**The effect of steaming and soaking on the respirable particle, bacteria, mould and nutrient content in hay for horses**

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**Abstract**

Forage is crucial for stabled horses, promoting gut health, supplying valuable nutrients and maintaining normal feeding behaviours. Forage can contain high levels of respirable dust pre-disposing horses to respiratory disorders. This study examined the effect of different treatments on the airborne respirable particles (ARP), microbial and nutrient content of hay for horses in 3 experiments. Experiment 1a 8 bales of meadow hay were subjected to 5 treatments n = 40; dry (D), 10-minute soak in water (W), steamed in a bin (TWB), steamed in a Haygain 600 (HG), steamed with a kettle of boiling water (K) on ARP content. Experiment 1b microbial contamination was measured in 5 bales of meadow hay after treatments D, TWB, HG in cold conditions (0-7oC) (n=15). Experiment 2 measured the nutrient content of 30 different hays after D and HG treatments, n = 60. Data in Experiments 1a and 1b were analysed using ANOVA and least significant difference test: hay and treatment as factors. Experiment 2 was analysed using paired t-test with significance levels accepted P<0.05. Results showed steaming in the HG reduced ARP and microbial contamination by 99%. TWB or K reduced ARP in hay by 88%. W, TWB or K did not reduce microbial contamination. HG treatment preserved mineral and protein contents but reduced WSC by 18.3%. Steaming using a HG steamer is a feasible long-term strategy for reducing ARP and microbial contamination while conserving mineral and protein content in hay and is thus suitable for providing hygienically clean forage to stabled horses.

**Key words:** hay, dust, soaking, steaming, nutrients.

**1. Introduction:**

Grass hay is the most common fodder fed to stabled horses in the UK [1] and USA [2]. The nutrient content of both seed hay (mono-species) and meadow hay (multi-species) is mainly determined by the grass mixture and stage of growth at harvest, while the hygienic quality is more influenced by weather during the conservation process and storage conditions [3]. Feeding long forage to stabled horses can help maintain normal time budgets by satisfying the animal’s innate need to chew [4]. Good hay or haylage can also supply a significant proportion of the daily nutrient requirements, although many owners find haylage too energy dense to be offered *ad libitum* and thus prefer to feed their horses a higher fibre lower energy forage such as grass hay.

Traditionally farmers and horse owners assess hay visually and by smell, however, even well conserved hay can contain significant levels of respirable dust and therefore visual assessment is not a recommended method for selecting hygienic hay [5]. Respirable dust is composed of particles less than 5µm in size and is referred to by Hessel et al [6] as the thoracic fraction, and by Art et al [7] and Clements and Pirie [8] as particles that are sufficiently small 0.5 – 5µm to penetrate the peripheral airways. Respirable dust contains potentially allergenic particles, such as mould and bacteria spores, mite faeces, endotoxins and beta glucans, all of which have the potential to contribute to the aetiopathogenesis of equine environmental airway diseases including Recurrent Airway Obstruction (RAO) in stabled horses [9,10]. While administration of corticosteroids and bronchodilators can alleviate the symptoms of RAO, these drugs contravene competition rules, and long-term use is expensive. Maintaining an RAO horse in an asymptomatic condition is best done by reducing the dust /animal interaction [11, 12, 13]. Woods et al [14] and Moore-Colyer and Auger [15] both demonstrated that during feeding, dust in the breathing zone of the horse can be significantly higher than in the general stable environment thus minimising dust released from feed is paramount. To reduce the dust released from hay fodder many owners soak or steam their hay before feeding [16].

Soaking reduces the number of airborne respirable particles (ARP), but has undesirable consequences as it leaches valuable minerals [17,18] and water soluble carbohydrates (WSC) [19] from the hay, increases bacterial concentrations by 1.5 – 5 fold [20,21] and can produce post-soak liquor with a very high biological oxygen demand [22]. Poor forage hygiene derived from bacterial and mould proliferation has been associated with colic in horses [23] thus reducing the quality of forage by soaking is highly undesirable.

