### **HAYGAIN**°

### **Published Research**

Supporting the Use of Haygain Hay Steamers



#### INTRODUCTION

The ultimate goal in horse sports—and the one which generally leads to the best performance—is obtaining a horse that is, to borrow the words of the Fédération Equestre Internationale, a "happy athlete." Achieving that goal means providing our horses with optimal training, healthcare, and management conditions that meet his or her unique needs as a horse.

Proper nutrition falls at the crux of this effort. It provides the energy horses need for their training regimens; it ensures the good health of the entire horse inside and out; and it responds to the significant primordial need of this large herbivore to spend long periods of time consuming fiber. Horses must eat forage—and lots of it—for both their physical and their mental health. When grass pastures aren't available, stored forage is the solution.

Unfortunately, stored forage can also be a serious problem. In particular, dry hay—no matter how nutritious—is a menace to horses' respiratory systems. It even poses health risks to the horses' handlers working in dust-filled barns.

Studies indicate that the long-standing tradition of watering down and soaking hay does, effectively, get rid of most of the airborne dust associated with dry hay. And it leaves a wet, glistening green forage that gives us impression that we're feeding a much healthier fodder.

That's a deception, though, as recent microanalyses have shown us. **Deep down in the forage, soaked hay quickly becomes a massive, highly productive breeding ground for bacteria and mold.** Multiplying 150% within the first 10 minutes after soaking, these germs grow at alarming rates even while the horse is still eating the ration. And perhaps unsurprisingly, horses tend to turn their noses away from soaked hay, eating it only when there are no other options, and consuming it slowly while its invisible pathogens proliferate.

Meanwhile, the multiple gallons of water used for soaking pick up some of the hay's noxious micropathogens while robbing it of its nutritional qualities. The result is a poor quality, poor-tasting, germ-packed meal for your horse, and a voluminous toxic waste soup for the planet.

Evidence-based high-temperature hay steaming techniques, however, provide a powerful solution to this conundrum, allowing for healthier airways, healthier guts, and a healthier environment. Recent studies have shown that the Haygain Hay Steamer eliminates 99% of airborne particles, bacteria, and mold, while preserving the forage's nutritional qualities and natural probiotics—the "good" microorganisms that promote healthy digestion. Haygain steaming also dramatically reduces the presence of fungi in the lungs and the risk of having inflammatory airway disease compared to dry hay—which can provoke respiratory signs within a matter of days in asthmatic horses.

And horses apparently really like the taste of Haygain-steamed hay, preferring it to dry hay, soaked hay, and even haylage. As for the environment, Haygain Hay Steamers use minimal water, and the steaming process kills toxins rather than letting them seep into the waste water.

Haygain Hay Steamers are designed based on scientific evidence. They respond to a clear need in the industry to provide healthy hay that promotes optimal health, welfare, and performance. And they're produced by a company that stands strongly behind the importance of equine science in general: Haygain is at all times committed to on-going scientific studies in a variety of equine-related fields, including nutrition, inflammatory airway disease, microbiota, bedding, equine metabolic syndrome, novel allergy tests, and more.

The science is clear: if we want to do right by our horses, we can no longer just accept doing things the way we've always done them before. As our knowledge about horse health, welfare, and performance improves through scientific research, so should our practice. And that includes the way we feed them, and the quality and cleanliness of the forage we provide.

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### 1 THE EFFECT OF STEAMING AND SOAKING ON THE RESPIRABLE PARTICLE, BACTERIA, MOULD, AND NUTRIENT CONTENT IN HAY FOR HORSES.

by Moore-Colyer, M.J.S. Taylor, J. and James, R. (2016) Journal of Equine Veterinary Science. Vol 39. 62-68, ISSN 0737-0806

The Haygain Hay Steamer significantly outperforms other hay-soaking and steaming techniques in comparative testing, with a 99% drop in bacteria, mold, and airborne dust without any loss of protein or mineral content. Although soaking and home steaming techniques also reduce airborne dust, they do nothing for microbial contamination. On the contrary, unlike Haygain, these processes appear to actually incubate germs, thereby encouraging their proliferation.

**Introduction:** 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 predisposing horses to respiratory disorders.

**Method and Results:** This study examined the effect of different treatments on the airborne respirable particles (ARPs), microbial and nutrient content of hay for horses in three experiments. Experiment 1a eight bales of meadow hay were subjected to five treatments n = 40: dry (D), 10- minute soak in water (W), steamed in a wheelie bin (TWB), steamed in a Haygain (HG) 600, and steamed with a kettle of boiling water (K) on ARP content. Experiment 1b microbial contamination was measured in five bales of meadow hay after treatments D, TWB, and HG in cold conditions  $(0-7^{\circ}C)$  (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 analyzed using analysis of variance and least significant difference test: hay and treatment

as factors. Experiment 2 was analyzed using paired t- test with significance levels accepted P < .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 water- soluble carbohydrate by 18.3%.

**Conclusion:** Steaming using an 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.



#### PUNGI IN RESPIRATORY SAMPLES OF HORSES WITH INFLAMMATORY AIRWAY DISEASE.

by Dauvillier, J, ter Woort, F, van Erck-Westergren, E. (2019) J Vet Intern Med. 33: 968-975

Dry hay contains mold spores, which could be a significant culprit in the development of inflammatory airway disease (IAD). This investigative study reveals that horses with fungal elements in their airways are more than twice as likely to have IAD. Those fed Haygain-treated hay, though, are significantly less likely to have fungi in their respiratory systems and 65% less likely to have IAD. By contrast, home steaming and soaking increases the risk of airway-based fungi—and of an IAD diagnosis—making these options poor substitutes for Haygain technology in the fight against IAD.

