 20
Standing	Frequency	6.22 ± 1.66 [a]	4.10 ± 1.49 [b]
	Duration (sec)	4082.73 ± 775.46 [a]	2388.42 ± 801.11 [b]
Lying	Frequency	2.26 ± 1.29 [a]	4.96 ± 1.33 [b]
	Duration (sec)	1959.6 ± 762.84 [a]	3950.75 ± 744.48 [b]
Walking	Frequency	0.2 (0.15 – 0.45)	0 (0 – 0.2)
	Duration (sec)	211.18 ± 96.87 [a]	95.66 ± 50.25 [b]
Feeding	Frequency	0.3 (0 – 1.95)	0.6 (0.2 – 1.35)
	Duration (sec)	820.28 ± 261.32	742.74 ± 322.56
Drinking	Frequency	0 (0 – 0.5)	0 (0 – 0.2)
	Duration (sec)	142.71 (0 – 213.86) [a]	6.64 (0 – 36.62) [b]

[a, b] different superscript within row indicates significance at $p < 0.05$.

$p < 0.05$. There were no significant differences ($p > 0.05$) in the duration and frequency of feeding behaviour between the groups. In addition, cows housed on CF had a significantly higher frequency of standing behaviour compared to cows housed on RM: t (18) = 3.00; $p < 0.05$. Cows housed on RM spent more time lying on the ground during the observation period, as shown by the frequency of lying behaviour that differed significantly compared to cows housed on CF: t (18) = 4.60; $p < 0.01$.

Glucose Tolerance Test (GTT)

Figure 3 shows the plasma glucose concentration relative to the infusion of glucose (0.25 g/kg of BW) for cows housed on CF and cows housed on RM. Similar glucose concentration trends can be seen for both groups. There was no significant interaction between the type of floor and time relative to glucose infusion on plasma glucose concentration, F (1.63, 26.02) = 3.51; $p > 0.05$. Prior to the infusion of glucose, the baseline glucose concentration at -15 min for both CF and RM floors were non-significant (CF: 4.1 ± 0.6 mmol/L, RM: 4.0 ± 0.4 mmol/L, $p > 0.05$). However, the plasma glucose concentration increased immediately after the infusion of glucose at 0 min and dropped significantly 15 minutes post glucose infusion (CF: 27.8 ± 10.7 mmol/L vs. 11.1 ± 4.9 mmol/L, RM: 34.3 ± 2.1 mmol/L vs. 9.3 ± 1.7 mmol/L, $p < 0.05$). At 0 min, cows on RM had higher blood glucose concentrations than cows housed on CF; however, it was not statistically significant ($p > 0.05$). Furthermore, the plasma glucose concentrations in cows housed on CF and RM at 15 min, 30 min, 60 min, and 120 min collection mark were not significantly different ($p > 0.05$). The CR_{0-15} observed during GTT is significantly different (CF: 5.93 ± 3.17 %/min, RM: 8.78 ± 1.21 %/min; $p < 0.05$). However, the AUC_{120} (CF: 55.56 ± 14.8 mmol/L x 120 min, RM: 60.13 ± 6.3 mmol/L x 120 min; $p = 0.44$) was not significantly different between the two floor types.

