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Cacao bean husk: an applicable bedding material in dairy free-stall barns

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Objective: The objectives of the study were to assess the effect of cacao bean husk as bedding material in free-stall barn on the behavior, productivity, and udder health of dairy cattle, and on the ammonia concentrations in the barn.
Methods: Four different stall surfaces (no bedding, cacao bean husk, sawdust, and chopped wheat straw) were each continuously tested for a period of 1 week to determine their effects on nine lactating Holstein cows housed in the free-stall barn with rubber matting. The lying time and the milk yield were measured between d 4 and d 7. Blood samples for plasma cortisol concentration and teat swabs for bacterial counts were obtained prior to morning milking on d 7. The time-averaged gas-phase ammonia concentrations in the barn were measured between d 2 and d 7.
Results: The cows spent approximately 2 h more per day lying in the stalls when bedding was available than without bedding. The milk yield increased in the experimental periods when cows had access to bedding materials as compared to the period without bedding. The lying time was positively correlated with the milk yield. Bacterial counts on the teat ends recorded for cows housed on cacao bean husk were significantly lower than those recorded for cows housed without bedding. Ammonia concentration under cacao bean husk bedding decreased by 6%, 15%, and 21% as compared to no bedding, sawdust, and chopped wheat straw, respectively. The cortisol concentration was lowest in the period when cacao bean husk bedding was used. We observed a positive correlation between the ammonia concentrations in the barn and the plasma cortisol concentrations.
Conclusion: Cacao bean husk is a potential alternative of conventional bedding material, such as sawdust or chopped wheat straw, with beneficial effects on udder health and ammonia concentrations in the barns.

Keywords: Dairy Cattle; Bedding Material; Cacao Bean Husk

INTRODUCTION

Lying behavior is important for health of dairy cattle. Lying time has been shown to have a higher behavioral priority than eating time and social contact [1]. Dairy cattle show decreased concentration of growth hormone [2], and altered hypothalamic-pituitary-adrenal activity [3-5] when prevented from lying down.

Lying behavior is influenced by several factors such as stall size [6], and the health and production status of the cow [7,8]. In the barn, the type of the lying surface is especially important. Cows spend more time lying down when thick [9], soft [10], and dry [11] bedding is provided. There is increasing evidence that bedding plays a key role in increasing the productivity of dairy enterprises. Leg injuries are lower [12] and milk yields are higher [13] when dairy cattle are housed with bedding than when housed without bedding.

Udder health is also influenced by bedding material. Bacterial populations in bedding are positively correlated with the bacterial load in teat skin [14] and with the incidence of clinical mastitis in lactating cows [15]. Therefore, reducing the number of pathogenic bacteria in the bedding could reduce the risk of mastitis. The rate at which bacteria grow in the bedding varies with the type of its material [14].

Ammonia emission leads to the formation of secondary particulates, which are a potential hazard for human health, and causes eutrophication and acidification of natural ecosystems [16]. Agriculture is the major source of global ammonia emission [17]; livestock houses are one of the largest sources. Ammonia emissions from livestock housing are influenced by interactions between the materials used for bedding and the urine deposited in it [18,19].

Thus, the presence of bedding and the type of bedding material have a great impact on the performance and health of dairy cows, and on the ammonia emission from the barns. However, in some regions, alternative bedding materials are in great demand because of increasing costs of the conventional materials such as sawdust and wheat straw.

Cacao bean husk, mainly consisting of the seed coat and the embryo of the cacao bean, is a waste product of chocolate and cocoa milling industries. Cacao bean husk accounts for approximately 10% to 12% in weight of total weight of cacao bean [20]. The husk is dry and crisp, and has been reported to display antibacterial [21] and deodorizing effects [22]. Therefore, it might be possible to use cacao bean husk as a bedding material in the dairy barn.

The objectives of the present study were to assess the effect of cacao bean husk as a bedding material in free-stall barn on the behavior, productivity, and udder health of dairy cattle, and on the ammonia concentrations in the barn. For these purposes, we compared four different stall surfaces (no bedding, cacao bean husk, sawdust, and chopped wheat straw) in this study.

