AIRBORNE DUST AND AEROALLERGEN CONCENTRATIONS IN DIFFERENT SOURCES OF FEED AND BEDDING FOR HORSES

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SUMMARY

Standardized methods were used to make quantitative and qualitative assessments of respirable dust and aeroallergens in feed and bedding for horses. Concentrations of airborne dust were measured by using a Rion particle counter, and levels of major aeroallergens implicated in chronic obstructive pulmonary disease were measured by using an Andersen sampler. Laboratory conditions allowed comparison of the different sources of forage, supplements, and bedding without external influences such as ventilation, external temperature and horse activity affecting the result. Grass silages of approximately 50 % dry matter and alfalfa pellets appeared to be very good sources of forage with low levels of dust and aeroallergens. The studied good quality straw was significantly less dusty with fewer allergens than the wood shavings. Supplements, such as whole grains and molassed concentrates, contained many respirable particles and aeroallergens. Rolled grains were significantly more dusty than good hay.

INTRODUCTION

Breathing exposes the lungs to a complex and dynamic mixture of gaseous and particulate pollutants. The potential mechanisms of injury are diverse, as are the resulting diseases, and the adverse effects on health may be immediate or delayed. The short-term athletic ability and long-term welfare of horses are largely dependent on their respiratory well-being.

Health hazards arising from the inhalation of airborne dust depend on the concentration of particles as well as on their physical, chemical, and/or biological properties (22). Correlations between concentrations of airborne dust in horse stables and pulmonary diseases have been widely studied in recent years. High concentrations of airborne dust are reported to increase the severity (5) and duration (3) of subclinical lower respiratory tract inflammation (LRTI), a covert disease frequently associated with the poor performance syndrome. In particular, horses suffering from chronic obstructive pulmonary disease (COPD) require strict environmental control. For these horses, acute exacerbation of the disease is induced by contact with the stable environment and hay. This common syndrome is probably an allergic response to airborne antigens which are constituents of stable and hay dust, including *Faenia rectivirgula* (previously named *Micropolyspora faeni* (30)) and *Thermoactinomyces vulgaris*, two thermophilic actinomycetes, and *Aspergillus fumigatus* (8, 25, 26).

Environmental management to control equine respiratory disease aims to minimize exposure to stable dust throughout the horse's life. Different studies have determined the concentrations of dust and allergens in stables (11, 27, 32). It is clear that airborne dust concentrations are significantly greater around the horse's nostrils (breathing zone) than in the stall (32). Because ventilation does not remove the dust challenge from the horse's nostrils when the animal is eating and sniffing, changing the source material is the only way to decrease respirable dust levels (32). Bearing this in mind, studying respirable dust and aeroallergen concentrations in different sources of feed and bedding seems to be the best approach to evaluate alternative materials to decrease toxic, irritant, and allergic reactions to stable conditions.

The aim of this study was therefore to report a standardized method to assess, both qualitatively and quantitatively, aerosol pollution from forage, bedding, and supplements, that are commonly used in equine management. Respirable dust is, by definition, the fraction of airborne particles that reaches the peripheral airways (22). It is therefore considered as a good index to evaluate the health hazard of airborne dust inhalation (22). In this study, the concentration of dust liberated from samples was measured under standard conditions and the antigenic burden, represented by the three major allergens incriminated in COPD, was assessed.

MATERIAL AND METHODS

Samples analysed

The materials studied were chosen because of their frequent use in equine management. The forages studied were grass hays (both good and dusty), grass silages, and alfalfa pellets. The good quality grass hay contained approximately 84 % dry matter (DM) and was not dusty when shaken. Dusty hay came from the same source as good quality hay, but the bale had been moistened twice, then left for two months, after which, large areas of moisture were observed inside the bale and a dust cloud was liberated on handling. A grass silage, containing approximately 78 % DM, was compared with three commercial grass silages, approximately 50 % DM (HorseHage®, Mark Westaway and Son, Devon, England; Horse Dinner®, Jopack, Moorsele, Belgium, and Préfané Liégeois®, Mortier, Belgium). The characteristics of these three commercial silages are shown in table 1. Although they may have been some quantitative and qualitative differences between these three silages, they were considered as a group because they were markedly different from the other

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Table 1. Desciption of the commercial grass silages used for quantitative and qualitative measurements (Mean ± standard deviation).

