

# **The effect of steam treatment on the total viable count, mould and yeast numbers in hay using the Haygain hay steamer.**

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## **Abstract**

Five bales of English rye grass mix meadow small baled hay were steamed in the Haygain hay steamer using its unique method to introduce steam through a manifold system for 50 minutes. Microbiology test results showed that steaming in this way was very effective at reducing total viable count of bacteria, 99.08% and 98.84%, respectively and 100% reduction in fungi and yeast of the hay. This drastic improvement in the hygiene quality of whole bales of hay makes it a safe and suitable fodder for all types of horses.

Keywords: hay, soaking hay, steaming hay, hay steamer, stable environment, dust, respirable particles, fungal spores, *Aspergillus*, respiratory disorders, RAO, IAD.

## **Introduction**

Domestication of the horse has resulted in the combination of eating conserved forage in a stable environment, which has subjected the horse to dust (bacteria, fungal spores, plant particles and insect fragments) which can produce respiratory disorders (Clark, 1992). It is widely regarded that even good quality hay contains mould and fungal spores along with bacteria and other organic dust and this is due to the nature in which hay is made in the field. Moulds such as *Aspergillus fumigatus* are linked to the development of conditions such as Recurrent Airway Obstruction (RAO) formerly known as COPD in horses (McGorum *et al* 1993) and Farmer's Lung in humans (Kotimaa *et al* 1991).

The incidence and significance of the resultant respiratory disorders is notably high for all types of horses. It was reported that 1 in 6 horses in the UK are diagnosed with Recurrent Airway Obstruction and 80% of horses stabled part of the time suffer from some degree of airway inflammation (Horse and Hound, 2006). It is also a particularly large problem in the racing industry, an epidemiology study by Rossdale *et al* (1985) found respiratory problems in race horses to be the second highest reason for lost training days after lameness. In concurrence with

this finding, it is also reported that up to 80% of race horses are affected at some point in the first years of training in the UK (Wood *et al* 1999) and Australia (Christly *et al* 2001). In addition a study investigating the occurrence of IAD in Japanese Thoroughbred race horses found 73.3% of horses who presented with a cough or poor performance were diagnosed with IAD.

While dust extracted shavings and cardboard can be used to minimise dust from bedding, it is more difficult to reduce the dust levels in fodder without negatively affecting the nutrient quality of the feed. Hay is an ideal conserved forage to feed stabled horses, however, hay contains significant levels of respirable mould and fungal spores especially when made in less than ideal conditions due to climatic limitations. In fact, hay has the potential to contain the highest concentration of all sources in the stable environment (Webster *et al* 1987; Raymond *et al* 1994; Robinson *et al* 2001).

Many owners soak hay to reduce airborne particles, but soaking hay nets leaches nutrients (Moore-Colyer, 1996) is laborious and heavy to handle and results in post-soak liquor that has a very high Biological Oxygen Demand (BOD) (War and Petch, 1992). Art *et al* (2002) suggested that soaking bales of hay is only efficient when done for several hours and with the strings cut, thus permitting water penetration to the centre of the bale. This is neither suitable nor practical for larger establishments or racing yards.

Steaming hay has previously been shown by Blackman and Moore-Colyer (1998) to have none of the above disadvantages and is very effective at reducing the airborne respirable particles. Horse owners have attempted to steam with home-made steamers using plastic containers with boilers, and the equivalent is also currently sold in the UK as hay steamers. However, there has been no published data on their efficacy and they are thus not widely used by horse owners. In addition this method is only capable of steaming loose-hay or hay-nets which is not practical or economical for the larger yards.

The objective of the current study was to test how well the Propress Equine Haygain hay steamer reduced bacteria, mould and yeast numbers when complete bales of hay were steamed for 50 minutes.

## Materials and Methods

The Haygain hay steamer was made up of two components, firstly a steam generator; which heated the water to boiling point to produce steam. The steam escaped from the heating vessel through the elbow, specifically designed to create a small amount of back pressure (75-100mb). The steam travelled down a multi layer construction hose with braided reinforcing and due to the back-pressure, distributed the steam evenly through the manifold system. The second component, the insulated chest contained the steam distribution manifold inside, referred to as the hay chest, which infused the steam into the hay shown by illustration 1.

### Illustration 1



### Operation of the Haygain hay steamer

A fully strung bale was placed onto the steam manifolds inside the hay chest and pushed down evenly and firmly so the steam lances pierced the hay to their full length. The lid containing a thermometer which read the atmospheric temperature inside the hay chest was secured.

The filler cap on the steam generator was then removed and filled to the maximum level as directed by the side-glass (approximately seven litres) with clean water, plugged into an electrical supply and switched on. The hay steamer was then left to steam the hay for 50 minutes.