MATERIALS AND METHODS

Animals and housing

The experiment was conducted at the experimental free-stall barn of Tokyo University of Agriculture and Technology (Fuchu, Tokyo, Japan; Figure 1), from November 4 to December 2, 2014. The temperatures during the study period ranged between 6.2°C and 20.5°C. The free-stalls were 120 cm wide and 255 cm long with neck railing. Nine lactating Holstein cows (day in milk, 222.6±58.2; parity, 1.7±0.7) were used in this experiment. Before the experiment, solid blocks made of wood chips have had been used as the lying surface. The floorings of the alley were of solid concrete, and automatic scrapers were used twice daily, during milking, for their cleaning. Animals had *itum* access to water and total mixed ration, consisting mainly of corn silage, and were milked twice a day at 0900 and 1700. The experimental protocol was approved in accordance with the Guide for the Care and Use of Laboratory Animals prepared by Tokyo University of Agriculture and Technology.

Bedding materials

Three different bedding materials were used in the experiment; cacao bean husk (particle size of approximately 1 cm in diameter; Japan Chocolate Industrial Corporation, Itabashi, Tokyo), sawdust (Japanese cedar, particle size of approximately 1 mm in diameter; Ome, Tokyo), and chopped wheat straw (approximately 3 cm; experimental farm, Tokyo University of Agriculture and Technology, Fuchu, Tokyo). Bedding materials were analyzed for pH, total nitrogen, total carbon, and moisture content (Table 1). The pH of a demineralized water/bedding mixture (1:2 ratio by weight) was measured using a pH mater (PHL-40). Bedding materials were dried at 80°C for 3 d and ground through a 1 mm screen before total N and C analyses by combustion assay (NC analyzer, SUMIGRAPH NC-80 AUTO, Sumitomo Chemical, Tokyo, Japan). The moisture contents of bedding materials were

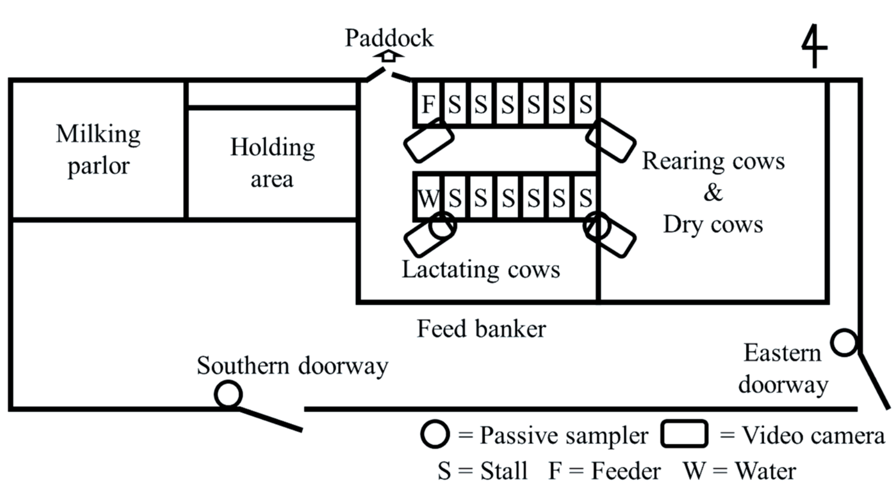

Figure 1. Layout of the free-stall barn showing positions of the passive samplers and the video cameras.

Table 1. Characteristics of the bedding materials used in the study (means of duplicate samples)

Bedding material	Measurement item			
	pH	Total C (g/kg)	Total N (g/kg)	Moisture content (%)
Cacao bean husk	5.3	322	29	10.9
Sawdust	6.1	342	2	21.2
Chopped wheat straw	7.4	293	5	19.8

determined by drying a 25 g composite sample at 80°C for 3 d or until constant weight was achieved.

The rubber mats (KKM feather soft; 1,200 mm×1,830 mm×30 mm; GEA Farm Technologies GmbH Co., Ltd., Nagano, Japan) were placed in the free-stalls during the experiment. Four free-stall surfaces were tested over the following periods: control (no bedding materials), November 4 to 10; cacao bean husk, November 11 to 17; sawdust, November 18 to 24; and chopped wheat straw, November 25 to December 1. Visible fecal matter, urine, and soiled bedding were removed and fresh bedding was added twice daily, during milking to maintain a total thickness of 1 cm. The rubber mats were removed, washed with water, and sterilized with slaked lime between the experimental periods.

Behavior

The behavior of the cows was videographed between d 4 and d 7 of each experimental period using 4 cameras (CCD VIDEO CAMERA Excellent Quality, Veilnet, Osaka, Japan) and an HD recorder (H-264 DVR, Japan). The cameras were mounted at positions from where all 12 stalls could be observed (Figure 1). All animals were marked uniquely on their backs with a paint sticks. The recordings were scored using scan sampling at 10-min intervals. During each scan, we recorded which cows were lying down in each stall. This sampling regime allowed us to estimate the amount of time each cow spent lying in each stall. The lying events outside the free-stall were not recorded.