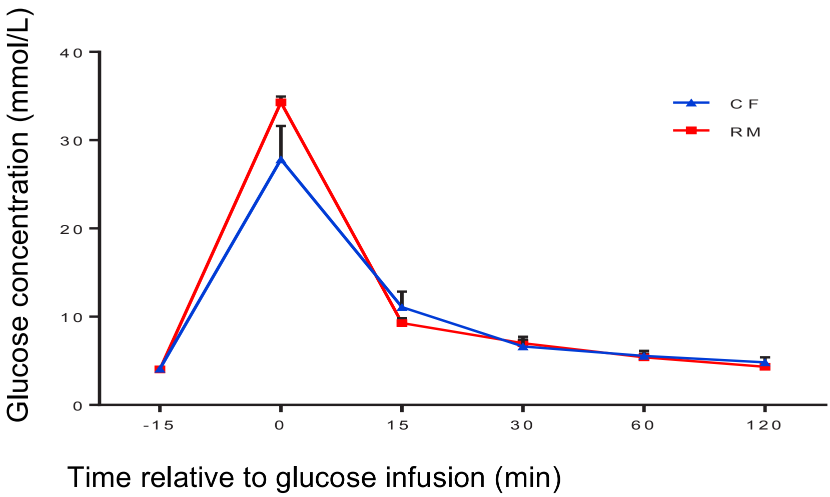

Figure 3. Plasma glucose concentration in response to glucose infusion (0.25 g/kg of BW) at various time (min) intervals for cows housed on concrete flooring (CF), and cows housed on rubber mats (RM). Values are presented as mean ± S.D.

Adrenocorticotropic (ACTH) Challenge

Results for the plasma cortisol concentration of cows housed on CF and cows housed on RM in response to the injection of ACTH (0.16 µg/kg of BW) is shown in Figure 4. Compared to the baseline reading (at 0 min), the plasma cortisol concentration for both CF and RM increased significantly at 30 min after ACTH administration (CF: 38.1 ± 23.9 ng/ml vs. 62.3 ± 18.8 ng/ml, RM: 17.2 ± 10.8 ng/ml vs. 48.4 ± 12.1 ng/ml, $p < 0.05$). However, the cortisol concentration of cows housed on CF started to decrease gradually at 60 min (and continued to decrease until 120 min) while the cortisol concentration of cows housed on RM started to decrease only at 90 min. There was a significant interaction between the effects of flooring types and time relative to ACTH administration on plasma cortisol concentration [$F(2.537, 45.667) = 26.95$; $p < 0.05$]. Specifically, the mean cortisol concentration (at 0 min) of cows housed on CF (38.1 ± 23.9 ng/ml) was significantly higher than that of those housed on RM (17.2 ± 10.8 ng/ml), $p < 0.05$. At 30 min, cows on CF tended to have higher cortisol concentrations than cows housed on RM. However, no significant differences were indicated for cortisol concentrations at 30 min, 60 min, 90 min, and 120 min ($p > 0.05$) between the flooring types. During the ACTH challenge, no significant differences for $CR_{60\text{-}120}$ (CF: 0.93 ± 0.79 %/min, RM: 0.62 ± 0.4 %/min; $p = 0.3$) and AUC_{120} (CF: 201.3 ± 75.3 mmol/L x 120 min, RM: 181.5 ± 40.4 mmol/L x 120 min; $p = 0.48$) were observed between the cows housed on CF and cows housed on RM.

