ABSTRACT: The aim of this study was to compare different types of bedding and mucking regimens used in horse stables on the generation of airborne particulate matter <10 µm (PM10) and 3 biogenic gases (carbon dioxide, nitrous oxide, and especially ammonia). Three separate experiments were undertaken. The experiments were carried out in an enclosed stable (9.7 m long, 8.7 m wide, and 3.5 m high) that had 5 single boxes housing 4 horses. The measuring instruments were set up in the middle of one side of the stable. In Exp. 1, 3 types of bedding material (wheat straw, straw pellets, and wood shavings) used for horses were assessed according to their ammonia generation. Each type of bedding was used for 2 wk, with 3 repetitions. The mean ammonia concentrations within the stable were 3.07 ± 0.23 mg/m³ for wheat straw, 4.79 ± 0.23 mg/m³ for straw pellets, and 4.27 ± 0.17 mg/m³ for wood shavings. In Exp. 2, the effects of the mucking regimen on the generation of ammonia and PM10 from wheat straw (the bedding with the least ammonia generation in the previous experiment) were examined using 3 different daily regimens: 1) no mucking out, 2) complete mucking out, and 3) partial mucking out (removing only feces). The mean ammonia concentrations in the stable differed significantly among all 3 mucking regimens (P < 0.05). The greatest values were recorded when the stalls were mucked out completely every day [least squares means (LSM) = 2.25 ± 0.1 mg/m³]. No mucking out resulted in an LSM of 1.92 ± 0.1 mg of ammonia/m³, whereas an LSM of 1.54 ± 0.1 mg of ammonia/m³ was found when the partial mucking out method was used. No mucking out also resulted in significantly less average PM10 (124.4 ± 13.4 µg/m³) than in the other 2 regimens (P < 0.05). In Exp. 3, a 6-wk bedding regimen without mucking out was evaluated with regard to gas and airborne particle generation. The ammonia values were found not to increase constantly during the course of the 6-wk period. The average weekly values for PM10 also did not increase constantly but varied between approximately 90 and 140 µg/m. It can be concluded from the particle and gas generation patterns found in the results of all 3 experiments that wheat straw was the most suitable bedding of the 3 types investigated and that mucking out completely on a daily basis should not be undertaken in horse stables.

Key words: airborne particle, ammonia, bedding material, equine husbandry, mucking interval

INTRODUCTION

The equine respiratory tract reacts very sensitively to airborne particles (McPherson and Thomson, 1983; Burrell, 1985; Leadon, 1986; Clarke et al., 1987; Vandenput et al., 1998) and some biogenic gases (Katayama et al., 1995). Holcombe et al. (2001) established that stabling is associated with less airway inflammation and the persistence of upper airway inflammation in young horses. Gerber et al. (2003) found that horses housed in a stable environment showed evidence of inflammatory airway disease, even though they were clinically healthy and performed well.

Among other factors, bedding has an influence on the climate in a stable in terms of airborne particle generation, water-binding capacity, and ammonia binding (Webster et al., 1987; Woods et al., 1993; Clarke, 1994; Raymond et al., 1994; Dunlea and Dodd, 1999; Banhazi et al., 2002). Suboptimal air quality and hygiene problems within a stable are often linked to bedding and bedding management. Many biogenic gases are generated in livestock housing because of metabolic transformation processes in the animal and in excrement. Most of these gases, ammonia, carbon dioxide, and the nitrogen oxides (especially nitrous oxide), are generated
by fecal material. Ammonia is the most important gas in the stable air with respect to animal health, particularly of the respiratory tract.

The aim of this study was to compare the in situ generation of 3 biogenic gases and airborne particulate matter of \(<10\ \mu m\) (PM\(_{10}\)) in horse boxes under different conditions in 3 separate experiments to assess the suitability of different bedding materials (wheat straw, straw pellets, and wood shavings) and mucking regimens for horses. In addition, in Exp. 1, three different bedding materials (wheat straw, straw pellets, and wood shavings) were compared.

**MATERIALS AND METHODS**

The procedures involving animals were approved by a local care committee. The study was supported by the German Federal Ministry of Food, Agriculture and Consumer Protection. The horses involved were kept in boxes in consideration of the guidelines for evaluation of horse keeping under aspects of animal welfare (Zeitler-Feicht et al., 2004).