	Composition	Cutting stage	Respirable dust	*A. fumigatus*	*F. rectivirgula*	*T. vulgaris*
			particles/litre of air	cfu/42.45 l of air	cfu/42.45 l of air	cfu/42.45 l of air
Horse Hage®	rye-grass ± 49% DM	growth	3556±1840[a]	102±49[a]	14±4[a]	73±24[a]
Horse-Dinner®	rye-grass ± 48% DM	flowering	4373±1992[a]	463±182[b]	19±11[a]	35±17[ab]
Préfané Liégeois®	grass ± 50% DM	growth	5376±1305[b]	242±49[c]	11±9[a]	18±5[b]

Values with no common designations are significantly different ($p<0.05$) (Mann-Whitney test); cfu = colony forming units.

forages studied. The three commercial silages represented different qualities inherent to different production systems.

The bedding materials studied were straw, flax straw (Equilin®, Lamerant, Leneubourg, France), and wood shavings (Plomp®, Waarden, the Netherlands). Straw from two different sources was investigated. Both were of good quality, well stored, and not dusty when handled. The flax straw and wood shavings were commercially prepackaged especially for horses. Each of the studied samples was collected from a different package or bale.

Three types of supplement were studied : whole grains of spelt, a mixture of rolled grains of barley (50%) and oats (50%), and molassed concentrates (Mélange Chevaux Extra n°1, Ets Mathy, Remouchamps, Belgium).

Sampling technique

The liberation and sampling of airborne dust and allergen from samples was standardized as follows : for each measurement, 100 g of the sample was placed in a sample chamber (1 m high, 25 cm inside diameter) through which there was a constant flow of air (200 litres/min). The samples were taken directly from the batches and very carefully placed in the sample chamber to avoid differences due to handling. The liberated dust cloud passed into a 2 m^3 hermetic chamber containing the measuring apparatus for 3 minutes before sampling started in the fourth minute. The airflow was maintained throughout the measurement period. The entire apparatus was placed in a room with a temperature of 18 ± 2°C and a relative humidity of 60 ± 5 %.

Between samples, the chambers were cleaned with a vacuum cleaner. Thus, air was totally removed and changed. Before each study day, the residual airborne dust in the hermetic chamber was sampled to ensure a constant basal level of airborne dust. Only one type of material was studied each day to avoid cross-contamination. Dust and spore concentrations from each material were measured simultaneously.

Measurement of total respirable dust

Dust particles were counted and sized with an optical particle counter, the Rion KC-01B (Hawksley & Sons, Lancing, Sussex). Particles were identified by using a light scattering technique; there were five size ranges : 0.3 - 0.5 μm, 0.5 - 1 μm, 1 - 2 μm, 2 - 5 μm, and 5 μm and above. The counter was positioned at the centre of the 2 m^3 chamber. One litre of air was sampled for each measurement. The experiment was repeated 17 to 22 times for each type of material. Only the respirable dust fraction (between 0.5 and 5 μm) was analysed. This fraction corresponds to the portion of the inspirable dust particles that are able to penetrate beyond the terminal bronchi. The Rion counter was recalibrated by Hawksley & Sons after every 1,000 hours of use, using reference particle aerosols of known size.