Steaming hay is rapidly becoming an acceptable alternative to soaking. Blackman and Moore-Colyer [18] reported that steaming hay for 80 minutes in 5 kg hay nets in a plastic dust bin fitted with a kettle element in the bottom, reduced ARP by 95% while conserving the mineral content of the hay. However, the impact of steaming in a dust bin on the bacterial and mould concentrations in the hay has not been established. Steaming using the specifically designed hay steamers such as the Haygain1000 (HG 1000) and HG 600 (Propress Equine Ltd, Hungerford, UK) has been shown to reduce ARP and microbiota in hay [20,24,25] but to date there is no published information on the effect of high-temperature steaming on the nutrient content of hay.

The objective of the Experiment 1a was to determine the effect of soaking, and three different steaming techniques on the ARP numbers. In Experiment 1b the objective was to measure the effect of two different steaming techniques on the bacteria and mould content in hay; while Experiment 2 determined the effect of high-temperature steaming on the nutrient content of hay for horses.

**2. Materials and Methods**

*2.1 Experiment 1a*

Eight square bales of field-dried UK meadow hay conserved in 2011 weighing approximately 25 kg were subjected to 5 different wetting treatments. Each bale was divided into 5 equal sections of approximately 5 kg and placed into small-holed (50mm) hay nets. Before the steaming treatment took place three wooden rulers containing non-reversible temperature strips (555-409, RoHS Scale B Self-adhesive, testo. WWW.testo.com), were pushed firmly into three different areas of the hay so that steam distribution and the temperature reached inside the hay could be measured. The hay was then placed into the steamer or bag and the treatment applied.

*2.1.1. Treatments and dust sampling*

1. Dry (D); 2. Soaked in 30 litres of clean tap water for 10 minutes at 16oC (W); 3. Steamed using a Haygain 600 steamer (Properss Equine Ltd, Hungerford, UK) (HG); 4. Steamed in a home-made steamer which consisted of a standard domestic 240 litre Wheelie Bin (model CNK/GREWB2; Amazon.co.uk) and a plastic steam-producing wall-paper stripper (Earlex SS77 2300W, Screwfix, Newbury, Berkshire, UK) in the bottom of the bin (TWB); 5. Steamed by pouring a kettle of boiling water the over hay in a bag (K). Post-treatment the airborne respirable particle content was sampled using the method of Moore-Colyer [17]. A large white sheet was placed on the floor in a clean class room and the hay emptied onto it. A cyclone dust sampler (Munro personal sampler AS 200, Woodford Green, Essex, UK) was hung 1 metre above the floor and switched on for 3 minutes. Hay samples were manually shaken with a fork for three minutes. Airborne respirable particles (ARP) were captured on nitrocellulose membrane filter papers. Post-shaking the filter papers were carefully mounted in triacetate and stored for 48 hours by which time the filter paper had dissolved leaving the ARP clearly visible. Counting was performed using an eye piece graticule (NE 11A-19 mm1mm ind.X grid, Hatfield UK) and a binocular microscope according to the method of Moore-Colyer [17]. ARP numbers per litre of air from 1 kg hay were calculated before being subjected to data analyses.

*2.1.2. Data analyses*

Differences between treatments for this randomised block experiment were determined using 1-way ANOVA with main effects being treatment (5) and replicates (8) thus n = 40. Differences between means were calculated using least significant difference (LSD) test where LSD = t (error df) x s.e.d. Due to the skewing of the data ANOVA was performed on log10 transformed data [26,27] as per the accepted procedure for right-handed skewed data [21,28]. Results were expressed as geometric mean particle numbers / litre air / kg hay as this value approximates closely to the median which is the most accurate expression of the distribution of the ARP in hay samples [21,28].

*2.2. Experiment 1b*

2.2.1. Treatments

Five 25 kg small square bales of UK conserved meadow hay conserved in 2011 were subjected to 3 different treatments. Each bale was sub-divided into 3 equal sections of approximately 8kg each and placed into small-holed (5cm diameter) hay nets. Treatment 1 was Dry (D); Treatment 2 hay was steamed in HG 600 (HG); Treatment 3. hay was steamed in the Wheelie Bin (TWB) as detailed in Experiment 1a above. All treatments were carried out in a cold room to replicate UK winter conditions (i.e., 0 - 7oC). Post treatment total viable bacteria (TVC) and mould (CFU/g) were determined by culturing techniques as detailed by Moore-Colyer [21].