**Introduction:** 731 horses which had been referred for a regular health check, poor performance or respiratory issues, were scoped and their case history and results recorded.

**Method:** An observational study was performed, collecting clinical data, environmental conditions, and results of a tracheal wash (TW; cytology, fungal culture, and bacterial culture), and bronchoalveolar lavage (cytology).

**Results:** The results found 84% of the horses examined were suffering from IAD and that in 72% of all horses the presence of different types of fungi in the airways was established. This ranged from Aspergillus through Penicillium to Mucor, Absidia, Geotrichum and Candida.

Risks of being diagnosed with IAD and likelihood of fungi in TW were higher when horses were bedded on straw or fed dry hay. They discovered that horses had a

3.8 times greater chance of being diagnosed with IAD if fungi were found in their airways.

An important outcome of the study was finding that after analyzing all the forage options (dry hay, soaked hay, haylage or Haygain steamed hay) steamed hay not only had the lowest risk but was the ONLY method which significantly decreased the risk of IAD.

This means feeding horses Haygain steamed hay alone could reduce the incidence of IAD by a remarkable 65%. Or, in other words, it reduced the chances of being diagnosed with IAD by an impressive 2.7 times.

In fact, this was found to be true even if hay-steaming were the only environmental management change made in the horse's care regime.

Further, the study showed that while soaking hay or feeding haylage showed a limited reduction in respirable particles, only steamed hay significantly reduced the risk of disease [by almost two-thirds].

Conclusion: IAD is highly prevalent from environmental conditions, in particular from bedding and forage. The presence of fungi was very noxious to horses' health. Feeding steamed hay lowered the risk of finding fungi in the airways and diagnosing IAD in horses.



#### **3** STEAMING OR SOAKING

by Wyss, U. and Pradervand, N. Agroscope Science. Nr 32 p32-33 (2016)

Haygain steaming dramatically reduces the presence of dust particles, mold, bacteria, and yeast—even after three days. Soaking hay, however, leads to quite the opposite effect, with germ concentrations dramatically and gradually increasing the longer the hay is soaked, and the longer the hay sits after soaking. Soaking also significantly reduces nutritional content, especially sugar, due to leaching and greater amounts of yeast, whereas Haygain treatment conserves hay's nutritional qualities.

**Introduction:** Sensitive horses are already allergic to a small amounts of dust particles and mould in hay (Meyer and Coenen 2014). Soaking hay by submersing it in water for a period of time and specially designed hay steamers are both used to treat hay fed to these sensitive horses. By soaking or dampening hay, the volatile dust particles are wetted and the inhalation of dust is reduced. To what extent the microbiological quality can be improved with steaming or soaking and the ingredients can be influenced, was examined in two different hay samples.

**Method:** Two different hay samples were used for this experiment. For steaming, the apparatus Haygain HG 1000 was used. After heating the steam generator, the hay was steamed for 50 minutes. Samples were taken from the starting material, immediately after steaming, and after the steamed hay was stored for three days, analyses were carried out. In addition, hay was soaked from the same raw material. The hay was soaked for 5 minutes, 1 hour, 6 hours and 24 hours. In addition, a further sample was taken in the hay soaked for five minutes three days later. The microbiological quality (aerobic mesophilic bacteria, mould, fungi, yeasts) as well as the dry matter (DM) content and the ingredients were investigated in the samples using the near infrared light (NIRS) method.

**Results and Discussion:** By steaming, the DM content of the hay dropped from an average of 89 to 80%. The dry matter content of the hay decreased much more strongly by watering. Depending on the duration of the soaking, the DM values were still between 32 and 17%.

After steaming, aerobic mesophilic bacteria, moulds and yeasts decreased. The values

did not increase even during the sampling period three days after steaming.

It looked different in soaking. Here the microbial and bacterial contents did not decrease significantly with a watering of 5 minutes. The microbial and bacterial contents and yeasts, increased sharply with increasing duration of soaking. In the soaked hay samples, in which the bacterial content was determined only after three days, the feed was warm during sampling. These samples showed very high bacterial counts and were considered to be highly spoiled. Steaming had little effect on the ingredients, especially the sugar content. On the other hand, the sugar content decreased as a result of the soaking. This is likely to be due to leaching and the activity of the yeasts, which have reduced a portion of the sugar.

The results of this study coincide with the results obtained from Moore-Colyer et al. (2016). Regarding the two mycotoxins zearalenone and deoxynivalenol, the two feeds had very low values before the treatment. They were clearly below the tolerated limit concentrations (DLG 2000). It should be noted that these mycotoxins are heat-stable and are not degraded by steaming, which has been confirmed by additional analyzes.

**Conclusion:** By steaming, the bacteria content can be reduced. As a result of soaking, the microbial and bacterial content increases and the sugar content decreases. Hay soaked for long periods is no longer suitable for feeding.