**Experimental Design**

The investigation was divided into 3 separate experiments. Experiment 1 was carried out from February 10, 2006, to June 9, 2007. Wheat straw, straw pellets, and wood shavings were compared with regard to gaseous ammonia, carbon dioxide, and nitrous oxide generation in the horse stable. The results of the particle analyses of Exp. 1 have been published elsewhere (Fleming et al., 2008b) and only the gas generation is presented.

The dates for Exp. 2 are shown in Table 1, whereas Exp. 3 was carried out from November 8 to December 20, 2006. In addition to the gas analyses, as in Exp. 1, the PM\(_{10}\) generation was assessed in these experiments. In Exp. 2, three different mucking regimens were investigated, each over a period of 2 wk, using wheat straw bedding: regimen 1 = no mucking out; regimen 2 = mucking out completely on a daily basis; regimen 3 = removing feces on a daily basis (designated “skipping out”). In Exp. 3, a 6-wk period of wheat straw bedding without mucking out was evaluated with regard to the long-term course of gas and airborne PM\(_{10}\) generation.

**Bedding Materials.** The following bedding materials were analyzed and compared: wheat straw (not chaffed, blade length 20 to 30 cm, harvest 2006; water-binding capacity 320.8%; Fleming et al., 2008a), dry wood shavings (spruce wood; Goldspan, Brandenburg Group, Goldenstedt, Germany; water-binding capacity 315.9%; Fleming et al., 2008a), and wheat straw pellets (the straw was ground and made into pellets, diameter 1 cm; Biolan, RWZ Rhein-Main eG, Köln, Germany; water-binding capacity 419.1%; Fleming et al., 2008a).

**Table 1.** Experimental design and operating schedule of Exp. 2

<table>
<thead>
<tr>
<th>Item</th>
<th>Mucking regimen(^1)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Time period, wk</td>
<td>2</td>
</tr>
<tr>
<td>Type of mucking</td>
<td>None</td>
</tr>
<tr>
<td>Fresh bedding</td>
<td>Daily</td>
</tr>
<tr>
<td>Bedding strewn on d 1, kg/m(^2)</td>
<td>3.5</td>
</tr>
<tr>
<td>Fresh bedding, kg/(m(^2)∙d)</td>
<td>1</td>
</tr>
</tbody>
</table>

\(^1\)Regimen: 1 = November 8 to 22, 2006; 2 = January 11 to 25, 2007; 3 = January 26 to February 9, 2007.

**Table 2.** Amount of bedding materials used in each horse box and the carbon available at the beginning and end of each 14-d trial period, as well as the total carbon content of the different bedding materials (Exp. 1)

<table>
<thead>
<tr>
<th>Bedding material</th>
<th>Total carbon content, % of DM</th>
<th>Bedding strewn on d 1, kg/m(^2)</th>
<th>Carbon available on d 1, kg/m(^2)</th>
<th>Carbon available on d 14, kg/m(^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheat straw(^1)</td>
<td>40.69</td>
<td>3.5</td>
<td>1.43</td>
<td>5.63</td>
</tr>
<tr>
<td>Straw pellets</td>
<td>42.94</td>
<td>17.0</td>
<td>7.15</td>
<td>7.15</td>
</tr>
<tr>
<td>Wood shavings</td>
<td>45.32</td>
<td>12.0</td>
<td>5.44</td>
<td>5.44</td>
</tr>
</tbody>
</table>

\(^1\)Fresh bedding added each day = 1 kg of wheat straw/m\(^2\) (equivalent to 0.4 kg of carbon/m\(^2\)).
other buildings were protecting it. It could therefore be assumed that air movements had no effect on the concentration of either the gases or the airborne particles. In addition, the conditions within the stable were kept as constant as possible during the experiments. Door 2 (width 0.9 m, height 2.0 m) was kept closed throughout the whole series of experiments, apart from a 30-cm-wide gap. One-half of door 1 (width 1 m, height 2.5 m; entrance for the horses) remained wide open throughout the whole series of experiments.

**Horses and Feeding.** Throughout the entire period of Exp. 1, two mares (average BW = 550 ± 15 kg) with foals and 2 riding mares (average BW = 500 ± 20 kg) were housed in the stable: boxes 1 and 2 (mare with foal); boxes 3 and 4 (riding mares). These 4 mares were again housed in the stable throughout the entirety of Exp. 2 and 3, although mares in box 1 had their foals on February 2 and 4, 2007, respectively (both at the end of the series of experiments, which finished on February 9, 2007).