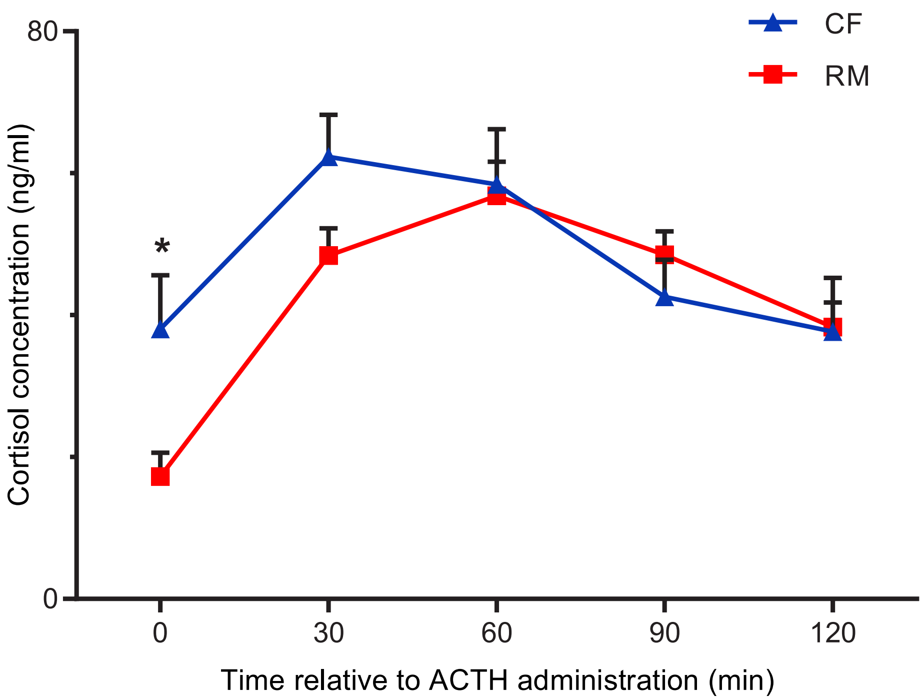

Figure 4. Plasma cortisol concentration in response to ACTH injection (0.16 µg/kg of BW) at various time intervals (min) for cows housed on concrete flooring (CF), and cows housed on rubber mat (RM). * Significantly different at $p < 0.05$. Values are presented as mean ± S.D.

Table 3. Total white blood cell count of cows housed on CF and cows housed on RM–enriched farms. Values are presented as mean ± S.D. Normal reference values derived from Wood and Quiroz–Rocha (2010).

Parameter	Reference value	CF	RM
WBC ($x10^9$ cells/L)	5.1 – 13.3	10.35 ± 4.1	15.11 ± 8.1
Lymphocytes ($x10^9$ cells/L)	1.8 – 8.1	5.30 ± 2.2	8.85 ± 5.1
Neutrophils ($x10^9$ cells/L)	1.7 – 6.0	3.37 ± 1.8	4.0 ± 2.1
Monocytes ($x10^9$ cells/L)	0.1 – 0.7	0.63 ± 0.3	1.0 ± 0.6
Eosinophils ($x10^9$ cells/L)	0.1 – 1.2	0.89 ± 0.5	1.08 ± 0.8
Basophils ($x10^9$ cells/L)	0 – 0.2	0.05 ± 0.1	0.03 ± 0.1

Stress Leukogram

The total white blood cell counts (lymphocytes, neutrophils, monocytes, and eosinophils) for cows housed on CF and RM are shown in Table 3. The total counts of white blood cells were not significantly different between the two groups ($p > 0.05$). Even though cows housed on CF had a higher ratio of neutrophils to lymphocytes (N: L), (CF: 0.7 ± 0.4, RM: 0.48 ± 0.2, $p > 0.05$), the differences observed were not significant.

Milk Yield

The average milk yield (litre/cow/day) of cows housed on RM was significantly higher than cows housed on CF (RM: 17.8 ± 0.84 L vs CF: 7.55 ± 0.72 L, t (1.95) = 13.02, $p < 0.05$). However, within the group of flooring types, there was no significant difference in average milk yield.

DISCUSSION

Behavioural observations indicate that the type of floor material used in the farm may influence the duration and frequency of lying and standing behaviour of dairy cows. The frequency and duration of lying behaviour were significantly higher for cows housed on RM than cows housed on CF, and cows housed on CF had a significantly higher frequency and duration of standing behaviour than those on RM. These results are consistent with those obtained by Rushen *et al.* (2007), and Jain *et al.* (2013) in which cows housed on RM spent less time standing and more time lying down possibly due to the softness of the RM. In addition, behavioural observations in this study were performed during the resting period, during which no dairy cattle operations such as milking, or treatment were conducted. This result is corroborated by Grant (2003) who suggested that cows need to spend at least 12 hours per day (up to approximately 50 % of their daily activities) lying down. Moreover, Jain *et al.* (2013) stated that cows tend to lie down more rather than standing during the resting period, especially when they are provided with comfortable flooring. This activity may be beneficial to cows as they typically prefer to ruminate while lying down (Cooper *et al.,* 2007). Phillips and Leaver (1986) reported that cows normally spend approximately an hour per day ruminating while standing, but approximately six hours per day while lying down, indicating a strong relationship between lying down and rumination.