## **Methodology**

Five bales of good quality rye grass mix meadow hay were randomly selected from hay purchased from a 'horse hay' merchant in Gloucestershire. Each bale was subjected to the following treatment. Dry samples were taken from five areas of the bale using large tongs and temporarily stored in a sterile glass beaker. The bale was then placed onto the spiked manifold in the steamer and the boiler (containing approx 7 litres of water) turned on. The bale was left in the steamer for 50 minutes, where upon the boiler was switched off and the lid was carefully removed. Tongs were then used to take samples from another five areas of the bale and also placed into a sterile glass beaker. The dry and steamed samples were then separately prepared using the following procedure.

1 g hay was roughly chopped with sterilized scissors into a stomacher bag and 79 ml of maximum recovery diluent (MRD) added. The mixture was then 'stomached' for 2 minutes. 1ml of the sample was then taken and put into 9ml of MRD in a sterile screw-top tube. Sequential dilutions were then prepared down to  $10^{-4}$ . 1 ml was taken from each of  $10^{-1}$ ,  $10^{-2}$ ,  $10^{-3}$ ,  $10^{-4}$  dilutions and placed onto 3M<sup>TM</sup> petrifilms for Total Viable Count (TVC), mould and yeast, two replicates were prepared for each dilution. The 3M<sup>TM</sup> Petrifilm<sup>TM</sup> Aerobic Count (AC) Plate, a sample-ready-culture-medium system which contains nutrients, a cold-water-soluble gelling agent, and a tetrazolium indicator that facilitates colony enumeration of aerobic bacteria was used for TVC. The dilutions were plated out, the top film was lifted and 1ml of the diluent was dispensed using a Volac high precision Ultra micropipette, the top film was dropped onto the sample and then the plastic spreader was used to distribute the sample evenly over the entire Petrifilm plate growth area before the gel was formed. These were then incubated at 33°C for 3 days. The 3M<sup>TM</sup> Petrifilm Yeast and Mold (YM) count plate, a sample-ready culture medium system which contains nutrients supplemented with antibiotics, a cold-water-soluble gelling agent and an indicator that facilitates yeast and mould enumeration was used to determine yeast

and mould counts using the same preparation as described for TVC. The mould and yeast Petrifilms were then incubated at 20°C for 5 days.

## **Data Analysis**

Colony numbers were counted following the 3M™ interpretation guide and using a standard colony counter, an illuminated magnifier. For TVC all red colonies regardless of size or intensity were counted. The circular growth area was approximately 20cm<sup>2</sup>, on plates that contained more than 300 colonies, three representative squares were counted and used to determine the average number per square. The average was then multiplied by 20 to determine the estimated count per plate. High concentrations of colonies on the Petrifilm are known to cause the entire growth area to become red or pink. These are recorded as too numerous to count (TNTC) and the higher dilutions were used for actual counts.

Mould and yeast counts were also determined using a standard colony counter and the 3M™ interpretation guide was used to distinguish between the yeast and mould based on their characteristics:

Yeast colonies were small, had defined edges, were pink-tan to blue-green in colour, appeared raised and had a uniform colour. The Mould colonies were large with diffuse edges, they were variable in colour, appeared flat and had a dark centre. The circular growth area was approximately 30cm<sup>2</sup>, on plates that contained more than 150 colonies, representative squares were used to determine the estimated count per plate by multiplying the average number by 30. High numbers of yeast colonies are known to cause the entire area to turn blue and mould to turn the area black or yellow, this was recorded as TNTC and the higher dilutions were used to determine actual counts.

All counts were recorded into an excel spreadsheet and for every bale, each dilution and repetition was recorded alongside each treatment (dry or steamed). Differences between dry and steamed hay were determined using the non-parametric Wilcoxon signed rank test.

## **Results**

Table 1 below shows the microbiological test results of dry and hay steamed in the production model of the Haygain hay steamer for 50 minutes.

**Table 1** Total colony forming units (CFU/g) from dry hay and hay steamed in the HAYGAIN hay steamer production model (HG1000) for 50 minutes.

| <b>Parameter</b> | <b>Dry</b>           | <b>Steamed</b> | <b>Probability</b> |
|------------------|----------------------|----------------|--------------------|
| TVC              | 381573               | 4453           | 0.008              |
| Fungi            | 1.85x10 <sup>8</sup> | 0              | 0.008              |
| Yeast            | 6893333              | 0              | 0.008              |

## **Discussion**

The results of this experiment clearly show that steaming for 50 minutes in the Haygain hay steamer produced hay devoid of fungi or yeast (100% reduction) and a 98.84% reduction in bacterial contamination. Steamed bales were fed to horses within 12 hours of being steamed and the palatability of the hay was described as very good.

## **Conclusions**

Steaming hay in the Haygain hay steamer drastically improved the hygiene quality of whole bales of hay. The microbial contamination was reduced to zero for fungi and yeasts and by 98.84% for bacteria. Minimising exposure to these respirable particles is hugely beneficial to the health of the horse and in particular the respiratory system. Pathogenic challenge to both the respiratory and digestive systems is therefore all but eliminated making the steamed hay an excellent feed for all performance horses.

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