

This is an author produced version of a paper published in Animal.

This paper has been peer-reviewed but may not include the final publisher proof-corrections or pagination.

Citation for the published paper:

P. A. Harris, A. D. Ellis, M. J. Fradinho, A. Jansson, V. Julliand, N. Luthersson, A. S. Santos and I. Vervuert. (2017) Review: Feeding conserved forage to horses: recent advances and recommendations. *Animal.* Volume: 11, Number: 6, pp 958-967. http://dx.doi.org/10.1017/S1751731116002469.

Access to the published version may require journal subscription. Published with permission from: Cambridge University Press.

Epsilon Open Archive http://epsilon.slu.se

- Review: Review of feeding conserved forage to horses: recent advances and
   recommendations
- 3 P.A. Harris <sup>1</sup>, A.D. Ellis <sup>2</sup>, M.J. Fradinho <sup>3</sup>, A. Jansson <sup>4</sup>, V. Julliand <sup>5</sup>, N. Luthersson <sup>6</sup>,
- 4 A.S. Santos <sup>7</sup> and I. Vervuert <sup>8</sup>.
- <sup>5</sup> <sup>1</sup> Equine Studies Group, WALTHAM Centre for Pet Nutrition, Leics LE14 4RT, UK
- <sup>2</sup> UNEQUI, Research~Education~Innovation, Southwell, Nottinghamshire, NG25 0DS,
  7 UK
- <sup>3</sup> CIISA, Faculdade de Medicina Veterinária, Universidade de Lisboa, Av.
  <sup>9</sup> Universidade Técnica, 1300-477 Lisboa, Portugal
- <sup>4</sup> Dept of Anatomy, Physiology and Biochemistry, Swedish University of Agricultural
- 11 Sciences, 750 07 Uppsala, Sweden
- 12 <sup>5</sup>AgroSup Dijon, 21079 Dijon Cedex, France
- <sup>6</sup> Hestedoktoren I/S, Bukkerupvej 195, 4360 Kr. Eskilstrup, Denmark
- 14 <sup>7</sup> Department of Veterinary Medicine, Escola Universitária Vasco da Gama, 3020-
- 15 210 Coimbra / CITAB-UTAD Center for Research and Technology of Agro-
- 16 Environmental and Biological Sciences, Vila Real, PA Box 1013, 5001-801 Vila
- 17 Real, Portugal
- <sup>8</sup> Institute of Animal Nutrition, Nutrition Diseases and Dietetics, Faculty of Veterinary
- 19 Medicine, University of Leipzig, An den Tierkliniken 9, D-04103 Leipzig, Germany.
- 20 Corresponding Author: Pat Harris. E-mail: pat.harris@effem.com
- 21 Short Title: Feeding conserved forage to horses

22 **Abstract:** The horse is a non-ruminant herbivore adapted to eating plant-fibre or forage 23 based diets. Some horses are stabled for most or the majority of the day with limited or no 24 access to fresh pasture and are fed preserved forage typically as hay or haylage and sometimes silage. This raises questions with respect to the quality and suitability of these 25 26 preserved forages (considering production, nutritional content, digestibility as well as 27 hygiene) and required quantities. Especially for performance horses, forage is often 28 replaced with energy dense feedstuffs which can result in a reduction in the proportion of the 29 diet that is forage based. This may adversely affect the health, welfare, behaviour and even 30 performance of the horse. In the past 20 years a large body of research work has 31 contributed to a better and deeper understanding of equine forage needs and the 32 physiological and behavioural consequences if these are not met. Recent nutrient 33 requirement systems have incorporated some, but not all, of this new knowledge into their 34 recommendations. This review paper amalgamates recommendations based on the latest 35 understanding in forage feeding for horses, defining forage types and preservation methods, 36 hygienic quality, feed intake behaviour, typical nutrient composition, digestion and 37 digestibility as well as health and performance implications. Based on this, consensual 38 applied recommendations for feeding preserved forages are provided. 39 40 41 **Key words:** forage, health, hygiene, behaviour, requirements

42

43 Implications: Improved consistency in the terminology used for preserved forages
44 and the adoption of consensual recommended minimal forage intake levels.

#### 45 Introduction

46 The horse is a non-ruminant grazing herbivore adapted to eating plant-fibre or forage based diets. A wide range of plant species can be hydrolyzed and/or fermented within 47 its specialized gastro-intestinal tract (GIT), thanks to the presence of a mixed microbial 48 symbiotic population throughout the whole GIT, but particularly in the hindgut, which 49 facilitate fibre digestion (de Fombelle et al., 2003; Dougal et al., 2013). Gastrointestinal 50 tract (GIT) digestion provides energy mainly through the production of short-chain fatty 51 acids (SCFA), mostly acetate, propionate and butyrate, also often described as volatile 52 fatty acids (VFA's) (Merritt and Julliand, 2013). The whole digestive system is well 53 adapted to a trickle (almost continuous intake of small amounts) feeding intake pattern, 54 with the horse naturally foraging for around 10 - 15 hours a day (Ellis, 2010). 55 Historically the energy demands of working horses were too high, and time too short, 56 57 to be met by forage alone, especially as forage quality was often poor, grazing limited and it was difficult to distribute and transport bulky forages. Therefore, oats, barley, 58 59 beans and root vegetables were used as a major component of the diet (Stewart, 60 1838). The inclusion of energy rich feedstuffs (particularly cereals and vegetable oils) in the diets of many horses, especially performance horses, continues today despite 61 the availability of better quality forages (Richards et al., 2006, Lindburg 2013). An 62 increase in starch intake, often in combination with a reduction in the amount of forage 63 provided, can have health, welfare and performance consequences. 64