## **4** THE EFFECT OF FIVE DIFFERENT WETTING TREATMENTS ON THE NUTRIENT CONTENT AND MICROBIAL CONCENTRATION IN HAY FOR HORSES.

by Moore-Colyer MJS, Lumbis K, Longland A, Harris P (2014) PLOS ONE 9(11)

Horses at risk of metabolic disorders can get the starch-reduction benefits of soaked hay without the risks of increased mold and bacteria through Haygain treatment after soaking. Steamed or not, soaked hay has 34% lower water-soluble carbohydrate concentrations. But without Haygain steaming after soaking, germ quantities rise five-fold and spoil the hay. Haygain treatment immediately after (but not before) hay-soaking dramatically reduces its fungal and bacterial concentrations.

**Introduction:** Ingestion of high levels of water soluble carbohydrates (WSC) by horses from either forage or cereal based feeds increases the risk metabolic disorders such as insulin resistance, equine metabolic syndrome (EMS) polysaccharide storage myopathy (PSSM) and laminitis. Hay or forage replacers with WSC level < 100g/kg DM are best for such animals. WSC levels in UK hays are commonly between 100 and 310g/kg DM. Recent research has shown that soaking hay increases the bacterial content of the fodder and this could compromise the health of the horse (Moore- Colyer and Fillery, 2013). The aim of this study was to measure the effect of soaking, steaming and a combination of both treatments on the WSC content and microbial contamination of 5 different UK hays.

**Materials and Method:** Five different hays were used to determine the effect of 5 different soaking and steaming treatments on the WSC and microbial contents of UK hay. Hays were subjected to the following treatments: Dry (D), steamed for 50 minutes in the HG 600 steamer (S), soaked in water at 16oC for 9 hours (W),

steamed then soaked (SW) and soaked then steamed (WS). Post treatment hays were tested for WSC, bacteria and mould. Differences between means were determined using ANOVA and least significant difference with hay (5), bale (3) and treatment (5) as fixed factors, thus n = 75.

**Results:** W, SW and WS treatments were all equally effective at reducing WSC with significantly (P<0.05) lower mean contents (79-83g/kg DM) compared with the 126 and 122 g/kg DM for D and S respectively. S and WS had significantly (P<0.05) less bacteria (1046 and 490 cfu/g DM) compared with W which increased cfu/g DM from 60256 in D up to 354813. Mould contents cfu/g DM were significantly (P<0.05) reduced by S (2) and WS (1.9) but no difference was seen between D (1148), W (692) or SW (501).

**Conclusion:** Steaming increased the moisture content across all of the hays 2 fold and soaking by > 5 fold. The mean loss of WSC with S was only 3 % whereas soaking caused an average WSC loss of 34% (range 23% to 53%). Submerging hay in water caused rapid and extensive proliferation of bacteria up to 5 fold and this could compromise the health of the horse. S and WS on the other hand reduced (P).



## **5** THE HAYBIOME: CHARACTERISING THE VIABLE BACTERIAL COMMUNITY PROFILE OF FOUR DIFFERENT HAYS FOR HORSES FOLLOWING DIFFERENT PRE-FEEDING REGIMENS.

by Daniels S, Hepworth J, Moore-Colyer M (2020) PLoS ONE 15(11)

Genetic sequencing of meadow and ryegrass hay reveals that Haygain treatment kills off "bad" bacteria that cause respiratory and dental disease but maintains "good" bacteria that break down plants, benefiting horses' digestion, all the while preserving nutritional content. Soaked hay, meanwhile, increases "bad" bacteria, reduces nutritional value, and creates biohazardous waste water presumably contaminated with, among other pathogens, blue-green algae.

**Conclusion:** Collectively these findings add to a body of evidence that suggest high temperature steaming is the most suitable pre-feeding regimen of hay for equid health. Steaming reduces potential disease -causing bacteria associated with respiratory and dental problems without changing the bacterial diversity within hay.

**Introduction:** Respirable dust in conserved forage can pose problems for respiratory health, thus soaking and high temperature steaming are employed to reduce the levels in hay. This research aimed to determine the bacterial community 'haybiome' following different pre-feeding regimes.

**Methods:** Genetic sequencing was conducted on samples of 4 types of meadow and ryegrass hay after either 12 hours of soaking in water at 16°C (61 F) or one hour in a commercial steamer (HG600) which heated the hay to at least 95°C (203 F) for 10 minutes. This was compared to the same hay left dry. Dry hay contained a variety of good bacteria, respiratory and dental disease-causing bacteria and cyanobacteria (toxic) bacteria. Good bacterial populations in the dry hay which aid digestion of plant materials survived the steaming process whilst all disease causing bacteria were successfully eliminated. Soaking increased Gram-negative bacteria and reduced bacterial diversity. Overall the greatest reductions were from high temperature steaming without reducing bacterial diversity.



### **O** NUTRIENT CONTENT CHANGES FROM STEAMING OR SOAKING TIMOTHY-ALFALFA HAY: EFFECTS ON FEED PREFERENCES AND ACUTE GLYCAEMIC RESPONSE IN STANDARDBRED RACEHORSES 1.

by Owens .T. G, Barnes. M, Gargano V.M, Julien. L, Mansilla . W. D, Devries. T.J, Mcbride .B.W, Merkies. K, Shoveller. A.K. (2019)

Journal Animal Science. 3. 97.

Standardbred racehorses spend more time eating Haygain-treated hay, and eat more of it, than soaked hay, suggesting they like it more. Unlike soaked hay, Haygain-steamed hay maintains the dry hay's original levels of non-structural carbohydrates, protein, and potassium. Even so, post-meal glucose levels are no higher after eating Haygain-treated hay than what they are after eating dry or soaked hay.