Determination of spore concentrations

An Andersen sampler (Andersen samplers, Inc., Atlanta, Georgia), with appropriate nutrient media, was used for determining the number of viable airborne particles, as described below. It consists of a series of six impactor plates, each perforated with a regular pattern of 400 holes, spaced evenly above the surface of a nutrient agar medium in a Petri dish (1). The holes were of uniform size within each stage, but decreased in size with each succeeding stage. Air was drawn through the device at the rate of one cubic foot per minute (28.3 l/min) for 90 seconds precisely, using a small vacuum pump. The particle-bearing air was impacted directly onto the media surface. The sampling period was chosen to prevent overloading of the plates.

After a sample was drawn through the instrument, the Petri dishes were incubated at 46°C for 3 days for *A. fumigatus* and at 56°C for 5-6 days for the two actinomycetes before colonies were counted. *Aspergillus fumigatus* was isolated on 2 % malt extract agar containing 20 iu penicillin/ml, 40 iu streptomycin/ml (14) and 0.05 per cent Triton N-101 (Sigma-Aldrich Corp., USA). The non-ionic surfactant Triton N-101 was used to decrease the radial growth rate of fast-growing fungi (23). The two thermophilic actinomycetes were isolated on nutrient agar supplemented with 0.2 % casein hydrolysate and with cycloheximide, at 50 μg/ml, to inhibit fungi (20).

Table 2. Respirable dust particles per litre of air and concentrations of viable spores (colony forming units (cfu) in 1.5 cubic foot of air) of *Aspergillus fumigatus (A. fumigatus)*, *Faenia rectivirgula (F. rectivirgula)* and *Thermoactinomyces vulgaris (T. vulgaris)* in different types of bedding and supplement (Mean ± standard deviation).

	Respirable dust particles/litre of air	***A. fumigatus*** cfu/42.45 l of air	***F. rectivirgula*** cfu/42.45 l of air	***T. vulgaris*** cfu/42.45 l of air
Good hay	63038±30033[a]	852±238[a]	131±52[a]	139±51[a]
Silage 78% D.M.	8775±2514[b]	489±276[ab]	73±50[a]	93±30[a]
Silages ± 50% D.M.	4488±1932[c]	193±179[bc]	15±9[b]	49±36[b]
Alfalfa pellets	9510±4404[b]	110±108[c]	5±2[c]	18±8[c]
Wood shavings	31492±12910[a]	710±124[a]	53±29[a]	79±59[ab]
Good straw	11571±4897[b]	402±214[a]	18±17[ab]	33±17[b]
Flax straw	9251±1776[b]	104±23[b]	10±9[b]	60±13[a]
Rolled grains	120321±30617[a]	431±27[a]	76±68[a]	47±45[ab]
Whole grains	4109±858[b]	189±64[a]	5±2[b]	43±5[a]
Molassed concentrates	2093±613[c]	33±14[b]	12±7[ab]	128±78[b]

Values with no common designations are significantly different ($p<0.05$) (Mann-Whitney test); cf 4= colony forming units.

Identification and counting of colonies

A simple microscopic examination does not allow the specific identification of spores (19). Culture techniques with special media and a high incubation temperature allow only a small proportion of potentially relevant species to grow (24). For the two actinomycetes, in addition to cultural and microscopic morphology, each strain was subjected to an array of biochemical tests. These tests were carried out according to the methods of Kurup and Fink (18) and included decomposition of hypoxanthine, casein, starch, and esculin. These tests allowed identification of strains of *F. rectivirgula* and *T. vulgaris* from other thermophilic species. *Aspergillus fumigatus* is known to grow at temperatures between 12 and 65°C but has optimum growth at 42°C (21). To minimize the growth of other thermophilic *Aspergillus* species, we incubated plates at 46°C. The selective temperature , macroscopic colour and appearance of the colony, and a simple examination under light microscopy were sufficient to differentiate this species from others.

Statistics

Since airborne dust concentrations were not normally distributed (2), the data are expressed as geometric means and non-parametric statistics were applied. The Mann-Whitney rank-sum test was used with confidence limits of 0.05.