Due mainly to environmental conditions and the lack of availability/undesirability of grazing, many horses are fed preserved forages (hay, haylage and sometimes silage), in particular preserved grass, either at specific times of the year or all year round and therefore preserved forages provide all or part of their forage intake. During the 2012 meeting of the European Workshop on Equine Nutrition (EWEN) it was agreed that

there was a need for a consensus paper on the topic of preserved forage feeding to
horses. The aim of this review paper is therefore to summarize recent findings and to
provide consensual applied recommendations for feeding preserved forage.

73

# 74 Forage types and preservation methods

Roughages are high fibre feeds (Morrisson, 1956) generally obtained as a crop residue or a by-product (Ellis *et al.*, 1988) e.g. straw and cereal hulls. Forages are also high fiber feeds obtained by cutting and preserving the whole plant (except roots). Although they define different products, the words forage and roughage are typically used interchangeably. From a behavioural aspect the term 'foraging' encompasses all feed intake activities of horses both on pasture and in housing situations.

81 Cut grass air dried/wilted in the field or a barn is termed hay, whereas silage is forage 82 preserved moist and airtight, and thus fermented (McDonald et al., 1991) The term 83 haylage was originally used to describe silage with a DM content of around 50 % 84 (Gordon et al., 1961) and although haylage (and hay/silage) can be harvested at any 85 stage of plant maturity today *haylages* are typically grown and cut at later growth 86 stages similarly to hay, but baled before becoming dry resulting in DMs typically >50 but <70% although occasionally up to 85% (see Fig. 1 and supplementary Table S1). 87 Hay baled with too high a moisture content due to unsuitable weather conditions at 88 cutting time and especially during wilting, insufficient turning especially if in rows 89 90 (rather than being wilted widespread which helps speed up wilting, thereby helping to 91 reduce protein degradation and loss of nutrients) etc. allows the development of fungal 92 spores/bacteria and increases the risk of mycotoxin development. To stop undesired microbial growth, roughage/forage therefore has to be preserved under controlled 93 94 conditions (Fig. 2). Hay and straw should be preserved during conditions of low water

95 activity in the crop (i.e. preserved at a DM content ideally above 85%) and given that the bales will be exposed to air, relative humidity must be lower than required for mould 96 growth at the given temperature (Fig. 2) and therefore ideally <70%. Forages may also 97 98 be preserved utilizing airtight conditions, with or without lactic acid fermentation. In the water soluble carbohydrates in the forage are fermented by anaerobic 99 silage 100 lactic acid bacteria (naturally occurring on the crop or added as inoculants (Fig. 1). For 101 sufficient lactic acid production to occur, the crop DM content must ideally be around 30% or less and certainly <50 % (Fig. 3). If the acids produced sufficiently increase 102 hydrogen ion concentrations, undesirable microbial growth will be inhibited. To confirm 103 104 proper ensiling of forages (without butyrate) with a DM 15-50%, pH can be used as an 105 indicator. Weissbach, 1996 and Spörndly et al., 2003 suggest that the pH should be 106 less than: 0.0257 x DM% + 3.71 (Weissbach, 1996) but according to Field and Wilman, 107 1996 a higher pH (0.0028 x DM(g/kg) + 4.209 (equation estimated from their figure) might be allowed if silage is preserved in bales and not in bunkers. Preservation at 108 109 higher DM contents relies on maintenance of airtight packaging not a low pH (Mihin, 110 1940). In haylage/silage bale production, at least 4 layers of film are needed to 111 promote good preservation (Keles et al., 2009; McEniry et al., 2011) but adding layers 112 (6-10) increases the CO<sub>2</sub> content (Müller, 2005) and for bales to cope with handling, transportation, birds and long-term storage 8 layers or more are recommended 113 (Jacobsson, 2002; Spörndly pers. com.). In haylages with very high DM contents there 114 115 may be an increased risk of perforation (allowing air entrance and mould growth) by 116 stiff and sharp stems. To facilitate safer preservation and storage such haylages require more layers of film than forages with a lower DM content. Some professional 117 118 horse haylage producers may use 12-20 layers (Jansson A, pers. com.). More research is needed into the impact of the number of layers used. Mature and rough 119

