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Original Research

# 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 behaviors. Forage can contain high levels of respirable dust predisposing horses to respiratory disorders. 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°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%. 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.



## 1. Introduction

Grass hay is the most common fodder fed to stabled horses in the UK [1] and United States [2]. The nutrient content of both seed hay (monospecies) and meadow hay (multispecies) is mainly determined by the grass mixture and stage of growth at harvest, whereas 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 fiber 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 5 μm in size and is referred by Hessel et al [6] as the thoracic fraction and by Art et al [7] and

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Clements and Pirie [8] as particles that are sufficiently small (0.5–5 μm) to penetrate the peripheral airways. Respirable dust contains potentially allergenic particles, such as mould and bacteria spores, mite feces, endotoxins, and beta glucans, all of which have the potential to contribute to the etiopathogenesis of equine environmental airway diseases including recurrent airway obstruction (RAO) in stabled horses [9,10]. Although 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 [11–13]. Woods et al [14] and Moore-Colyer and Auger [15] demonstrated that during feeding, dust in the breathing zone of the horse can be significantly higher than in the general stable environment thus minimizing 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 (ARPs) but has undesirable consequences as it leaches valuable minerals [17,18] and water-soluble carbohydrates (WSCs) [19] from the hay, increases bacterial concentrations by 1.5-fold to fivefold [20,21], and can produce postsoak 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 dustbin 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 dustbin 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, whereas 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 five different wetting treatments. Each bale was divided into five equal sections of approximately 5 kg and placed into small-holed (50 mm) hay nets. Before the steaming treatment took place, three wooden rulers containing nonreversible 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 L of clean tap water for 10 minutes at 16°C (W); (3) steamed using a Haygain 600 steamer (Properss Equine Ltd, Hungerford, UK) (HG); (4) steamed in a homemade steamer which consisted of a standard domestic 240-L 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 wheelie bin (TWB); and (5) steamed by pouring a kettle of boiling water over the hay in a bag (K). After treatment, the ARP 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 meter above the floor and switched on for 3 minutes. Hay samples were manually shaken with a fork for 3 minutes. Airborne respirable particles were captured on nitrocellulose membrane filter papers. After 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]. Airborne respirable particle numbers per liter of air from 1-kg hay were calculated before being subjected to data analyses.

#### 2.1.2. Data Analyses

Differences between treatments for this randomized block experiment were determined using one-way analysis of variance (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 $LSD = t_{(\text{error df})} \times SED$. Because of the skewing of the data, ANOVA was performed on log 10 transformed data [26,27] as per the accepted procedure for right-handed skewed data [21,28]. Results were expressed as geometric mean particle numbers/liter 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 three different treatments. Each bale was subdivided into three equal sections of approximately 8 kg each and placed into small-holed (5 cm diameter) hay nets. Treatment 1 was dry (D); treatment 2 hay was steamed in HG 600; 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., 0–7°C). After treatment total bacteria, as measured by total viable bacterial count (TVC) and mould (colony forming unit [CFU]/g) were determined by culturing techniques as detailed by Moore-Colyer et al [21].