Pellets [3 kg/(mare·d)] and oats [1 kg/(mare·d)] were fed twice daily in a trough located 1 m from the ground. The pellets (Derby Zucht, Derby Spezialfutter GmbH, Münster, Germany) contained 12.5 MJ of ME/kg and 15% CP. The oats contained 11.5 MJ of ME/kg and 8.5% CP. Hay (8 MJ of ME/kg and 5.4% CP), which was placed on the ground in the aisle, was also fed twice a day at a rate of 6 kg/(mare·d). The feed quality and type remained constant over the course of the experiments.

**Measuring Instruments**

The measuring instruments were set up in the central box between box stalls 3 and 4 (indicated by the concentric circles in Figure 1). The concentrations of ammonia, carbon dioxide, and nitrous oxide were measured continuously online with an Innova 1312 multisensor monitor (Innova AirTech Instruments A/S, Ballerup, Denmark), Innova 1309 multiplexer (AirTech Instruments A/S), TEOM 1400a particle analyzer (Thermo Scientific, Waltham, MA), Tinytag measuring cylinders [Gemini Data Loggers (UK) Ltd., Chichester, West Sussex, UK] for detection of air temperature and relative air humidity. PM10 = airborne particulate matter <10 µm.
of the boxes at a height of 1.30 m and directly above the bedding (0.50 m). These measurement heights were chosen to take into account the area of inspiration (nose-trill height) of the horse when standing and when lying, respectively. The measuring points were situated above one another on the wall between the boxes (marked as crosses in Figure 1). In addition, 2 other gas measuring points were used: one fixed above the aisle in the middle of the stable (height 3.50 m), and the other on the outer wall of box 4 to measure the constituents of the outside air. The air sampled from the 10 measuring points was conveyed through the measuring tubes and the multiplexer to the gas monitor, where detection took place, with the individual gases being analyzed simultaneously. The measuring cycle enabled the values to be determined for each gas, at each measuring point, within 1 h.

The airborne particle measuring device was also set up in the central box (represented by the concentric circles in Figure 1). The airborne particle concentrations were detected continuously online by using a TEOM 1400a gravimetric particle analyzer (Thermo Scientific, Waltham, MA), which uses a microweighing technology that provides true mass measurements (Patashnick and Rupprecht, 1991). Because the TEOM 1400a particle analyzer is a stationary, highly sensitive measuring instrument, it had to be set up in the empty box without any contact with the horses. Despite its location, the values measured by the TEOM 1400a instrument did reflect the particle concentrations in the box because airborne particulate matter distributes itself uniformly throughout the air within a short period of time (Cox, 1995).

The TEOM 1400a instrument consists of 2 components, a control unit and a sensor unit that contains the air inlet, as well as a microscale. This scale is made up of an oscillating conical tube with a filter attached at the top. The incoming air flows through this tube. The rate of oscillation of the tube is influenced by the dust load and can be used for weighing over the period under investigation. The airborne particle analyzer was equipped with a sample inlet that measured only the particle fraction PM10 because the particles of this fraction are known to be capable of entering the lungs (CEN standard EN 481; Comité Européen de Normalisation, 1993). The sample inlet was set up at a height of 1.80 m. The particle analyzer measured the effective PM10 concentration every minute. In addition, the analyzer calculated the mean values for each 30-min period and saved them. These values were then used in the statistical analyses.

The air temperature and relative air humidity were also measured routinely (1 value/h) at 2 points within the stable and at 1 point outside (marked by the black stars in Figure 1) by using Tinytag measuring cylinders (Gemini Data Loggers (UK) Ltd., Chichester, West Sussex, UK). The external measuring point was located on the outside wall of box 1.

### Experimental Procedure

To create similar conditions for each of the 3 types of bedding material (Exp. 1) and every mucking regimen (Exp. 2 and 3), a standardized daily routine was followed. Feeding times were 0800 and 1800 h. The horses were put out to pasture between 1100 and 1800 h; the rest of the day, they remained in their boxes. At 1100 h, after the horses had left the stable, the boxes were cleared of feces if the regimen dictated this. New wheat straw was then strewn on the damp or dirtied spots if allowed by the mucking regimen. Subsequently, the boxes were cleaned and the stable aisle was swept. The aisle was swept once again at 1800 h after feeding.