2.2.2. Culturing technique

Post treatment the 8 kg of hay was emptied onto a clean cotton sheet in a clean classroom and thoroughly mixed by hand. A 100g sub-sample was taken and roughly chopped with scissors, (previously wiped with ethanol, and allowed to dry) and mixed again after chopping. A 1 gram sub-sample was then weighed into a sterile plastic bag (Seward BA6040) to which 79ml of peptone saline solution (MRD) was added. The bag was then placed into a Lab Blender 80 model (Steward Laboratory, Blackfriars Rd, London). The mixture was then ‘blended’ for 2 minutes in order to wash mould and bacteria from the hay into the solution as per instruction manual for 3M petrifilms (3M Microbiology, 2013). One millilitre of the blended solution was placed into a sterile screw-cap tube (VWR, UK) pre-loaded with 9ml MRD. Serial dilutions were prepared to 10-4. A 1ml sample was then taken from10-2, 10-4 dilutions and separately placed onto pre-labelled 3M Aerobic TVC 20cm2 petrifilm, and 3M Yeast and Mould 30cm 2petrifilm (3M Microbiology, Carl-Schurz-StraBe 1, Germany). Petrifilms were a sample ready, culture medium containing nutrients, a cold water soluble gelling agent, a tetrazolium indicator for the TVC films, and antibiotics for determination of yeasts and moulds. Four petrifilms (2 TVC and 2 yeast and mould) were prepared for each sample. TVC samples were incubated for 3 days at 32°C, yeast and mould films were incubated for 5 days at 20°C.

2.2.3. Microbial enumeration

Colony numbers were enumerated using an illuminated magnifier following the 3MTM interpretation guide. For TVC films, all vital stained colonies were counted. When colony numbers were particularly dense, and small and > 100 per film, three representative 1 cm squares were counted. The average was determined, and scaled up 20-fold as an estimation of the count per film. Mould colonies were identified as described by Moore-Colyer and Fillery [20] by their large flat areas with diffuse edges and dark centres. Mould colonies were counted as total colonies grown per film, or when numbers were greater than 200 representative squares were counted and scaled up 30-fold as an estimation of the count per film.

2.2.4. Data analyses

Difference between treatments were determined by 1-way ANOVA with treatment (3) and replicate (5) thus n = 15. Differences between means were calculated using least significant difference (LSD) test where LSD = t (error df) x s.e.d. Skewed data was subjected to log10 transformation [21,27]. Results were expressed as geometric mean CFU/g of hay which approximated closely to the median and is accepted to be the most accurate expression of the distribution of CFU in the samples [21].

*2.3. Experiment 2*

*2.3.1. Treatments*

Thirty different small square bales (approximately 25 kg each) of a range of meadow and seed hays collected from all over the UK were sampled before steaming i.e., dry (treatment 1) and after steaming in a HG 1000 (Propress Equine Ltd, Hungerford, UK) (treatment 2) thus n = 60. Samples (approximately 500g) were compiled from intact bales using long (300mm) tweezers from 5 different areas and depth of the bale and placed into pre-labelled plastic bags. The bale was then steamed in the HG1000 for 50 minutes. Post steaming the bale was removed from the steamer and another 500g composite sample was taken in the same manner from five different areas of the bale and placed into a separate pre-labelled plastic bag. Samples were immediately stored at -20oC before being dried in a force-draught oven at 60oC and milled in preparation for nutrient analyses.