Milk yield

Milk yield was measured daily in all cows using an automatic system (Metatron, GEA Farm Technologies GmbH Co., Ltd., Japan). The milk yields on the 4 days from d 4 to d 7 of each experimental period were chosen for analysis from these recordings.

Blood samples

Blood samples were taken from jugular vein on d 7 of each experimental period in lithium heparin Vacutainer tubes just before the morning milking. The blood was centrifuged at 3,000 rpm for 10 min and the plasma thus obtained was frozen at –20°C until further analysis. Plasma cortisol was analyzed through enzyme immunoassay (EIA) using a commercial kit (Cortisol EIA Kit 500360, Cayman Chemical, MI, USA). The detection range of the assay was 6.6 to 4,000 pg/mL.

Teat samples

Bacteriological samples were collected on d 7 of each experimental period using sterilized swabs (Becton, Dickinson and Company, Tokyo, Japan) before the morning milking. Teat swabs were collected individually from the anterior right teat by rotating a swab around the exterior of the teat orifice; samples from two cows were excluded due to sampling mistakes. The swabs from the cows were pooled in a single test tube containing 4 mL of phosphate-buffered saline and shaken in a vortex for 60 s. The rinse solution and its dilutions (10^{-1}, 10^{-2}, and 10^{-3}) were plated on the surface of blood agar (Kanto Chemical Co., Inc., Tokyo, Japan) supplemented with defibrinated horse blood (Cosmo Bio co., ltd. Tokyo, Japan). Prior to plating, each Petri dish was divided into four equal parts by drawing two perpendicular lines on the plastic base of the dish using a black felt maker so that the four dilutions could be plated simultaneously on a single Petri dish. In brief, 25 µL of inoculum was spread on the agar plates with a sterilized glass rod and the plates were incubated for 24 h at 37°C before bacterial colonies were counted. Only those plates that contained 10 to 100 colonies were used to estimate the bacterial counts, and all plates showing visible signs of cross contamination were discarded. Bacterial counts were expressed as log10 colony-forming unit (cfu) per swab.

NH_3 emission

The time-averaged gas-phase ammonia concentrations in the barn were measured between d 2 and d 7 of each experimental period using Ogawa passive samplers (Ogawa & Co., Ltd., Kobe, Japan). The sampler is a double-sided passive diffusion sampler equipped with a diffusive end cap, followed by a stainless steel screen, and a 14-mm quartz filter impregnated with citric acid. Two samplers were deployed inside the barn (approximately 3 m height; Figure 1), and two samplers were deployed in the doorway (approximately 2 m height; Figure 1). Each sampler contains two filters for the purpose of replication at each location. After collection, ammonia was eluted from the passive sampler filters with 10 mL of Milli-Q water for 30 min and syringe-filtered (GL chromatodisk; 13 mm diameter, 0.2 µm, GL Sciences Inc., Tokyo, Japan). The syringe and the filter were pre-rinsed with deionized water and 1 to 2 mL of sample. Filtration removes filter particles, which could cause a positive absorbance artifact during analysis. Ammonia was analyzed as ammonium ion using ion chromatography system (ICS-1100, Thermo Fisher Scientific Inc., Yokohama, Japan). Ammonia concentrations in air were calculated according to the Ogawa protocol (Ogawa & Co., Ltd., Japan).

Statistical analyses

Data were analyzed using the one-way analysis of variance. If the one-way analysis of variance was significant, the Tukey-Kramer multiple comparisons test was applied to identify the significant differences between treatments. Extremely high or low values of plasma cortisol concentration were excluded by the Smirnov-Grubbs' outlier test (p<0.01). All analyses were conducted with Statcel 3 (OMS Publishing Inc., Tokorozawa, Japan). Pearson correlation was used to estimate the associations among lying time (h/d), milk yield (kg/d), plasma cortisol (ng/mL), bacterial counts on teat ends (log_{10} cfu/swab), ammonia concentrations in the barn (ppm), and temperature (°C) based on the average values over each experimental period.

RESULTS AND DISCUSSION

The presence of bedding and the type of material used have a great impact on dairy farms in every respect; behavior [10], productivity [13,23], and udder health [14] of dairy cattle, and NH_3 emission from the barns [16,17]. In this study, we assessed the effect of cacao bean husk as bedding material in free stall barn compared to conventional stall surfaces; no bedding, sawdust, and chopped wheat straw. Our results showed that cacao bean husk is a potential alternative of conventional bedding material with beneficial effects on udder health and ammonia concentrations in the barns.