**Introduction:** Soaking hay and steaming hay are strategies that are used to reduce respirable dust particles for horses but may result in variable nutrient losses, including non-structural carbohydrates (NSC) and minerals.

The aims of this study were:

- To identify nutrient losses from first-cut timothy-alfalfa hay grown in southern Ontario, Canada, after soaking for 30 min or steaming for 60 min.
- Establish whether horses prefer hay when it is dry, soaked, or steamed.
- Examine acute glycemic response in horses after being fed dry, soaked, and steamed hays.

**Method:** 13 Standardbred racehorses were offered 2 of these hays side by side for 30 min on 6 consecutive occasions until all possible combinations had been

offered. Quantity of hay eaten was determined and horses were video recorded during feedings to assess time spent eating and investigating hay.

Blood glucose was measured every 30 min from 9 Standardbred racehorses for 6 h following a meal of 0.5% of their body weight of treatment hay on a dry matter basis (DMB).

Soaked, but not steamed, hay had lower concentrations of soluble protein, NSC, and potassium in contrast to the same dry hay (P < 0.05). Peak glucose, average blood glucose, total area under the curve, and time to peak did not differ among treatments (P > 0.05).

**Conclusion:** Acute glycemic response of racehorses was not influenced by soaking or steaming hay. Horses also consumed less soaked hay than dry or steamed hay and spent less time eating soaked hay than dry or steamed hay. This further supports previous studies that found horses prefer steamed hay to soaked or dry.



### THE EFFECT OF MANAGEMENT REGIME ON AIRBORNE RESPIRABLE DUST CONCENTRATIONS IN TWO DIFFERENT TYPES OF HORSE STABLE DESIGN.

by Moore-Colyer, M J S. Auger, E.J. (2017)

Journal of Equine Veterinary Science. Vol 51. 105-109.

Managing performance horses in stables with dry hay and straw bedding is common, but it creates significant dust in stalls and walkways, especially in barns with several stalls sharing the same airspace. Reduced-dust food and bedding like haylage and wood shavings can help. Better yet, though, as this study shows, is a combination of wood shavings and Haygain-steamed hay, which provides up to a 17-fold reduction in airborne dust levels compared to multi-stall barns using dry hay and straw.

**Introduction:** It is widely acknowledged that dust, particularly respirable particles (RP) of <5 µm in size, found in the stable environment has a major negative impact on respiratory problems in horses and can cause the debilitating allergic condition known as Recurrent Airways Disorder (RAO). The level of dust within the stable environment is influenced by the management regime, namely the choice of bedding and forage and the ventilation rate. The aim of this study was to investigate the relationship between respirable dust in the breathing zone (BZ) of the horse and the general stable zone (SZ) when horses were kept on different management regimes, in either American Barns or straight-block stables.

**Methodology:** Thirty eight individual stables in 10 different American barns and 34 stables in 9 different straight stable blocks were used for data collection.

Samples of respirable dust (RD) were collected from the area close to the horse's nose (breathing zone - BZ) and the middle of the stable (stable zone -SZ) using a cyclone personal air sampler that captured RD on nitrocellulose membrane filter

papers. Particle numbers were counted using a microscope and expressed as RP per litre of air sampled.

Management regimes were either 1, shavings and steamed hay; 2, straw and haylage; 3, straw and dry hay; 4, shavings and dry hay. An average of 8 replicate stables was sampled for each regime. Stables were sampled during quiet periods in the yards. Data were analysed using a Wilcoxon Matched-Pairs test (Genstat 15) with significant differences between means set at P<0.05.

**Results:** The lowest respirable particle concentrations (< 360 RP/l air) for both stable designs across both zones were with shavings and steamed hay. Straw and dry hay produced the most amount of dust in the SZ and the BZ of 6250 and 5079 RP/l air in American Barns respectively and was significantly greater than the 2901 and 942 RP/l air measured from the straight stables. In contrast, straw and haylage produced significantly more respirable dust in both zones in straight stables compared with American barns. Shavings and dry hay produced significantly more dust in the BZ than in the SZ in both stable types, while straw and haylage produced more dust in the SZ compared with the BZ across both stable designs.

**Conclusion:** Forage and bedding have major impacts on dust concentrations in horse stabling. Shavings and steamed hay produced the lowest level of respirable dust across both zones and stable types and thus is the preferred management regime for stabled horses. Feeding dry hay or bedding on straw significantly increases the dust in both zones particularly in American Barn stables and could put horses at risk of developing respiratory disorders.



### **13** THE EFFECT OF SOAKING OR STEAMING TIMOTHY HAY ON VOLUNTARY INTAKE AND DIGESTIBILITY BY THOROUGHBREDS.

by Pagan, J.D. Whitehouse, C. Waldridge, B. M. Grev, A.M. Garling, S.W. Yates, O.L. Davis. S. James, B. (2015)

Kentucky Equine Research

Hay loses twice as much dry matter when soaked compared to when it's steamed in a Haygain device, according to this preliminary study. Thoroughbred horses might prefer steamed hay to soaked hay since they eat significantly more of it; they even eat slightly more hay when it's steamed in a Haygain device compared to when it's dry. Based on the results from three horses, Horses digest fibers, carbohydrates, fat, protein, and other kinds of dry matter in steamed hay just as well as they do in dry hay.

**Introduction:** Three mature Thoroughbred geldings were used in a 3x3 Latin square trial to compare the intake and digestibility of dry timothy hay to the same hay that was either soaked or steamed in a hay steamer.