The time spent by dairy cows standing on the different types of floors is similar to that found in previous studies by Haley *et al.* (2001) and O'Callaghan (2002). The results from both studies indicated that cows on concrete flooring spent more time standing idly, resulting in less lying down time. However, increased standing duration may predispose cows to lameness, especially on hard flooring such as concrete (Cook & Nordlund, 2009). The abrasive feature of CF may initiate uneven hoof wear (Telezhenko *et al.,* 2008), which can develop through pressure on the soft part of the cow's hoof (Hinterhofer *et al.,* 2005). Dairy cows that succumb to hoof problems as a result of being housed on CF seem to be more hesitant to change their posture from lying to standing (or vice versa) due to pain (Rushen *et al.,* 2007). Even though the claw health and lameness status of cows were not determined in this study, Sadiq *et al.* (2017) reported that the prevalence of lameness and claw lesions in the state of Selangor, Malaysia were 19.1 % and 31 %, respectively. Although they reported no significant differences in the lameness incidence rate between cows housed on different flooring types, the state of Selangor (Sadiq, 2018) had a higher prevalence of lameness (43.6 %) in cows on CF than cows housed on RM (24.6 %). Regardless, the disruption of lying behaviour generally occurs when the cows feel restless in indoor confinement (Connell *et al.,* 1989). However, other factors such as fear of traumatic injury acquired from falling, slipping, or fighting with other cows (Azhar *et al.,* 2016) could also account for the decreased time spent lying down.

It has been reported that dairy cows prefer to walk on RM compared to CF (Bergsten & Telezhenko, 2005; Telezhenko *et al.,* 2007; Haufe *et al.,* 2009). A possible explanation for this might be that the use of RM as flooring material provides a comfortable surface that may help reduce the compression of the claw as the cows walk (Schmid *et al.,* 2009), hence, improving their gait (Telezhenko & Bergsten, 2005). However, this study's results show that cows housed on CF tend to walk more, and the duration spent on this activity was significantly different than that of the cows housed on RM. This result may indicate that cows may prefer to seek comfortable places such as the RM to lie down under the hot weather during the rest period, which is not offered by uncomfortable flooring materials such as concrete. Moreover, the behavioural patterns observed could be influenced by an increase in ambient temperature on the farm. For example, the standing behaviour of dairy cows was reported to increase to about 34 % when the air temperature rises (Tapki & Sahin, 2006). The provision of RM in dairy farms, therefore, may reduce the overall time spent by cows moving around the stall searching for a comfortable surface to lie down as often seen displayed by cows on concrete floors.

In contrast, the time spent feeding was not affected by the use of RM, even when mats were placed in front of the feed bunk (Fregonesi *et al.,* 2004). Similarly, this study's results show that there is no significant difference in the feeding behaviour of the cows in the CF and RM farms. In contrast, Tucker *et al.* (2006) reported that the total time spent on feeding was significantly greater when the RM were placed on the farm. A possible explanation for the results obtained in this study could be the limited observation time and other restrictions imposed by the owners of the farms. In addition, the observation was performed when most of the cows were resting after the initial daily feeding period. Hence, the duration of behavioural observation needed to be prolonged to several hours per day, as many related studies were conducted for at least 24 hours per day (Fregonesi *et al.,* 2004; Winckler *et al.,* 2015).

Apart from behavioural parameters, physiological data are useful indicators in evaluating the effects of flooring types on dairy cows in greater depth. The combination of behavioural study and hormone evaluation could be used to more accurately interpret the data than using either the behavioural or hormone study alone. The GTT was performed to measure the body's response to the administration of a specific dosage of glucose solution, either given orally or intravenously. This test was implemented to explore energy metabolism in response to the potential stress experienced by the animals (Huzzey *et al.,* 2012; De Koster *et al.,* 2017). Furthermore, overstocking on farms can cause changes in the energy metabolism of dairy cows, possibly due to changes in glucocorticoid secretion in response to stress (Huzzey *et al.,* 2012). Acute stress has been known to stimulate the production and secretion of glucose through the expression of glucose-6-phosphatase in the liver via activated glucocorticoid receptors, in response to the elevation of cortisol concentration (Wilcox *et al.,* 2017). However, the farms under study were not overstocked and hence changes in the GTT results may be attributed to other confounding factors present in the farms.