Cows spent more time lying down when beddings were provided (Figure 2A). The time cows spent lying in the stalls was approximately 2 h more per day on cacao bean husk (11.5 h/d), sawdust (11.5 h/d), and chopped wheat straw (12.1 h/d) than on no beddings (9.6 h/d). Previous studies showed that lying times increase when stalls contain more bedding, such as sawdust, shavings, and straw [9,10]. Therefore, our results indicate that cacao bean husk is usable as a substitute for the conventional bedding material.

Though most of the cows under this experiment were in the late lactation stage (DIM; 222.6±58.2), their milk yield increased during the experiment (Figure 2B); control (24.1 kg/cow/d), cacao bean husk (24.9 kg/cow/d), sawdust (25.3 kg/cow/d), and chopped wheat straw (26.0 kg/cow/d). We observed a positive correlation between lying time and milk yield (r = 0.92, p<0.10). Temperature had no significant correlation either with lying time or with milk yield. Although it is unknown how lying behavior affects milk production, the comfort of the resting areas or lying time may be key factors in increasing milk yield [9,13,23].

The bacterial counts on the teat ends of the cows were lowest in the period when cacao bean husk was used (Figure 2C); those of the cows housed on cacao bean husk (3.69 log_{10} cfu/swab) were significantly lower (p<0.05) than those on no beddings (4.77

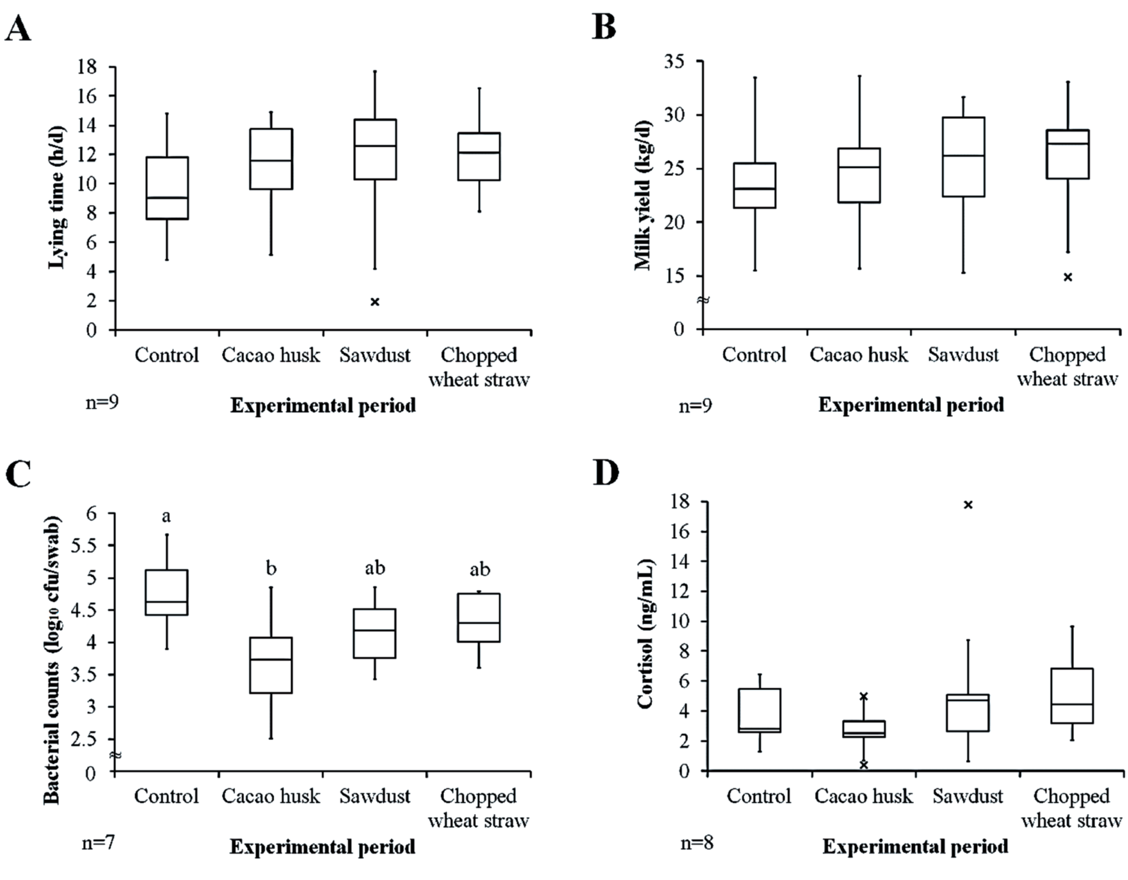

Figure 2. Lying time (A, n = 9) and milk yield (B, n = 9) between d 4 and d 7, and bacterial counts (C, n = 7), and plasma cortisol concentration (D, n = 8) on d 7. Boxes contains the 50th (median), 75th (upper), and 25th (lower) percentiles. Observations at more than 1.5× the inter-percentile range are plotted individually as possible outliers (×). The whiskers indicate maximal and minimal values that are not suspected outliers. [a-b] p<0.05.