**Exp. 1.** Each of the different bedding materials was used for a period of 14 d. During each 14-d trial period, all 4 boxes were strewn with the same bedding material. After 14 d, the stable was completely mucked out and aerated for 5 h (both doors were kept open). Gas measurements were not made during the time when bedding was not present in the boxes (the airing between removal of the old bedding and the laying down of the new). Recording of the gas measurements was resumed only after new bedding had been strewn in all the boxes. The materials were used and investigated in the following sequence: wheat straw (14 d), straw pellets (14 d), and wood shavings (14 d). This cycle was repeated 3 times.

When wheat straw was used as bedding, it was replenished by hand every day (1 kg/m²). Wood shavings and straw pellets were used according to the instructions of the manufacturers. The manufacturers recommended that both the straw pellets and the wood shavings be strewn only once and that a complete exchange of bedding was not necessary until after 4 to 6 wk. The boxes were not completely mucked out during the 14-d trials with any of the 3 types of bedding materials; the excrement was simply removed and the bedding was tidied up every morning at 1100 h. The amount of bedding material used initially and the total carbon content at the beginning and at the end of the trial period within each of the boxes are shown in Table 2. As stated above, the results of the particle analyses in Exp. 1 were published previously (Fleming et al., 2008b) and are not considered further in this publication.

**Exp. 2.** The influence of the mucking regimen on gas and airborne particle formation within the stable was analyzed in a second experiment in which only wheat straw was used as bedding. Wheat straw is the material most commonly used in Germany for bedding horses and is generally used as deep litter bedding. All 4 boxes were strewn with the same wheat straw.

Three different mucking regimens were tested. In regimen 1, the boxes were not mucked out for 2 wk and no feces were removed. A predefined amount of wheat straw (1 kg/m²) was strewn once a day. In regimen 2, the boxes were mucked out completely and rebudded every day over a period of 2 wk, using 3.5 kg/m² per
day. In regimen 3, the boxes were not mucked out for 2 wk, although the feces were removed every day (termed “skipping out”) and new bedding was laid down at a rate of 1 kg/m². Table 2 shows the time schedule for Exp. 2.

**Exp. 3.** In Exp. 3, the use of wheat straw bedding over a 6-wk period without any mucking out was evaluated with regard to gas and particle generation. New straw was strewn each day at a rate of 1 kg/m².

**Data Analysis**

The statistical evaluation was carried out with SAS software (SAS Inst. Inc., Cary, NC). The airborne PM10 and gas data (ammonia, carbon dioxide, and nitrous oxide) from all 3 experiments were transformed into a Gaussian distribution. The data were analyzed using the GLM procedure. The results are presented as least squares means (LSM) with SE (t-test). The experimental unit for all the calculations in each experiment was the stable. Analyses of the ambient temperature and humidity data were undertaken using means and SE.

**Exp. 1.** A data set consisting of 4,032 observations per bedding material was available for the statistical analysis of Exp. 1. For ANOVA using the GLM procedure, the daily means of the gas values were calculated. These were used to analyze the gas concentration development within the course of the 14-d assessment period. In comparison, hourly means were calculated to analyze the gas concentration development within the course of a day. The fixed effects of material and repetition were considered. The relative humidity and air temperature in the stable were evaluated as covariables. The same statistical model was used for all 3 gases.

A data set of 1,008 observations was used for statistics regarding the airborne PM10 data. Daily means of the airborne PM10 were calculated. An ANOVA was carried out using the GLM procedure, taking into account the fixed effect of week. The relative humidity and air temperature in the stable were evaluated as covariables.

**RESULTS**

**Exp. 1**

The mean gaseous ammonia concentrations within the stable differed between the 3 bedding materials. The mean stable ammonia concentrations over the whole 14-d trial period were 3.07 ± 0.23 mg/m³ for wheat straw, 4.79 ± 0.23 mg/m³ for straw pellets, and 4.27 ± 0.17 mg/m³ for wood shavings. The differences were significant (P < 0.05) only between wheat straw and straw pellets. The mean values for all 3 gases are shown in Table 3.

The day of the trial had a significant influence on the gaseous ammonia concentrations in the stable. Figure 2A, 2B, and 2C shows the daily mean ammonia concentrations in the boxes and external air for wheat straw, straw pellets, and wood shavings, respectively. The mean ammonia concentrations in the stable increased during (P < 0.05) the first few days of the trials with all 3 types of bedding material. However, the mean ammonia concentrations of the straw pellet and wood shaving treatments were less during (P < 0.05) the first days of the trial than were those for wheat straw. In comparison, after d 4, the mean ammonia concentrations of the straw pellets and wood shavings were greater (P < 0.0001) than those for wheat straw because they increased constantly until d 6. Thereafter,
the ammonia concentrations in the straw pellets and wood shavings decreased ($P < 0.05$) and varied between 4 and 7 mg/m$^3$ until the end of the trial period (d 14). In comparison, the ammonia concentration of the wheat straw remained within a smaller range during this time, between 2 and 4 mg/m$^3$.