120 crop may be difficult to preserve correctly since more air can be trapped in such bales, 121 increasing the risk of localized mould growth. Higher density baling, late tossing, lower 122 dry matter (75%), as well as rain occurring after cutting strongly increased mould 123 counts in hay (Seguin *et al.*, 2010) but baling for haylage when too dry (86-88% DM) also increased mould counts (Martinson et al., 2011).. Bales of forage with very low 124 125 DM contents (<30 %) are also at risk of losing airtightness due to their heavy weight, plant structure collapse during fermentation, formation of effluent etc. all of which result 126 127 in increased pressure on the film. In forage with DM content < 40 % clostridial 128 fermentation can also occur (McDonald et al., 2002) and the prevalence of clostridial 129 fermentation seems to be higher in bales with unchopped vs chopped forage (Pauly, 130 1999). In well-made haylage, whilst there will still be some microbial activity, reduction 131 in WSC and production of by-products of fermentation this will be much more limited compared with that for silage (Müller et al., 2007; Muhonen et al., 2009). 132

133 Figures 1, 2 and 3 here

# 134 Nutritional composition

The nutritive value of forage to the horse is determined predominantly by its nutrient 135 content and digestibility, which in turn may be influenced by level of intake and feed 136 interactions. Nutrient content depends on many plant related factors as well as the 137 138 environment (Buxton, 1996). Herbage maturity at harvest, however, is a key influencer 139 (Virkajärvi et al., 2012). As the plant matures, the fibre fraction increases and crude protein decreases, leading to a decline in digestibility plus overall energy availability 140 (Ragnarsson and Lindberg, 2008; 2010; Müller, 2012) (Fig. 4). Nutrient content is also 141 142 closely related to plant species and morphology, with legumes typically higher in 143 protein and calcium than grasses. The proportion of leaf to stem will also affect 144 nutritive value, even between cultivars of the same species (Van Soest, 1994;
145 Bélanger and McQueen, 1997).

146 Figure 4 Here

Forage quality is also influenced by management factors especially fertilizer 147 applications, harvest techniques and storage conditions (Van Soest, 1994; Cookson 148 149 et al., 2000; Rotz, 2003). Hay nutrient losses are normally higher during harvest (e.g. 150 plant leaves left in the field due to mechanical handling of the dried crop) in contrast 151 with silage where losses are larger during storage, mostly due to fermentation activities, air infiltration and silage effluents (McGechan, 1989, 1990; Dürr, 2004; 152 Müller, 2012). Environmental factors affecting herbage development (including 153 154 temperature, precipitation, solar radiation and soil nutrient availability) obviously can vary with geographical location resulting in a range of climatic and soil conditions. 155

156 Not surprisingly forage chemical composition and consequent nutritional value therefore shows a great variation between regions, crop years, harvest time, seasons 157 158 and even within farming places (see Supplementary Table S1). The variability 159 highlights the importance of forage analysis when designing forage based rations, especially when feeding brood mares, growing and performance horses (Jansson et 160 161 al., 2012) or those with particular clinical conditions (such as laminitis, HYPP etc.). Ideally mineral content should also be analysed. Legume forages tend to have higher 162 protein and calcium contents than grass forages and this needs to be taken into 163 consideration when formulating the total ration (which should preferably be based on 164 nutrient analysis). 165

166

# 167 Digestion and Digestibility

168 Digestion can be defined as the process in the gastrointestinal tract by which forage constituents are converted by physical and chemical breakdown into substances 169 (mainly SCFA) that can be absorbed and assimilated by the body (Argenzio et al., 170 171 1974). Water soluble, as well as enzymatically digestible carbohydrates (WSC, starch non-starch polysaccharides, soluble fibre) are digested from the stomach onwards 172 173 along the whole GIT as long as they are not trapped within insoluble fibre. Insoluble fibre digestion depends exclusively on microorganisms that have the ability to 174 175 hydrolyze the predominant  $\beta$ 1-4 linked polysaccharides of cell walls. It occurs primarily in the horse's hindgut with a limited amount of fermentation in the foregut (de Fombelle 176 177 et al., 2003; Jouany et al., 2009). The extent of any forage digestion depends on both 178 the fibrolytic microbial activity of the hindgut ecosystem and the total time during which 179 parietal components are exposed to this activity (Merritt and Julliand, 2013). This duration is defined as the mean retention time (MRT) of digesta (Miyaji et al., 2008b). 180 181

182 Several intrinsic and extrinsic factors can affect forage digestion and digestibility (digestibility = total nutrient/energy ingested minus nutrient/energy excreted and 183 expressed as % of total ingested). The Influence of intrinsic factors such as horse 184 185 breed, individuality and age has been studied on forage digestion and digestibility. It has been anecdotally suggested, for example, that 'easy keepers' may have higher 186 187 digestion efficiency than other horses. However, in a controlled study (Ragnarsson and Jansson, 2011) where two haylages (cut at early and late maturity stage) were 188 189 fed to both Icelandic horses (easy keepers) and Standardbred horses no such effect could be observed (Table 1). Recent studies also showed that DM, organic matter and 190 191 neutral detergent fibre fraction apparent digestibility were not different between weaned foals (6 and 12 months) and adults (14 years) (Ringler et al., 2009; Earing et 192

*al.*, 2013), which complemented and confirmed previous data reporting no variation of
total apparent digestibility of DM between weanlings (5 months), and those aged 8
and 12 months old (Cymbaluk *et al.*, 1989). At two months of age, the SCFAs profile
of foal's faeces remained constant and cellulolytic bacterial concentration was
comparable with adult values suggesting that the fibre-degrading capacity in foals was
established by two months of age (Faubladier *et al.*, 2013).