Based on our results for the GTT, there was no significant interaction between the effects of flooring types and time relative to glucose infusion on plasma glucose concentration between the cows housed on CF and those on RM. The spike in plasma glucose concentration at 0 min only indicated that the sum of exogenous glucose recently administered into the animal's system and the glucose level already present in the body had not undergone metabolism at that point. Although cows housed on RM had higher glucose concentrations upon the infusion of glucose compared to cows housed on CF, the differences were not statistically significant. Despite this, the clearance rate (CR) for glucose response was significantly higher for cows housed on RM compared to those on CF. This result indicates that cows housed on RM had either a higher rate of glucose uptake or disposal through an increase in insulin secretion (Hayirli, 2006). As a potent glucose homeostasis regulator, insulin is necessary for lowering the blood glucose level by stimulating glucose uptake from the blood into the tissues or storing glucose as glycogen, particularly when the blood sugar level is high. Therefore, more research concerning the effects of different types of flooring (CF and RM) on insulin concentration in cows is needed to understand fully the mechanism involved.

The results from the ACTH challenge indicate that the basal plasma cortisol concentrations following administration of exogenous ACTH were significantly higher in cows housed on CF compared to cows housed on RM, suggesting that the animals in this group were already experiencing acute stress prior to the experiment. Furthermore, the plasma cortisol concentration showed a 2 to 3-fold elevation at 30 minutes after the ACTH injection in both groups (CF: 38.1 ± 23.9 ng/ml to 62.3 ± 18.8 ng/ml, RM: 17.2 ± 10.7 ng/ml to 48.4 ± 12.1 ng/ml), indicating that regardless of the type of flooring, both groups of animals experienced acute stress at the initial stages of the test, such as the blood sampling activity (Hopster *et al.,* 2002). Even though cows on CF had a greater cortisol response compared to the cows housed on RM, no significant differences in AUC and CR of cortisol response were observed between the two groups. However, cows housed on CF had a slightly higher clearance rate compared to cows housed on RM which may indicate that the cows housed on CF adapt more easily in response to acute stress. It is known that animals display a habituated response or adaptation towards uncomfortable and long-existing housing conditions that are presumed to be stressful (van Borell, 2001; Simpkiss & Devine, 2003). Thus, results from the GTT and ACTH challenge in cows housed on CF may reflect a state of physiological desensitization to stressors associated with the flooring.

In addition, factors such as the ambient temperature and relative humidity in these farms during the entire period of study may also affect the stress response of these animals. Based on the meteorological data for the year 2017 and 2018, the average monthly temperature was similar (27 °C) throughout the period of study while the percentage of relative humidity was 81 % (for April 2017 and April 2018) and 69 % (for August 2018). According to Habeeb *et al.* (2018), moderate signs of heat stress may be triggered when the temperature reaches between 26.7 to 32.2 °C and with the percentage of humidity ranging from 50 to 90 %. However, the temperature in the sheds may vary slightly from the ambient temperature outside the sheds based on the designs of house/stalls. Therefore, a slight difference in the temperature and humidity in these farms may also contribute to differences in the results observed.

According to O'Loughlin *et al.* (2014), a stress leukogram or the total number of leukocytes and its differential count could be used as a sensitive indicator in assessing stress response in cows. Apart from stress, an increase in the number of white blood cells or leukocytosis has been associated with inflammation or trauma (Cerny & Rosmarin, 2012) such as in the case of lameness or physical injuries. On the other hand, an elevation in glucocorticoids brought on by stress or glucocorticoid treatment also causes a shift in the peripheral blood cells, identifiable as neutrophilia and lymphopenia (Burton *et al.,* 2005). In the present study, there were no significant differences in the number of leukocytes between cows housed on CF and RM. In comparison to the standard reference values proposed by Wood and Quiroz-Rocha (2010), the number and percentage of leukocytes for both groups of cows on different flooring types were within the normal range. According to Calamari *et al.* (2004), the neutrophils to lymphocytes ratio could be used as an indicator of stress when the ratio is greater than 1. Similarly, Wood and Quiroz–Rocha (2010) suggested that the normal neutrophils to lymphocytes ratio of the cows must be in the ratio of 1:2. In the present study, both groups of cows housed on different types of flooring had a neutrophils to lymphocytes ratio of 1:2 and were within the normal range, indicating that the animals were not in a state of stress. Jones and Allison (2007) suggested that an increase in the number of monocytes may also indicate physiological stress. Even though the results of this study showed that cows housed on RM had a slightly higher monocyte count than the cows housed on CF, the difference between the groups was not statistically significant. In contrast, results from another study conducted on purebred Holstein dairy cows indicated that the provision of rubber flooring did not improve dairy cow locomotion although cows that were assigned with rubber flooring had a greater neutrophil and lesser lymphocyte number than cows that were assigned to concrete flooring (O'Driscoll *et al.,* 2009). Thus, breed predisposition may also play a role in the physiological response towards stress; however, the possibility needs to be explored further. Nevertheless, taken together, results from the present study may indicate that the cows housed on CF and RM might have adjusted and acclimatized to the long-existing housing conditions that are predetermined to be stressful (Andrews & Walker, 1999).