The mean ammonia concentration of the external air remained constant throughout the use of each of the different bedding materials, and there were no obvious differences ($P > 0.05$) in this factor between the 3 bedding materials: wheat straw, 1.06 ± 0.1 mg/m$^3$; straw pellets, 1.09 ± 0.1 mg/m$^3$; and wood shavings, 1.06 ± 0.1 mg/m$^3$. As can be seen, the external ammonia concentration was less than that found in the stable.

Small but significant differences in the stable air temperature were present. The mean air temperature was greatest with the straw pellets (15.7 ± 1.1°C), followed by the wood shavings (13.5 ± 1.0°C) and then the wheat straw (12.6 ± 1.5°C). In general, the mean stable air temperature remained relatively constant throughout each of the 14-d trials. However, during the wheat straw trial, the mean stable air temperature and the mean ammonia concentration showed an almost parallel course over the 14-d trial period (Figure 2A).

When the course of the ammonia concentration within a day was followed (Figure 3), it could be seen that the concentrations with the straw pellets and wood shavings decreased constantly from ($P < 0.05$) 1100 h until the horse was returned to its box at 1800 h. Afterward, there was a tendency for the ammonia concentration to increase. For wheat straw, the daily decline in ammonia between 1100 and 1800 h was slight.

**Exp. 2**

Table 4 shows the mean gaseous ammonia and nitrous oxide concentrations as a function of mucking regimen. The greatest ammonia concentrations were measured when the boxes were mucked out completely every day (regimen 2): LSM = 2.25 ± 0.1 mg/m$^3$. The smallest LSM (1.54 ± 0.1 mg/m$^3$) was found when only the feces were removed every day (regimen 3). Regime 1 (no mucking out) resulted in mean concentrations of 1.92 ± 0.1 mg/m$^3$. The mean gaseous ammonia concentrations in the stable in regimen 2 were significantly greater than those in regimens 1 and 3 ($P < 0.05$).

The mean nitrous oxide concentrations were similar in all 3 regimens; however, the LSM of regimen 2 (daily mucking out) was greater than those of the other 2 ($P < 0.05$). Table 4 also shows the average airborne PM10...
concentrations as a function of the mucking regimen. The PM10 LSM for regimens 2 (mucking out completely every day) and 3 (removing feces every day) did not differ significantly (248.86 ± 10.7 µg/m³ and 281.68 ± 16.5 µg/m³, respectively). Regimen 1 (no mucking out), however, had significantly smaller average PM10 concentrations than the 2 other regimens with 124.36 ± 13.37 µg/m³ (P < 0.05). The mean PM10 concentration was 248.86 ± 10.7 µg/m³ for regimen 2 (mucking out completely every day) and 281.68 ± 16.5 µg/m³ for regimen 3 (removing feces every day).

The mean ammonia concentrations in the boxes and the external air, in addition to the mean air temperatures in the boxes during each of the 14-d trials for all 3 mucking regimens, are shown in Figure 4A, 4B, and 4C. If the individual trends for the mean ammonia concentration in the stable are considered, clear differences between mucking regimens can be seen. Increasing (P < 0.0001) concentrations of ammonia were measured in regimen 1 (no mucking out), up to and including d 7 (on average, up to 3.7 mg/m³). The ammonia concentration then declined (P < 0.0001) and remained constant at 1.5 mg/m³ from d 10 to 14. In regimen 2, complete mucking out was carried out on a daily basis, and there was a clear and repeated increase and decrease (P < 0.05) in the average daily ammonia concentrations throughout the 14-d trial. However, the ammonia concentration decreased slightly over the entire 14-d period with this regimen. The ammonia concentration was least with regimen 3, but the average daily value fluctuated over the 14 d. Until d 7, the average daily values increased (P < 0.0001) and then decreased (P < 0.05) slightly, where they remained at an average concentration of 1.5 mg/m³ until the end of the trial.