199

200 Extrinsic factors such as botanical characteristics are known to influence growth and 201 metabolism of plants, and therefore their nutritive value as forages. In horses, the average DM digestibility decreased significantly in forages having higher NDF and 202 203 lower crude protein contents (Edouard et al., 2008). More specifically, alfalfa has 204 higher DM and CP digestibility coefficients than tall fescue, caucasian bluestem (Crozier et al., 1997), coastal Bermuda grass (Sturgeon et al., 2000; Potts et al., 2010) 205 and Matua grass (Sturgeon et al., 2000). Alfalfa also had higher OM digestibility than 206 207 grass hays (LaCasha et al., 1999) and more specifically coastal Bermuda grass (Potts 208 et al., 2010). Digestion and energy and crude protein content of forage is greatly affected by the stage of maturity of plants (Fig 4; Table 2). 209

210 The type of forage preservation, however, appears to have limited impact on apparent digestibility. When hay and haylage originating from the same crop (same 211 harvest/batch) were compared, digestibility did not differ (Bergero and Peiretti, 2011) 212 213 although when hay and silage from the same crop was compared, digestibility was 214 slightly higher in silage compared to hay (Muhonen et al., 2008b). Feed changes from hay to silage or haylage (even from the same harvest/batch) may, however, affect the 215 216 microflora as well as the DM of the hindgut contents (Muhonen et al 2008, Muhonen 2009) Similarly changes between forage batches with different CP contents should 217

be made slowly, as colonic pH, for example, has been shown not to be stable within 3 weeks after such a change (Muhonen *et al.*, 2008a) although further studies are needed to assess the importance of these alterations. Until we do know more it is recommended that changes between forage batches should therefore be made carefully to minimise the risk of disturbances. In support of these recommendations the incidence of certain types of colic has been shown to increase especially in the first 7 days (but up to 28days) after a change in forage feeding (Hillyer et al 2002).

225

Insert Table 1 and 2

227

## 228 Hygienic quality

This is a key issue, as forages fed to horses have often been reported to be of poor 229 230 hygienic quality (Wichert et al., 2008) which can lead to significant health problems for example, mould spore exposure, especially from Aspergillus fumigatus has been 231 232 implicated in the aetiology of recurrent airway obstruction in horses (Pirie 2014). 233 Although the term "poor hygienic quality" is not well defined, it may include biological 234 contaminants (e.g. pests, microorganism and their related toxins), chemical contaminants (e.g. fertilizer, heavy metals), and physical contaminants such as soil. 235 236 In that context, feed hygiene, as stated by EU legislation (Regulation (EC) No 183/2005), includes all aspects that must be considered in order to produce, sell and 237 feed a safe feedstuff that will not result in any harmful effects on the animal, and 238 therefore applies to forage as well. EU legislation (EC No 32/2002) defines maximum 239 acceptable levels for a variety of contaminants in feedstuffs including for heavy metals, 240 241 aflatoxin B1, rye ergot and substances such as pesticides.

242

243 Several parameters can be used to evaluate hygienic quality including feel (e.g. dry, clammy), smell (e.g. typical, mouldy), colour (e.g. green, bleached), macroscopic 244 findings (e.g. presence of sand, soil, dead animals) and/or microbial evaluation 245 246 (Wichert et al., 2008, Kamphues 2013, Wolf et al., 2014). Knowing the DM content is key, as discussed above, as microorganisms need water for survival and multiplication 247 248 (Kamphues 2013). A macroscopic evaluation should routinely be undertaken for obvious moulds, as well as the presence of sand/soil and other potential contaminants 249 250 especially poisonous plants. Senecio spp., and Taxus baccata have been suggested 251 to be the main poisonous plants for horses (Berny et al., 2010) although regionally other poisonous plants may be important e.g. vitamin D-intoxication via Golden oat 252 253 grass in parts of Germany (Bockisch et al., 2015).

254 Much attention is given to the microbiological analysis of feedstuffs but there are significant effects of the analytical methods used. Pre-treatment, incubation 255 256 temperature, type of incubation substrate and incubation duration can all significantly 257 influence what is cultivated (Raymond et al., 2000; Müller et al., 2011). For example, the growth of thermophilic bacteria such as actinomyces is supported by an incubation 258 temperature of 55°C for three days, whereas the growth of mesophilic aerobic bacteria 259 260 is supported by a lower incubation temperature (25°C), but a longer incubation time (7 days, Raymond et al., 2000). In some European countries, the characterization and 261 benchmarking for bacteria, moulds and yeasts in feedstuffs has been standardized 262 263 (Tables 3 and 4) to provide upper acceptable levels of contamination which are thought not to have any adverse effects on horse health. For this purpose 264 microorganisms are classified as being either epiphytic (i.e. normal contaminants that 265 are present even under optimal conditions) or spoilage inducing. Importantly, both 266 types may impact health (e.g. respiratory problems by moulds). However, it should be 267

emphasised that whilst these benchmark levels can be used to describe spoilage, the
consequences on equine health through exceeding the benchmark levels requires
more in-depth evaluation.