*2.3.2. Nutrient analyses*

Water soluble carbohydrates (WSC) were measured by the phenol-sulphuric acid method [29]. Nitrogen was analysed by a rapid combustionmethod using a LECO FP-428 analyser (LECO Corp., St. Joseph, MI).Trace elements and extractable P was measured by destroying the organic matter in the sample by dry ashing at a temperature not exceeding 550°C and the soluble residue was dissolved in 25% v/v hydrochloric acid [30]. The extracts were filtered and P determined colorimetrically at 420 nm [31]. Mg, K, Ca and Na were measured by inductively coupled plasma atomic emission spectroscopy (ICP-AES) (Varian Liberty AX, Agilent, UK). Nitrogen was analysed by a rapid combustionmethod using a LECO FP-428 analyser (LECO Corp., St. Joseph, MI) and crude protein calculated by N x 6.25.

*2.3.3 Data Analyses*

Differences between dry and steamed samples were determined by paired t-test using Genstat 15 [26] with P<0.05 as significant.

**3. Results**

Hays used in all experiments were either meadow hay conserved from permanent pasture and contained a range of different grass species, or seed hay which was all single species perennial rye grass (*Lolium perenne*). Hays were collected from across the UK and were thus a cross-section in terms of hygienic quality and nutrient content from the 2009 hay-making season.

*3.1 Experiment 1a*

Results in Table 1 show that all the soaking and steaming treatments reduced the ARP numbers in post-treated hay compared with the dry (D) hay. Soaking (W) and steaming in the HG 600 (HG) reduced ARP numbers by 99% showing that these two treatments were more effective at reducing ARP than steaming with TWB or K which reduced ARP numbers by 88%.

*3.2. Experiment 1b*

Mould and bacterial concentrations (TVC) expressed as geometric mean CFU/g hay (Table 2) were reduced by 99% (P<0.001) after steaming in the HG 600. In contrast steaming in the TWB, did not significantly reduce mould or bacteria concentrations compared with the dry hay. Temperatures reached inside the hay as determined by 3 non-reversible temperature strips per replicate averaged 102 ±4.7 oC across the 5 replicates for the HG 600 steamer. When removing the hay from TWB steamer there were dry cool sections indicating that the steam had not penetrated all of the hay. The results from the temperature strips showed an average temperature of 58 ± 45oC indicating the variability of penetration of steam throughout the hay.

*3.3. Experiment 2*

The nutrient contents of 30 samples of hay before and after a 50 minute steam in the HG 1000 are shown in Table 3. There were no reductions in nutrient content between dry and steamed hay for crude protein (CP), Ca, Mg, Na, P, Cu, Mn, N, K and Zn. The only nutrient to show loss after steaming was WSC, which reduced the content from 126 to 103 g/kg DM, representing an 18% loss of the WSC present.

**4. Discussion**

Across all the treatments examined the results showed that steaming a range of hays in a high-temperature hay steamer conserved mineral and crude protein contents while being the most effective method for reducing airborne respirable particle (ARP) and viable microbial numbers.

*4.1. Airborne respirable particle content*

The results from Experiment 1a are in agreement with the previous findings of Moore-Colyer and Fillery [20] and clearly show that soaking hay in water for 10 minutes or steaming using the HG 600 hay steamer were equally effective at reducing ARP numbers in hay. The results from the HG 600 confirmed that the spiked manifold system distributed steam evenly throughout the bale as all the temperature strips in every replicate reached at least 99oC. The consistent rise in temperature is likely facilitated by the insulating nature of the double-skinned container which allows the temperature inside the box to rise quickly even when external conditions are cold i.e. 0 – 7oC as was the case in this experiment.

On the other hand steaming using the home-made steamer (TWB) or a kettle of hot water (K) was less effective at reducing ARP, although the reductions noted of 88% were similar to those previously reported by Blackman and Moore-Colyer [18] who also used a dustbin type container to steam hay. The lower reductions in ARP content reported in TWB can be attributed to the fact that not all of the hay was effectively steamed. Several of the temperature strips, a marker of relative humidity inside the hay, and thus efficacy of steam penetration, did not register any increase in temperature, showing that when steam was released into the bottom of TWB it did not reach all of the hay. Penetration and even distribution of steam throughout the hay, was also not achieved in the study reported by Earing et al [27] who used a Happy Horse Professional Steamer to steam bales of alfalfa and orchard grass hay. They reported that after 90 minutes of steaming, temperatures reached inside the hay were 46oC which produced a maximum reduction in total suspended particulate matter of only 55%.