$\log_{10}$ cfu/swab), though had no significant difference with those on other beddings (sawdust, 4.13 $\log_{10}$ cfu/swab; chopped wheat straw, 4.28 $\log_{10}$ cfu/swab). We did not compare the bacterial loads in bedding in this study. However, previous studies have shown a positive correlation between the number of mastitis pathogens in the bedding and their number on the teat ends of lactating dairy cows [14,15]. Therefore, cacao bean husk may be more effective in reducing bacterial counts both in the bedding and on the teat ends than other materials studied, which could reduce the risk of mastitis.

Previous studies have shown cacao bean husk to exert antibacterial activities by virtue of its unsaturated fatty acid content [21]. The process by which cacao bean husk is produced may also contribute to its ability to resist bacterial growth. Cacao bean husk is separated from the cotyledons, which is the edible part, by roasting for about 40 min at 100°C to 220°C [20]. Therefore, it is likely to be less contaminated with bacteria initially than sawdust or straw. Its lower moisture content (Table 1) could also be responsible for lower bacterial counts as suggested by other studies [14].

Among all the beddings tested, on all locations, except west in the barn, the ammonia concentration was lowest during the period when cacao bean husk bedding was used (Figure 3). The average ammonia concentration during the period with cacao bean husk bedding (0.99 ppm) was lower by 6%, 15%, and 21% as compared to the periods with control (1.06 ppm), sawdust (1.17 ppm), and chopped wheat straw (1.26 ppm) beddings, respectively. The physical structure of cacao bean husk, which is dry and crisp, may be one reason for lower ammonia concentrations. Previous studies have indicated that the ability of beddings to separate feces and urine was the most important factor in determining ammonia emission rates [18,19]. In addition to its interactions with feces and urine, the deodorizing activity of cacao bean husk may also contribute to the lower ammonia concentrations in the air. A mixture of polyphenolic compounds contained in cacao bean husk confers high deodorizing activity [22].

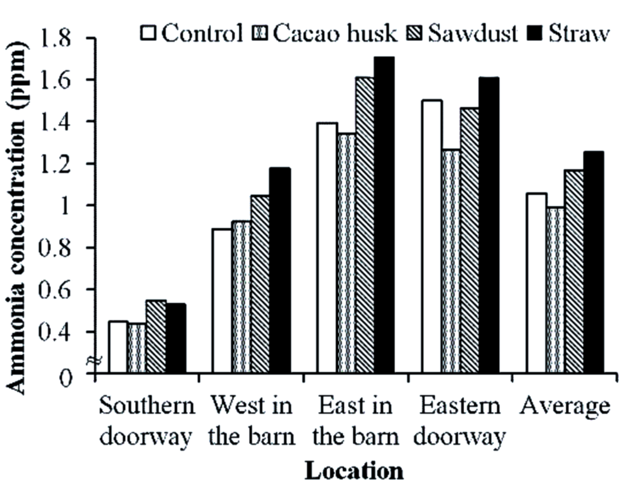

Figure 3. Ammonia concentration in the barn between d 2 and d 7 of each experimental period. Data indicate means of duplicate samples.

We measured concentration of plasma cortisol in cows as a biochemical marker of their stress. The cortisol concentration was lowest in the period when cacao bean husk bedding was used (Figure 2D). Furthermore, ammonia concentrations in the barn were positively correlated with plasma cortisol concentrations of the cows ($r = 0.90$, $p<0.10$). Previous study showed, in simulated ship transportations, that high concentrations of ammonia could induce temporary stress responses in cattle [24]. This could be one of the reasons for the lowest concentration of cortisol being observed in the period with cacao bean husk bedding.

In conclusion, cacao bean husk has potential as an alternative to conventional bedding material, such as sawdust or chopped wheat straw, with beneficial effects on udder health and ammonia concentrations in the barns.

CONFLICT OF INTEREST

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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