271

Undesirable bacteria, moulds and yeasts multiply under adverse conditions such as
rainfall, high humidity, and high ambient temperatures or due to vectors like mites. As
outlined above a DM content of >85% reduces microbial activity

275

Mycotoxins such as ergot alkaloids, , zearalenone, deoxynivalenol, fumonisin B1 and 276 277 B2, ochratoxin A or aflatoxin B1 are secondary metabolites produced by fungi that are capable of causing toxicity (see review Riet-Correa et al., 2013). However, little is 278 known about the impact of mycotoxins on equine health status. Several mycotoxins 279 280 are designated as undesirable substances with maximum levels in human food, whereas only aflatoxin B1 and rye-ergot are currently designated as undesirable 281 282 substances with maximum levels for animal feedstuffs. Further analyses, therefore, 283 may be required including detailed mycotoxin determinations e.g. clinical signs of leukoencephalomalacia requires fumonisin analysis especially when corn silage with 284 corn cobs is being fed to the affected horses. Whilst it is impossible to fully eliminate 285 286 mycotoxins, it is important to reduce contamination by optimizing harvesting and storage conditions. 287

Botulism occurs following exposure to toxins (8 different serotypes) produced by anaerobic spore-forming bacterium *Clostridium botulinum* and other botulinum toxinproducing clostridia (Galey 2001). In adult horses, food-borne botulism is acquired by the ingestion of preformed toxins (mainly type B, C or A). Feeding big bale silage or haylage have been often associated with botulism outbreaks in horses, mules or

cattle (e.g. Ricketts *et al.*, 1984; Divers *et al.*, 1986; Wollanke 2004; Myllykoski *et al.*,
2009), although there have been outbreaks linked with hay feeding (Wichtel and
Whitlock 1991; Johnson *et al.*, 2010). Botulinum toxin production typically occurs due
to contamination with animals, soil or poultry slurry. Equivocal results have been
reported regarding the potential risk of silage or haylage contamination with
Clostridium botulinum spores through using the wastage from biogas anaerobic
digesters for fertilization (Müller *et al.*, 2013; Neuhaus *et al.*, 2015).

300

#### 301 Methods of sampling

A good sampling procedure is crucial so that any analysis (e.g. nutrients, microbial counts) represents the mean value for the whole batch. For the official feed control EU legislation (EC No 691/2013) suggests for example that 5 up to 40 individual samples should be aggregated to form an initial overall sample of between 1 (low specific gravity, e.g. hay or straw) and 4 kg as fed (e.g. silage).

307 Table 3 and 4 here

# 308 Forage Intake Behaviour and Welfare considerations

Free-ranging horses perform 10-15 individual feed-bouts/day and forage for 12.5±2.5 hours per day with recordings as high as 18 hours (Ellis, 2010, Supplementary Table S2). 'Non-foraging' bouts are rarely longer than 3 hours duration (2±1.3) (Souris *et al.*, 2005; Van Dierendonck *et al.*, 1996; Hallam *et al.*, 2012, Ellis *et al.*, 2015;). Even when stabled, horses tend to spend a minimum time (8.5-12 hrs/day) on foraging related behaviours, at times 'topping up' their food intake behaviour by ingesting woodshavings and performing coprophagy for up to 3hrs per day (Ellis *et al.*, 2006, Ellis 316 2010). Curtis et al. (2011) suggested the potential for wood-shaving intakes of up to 317 3.5kg in a few individuals fed DM restricted diets as part of a weight loss programme and this is in line with considerable amounts of woodshavings reported in the stomach 318 319 of horses on a low forage diet (Boswinkel et al 2007). Intake times for chopped lucerne/alfalfa are about twice as fast as those for long hay (Ellis, 2010). Chopping 320 321 very late cut hay to 3.5 cm particle length did not shorten intake times in 8 horses but adding chopped forage (~2.5cm or 4cm) to pellets at 30% inclusion rate doubled 322 323 concentrate feed intake times (Ellis and Hill, 2002; Ellis et al., 2005). Replacing hay 324 with ground-hay pellets reduces chewing time/kg by 75% (Ellis et al., 2010) which may partially explain the increased intakes (to 4-5% of BW) seen by Argo et al. (2002) and 325 326 Henneke and Callaham (2009) when they fed only pellets compared to a chaff-feed or 327 hay. Elia et al. (2010) found that foraging through wood-shaving bedding increased by 3.5 hrs/day, when feeding just hay pellets compared to feeding hay. This strong 328 329 motivation to spend a minimum of 8 (stabled) to 12 (grazing) hours on intake 330 behaviours, irrelevant of the energy density or composition of feedstuffs is supported by many studies on voluntary intake behaviour (Supplementary Table S2) and night 331 time observations of stabled horses (Ellis et al., 2015). In addition stabled horses rest 332 333 for 3-4 hours maximum before re-commencing foraging-related behavior (Ellis et al., 334 2015).