*4.2. Microbial contamination of hay*

The meadow hay used in this experiment had no visible signs of bacteria or mould but the microbial analyses confirmed that 2.3 x 105 bacteria and 5.3 x104 mould CFU/g were present. Earing et al [27] classified their alfalfa-orchardgrass mixed hay to have low mould when containing 1x104 CFU/g and moderate mould when containing 2.7 x105 CFU/g, while others [21] reported mould contamination levels in 5 different types of horse hays to range from 0 to 4.6 x 106 CFU/g and bacterial levels of 120 – 3 x106 CFU/g. Based on these previous findings the average microbial burden for the hays used in this study can be classified as moderately contaminated. While some hays as reported by Seguin et al [32] can have either high bacteria or high mould levels, reflecting a potential ecological niche advantage of one organism over another, the hay used in this experiment was moderately contaminated with both. Bacterial and fungal contamination of hay is dependent on plant physiological status, weather conditions, harvest conditions and the speed and stabilisation of storage [33]. Some fungi contamination, notably *Aspergillus* spp, can occur as a result of soil contamination and the proliferation of these is associated with higher levels of moisture during cutting and storage [34], thus it is reasonable to conclude that the conservation process and the storage of the hay used in this experiment were sub-optimal.

Moreover, while some hays on visual assessment show no signs of bacteria and mould growth, this does not necessarily mean that ARP content was low. Indeed many subjectively assessed ‘good hays’ carry a high ARP load, thus visible assessment cannot be relied upon when assessing the hygienic quality of hay, a fact also noted by Clarke and Madelin [5]. Ideally microbial analyses and measurement of ARP content should be obtained before hay is purchased for consumption by stabled horses.

*4.3 Steam treatments and microbial contamination*

The complete distribution of high-temperature steam in the HG 600 as measured by the temperature strips (> 99oC), was responsible for the very high reductions in bacteria and mould concentrations noted in Experiment 1b where 99% of the bacterial and mould spores were killed. These results agree with those reported by Moore-Colyer and Fillery [20] who found that the high temperatures reached in the HG steamer reduced the concentrations of bacteria and mould compared with dry hay. In contrast the bacterial content in the hay steamed in TWB were not reduced compared with the levels found in the dry hay. As both HG 600 and TWB treatments involved putting steam into hay it can be concluded that the lack of efficacy of the steaming process in TWB was due to a combination of lack of penetration of steam through all of the hay and the average low temperatures of 58oC attained after 50 minutes of steaming. TWB was a non-insulated plastic container and in the cold conditions the temperature inside the hay did not increase to the desired level of 90oC for 10 minutes which has previously been shown to kill all the mould and a high proportion of the bacteria present in hay [35]. Effectively it appears that TWB acted as an incubator containing warm damp air which probably stimulated the growth of bacteria many of which thrive at temperatures between 18 – 40oC. Thermophilic bacteria are known to be major allergens for RAO sensitive horses [10], thus partial heating with steam or soaking in water which cause 1.5 – 5 fold increases in bacteria [20, 21] are highly undesirable and could compromise the respiratory health of the horse. The fact that post soak proliferation of bacteria in hay can occur rapidly, 10-minute soak + 2 hour processing time [20,21], has implications for feeding management. Soaked hay should be fed immediately, and hay nets of wet hay should not be left in stables for more than 2 hours as viable bacteria will thrive producing spores and deteriorating the nutrient and hygienic quality of the hay [36].

High-temperature steaming using a HG steamer has previously been shown to maintain the low-dust status of post-steamed forage for up to 4 days [37]. The fact that ARP levels remain low can be attributed to the reduction in viable microbial content and thus no more spores can be produced.

The reduction in viable bacteria will also benefit the digestive heath of the horse. Although no information exists to-date on the species profile or pathogenicity of the proliferating bacteria, species such as *Clostridia* [38] and enterobacteria [39] are commonly found in hay and haylage contaminated with soil, manure and cadavers. The endotoxins found in the outer cell wall of enterobacteria are implicated in laminitis [40], while general poor feed hygiene has been linked with digestive disorders and colic [23] thus proliferation of existing bacteria by soaking or partial steaming is highly undesirable.