**Method:** Each period lasted four weeks. The voluntary intake (kg/day), rate of intake (grams consumed/minute), chewing rate (number of chews/100 g) and apparent digestibility of dry, soaked and steamed mature timothy hay were investigated. The steamed hay was placed in a HAYGAIN HG-600 half-bale hay steamer.

Soaked hay was submerged for 1 hour in 18 gallons (~68L) of ambient temperature water and allowed to drain for 30 minutes in hay nets before feeding. During week 1, voluntary daily hay intake (air-dry hay equivalent) averaged 2.1% for the dry, 1.9% for the soaked and 2.2% BW/day for the steamed hay.

Steamed intake was significantly higher than soaked (p<.05). Rate of intake on an as-fed basis was significantly faster for the Soaked hay compared to the dry and steamed (p<.05). Conversely, the number of chews/100g of as-fed intake was significantly lower for the soaked compared to dry and steamed (p<.05).

The fiber components (ADF and NDF) of the soaked and steamed hays were greater than the dry hay (100% DM basis). This was due to a loss of other components from the soaked and steamed hays. If these compositions were recalculated assuming that the NDF content of the hays were equal then about 9% of the original DM of the soaked hay and 4% of the steamed hay dry matter were lost as a result of soaking and steaming. Much of the dry matter loss was from water-soluble carbohydrates (WSC).

**Conclusion:** Soaking or steaming reduced the WSC content of timothy hay, but it did not affect the digestibility of dry matter (DM), crude protein (CP), acid detergent fiber (ADF), neutral detergent fiber (NDF), crude fat, ash, non-structural carbohydrate (NSC) or residual WSC.

Steaming increased the free-choice intake of hay compared to soaking, but rate of intake and the amount of chewing was not affected when expressed on a DM basis.



### 2 PALATABILITY AND INGESTION BEHAVIOUR OF 6 POLO PONIES OFFERED A CHOICE OF DRY, SOAKED AND STEAMED HAY FOR 1 HOUR ON THREE SEPARATE OCCASIONS.

by Moore-Colyer, M.J.S. and Payne, V.

Advances in Animal Biosciences. Healthy Food from Healthy Animals. Vol 3 part 1. 127 (2012)

Horses consistently show a strong preference for Haygain-steamed hay compared to dry or especially soaked hay when given the choice of all three in small piles. They eat the steamed hay first, even if they nibble a little at the dry hay, and tend to finish up their ration of Haygain-treated hay before moving on to the dry hay. The rations of soaked hay, meanwhile, always rank last.

**Introduction:** Steaming has been shown to be an effective alternative to soaking for reducing respirable particles in fodder (Stockdale and Moore-Colyer, 2010). However, to date no information is available on the palatability of steamed vs soaked vs dry hay. The current trial sought to determine the palatability of dry, steamed and soaked hay when offered to 6 Polo ponies for 1 hour, previously fed haylage in a repeated measures design experiment.

**Method:** Replicate bales of hay were taken from first cut Timothy and Meadow Fescue hay which had been barn-stored for 6 months. Bales were divided into 2. One half was steamed in the HG 600 while 2 x 1 kg hay nets were taken from the other half for the dry and soaked treatments. Six Polo ponies were offered 1 kg each of the dry, soaked and steamed hay simultaneously. The hay was placed on the floor in 3 different corners of a rubber-matted stable, where water was available ad libitum. In order to eliminate positional preferences, the experiment was repeated 3 times for each horse with the position of each of the hays being rotated between the 3 corners of the stable. Data was subjected to a repeated

measures analysis of variance (Genstat 12). Observations were recorded on the first choice of forage eaten for a consecutive 5 minutes.

**Results:** Table 1. Average amount of forage consumed in kg (on 95% DM basis) when offered to 6 polo ponies on 3 separate occasions.

	STEAMED	SOAKED	DRY	S.E.D	SIG
Kg of Hay consumed	0.867c	0.050a	0.183b	0.0246	***

abc Values in the same row not sharing common superscripts differ significantly (P<0.001)

**Conclusions:** The results from this experiment clearly demonstrate that horses preferred to consume steamed hay to dry or soaked hay when offered free-choice in a stable environment. Observations of choice of feed revealed that steamed hay once tasted was always the first consumed. Some horses did nibble some dry hay, but quickly returned to the steamed hay until it was all consumed whereupon they then chose to eat the dry hay.



### **OUDE** COMPARISON OF AIRWAY RESPONSE OF RECURRENT AIRWAY OBSTRUCTION AFFECTED HORSES FED STEAMED VERSUS NON-STEAMED HAY.

by Blumerich, C.A., Buechner-Maxwell, V.A., Scrratt, W.K., Wilson, K.E., Ricco, C., Becvarova, I., Hodgson, J. Were, S. (2012)

Journal Equine Veterinary Medicine. 26. 744-744.

When horses already have recurrent airway obstruction (RAO), feeding them Haygain-steamed hay can make them less likely to have labored breathing and other manifestations of their disease. Testing the same horses on different feeding regimens shows that even within only 10 days, RAO-affected horses can develop significant clinical signs with dry hay that are essentially non-existent when they're fed Haygain-treated hay instead.

**Introduction:** Recurrent Airway Obstruction (RAO)-affected horses experience bronchoconstriction and airway inflammation in response to inhalation of aerosolized irritants including hay molds. Steaming hay reduces fungal content, but the effect on the antigenic potential of hay has not been investigated. The aims of this experiment were to test the hypothesis that RAO-affected horses develop less clinical disease when fed steamed versus non-steamed hay and this reduction coincides with decreased hay fungal content.