The mean ammonia concentration of the external air remained constant throughout each of the mucking trials. In addition, no differences could be determined among the 3 regimens: regimen 1 (no mucking out), 1.15 ± 0.2 mg/m³; regimen 2 (mucking out completely every day), 1.17 ± 0.1 mg/m³; and regimen 3 (removing feces every day), 1.10 ± 0.1 mg/m³.

As can be seen in the data in Figure 4D, 4E, and 4F, the trends in airborne PM10 were very similar when the 3 mucking regimens were compared with one another. The greatest concentrations (P < 0.0001) were measured with all 3 regimens on d 1, the day when the bedding was initially put down. The average daily values remained similar, to a great degree, over the course of the 14-d trials, but it could also be seen that in regimens 1 and 3, the average value declined slightly at the end of the trial period. This was not the case with regimen 2 (complete daily mucking out). Generally, the airborne PM10 concentrations were significantly less when mucking out did not take place (regimen 1).

Large variations in the mean stable air temperature occurred during the course of each regimen (Figure 4A to 4F). Despite this, slight differences in the mean stable air temperatures among the 3 regimens could be observed. The mean stable air temperature in regimen 3 (8.5 ± 2.5°C) was less (P < 0.05) than those in regimens 1 and 2 (12.3 ± 2.4 and 10.4 ± 3.2°C, respectively). There were no real differences in the relative air humidity in the stable among the 3 regimens. The humidity in regimen 3 (83.8 ± 4.0%) was greater (P < 0.05) than that in regimens 1 and 2 (79.2 ± 5.4 and 79.1 ± 5.7%, respectively). In general, the mean relative air humidity remained similar throughout each of the 14-d trial periods.

**Exp. 3**

In Figure 5A, the average ammonia, nitrous oxide, and carbon dioxide concentrations are shown as a function of the week of investigation. The mean weekly values of the box air temperatures were also calculated. The average weekly ammonia concentration value increased (P < 0.05) slightly until wk 4, and then remained constant until wk 6. The LSM of the ammonia concentrations of wk 1 to 3 did not differ significantly (P = 0.129), but a significant difference could be observed from wk 4 onward (P = 0.047). A similar development was also observed for both nitrous oxide and carbon dioxide. The mean nitrous oxide concentrations increased (P < 0.0001) until wk 4, and then remained constant and decreased (P = 0.1218) during wk 6. The mean carbon dioxide concentrations decreased (P = 0.0002) slightly from wk 4 until the end of the trial.
Figure 5B shows the average airborne PM10 values depending on the week, as well as the mean weekly values of the stable air temperature and the relative air humidity. The average weekly values for PM10 varied between approximately 90 and 140 µg/m³. Generally, however, the values tended to decrease ($P = 0.205$) in the period between wk 1 and 6. Both the mean weekly stable air temperature and relative humidity remained relatively similar throughout the 6-wk trial period (Figures 5A and 5B) and had no influence ($P > 0.05$) on the emission of PM10.

**DISCUSSION**

The main focus of Exp. 1 was the detection and quantification of 3 biogenic gases (ammonia, carbon dioxide, and nitrous oxide) released from 3 different types of bedding material. This study focused on ammonia. Carbon dioxide and nitrous oxide were also measured because these concentrations were needed to explain and discuss the ammonia concentrations within the stable (nitrification process).

**Figure 4.** Panels A to C: mean concentrations of gaseous ammonia in the stable and outside as well as the mean stable air temperature during the course of the 14-d trial period as a function of the mucking regimen. Panels D to F: mean airborne particulate matter <10 µm (PM10) concentrations, mean air temperature, and relative humidity in the stable during the course of the 14-d trial period as a function of the mucking regimen; n = 14 daily means (Exp. 2), $P < 0.05$. The bars represent the SE of the mean concentrations.
The results showed that when wheat straw was used, decreased ammonia concentrations were recorded in the box, compared with straw pellets and wood shavings. The values for ammonia generated with straw in this study were greater than those reported in many earlier investigations. Hessel et al. (2005) recorded average ammonia values of 1.9 mg/m³ in indoor boxes bedded with barley straw. Haake (1992) used different bedding substrates and measured average concentrations of 1.8 mg/m³ with straw and 2.4 mg/m³ with wood shavings in outdoor boxes. Jaggy (1996) measured average ammonia concentrations of 2.3 mg/m³ when using straw in indoor boxes, as well as 1.2 mg/m³ in outdoor boxes.