RESULTS

Use of the standardized methods allowed comparison of the respirable dust burden and the quantities of specific allergens present in different types of feed and bedding sources. All the results are presented in table 2.

Quantitative measurements

The number of respirable particles liberated by the dusty hay (mean $6.88 \pm 1.44 \times 10^5$ particles/litre of air) was tenfold greater than that from the good quality hay (mean $6.30 \pm 3.00 \times 10^4$ particles/litre of air). The different types of grass silage and alfalfa pellet feed all yielded significantly fewer respirable dust particles than the good hay, with grass silages of approximately 50 % DM yielding significantly fewer particles than all the others. Of all the beddings studied, wood shavings, even though specifically sold for horses, liberated significantly more respirable particles than many of the other materials studied. Nevertheless, wood shavings were significantly less dusty than good hay. There was no significant difference between good quality straw and flax straw. The mixture of rolled grains of barley (50 %) and oats (50 %) was significantly more dusty than the other feed supplements and the good hay. However, the quantity of respirable particles liberated from molassed concentrates was not significantly different from that liberated from wholes grains of spelt.

Qualitative measurements

Overall, there were no significant differences between the good hay and the grass silage that contained approximately 78 % DM with respect to the three COPD-incriminated allergens. No difference for *A. fumigatus* could be found between grass silage of approximately 78 % DM and grass silages of approximately 50 % DM However, for the two thermophilic actinomycetes, the number of colony forming units (cfu) liberated by silages with about 50 % DM was significantly smaller that of grass silage of 78 % DM or good hay, but was significantly greater than that for alfalfa pellets. The alfalfa pellets contained significantly less of the three studied allergens than the other feeds, except that the *A. fumigatus* content of alfalfa pellets did not differ significantly from that of grass silages with approximately 50 % DM.

Colonies from the dusty hay could not be quantified be-

cause the Petri dishes were overloaded, even when the sampling time was decreased to 5 sec.
Values for wood shavings did not differ significantly from those for straw of good quality. Concentrations of *A. fumigatus* and *F. rectivirgula* were lower for flax straw bedding than for the other sources.