#### 2.2.2. Culturing Technique

After treatment, 8 kg of hay was emptied onto a clean cotton sheet in a clean classroom and thoroughly mixed by hand. A 100-g subsample was taken and roughly chopped with scissors (previously wiped with ethanol and allowed to dry) and mixed again after chopping. A 1-g subsample was then weighed into a sterile plastic bag (Seward BA6040) to which 79 mL of peptone saline solution known as maximum recovery dilutant 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 to wash mould and bacteria from the hay into the solution as per instruction manual for 3M petrifilms (3M Microbiology, 2013). One milliliter of the blended solution was placed into a sterile screw-cap tube (VWR, UK) preloaded with 9 mL of maximum recovery dilutant. Serial dilutions were prepared to $10^{-4}$. A 1-mL sample was then taken from $10^{-2}$ and $10^{-4}$ dilutions and separately placed onto prelabelled 3M Aerobic TVC 20 $cm^2$ petrifilm and 3M yeast and mould 30 $cm^2$ petrifilm (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 (two TVC and two yeast and mould) were prepared for each sample. Total viable bacterial concentration 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 after the 3M 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 centers. 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 one-way ANOVA with treatment (3) and replicate (5) thus n = 15. Differences between means were calculated using LSD test where LSD = $t_{(\text{error df})} \times$ SED. Skewed data were subjected to log 10 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, that is, dry (treatment 1) and after steaming in a HG 1000 (Propress Equine Ltd, Hungerford, UK) (treatment 2) thus n = 60. Samples (approximately 500 g) were compiled from intact bales using long (300 mm) tweezers from five different areas and depth of the bale and placed into prelabelled plastic bags. The bale was then steamed in the HG 1000 for 50 minutes. After steaming, the bale was removed from the steamer and another 500-g composite sample was taken in the same manner from five different areas of the bale and placed into a separate prelabelled plastic bag. Samples were immediately stored at −20°C before being dried in a force-draught oven at 60°C and milled in preparation for nutrient analyses.

#### 2.3.2. Nutrient Analyses

Water-soluble carbohydrates were measured by the phenol-sulfuric acid method [29]. Nitrogen was analyzed by a rapid combustion method using a LECO FP-428 analyzer (LECO Corp., St. Joseph, MI). Trace elements and extractable P were 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% vol/vol 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 analyzed by a rapid combustion method using a LECO FP-428 analyzer (LECO Corp., St. Joseph, MI) and crude protein (CP) calculated by N × 6.25.

#### 2.3.3. Data Analyses

Differences between dry and steamed samples were determined by paired *t*-test using Genstat 15 [26] with $P < .05$ 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 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%.

**Table 1**
Geometric mean respirable particle (RP) numbers/liter of air/kg hay (±SD) in dry hay (D), hay soaked for 10 minutes in water (W), steaming in a Haygain (HG) 600 steamer, steamed in a homemade steamer (TWB), and steamed by pouring a kettle of boiling water over the hay in a bag (K).

| Particles | D | W | HG | TWB | K | SED |
|---|---|---|---|---|---|---|
| RP/L air/kg hay | 1180$^{c}$ (±1.35) | 2.8$^{a}$ (±2.52) | 4.0$^{a}$ (±4.01) | 63$^{b}$ (±5.18) | 142$^{b}$ (±2.19) | 1.81 |

Abbreviations: SD, standard deviation; SED, standard error of difference.
$^{abc}$Values in the same row not sharing common superscripts differ significantly ($P < .001$).

### 3.2. Experiment 1b

Mould and bacterial concentrations (TVCs) expressed as geometric mean CFU/g hay (Table 2) were reduced by 99% ($P < .001$) 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 three nonreversible temperature strips per replicate averaged 102 ± 4.7°C across the five 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 the hay. The results from the temperature strips showed an average temperature of 58 ± 45°C 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 minutes steam in the HG 1000 are shown in Table 3. There were no reductions in nutrient content between dry and steamed hay for 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 dry matter (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 CP contents while being the most effective method for reducing ARP and viable microbial numbers.

### 4.1. ARP 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 was 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 99°C. 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 that is 0–7°C as was the case in this experiment.

On the other hand, steaming using the homemade 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 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 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 46°C 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

**Table 2**
Geometric mean colony forming units (CFUs) per gram dry matter (±SD) of hay for bacteria (TVC) and mould found in dry hay (D), after steaming for 50 minutes in a homemade steamer (TWB) and in a Haygain (HG) 600 steamer.

| Particles | D | TWB | HG | SED |
|---|---|---|---|---|
| TVC (CFU/g) | 234422$^{a}$ (±2.80) | 549540$^{a}$ (±1.91) | 12$^{b}$ (±2.20) | 38.9 |
| Mould (CFU/g) | 53703$^{a}$ (±0.77) | 5012$^{a}$ (±3.13) | 5$^{b}$ (±1.37) | 16.2 |

Abbreviations: SD, standard deviation; SED, standard error of difference; TVC, total viable bacterial concentration.
$^{ab}$ Values in the same row not sharing common superscripts differ significantly ($P < .001$).