Furthermore, in a race horse population of 2900 animals, those fed <6.8kg/day of forage showed a significant increase in abnormal behaviours including oral stereotypies and weaving (McGreevy *et al.*, 1995). Lack of foraging opportunity has been directly linked to the onset of oral stereotypies in foals (Nicol *et al.*, 2002) and to possible stereotypic pre-cursor behaviour (increased water play and drinking, locomotion) in 3 out of 5 feed-restricted ponies (Dugdale *et al.*, 2010). Appetitive

behaviours have a positive feedback on motivation through the brain's pleasure
centres which become active as the horse pursues its goal (McBride and Hemmings,
2005). If the motivation to forage/chew is not fulfilled, other behaviours may replace
the original goal achievement behaviour as highlighted in several mammals (Hughes
and Duncan, 1988).

#### 346 Health considerations

Horses as previously stated are adapted to a slow and almost continual intake of a diet rich in structural fibre and low in rapidly hydrolysable carbohydrates. For many commonly encountered equine health issues, forage feeding management is an important factor in helping to reduce risk and maintain health.

351 Particle length influences motility and transit time within the GI tract, as larger particles move more slowly (Drogoul et al., 2000) and forage also influences gastric emptying 352 rate and/or the passage rate through the small intestine (Jensen et al., 2012). A more 353 354 stable (Willing et al., 2009) and diverse microbial population, with a larger core, is found when a forage only diet is fed especially compared to a sugar and starch rich 355 ration (Dougal et al., 2014). Stabling and feeding preserved forage results in a change 356 357 in gut motility and higher DM faeces despite an increased water intake (Williams et al., 2011, 2015). Horses have a lower pH in the proximal stomach during early morning 358 359 (1:00–9:00 AM), when stomach-fill tends to be lower especially in stabled animals (Husted et al., 2008). It is therefore not surprising that several nutritional risk factors 360 for equine gastric ulcer syndrome (EGUS), colic and diarrhea have been identified and 361 362 many of them are related to limited, reduced or a changed intake of forage (see table 363 5).

Recurrent airway obstruction (RAO) is the most common cause of chronic coughing in horses in temperate countries, with up to 14% prevalence in the UK (Hotchkiss *et al.*, 2007). This lower airway inflammatory disease results in a range of clinical signs from exercise intolerance/poor performance, to severe expiratory dyspnea (Pirie *et al.*, 2002; Pirie *et al.*, 2003; McGorum and Pirie 2008). Exposure to airborne organic dust (mostly endotoxins) via stabling and feeding of hay/straw with a high mould count plays a primary role (Couetil and Ward 2003) as discussed above.

371 The management strategies for horses at increased risk of several conditions, such as certain forms of laminitis (including those with the Equine Metabolic Syndrome and 372 373 Pituitary Pars Intermedia Dysfunction) as well as various muscular disorders including 374 the Equine Rhabdomyolysis Syndrome, includes reducing the intake of non-structural carbohydrates (NSC) (MacLeay et al., 2000;, McKenzie et al., 2003; Valentine et al., 375 376 2001; Hunt et al., 2008). Such horses are often put on restricted or no pasture access 377 (especially when the levels of fructans, starch and sugar are high or there is a high 378 herbage yield) and are fed a diet based on preserved forage or forage replacers (e.g. 379 commercial fibre based products) with a known and low content of NSC (<10-12% DM) to minimize post-feeding glycemic and insulinaemic responses (Borgia et al., 380 2011; Geor and Harris 2013; Harris et al., 2013). Recent observations also indicate 381 382 that forage CP content might be of importance for the insulin response (Ringmark and 383 Jansson 2013).

384 Table 5 here

# 385 **Performance considerations**

386 Historically, the use of forage in diets of performance horses has been limited 387 (Jansson and Harris 2013). Recent studies (Connysson *et al.*, 2006; Muhonen *et al.*,

388 2008b; Connysson et al., 2010; Essén-Gustavsson et al., 2010; Jansson and Lindberg 2012; Ringmark et al., 2012; Ringmark et al., 2015) show that forage-only diets can 389 meet the high energy requirements of horses in very heavy training (equal to or more 390 391 than twice maintenance requirements) if the energy density is high enough. The energy density of such forage must correspond to at least 10.5 MJ ME/kg DM 392 393 (Jansson and Lindberg 2012; Ringmark 2014). These studies indicate no adverse effects on performance with exception for slightly lower (ca -10 %) muscle glycogen 394 395 contents within 3 days after high intensity work compared to a high starch diet 396 (Jansson and Lindberg 2012). However, very high muscle glycogen contents and indications of rapid glycogen recovery have been documented on forage-only diets 397 398 providing crude protein intakes above current feeding recommendations and forage 399 WSC contents of 8.5-13.5 % of DM (Essén-Gustavsson et al., 2010; Ringmark, 2014). In the study by Jansson and Lindberg (2012) plasma lactate response was decreased, 400 401 venous pH and blood glucose was increased during exercise on the forage-only diet 402 compared to the traditional high starch diet, indicating a metabolic pattern that might improve performance. A recent study (Ringmark 2014) has also shown that it is 403 possible to get Standardbred yearlings into racing condition at the age of 3, and also 404 405 to win races, on a high-energy forage diet supplemented only with minerals and 406 vitamins. Horses also maintained plasma volume longer during 12 h of feed 407 deprivation on a forage only-diet compared to a high starch diet (Connysson et al., 2010). Altogether these studies suggest that high energy preserved forage can be an 408 alternative to high starch feeds in exercising horses and that such diets might promote 409 both health and performance. 410

# 411 Author's Recommendations for best practice

Based on the above and other published work (as well as personal views wherestated).