DISCUSSION

More and more practitioners and owners realize that strict control of the horse's environment is necessary to decrease the potential risk, severity, and duration of respiratory diseases. Several approaches can be taken to improve the quality of air breathed by stabled horses. Stable design and management are important for minimizing the release of dust, noxious gases, and infectious agents, and for their dilution (4, 28, 31), but attention needs to be paid to the horse's feed and bedding, which provide the principal sources of dust in stables (31). In addition, around the horse's nostrils, concentrations of respirable airborne dust are significantly greater than levels measured elsewhere in the stall (32). Because dust measurements *in situ* depend on many variables (5), such as previous activities, the design and ventilation of the stable, laboratory standardized conditions are necessary for qualitative and quantitative assessments of different types of feed and bedding in order to obtain an accurate estimation of their hygienic quality. Since only the air-dispersible part of the dust contained in the different sources is of interest for respiratory health, we chose to use a constant wind speed to simulate handling and shaking. This method was chosen over manual shaking as it provided a more standardized and reproducible method of creating the dust sample. Washing the samples in water gives a higher estimate of spore numbers than shaking does (19). Gregory and Lacey (13) found that the majority of air-dispersible spores are liberated from a sample during the first three minutes when samples are shaken in a wind tunnel. Therefore we chose to begin measurements at the fourth minute after the start of the flow of air through the sample chamber.
The quantity, composition, and aerodynamic size of dust particles in stable air determine, the inhaled dose, the pathogenicity, and their site of deposition in the respiratory tract, respectively (31). The respirable fraction of the dust is considered a good index of the respiratory hazards caused by airborne dust inhalation (9) and was chosen as a measure for the quantitative comparison between samples. Respirable particles (0.5 - 5 μm aerodynamic diameter) are small enough to avoid the protective filter of the upper airways and reach the deepest parts of the lung where they are able to initiate inflammatory and/or allergic responses. Qualitative comparisons were focused on the three airborne allergens most frequently implicated in the aetiopathogenesis of COPD. Their spores are of respirable aerodynamic diameter (19) and therefore have the potential to penetrate to the lower airways. The isolation of fungi and actinomycetes from suspensions of spores in air, using an Andersen sampler, seems the most satisfactory and reliable method to assess their numbers (19, 20), but it is essential to use optimal media, inhibitors, and incubation temperatures to obtain growth in culture without large contamination (7, 17).
Microbial and biochemical changes within self-heating hay (12, 15, 19), dependent on water content, lead to large increases in the number of thermophilic actinomycetes and fungi. This could explain the tenfold increase in respirable particles and the overload of colonies, which prevented qualitative measurements for the dusty hay studied, compared with the number in good quality hay from the same source. Even though the content of the respirable particles in grass silage of approximately 78 % DM was highly significantly lower ($p < 0.0001$) than that of good quality hay, qualitative measurements did not differ between these two types of forage. The alfalfa pellets contained fewer allergens than grass silages of ± 50 % DM but the use of alfalfa pellets is not readily accepted by horses and their owners .
In the present work, straw of good quality gave rise to fewer respirable particles than wood shavings, even though the studied samples of wood shavings came from commercial dust free shavings. The dust content of wood shavings was, however, lower than that of the good quality hay. But like hay or any materials of plant origin, the microflora of straw and wood shavings can change rapidly when a high moisture content is combined with a high temperature (4, 7, 16, 31). These factors may explain the discrepancy between our results and those previously reported by others (4, 6, 32).
A recent study showed that stabled horses spend 39 % of their time eating forage and concentrates. Close contact with the litter accounts for 9.5 % of the time when horses are sleep on floor and 4 % or 0.5 % when eat straw and wood shavings, respectively (29). Because of the time spent eating, alternatives to hay, such as silages, are necessary to control the internal environment. The present results demonstrate that there are alternative sources of forage which are less dusty and which contain fewer aeroallergens than hay. Supplements were not an important source of dust and allergens, except when grains were rolled. .
It is commonly recommended that hay and straw are excluded from the environment of horses with COPD and infectious pulmonary disease (10). While totally excluding hay, even of the best quality, is entirely justified, the choice of bedding seems to be more tricky. Indeed, commercial wood shavings are probably more constant in quality although they may sometimes be more dusty than commonly thought. The quality of straw is difficult to estimate and extremely variable, depending mainly on storage conditions. Further studies are necessary to show whether the use of good quality straw is likely to be help maintain COPD horses in clinical remission.
In summary, the present investigations (1) propose a standardized method for the assessment of the qualitative and quantitative content of dust in different feeds and bedding; (2) demonstrate that alternative materials may be used to decrease airborne dust and aeroallergen in the immediate equine environment and contribute to the improvement of horse management.

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COMPARISON OF AIR- AND BONE-CONDUCTED BRAIN STEM AUDITORY EVOKED RESPONSES IN YOUNG DOGS AND DOGS WITH BILATERAL EAR CANAL OBSTRUCTION

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ABSTRACT

Brain stem responses to air- and bone-conducted stimuli were analyzed in 11 young dogs, using an in-the-ear transducer and a vibrator designed for human hearing tests, respectively. The mean thresholds were 0 to 10 dB for air-conducted stimuli and 50 to 60 dB for bone-conducted stimuli. The wave forms and inter-peak latencies of the waves of the auditory evoked responses elicited by air-conducted and bone-conducted stimuli were similar. This indicated that the signals had the same origin and thus both the air-conducted and the bone-conducted responses could be considered to be auditory responses. Measurement of air-conducted and bone-conducted brain stem- evoked responses in five dogs with bilateral chronic obstructive ear disease revealed thresholds of 50 to 60 dB for air-conducted stimuli and 60 to 70 dB for

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