On average, decreased ammonia concentrations were measured during that part of the day when the horses were not in the boxes (1100 to 1800 h). It can be assumed that the increased ammonia values before and after this period resulted from activity of the horses in the boxes during feeding, when their movements and possible churning up of the bedding material would lead to a release of ammonia from the substrate.

The ammonia concentration of the outside air had no effect on the mean box ammonia concentration, although it should be noted that the mean box ammonia concentration during the first 3 d of all 3 types of bedding was barely greater than the ammonia concentra-

![Figure 5](image-url)
tion in the outside air. However, environmental influences cannot be totally excluded because of the results of the air temperature and relative air humidity measurements. It is known that ammonia concentrations and air temperature are positively correlated (Loehr, 1974; Käck, 1996). A virtually parallel course of mean stable air temperature and mean ammonia concentration was found to occur with wheat straw. For example, the increase in temperature from d 3 onward was associated with a concomitant rise in ammonia concentration. This phenomenon was only a tendency with the wood shavings. The mean stable air temperature increased by only approximately 1.5°C between d 1 and 6, whereas the mean box ammonia concentration increased greatly in the same period. In comparison, the reduction in mean ammonia concentration from d 7 onward could be explained by the slight decrease in air temperature. In contrast, no such relationship between air temperature and ammonia concentration occurred with the straw pellets.

Both carbon availability and the carbon-to-nitrogen ratio play an important role with respect to the emission of ammonia from bedding material. The availability of carbon is known to be improved by the processing of bedding materials (milling, chopping, and pelleting; Haug, 1980; Tam, 1995), such as with the straw pellets used in Exp. 1. Such processing leads to an enlargement of the surface area that is open to microbial attack, resulting in an increased conversion of the constituents of the material, thereby making carbon more freely available. In addition, the greater surface area supports gaseous exchange between the bedding and the surrounding air (Poincelot, 1975).

Various types of microorganisms, such as chemoheterotrophic bacteria, Actinomycetaceae, and fungi, are involved in the chemical conversion processes that occur in bedding. These microorganisms require nitrogen for their growth and metabolic processes (Focht and Verstraete, 1977; Schlegel, 1993) as well as carbon as an energy source and as a building block for their cells (Schlegel, 1993). The oxidation of organic compounds releases carbon dioxide, which is used as a source of energy for the synthesis of proteins by certain microorganisms, the nitrobacteria. Nitrifying microorganisms require 15 to 30 molecules of carbon (as an energy source) for the utilization of a single molecule of nitrogen in the production of protein. The optimal carbon-to-nitrogen ratio therefore lies between 15:1 and 30:1 (Poincelot, 1975; Haug, 1980). This relatively wide range is specified as the optimal carbon-to-nitrogen ratio because of the continual supply of feces and urine from the horse. With a very narrow carbon-to-nitrogen ratio (<15:1), the microorganisms cannot fix the nitrogen because of a lack of energy. This, then, results in a loss of nitrogen from the bedding in the form of ammonia (Mote and Griffiths, 1980).

Because of the increased surface area of the straw pellets, it can therefore be assumed that they would have an advantage with respect to binding ammonia compared with unprocessed bedding materials. This could not be confirmed in the present study because the greatest mean ammonia concentrations in the air were found when straw pellets were used as bedding. This greater ammonia concentration also occurred despite the fact that the straw pellets had the greatest carbon content at the end of the 14-d trial [7.15 kg/m³ vs. 5.63 kg/m³ (wheat straw) and 5.44 kg/m³ (wood shavings)], and in theory the greatest energy source for the conversion of nitrogen. To explain this discrepancy, other processes that occur in bedding need to be investigated.

The loss of nitrogen from the bedding is also influenced by the pore volume and oxygen content of the bedding. The available pore volume is substantially affected by the structure of the substrate and additionally by the degree of compaction of the substrate caused by the horse being in the box (Haug, 1980). In the present investigation, the degree of compaction was not measured, although it can be assumed that because of the mechanical processing of the straw pellets, this type of bedding was quickly compressed in the boxes so that the gaseous exchange between the substrate and the surrounding air was reduced. The resulting lack of oxygen would then lead to an inhibition of the nitrification processes, meaning that the nitrogen could not be oxidized to nitrate but would be emitted as ammonia.

Management of the 3 types of bedding differed. In contrast to the management of the straw pellets and wood shavings, for which no additional bedding was strewn, additional wheat straw was strewn every day on any damp and dirty areas in the boxes. Because of this, carbon was made directly available on those areas where it was needed and could be used. Management therefore appears to have had an important influence because the course of the ammonia concentration with both the straw pellets and the wood shavings was similar and both were different from that of the wheat straw.