**Method:** Six RAO-affected horses in clinical remission were divided into two groups and fed ad libitum steamed or non-steamed alfalfa hay for 10 days using a two-way cross-over design. All horses had ad libitum access to water and a mineral lick throughout the duration of the trial. Hay was steamed using the HG 1000 (Haygain Ltd). Clinical assessment was performed daily. Full assessment performed on days 1, 5 and 10, included upper airway endoscopy, assignment

of mucous scores and measurement of maximal change in pleural pressure. Bronchial fluid sampling and cytology were performed on days 1 and 10. Hay core samples were collected pre- and post-steaming and cultured to determine fungal and bacterial concentrations. Differences between treatments were determined using repeated measures ANOVA, mixed model ANOVA, Wilcoxon rank-sum and Wilcoxon two sample tests.

**Results:** Steaming significantly decreased the number of fungi colony forming units in hay. Horses fed non- steamed hay experienced a significant increase in clinical score (p<0.0001) and a trend towards total airway neutrophilia (p=.0834) during the feeding period, while parameters were unchanged in horses fed steamed hay.

**Conclusions:** These results indicate that steaming reduces the RAO-affected horse's response to hay which coincides with a reduction in viable fungal content of hav.



## THE EFFECT OF THREE DIFFERENT TREATMENTS ON THE RESPIRABLE PARTICLE CONTENT, TOTAL VIABLE COUNT AND MOULD CONCENTRATIONS IN HAY FOR HORSES.

by Moore-Colyer, M.J.S and Fillery, B.G. (2012)

6th European Workshop for Equine Nutrition, Lisbon, Portugal, June. 101-106.

Soaking hay effectively reduces airborne dust particles, but it increases bacterial concentrations by 150% within only 10 minutes. In this study with Lolium perenne hay, portions steamed with a Haygain device have about a third of the bacterial content of dry hay and a tenth of its mold content, and airborne dust particles are practically eliminated.

**Introduction:** Many horse owners soak hay to reduce airborne particles, but soaking is laborious, leaches nutrients (Moore-Colyer, 1996) and the resulting post-soak liquor is an environmental pollutant (Warr and Petch, 1992). In contrast, steaming hay in the HG 1000 has been shown by Stockdale and Moore-Colyer (2010) to reduce respirable particle numbers by 91% and fungal colony numbers by 98% (James and Moore-Colyer, 2010). However, the HG 1000 is too big for many owners and accordingly Propress Equine Ltd has produced the HG 600, a smaller version which steams half a bale and works on the same principles as the HG 1000. This study sought to compare the efficacy of the smaller steaming chamber in the HG 600, at reducing respirable particle numbers, bacteria, and mould concentrations in five bales of poor- quality perennial rye grass (Lolium perenne) hay and to compare steaming with dry hay and hay soaked in water for 10 minutes.

**Method:** Five replicate bales of barn-stored Lolium perenne hay made in Hungerford, June 2011, were treated as follows: Half was steamed for 40 minutes in HG 600. Two 4-6kg hay nets were weighed from the other half. One net was soaked in tap water (150C) for 10 minutes then drained for 10 minutes. Post treatment 1g from dry,

soaked and steamed hay were weighed into separate stomacher bags with 79 ml of maximum recovery solution and processed for 2 minutes. Sequential dilutions were prepared down to 10-4. Two x 1 ml from each were placed onto 2 x 3 M TM petrifilms, (3M Microbiology, St Paul, MN 55144-1000), and incubated for 3-5 days at 20oC (mould films) and 320C (bacteria), before counting using a standard colony counter. RP were determined according to the method of Moore-Colyer, (1996). Differences between treatments were determined using ANOVA on log transformed data.

	DRY	WET	STEAMED	S.E.D	SIG
RP (l air/kgDM)	1327a	0b	1.47b	1.719	P<0.001
TVC/g	21,877616a	37,153,522a	83,176b	2.55	P<0.001
Mould/g	1,174,897a	316,227a	1,072b	1.97	P<0.001

**Results:** Table 1. Geometric mean numbers of respirable particle (RP), total bacterial colonies (TVC) and mould colonies (Mould) from 5 bales of hay steamed for 40 minutes in the HG 600 (steamed), soaked for 10 minutes in water (wet) and dry hay (dry).

abc Values in the same row not sharing common superscripts differ significantly (P<0.001)

**Conclusions:** Steaming in the HG 600 was the most effective treatment for reducing RP, mould and TVC concentrations. Soaking reduced RP but increased TVC and thus cannot be recommended as a treatment for improving the hygienic quality of hay.



### THE EFFECT OF STEAM TREATMENT ON THE BACTERIA YEAST AND MOULD CONCENTRATIONS IN HAYLAGE FOR HORSES.

by Leggatt, P.Moore-Colyer, M.J.S (2013)

Proceedings of British Society of Animal Science Conference. Nottingham April. 103

Haylage is touted as a low-dust, low-allergen forage, but it has to be consumed quickly because it harbors rapidly growing bacteria and mold, which becomes 70% more abundant within only four days. Haygain-treated haylage, however, is so poor in pathogens that, even four days later, it's still 99% lower in bacterial content and 70% lower in mold content than freshly opened haylage.