414

415 A. The general use of the following terms is recommended

416

417 I. *haylage* for forages stored airtight and with DM content  $\geq$  50%.

418 II. *silage* for forages stored airtight with DM contents below 50%

419 III. *hay* for forage preserved at a DM content ideally above 85%

420

B. To fully understand the value of a forage, and its impact as part of the horse's
diet, nutrient analysis is recommended and an estimation of the energy content
should be made. This becomes particular important when feeding horses with health
disorders (e.g. low BCS, obesity, laminitis, PSSM).

425

C. Hygienic quality of forages must be a key consideration as poor hygienic
quality can lead to significant health problems. As a minimum, regular visual and
olfactory inspection should be undertaken and poor quality forage appropriately
disposed of. Benchmarks for microorganism contamination should be considered.

D. Changes in forage should be done gradually especially if the nutrient composition (energy, protein, WSC) is unknown or known to differ considerably due to changes in plant-species, growth stage and preservation technique. In such instances at least 2 and possibly more than 3 weeks adaptation period may be required.

436

437 In terms of forage requirements for horses it is recommended that:

The basis of any horse's diet should be fresh or preserved forage
 (recommended particle length >2.5 cm) offered ideally ad libitum or spread
 throughout the day to avoid prolonged periods (i.e. >4-5hrs) in a stable without
 foraging opportunity. However, in older horses with chewing difficulties, using ground
 high fibre products in a soaked mash form may be beneficial.

443 2. A horse requires sufficient forage fed in a form that supports species specific
444 feed intake behaviour ideally for a minimum of 8 hrs and preferably 10 hours/24hrs.

3. The amount of preserved forage in the ration should be calculated on a g DM
/kg BW rather than % of ration or an as-fed basis due to the great variation in DM
content of different forages.

448 4. The lower limit of daily forage intake should be 15g DM/kg BW in addition to 449 complementary (concentrate/compound) feeding, with an absolute minimum 450 recommendation of 12.5g DM/kg BW. Previously recommended minimums of forage 451 at 8-10g DM per kg BW/day are not acceptable according to the latest understanding 452 of equine ethological needs and health considerations.

5. Any lower amounts should only be fed <u>solus (i.e. no other fibre provision)</u>, under exceptional clinical circumstances (e.g. as low energy providing hays for animals undergoing severe restriction for weight loss purposes, post-surgery etc.), under veterinary supervision and with an appropriate forage vitamin/mineral/protein balancer.

6. Straw, if required, should be introduced into the diet very slowly, which helps
the horse to adapt its chewing behavior and reduce the risk of impaction, although this
remains a significant risk with certain individuals. The risk of gastric ulceration also

461 may increase when straw is the main roughage. Some of the authors (PH, AE, VJ, NL) 462 *personally recommend* that not more than 30% of the forage DM ration should be 463 straw (other than for donkeys). Others (AJ, IV) may recommend higher proportions 464 providing the straw is of a good hygienic standard and the overall ration is balanced 465 for protein vitamins, minerals and trace elements.

For horses requiring increased energy intake due to reproduction status or
work level, less mature forages that have a higher energy content, should be
considered. Forages cut at an earlier plant maturity stage are less likely to require
protein supplementation.

8. Intakes above those recommended here may be fed or consumed by horses,
as long as a healthy energy balance (body condition) is maintained. If body weight
increases even when feeding the minimum recommended intake, a forage with a
lower energy content should be fed rather than reducing the dry matter forage intake.

474

475

Acknowledgements: We thank all committee members, participants, sponsors and
organizers of the European Workshop in Equine Nutrition (EWEN) for their input and
support. In addition, special thanks are expressed to Manfred Coenen, Sarah
Ralston, Thomas Pauly, Rolf Spörndly, Cecilia Müller, Markku Saastamoinen, Dag
Austbø, Anne-Helene Tauson, Sveinn Ragnarsson, Joaquin Clotet, Samy Julliand,
Nicoletta Miraglia, Pier Giorgio Peiretti, Teresa Dentinho, Luis Ferreira, Rui Bessa
and Andreas Olt.