In Exp. 2, a constant increase in ammonia concentration up until d 7 could be identified in regimen 1 (no mucking out). Subsequently, the values fell steeply, and then remained relatively constant. Kaiser (1999) also showed that this development could be explained by microbial conversion in the bedding material. The initial, assimilative phase is characterized by several factors. There is a carbon breakdown phase mediated by thermophilic bacteria, fungi, and actinomycetes. In addition, there is a gradual increase in the nitrogen pool in the substrate because of an increasing accumulation of excrement. During this phase, there is little or no conversion of nitrogen, so the emission of ammonia
during the first few days is small. Subsequently, there is an increase in the emission of ammonia because of a shift in the balance of soluble ammonia to gaseous ammonia, with little or no formation of nitrous oxide. This increase in ammonia emission was also confirmed in the present study. The reduction in ammonia seen after d 7 can be explained by the fact that new bedding was scattered in the boxes on a daily basis. Because of the regular, daily addition of carbon, the carbon-to-nitrogen ratio was not restricted and, because of the adequate availability of energy carriers, the microorganisms could fixate nitrogen, which, in turn, resulted in less loss of ammonia in gaseous form from the bedding (Mote and Griffis, 1980).

The ambient air temperature did not appear to have any effect on the ammonia concentration in regimen 1, given that as the mean ammonia concentration decreased from d 7 onward, the air temperature continued increasing until d 10, and only afterward did the temperature decrease.

Marked variations in the mean daily ammonia values could be observed over each of the 14-d trials, especially with regimen 2 (mucking out completely every day). It can be assumed that a large amount of ammonia was released because of a daily churning up of the bedding with this regimen. In addition, nitrogen-binding conversion processes in the bedding were not possible because it was being changed daily. In regimen 3 (removing feces every day), the trend in ammonia concentration was similar to that for regimen 1 (no mucking out); however, the values were generally less. This reduced ammonia production can be explained by the absence of churning up of the substrate. Alternatively, with the daily removal of feces, a small source of nitrogen was eliminated. Another reason for the decreased concentrations in regimen 3 could be that the mean stable air temperature during this regimen trial (8.5 ± 2.5°C) was less than in the trials of regimens 1 and 2. Despite this latter possibility, the ambient air temperature had no effect on the course of the ammonia concentration over any of the 14-d trial periods.

Less airborne PM10 was found in the stable for regimen 1 (no mucking out) compared with regimens 2 (daily mucking out) and 3 (removal of feces). It can be assumed that as the strewn material became moister during the course of the trial because of a buildup of urine, the particles were increasingly bound and were not released into the atmosphere. Contrary to our original assumption that with increased humidity the generation of particles would be reduced, Butera et al. (1991) found that the relative humidity in the stable had no effect on the generation of airborne particles.

In Exp. 3, the PM10 concentration tended to decline over the 6-wk period in which no mucking out took place. This effect was also observed in earlier investigations (Fleming et al., 2008b), confirming the tendency shown in the non-mucking-out regimen (regimen 1) of Exp. 2.

**Conclusions**

With regard to the concentrations of ammonia in the stable, bedding with wheat straw had advantages over both straw pellets and wood shavings. Because of the results found in Exp. 1, it appears sensible to carry out work when there are no horses in the stables because activities in the stable (feeding, mucking out, and sweeping) cause both increased amounts of ammonia and increased airborne particle concentrations (see Fleming et al., 2008b). With regard to the management of bedding, it could be concluded from Exp. 2 that bedding for 2 wk on wheat straw with no mucking out had no negative influence on ammonia concentrations in the stable. Advantages were indeed offered by this regimen compared with mucking out daily. Even when wheat straw bedding was used over a longer time (6 wk), the ammonia values did not increase constantly with time. Based on these results, it would be sensible to dispense with daily mucking out, although additional bedding should be added regularly.

The choice of mucking regimen, however, was not just dependent on the ammonia and particle concentrations; other factors have to be taken into account. For example, the growth of pathogenic germs and fungi would certainly be favored if the same bedding were maintained for some weeks, which, in turn, would have a negative effect on animal health. Furthermore, there would be a greater risk of a reinfection of the horse by its own excrement (i.e., endoparasites) as well as a potentially greater development of insects within the stable.

**LITERATURE CITED**


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