Introduction: An increasing number of horse owners choose to feed haylage to their stabled horses. Good quality haylage if well conserved has a high nutrient value and low dust content and can make excellent long forage for performance horses. However, as conservation of haylage is more dependent on air-tight storage than lactic acid fermentation (Muller, 2005), once opened it must be used within 5 days. Exposure to air causes rapid bacterial and fungal growth rendering the forage unpalatable and potentially hazardous to feed. Previous work has shown that steaming hay in the Haygain range of steamers has reduced bacteria and fungi concentrations by >95% (James and Moore-Colyer, 2010; Moore-Colyer and Fillery, 2012) and so this study sought to determine if similar reductions in microbial concentrations could be achieved when steaming haylage.

**Method:** Five bales of commercially produced Lolium perenne haylage were randomly selected from a farm in Gloucestershire. Each bale was divided into 4 equal portions. Portion 1 was tested immediately, portion 2 left for 4 days then tested. Portion 3 and 4 were steamed in the HG 600 (Haygain Ltd). Portion 3 was tested immediately post steaming and portion 4 was left for 4 days before testing.

All 4 portions underwent the following procedure. One gram was weighed into separate stomacher bags with 79 ml of maximum recovery solution and processed for 2 minutes. Sequential dilutions were prepared down to 10-4. Two x 1 ml from each were placed onto 2 x 3 M TM petrifilms, (3M Microbiology, St Paul, MN 55144-1000), and incubated for 3-5 days at 20oC (mould films) and 2-3 days at 320C (bacteria), before counting using a standard colony counter. Differences between treatments were determined using ANOVA on log transformed data.

**Results:** Total bacterial counts (TVC) and fungi in fresh haylage, haylage opened for 4 days, freshly steamed haylage and steamed haylage left open for 4 days abc Values in the same row not sharing letters differ significantly (P<0.05)

CFU	FRESH	FRESH +4 DAYS	STEAMED	STEAMED +4 DAYS	S.E.D
Fungi/g	420	2786	12	128	
Log fungi	2.48c	3.38d	0.45a	1.58b	0.304
TVC/g	41,600	114,000	10	304	
Log TVC	4.556c	5.048c	0.823a	2.092b	0.2701

**Conclusion:** TVC and mould concentrations increase by 64 and 75% respectively in haylage opened for 4 days. Steaming significantly reduced microbial growth and this reduction was 10 maintained (99 and 70% lower respectively than freshly opened haylage) after 4 days aerobic exposure.





## **13** STEAMING HAY FOR HORSES: THE EFFECT OF THREE DIFFERENT TREATMENTS ON THE RESPIRABLE PARTICLE NUMBERS IN HAY TREATED IN THE HAYGAIN STEAMER.

by Stockdale, C. Moore-Colyer, M.J.S (2010)

European Workshop for Equine Nutrition, Cirencester, Sept 2010. The Impact of nutrition on the health and welfare of horses. EAAP publication No. 128. Ed Ellis, A., Longland, A.C., Coenen, M and Miraglia, N. p136-138.

Whether hay is very dusty or only slightly dusty, Haygain steaming drops airborne particle concentrations by an average of 94% in this study of four different hay sources. And even a day later, after the hay has had the chance to dry completely, airborne particle concentrations still remain 80% lower than the pre-steamed dry hay.

**Introduction:** It is widely known that even good quality hay contains a high number of respirable particles (those < 5  $\mu$ m in diameter) and that these particles can initiate the debilitating condition Recurrent Airways Obstruction (RAO) that causes respiratory distress, coughing and nasal discharge. This study sought to determine the efficacy of the HG 1000 steamer at reducing respirable particle numbers in 4 different hays, representing typical hay fed to horses across the UK.

**Method:** Two replicate bales of hay from four different sources (n= 8) were subjected to 3 treatments: dry, 50 minutes steam in the HG 1000 + shaken immediately, and 50 minutes steam and left to dry for 24 hours before shaking. Respirable particle (RP) numbers were measured from a 5 kg sample by shaking vigorously under a cyclone air sampler for 3 minutes. Differences in respirable particle numbers were determined using analysis of variance and lsd test = t (error df) x s.e.d.

**Results:** Table 1. Mean Respirable particle numbers (/kg hay / litre of air) from 2 replicates of four different dry hays.

	HAY 1	HAY 2	HAY 3	HAY 4	S.E.D	SIG
RP Numbers	51254c	28506b	12327a	10711a	3897,3	0.005

abc Values in the same row not sharing common superscripts differ significantly (P<0.05). Table 1 shows that significant differences (P<0.05) exist between respirable particle numbers in hays from different areas of the UK. These differences are most likely due to weather conditions during conservation and the dry matter of the hay when stored.

Table 2. Respirable particle numbers (/kg hay/litre of air) detected in dry, steamed and steamed +24 hours in 4 different hay.

	DRY HAY	STEAMED	STEAMED + 24 hours	S.E.D	SIG
RP Numbers	25699a	1586b	5398b	1937.5	0.001

Table 2 demonstrates that steaming a wide range of hays for 50 minutes in the HG 1000 significantly (P<0.001) reduced the respirable particle numbers by 94% compared with dry hay. Moreover steaming the hay and leaving it to rest for 24 hours did not significantly increase the respirable challenge indicating that even after being left for 24 hours steamed hay reduced the respirable challenge to the horse by 79%.

**Conclusions:** Steaming in the HG 1000 was an effective method for reducing respirable particles numbers in all hays, whether only slightly dusty (hay 4) or highly contaminated (hay 1).