The average milk yield for cows on RM was significantly higher than cows on CF. The result is inconsistent with previous findings whereby RM–enrichment had no significant effect on the milk yield of dairy cows (Kremer *et al.,* 2012; Eicher *et al.,* 2013). The contradictory results may be explained by the differences in study designs adopted by the current study and that by Kremer *et al.* (2012) and Eicher *et al.* (2013) whereby unlike the present study, the latter two studies explored the effects of flooring on milk production in an experimental setting. However, such differences may also be affected by the cow's ability to utilize energy from feed conversion efficiently enough to support milk yield while lying down (Botheras, 2007) congruent with the present study's results that cows housed on RM tend to lie down more than cows housed on CF. It is known that rumination time (Hassall *et al.,* 1993) and blood circulation to the udder (Rulquin & Caudal, 1992) are among the key factors in greater milk production. Results from several studies have indicated that the use of RM as a flooring material in dairy farms improved the milk yield possibly through the reduction in clinical mastitis (Ruud *et al.,* 2010). This is in agreement with the report by Valde *et al.* (1997) in that the risk of clinical mastitis in cows dropped by 14 % when these animals were housed on rubber mats. A possible explanation for this might be that RM surfaces are more hygienic (Herlin, 1997) compared to CF. In addition, RMs are easily drained and dried compared to concrete, thus providing a comfortable space which may encourage more cows to lie down. This suggests that aside from comfort and a non-slip surface, RM offers a more hygienic alternative to CF and thus decreases the risk of disease transmission, which may disrupt the milk production of dairy cows. It is worth noting that the hygiene of farms in the present study was well taken care of since the sheds and floor for both CF and RM farms were cleaned twice daily using high water pressure. However, it is possible that a high milk yield recorded in RM farms in the present study may not be associated solely to the use of specific flooring material alone or its hygienic conditions but may involve a more complex multifactorial interaction between the different environmental conditions, nutrition, and feed intake of the dairy cows. Therefore, it is recommended that the data regarding dry matter intake or proximate analysis of concentrates used in each farm which could contribute to a clearer explanation behind the significant results be taken into account in the near future.

CONCLUSION

In summary, the results of this study suggest that the use of anti-skid RMs may indirectly improve welfare conditions of dairy cows through encouragement on lying down activity, thus promoting the expression of natural behaviour such as rumination and consequently increasing their milk yield. Cows housed on RM may have been better able to the personnel involved in the procedure prior to the tests is important to minimize the occurrence of acute physiological stress. Although the significance of energy metabolism through the GTT was not evidenced in both RM and CF groups, future research is needed to determine whether the level of insulin secreted by cows under different types of flooring may play a role in the slight differences observed. Other factors such as the nutritional value of the feeds given, the nutrient intake of each cow, and the status of hoof health management in each farm and their interactions need to be considered in the interpretation of results obtained. Moreover, a slight change in the environmental conditions of the house (e.g., ambient housing temperature, and relative humidity) during the period of study may contribute to the overall outcomes of each farm. Therefore, inclusion of these data may further minimize the variability which could affect the overall results of similar studies in the future.

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