**Table 3**
Nutrient content (±SD) of 30 different samples of hay before (dry) and after steaming (steamed) for 50 minutes in the HG 1000 on an as fed basis.

| Nutrient (Units) | Dry | Steamed | SEM | Significance ($P$) |
|---|---|---|---|---|
| N (%) | 1.1 (±0.18) | 1.2 (±0.21) | 0.025 | .014 |
| Ca (%) | 0.4 (±0.09) | 0.4 (±0.12) | 0.027 | NS |
| K (%) | 1.4 (±0.28) | 1.5 (±0.26) | 0.068 | .041 |
| Mg (%) | 0.1 (±0.02) | 0.1 (±0.03) | 0.007 | NS |
| Na (%) | 0.1 (±0.07) | 0.1 (±0.09) | 0.025 | NS |
| P (%) | 0.1 (±0.03) | 0.2 (±0.03) | 0.008 | NS |
| WSC (%) | 12.6 (±3.12) | 10.3 (±3.60) | 0.827 | .009 |
| CP (%) | 7.0 (±1.11) | 7.4 (±1.31) | 0.025 | .014 |
| Cu (mg/kg) | 4.6 (±7.47) | 6.1 (±4.47) | 1.530 | NS |
| Mn (mg/kg) | 108 (±60.9) | 124 (±69.6) | 18.03 | NS |
| Zn (mg/kg) | 17.5 (±6.2) | 23.5 (±7.36) | 1.54 | .001 |

Abbreviations: CP, crude protein; HG, Haygain; NS, not significant; SD, standard deviation; SEM, standard error of the mean; WSC, water-soluble carbohydrate.

confirmed that $2.3 \times 10^5$ bacteria and $5.3 \times 10^4$ mould CFU/g were present. Earing et al [27] classified their alfalfa–orchard grass mixed hay to have low mould when containing $1 \times 10^4$ CFU/g and moderate mould when containing $2.7 \times 10^5$ CFU/g, whereas others [21] reported mould contamination levels in five different types of horse hays to range from 0 to $4.6 \times 10^6$ CFU/g and bacterial levels of $120–3 \times 10^6$ CFU/g. Based on these previous findings, the average microbial burden for the hays used in this study can be classified as moderately contaminated. Although 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 stabilization 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 suboptimal.

Moreover, although 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 on 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 (>99°C) 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 was 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 the hay and the average low temperatures of 58°C attained after 50 minutes of steaming. TWB was a noninsulated plastic container, and in the cold conditions, the temperature inside the hay did not increase to the desired level of 90°C 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°C–40°C. 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-fold to fivefold increases in bacteria [20,21] is highly undesirable and could compromise the respiratory health of the horse. The fact that postsoak 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 poststeamed 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], whereas 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.

### 4.4. Nutrient Content

The nutrient contents reported in Table 3 are the means from 30 samples of meadow and seed hay on an as fed basis collected from all over the UK 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 40–90 g/kg, whereas the average of 126 g/kg DM WSC content (range of 62–202 g/kg DM) was similar to previously reported values [21] but did not reach the high levels of 310 g/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 [18,41]. 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-fiber 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 behavior 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.

### 4.5. Steaming and Nutrient Content

The nutrient profiles detailed in Table 3 for hay after 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 poststeaming, 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 poststeaming 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, that is, 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. Although 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 fiber 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 present study show that when comparing dry, soaked, partially steamed, and high-temperature steaming, the most effective method for reducing ARPs, while conserving nutrients and improving the hygienic quality of hay fodder, is best achieved using the Haygain specifically designed high-temperature steamers. The present studies also show that partial steaming and soaking while effective at reducing ARPs in hay are contraindicated in terms of microbial contamination, and thus, either of these processes cannot be recommended as methods for producing hygienically clean fodder for stabled horses.

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