*4.3. Nutrient content*

The nutrient contents reported in Table 3 are the means from thirty samples of meadow and seed hay on an as fed basis collected from all over the United Kingdom and thus is an indication of the variation in the quality of hay conserved across the country in 2009. The average CP content was 70 g/kg DM with a range of 40 to 90g/kg while the average of 126 g/kg DM WSC content (range of 62 – 202 g/kg DM) were similar to previously reported values [21], but did not reach the high levels of 310g/kg DM noted in some UK hays by Harris and Geor [40]. The mineral profile of the hays was within the range for typical UK hays [18,37]. Hay is generally the preferred forage for stabled horses because it is conserved at a later stage of growth and thus has a lower nutrient content than silage or haylage. Allowing horses *ad libitum* access to high-fibre feed means they can trickle feed throughout the day without consuming too much energy and thus gaining excessive body weight. Trickle feeding also satisfies the horse’s innate need to chew and helps avoid the development of the stress-related aberrant behaviour crib biting [42, 43]. The hays tested in this study were generally at the lower end of the CP and WSC spectrums and thus would be suitable to be fed *ad libitum* to most stabled horses.

*4.4 Steaming and nutrient content*

The nutrient profiles detailed in Table 3 for hay post steaming clearly show that steaming using a HG 1000 conserves the minerals, trace elements and CP in hay. The fact that no losses were recorded between dry and steamed hay for N, Ca, P, K, Na, Mg, Cu, Fe and Zn are in agreement with previous results [18] supporting the finding that steaming conserved the mineral content of the hay. Several of the minerals appeared to increase post steaming, which is due to a proportional increase as a result of the loss of WSC from the hay. Steaming did reduce the WSC content dropping it by 18%. This drop is greater than the 3-7% noted by Moore-Colyer et al [21] and the 12% recorded by Earing et al [27]. However, the amount of WSC leached from the hay by either soaking in water [19] or steaming is highly variable and in the studies reported to date is not related to grass species or WSC content. The results of the present study confirm this with losses ranging from 2 to 54% of total WSC content.

Soaking hay in water has been reported to cause loss of soluble protein [21] however, results from this experiment indicate that wetting the hay with high-temperature steam does not have the same effect. The proportional increase in CP post steaming suggests that no CP was leached during the steaming process. These results are supported by reports from earlier studies [17, 18, 21, 27]. This is important nutritionally for the horse as it is only protein that is digested in the small intestine of the horse that is available for anabolic processes [33], thus any soluble protein present in the hay is worth conserving.

Most performance horses will require additional energy to that supplied by the more traditional type of long forage i.e., hay and haylage, although clearly forage type and maturity will play a major role in nutrient content and thus the ability of the forage to meet nutrient requirements. While many horse owners automatically add cereal-based concentrates to the diet Ringmark et al [44] and Ringmark and Jansson [45] have had success in terms of meeting energy requirements, maintaining body weights and performance characteristics by feeding high quality fibre such as haylage and alfalfa. Studies on voluntary food intake in a range of horses [46, 47] have reported that intake of steamed hay was higher than that of haylage and dry or soaked hay. Anything that encourages fit performance horses to eat forage and help them meet nutrient requirements from this portion of the diet will improve digestive health and time budgets for horses stabled for extended periods. When compiling diets for all categories of horses the effect that any processing has on the long forage nutrient content needs to be determined so that the diet can be balanced in the most appropriate way.

**5. Conclusions:**

The results of the current study show that when comparing dry, soaked, partially steamed and high temperature steaming, the most effective method for reducing airborne respirable particles, while conserving nutrients and improving the hygienic quality of hay fodder is best achieved using the Haygain specifically designed high-temperature steamers. The current studies also show that partial steaming and soaking while effective at reducing airborne respirable particles in hay are contra-indicated in terms of microbial contamination and thus either of these processes cannot be recommended as methods for producing hygienically clean fodder for stabled horses.

**Conflict of interests**

None of the authors recorded a conflict of interests.

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