### **4** AN INVESTIGATION TO DETERMINE THE PALATABILITY OF STEAMED HAY, DRY HAY AND HAYLAGE.

by Brown, E., Tracey, S and Gowers, I. (2013)

Proceedings of British Society of Animal Science Conference, Nottingham April 2013. p 104

Given the choice, horses prefer to eat Haygain-steamed hay over dry hay or even haylage, which is thought to be an equine favorite. According to trends set by the seven riding horses in this study, horses eat Haygain-treated hay first—and eat more of it in the first hour—compared to the two other forage options in their stall.

**Introduction:** In recent years, hay has been replaced by haylage in the diets of stabled horses. Haylage is reported to be highly palatable but can be too high in nutrients for some horses. Hay is more suitable fodder for many horses but can be dusty and induce respiratory disorders. Steamed hay is dust free and has been shown by Payne and Moore-Colyer (2011) to be more palatable than dry and soaked hay. The aim of this study was to determine which fodder, dry hay, steamed hay or haylage was the preferred fodder for stabled horses.

**Method:** Seven horses stabled at Writtle College Lordships Stud were simultaneously offered 3 hay nets containing either dry hay, haylage or hay that had been steamed for 50 minutes in the HG 600 steamer. Intake was determined by the weight difference in forage, before and after the one hour feeding period. Observations were taken for the first five minutes of each period to record the first, second and third choices of forage. Analysis of variance (ANOVA) followed by a Bonferroni post hoc test were used to determine differences in intake between the 3 forages and Chi squared test to determine differences in first choice of forage.

**Results:** The overall trial results demonstrated a significantly greater (P=0.003) consumption of no significant difference between steamed hay and dry hay or between haylage and dry hay. The Chi-squared goodness of fit test showed steamed hay to be chosen most often as the first forage to be consumed, followed by haylage and then dry hay (P=0.003).

**Conclusion:** The results of this experiment demonstrate that steamed hay was the first fodder chosen by horses and they went on to consumed more steamed hay than either haylage or dry hay within the 1 hour feeding period. Steamed hay is therefore a hygienically clean and palatable fodder for stabled horses.



## **DEVELOPMENT OF A COMPREHENSIVE PROTEIN MICROARRAY** FOR IMMUNOGLOBULIN E PROFILING IN HORSES WITH SEVERE ASTHMA.

by White S, Moore-Colyer M, Marti E, Coüetil L, Hannant D, Richard EA, Alcocer M. (2019)

Journal Veterinary Internal Medicine. 33. (5)

A new, affordable, comprehensive allergen test could make it easier for owners to find out what pathogens, in particular, are setting off their horses' severe equine asthma (SEA). In this promising protein microarray study, co-funded by Haygain, scientists identify various bacteria, mold, mites, and even pollen as sources of horses' asthma attacks.

**Introduction:** Severe equine asthma (sEA) is a prevalent, performance-limiting disease affecting 14% of horses in the Northern Hemisphere; associated with increased allergen-specific immunoglobulin E (IgE) against a range of environmental allergens. This study looked to develop a protein microarray platform to profile IgE against a range of proven and novel environmental proteins in sEA-affected horses.

**Method:** Six sEA-affected and six clinically healthy warmblood performance horses were used. A protein microarray was developed using protein extracts and purified proteins from many families including pollen, bacteria, fungi and arthropods associated with the equine environment.

**Conclusion:** This method identified a number of novel allergens, while also revealing an association between sEA and pollen sensitization. Protein microarrays can be used for large scale IgE mapping of allergens associated with the equine environment and provides a sound platform for specific diagnosis, management and treatment of sEA.



### 4 ANTIGEN ARRAY FOR SEROLOGICAL DIAGNOSIS AND NOVEL ALLERGEN IDENTIFICATION IN SEVERE EQUINE ASTHMA.

by White, S.J., Moore-Colyer, M., Marti, E. Hannant, D. Gerber, V. Couetil, L. Richard, E.A. Alcocer. M. (2019)
Scientific Reports. 9.

A new microarray technique detects asthmatic horses' sensitivity to up to 400 allergens through a single, inexpensive, and welfare-friendly test requiring just a few drops of blood. In this early field study, co-funded by Haygain, scientists reveal that horses can be allergic to bacteria, mold, mites, pollen, and—in a novel discovery—latex, a common arena footing material.

**Introduction:** It is common knowledge that the inhalation of stable 'dust' in predisposed horses results in SEA. This 'dust' contains bacteria, fungi, pollen and arthropods. This research aimed to establish the precise allergen/s that elicit this prevalent and debilitating condition.

**Materials and Methods:** Utilising advances in computational and robotic technologies, White developed a novel method which enabled the simultaneous assessment of almost 400 potential allergen in over 130 SEA-affected and healthy horses. This was the widest-scale allergen assessment in SEA horses to date and revealed many similarities with human allergic asthma. The results confirmed allergen previously identified in hay (e.g. Aspergillus fumigatus), as well as revealing a plethora of unidentified bacteria, fungi and arthropod.

**Conclusion:** The most significant and surprising allergens associated with SEA were from natural rubber latex. Latex has been traditionally ubiquitous to the horses environment In the form of riding surfaces and race tracks. The high level of respirable dust associated with training on these surfaces has already been linked with chronic bronchitis, inflammation and oxidative stress in riding instructors, and latex has long been associated with a variety of respiratory conditions in the human. These results indicate that exposure to latex may be detrimental to the respiratory health of the horse.

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