483

484

- 485 **References from 2010 onwards (The list of references from 2009 and before is given in**
- 486 **Supplementary Material S1).**
- 487 Andrews FM, Larson C and Harris P 2015. Nutritional management of gastric ulceration.
- 488 Equine Veterinary education (in press).
- 489 Bergero D and Peiretti PG 2011. Intake and Apparent Digestibility of Permanent Meadow Hay
- 490 and Haylage in Ponies. Journal of Equine Veterinary Science 31, 67-71.
- 491 Berny P, Caloni F, Croubels S, Sachana M, Vandenbroucke V, Davanzo F and Guitart R
- 492 2010. Animal poisoning in Europe. Part 2: Companion animals. Veterinary Journal 183,493 255-9.
- 494 Bockisch F, Aboling S, Coenen M, and Vervuert <u>1</u> 2015. Yellow oat grass intoxication in
- 495 horses: Pitfalls by producing hay from extensive landscapes? A case report.
- 496 Tierarztliche Praxis Grosstiere Nutztiere 43, 296-304.
- Borgia L, Valberg S, McCue M, Watts K. and Pagan J 2011. Glycaemic and insulinaemic
  responses to feeding hay with different non-structural carbohydrate content in control and
  polysaccharide storage myopathy-affected horses. Journal of animal physiology and
  animal nutrition *95*, 798-807.
- 501 Connysson M, Essén-Gustavsson B, Lindberg J E and Jansson A 2010. Effects of feed
- deprivation on Standardbred horses in training fed a forage-only diet and a 50:50 forageoats diet. Equine Veterinary Journal Suppl. 38, 335-340.
- 504 Curtis G, Barfoot C, Dugdale A, Harris P and Argo C 2011. Voluntary ingestion of wood
  505 shavings by obese horses under dietary restriction. British Journal of Nutrition 106, S178–
  506 S182.
- Dougal K, de la Fuente G, Harris PA, Girdwood SE, Pinloche E, Geor RJ, Nielsen BD, Schott
  II HC, Elzinga S and Newbold CJ 2014. Characterisation of the faecal bacterial community
  in adult and elderly horses fed a high fibre, high oil or high starch diet using 454
  pyrosequencing. *PloS one*, *9* p.e87424.

- 511 Dougal K, de la Fuente G, Harris PA, Girdwood SE, Pinloche E and Newbold CJ 2013.
  512 Identification of a core bacterial community within the large intestine of the horse. *PloS one*513 8, p.e77660.
- 514 Dugdale AHA, Curtis GC, Cripps P, Harris PA and Argo C 2010. Effect of dietary restriction
- on body condition, composition and welfare of overweight and obese pony mares. Equine
  Veterinary Journal 42, 600-610.
- 517 Durham A 2013. Intestinal disease In Equine Clinical and Applied nutrition. (ed. RJ Geor, PA 518 Harris and M Coenen), pp 568-581. Elsevier, Amsterdam, the Netherlands.

Earing JE, Lawrence LM, Hayes SH, Brummer M and Vanzant E. 2013. Digestive capacity in
weanling and mature horses. Journal of Animal Science 91, 2151-2157.

- Elia JB, Hollis N, Houpt KA 2010. Motivation for hay: Effects of a pelleted diet on behavior and
  physiology of horses. Physiology and Behavior 101, 623–627.
- Ellis AD 2010. Biological basis of behaviour and Feed Intake in horses. In The impact of
  Nutrition on the Health and Welfare of Horses (ed. AD Ellis, A Longland, M Coenen and
  N Miraglia), pp. 53-74. EAAP Publication No. 128, Wageningen Academic Publishers,
  Wageningen, the Netherlands.
- 527 Ellis AD, Redgate S, Zinchenko S, Owen H, Barfoot C and Harris P 2015. The effect of 528 presenting forage in multi-layered haynets and at multiple sites on night time budgets of 529 stabled horses, Applied Animal Behaviour Science 171, 108–116.
- Essén-Gustavsson B, Connysson M and Jansson A 2010. Effects of crude protein intake from
  forage-only diets on muscle amino acids and glycogen levels in horses in training. Equine
  Veterinary Journal 42, 341-346.
- Faubladier C, Julliand V, Danel J and Philippeau C 2013. Bacterial carbohydrate-degrading
  capacity in foal faeces: changes from birth to pre-weaning and the impact of maternal
  supplementation with fermented feed products. British Journal of Nutrition 110, 1040-1052.
- 536 Fedtke A, Pfaff M, Volquardsen J, Venner M and Vervuert I (2015): Effects of alfalfa chaff on
- 537 gastric mucosa in weanling foals. Pferdeheilkunde 31, 596.

- Geor RJ and Harris PA 2013. Laminitis. In Equine Applied and Clinical Nutrition, Health,
  Welfare and Performance. Saunders, Elsevier (ed. RJ Geor, PA Harris and M Coenen), pp.
  469-486. Elsevier, Amsterdam, the Netherlands.
- 541 Hallam S, Campbell EP, Qazamel M, Owen H and Ellis AD 2012. Effects of traditional versus
- 542 novel feeding management on 24 hour time budget of stabled horses. In Forages and
- 543 Grazing in Horse Nutrition (ed. M Saastamoinen, MJ Fradinho, AS Santos, N Miraglia), pp.
- 544 319-321. EAAP Publication No. 132, Wageningen Academic Publishers, Wageningen, the545 Netherlands.
- Harris PA, Coenen M and Geor RJ 2013. Controversial areas in equine nutrition and feeding
  management : the editors' views. In Equine Clinical and Applied nutrition (ed. RJ Geor,
  PA Harris and M Coenen), pp 455-468. Elsevier, Amsterdam, the Netherlands.
- Jansson A and Harris P 2013. A bibliometric review on nutrition of the exercising horse from
  1970 to 2010. Comparative Exercise Physiology 9, 169-180.
- Jansson A and Lindberg JE 2012. A forage-only diet alters the metabolic response of horses
  in training. Animal 6, 1939–1946.
- 553 Jansson A, Saastamoinen M and Lindberg JE 2012. Forage feeding systems. In Forages and
- 554 grazing in horse nutrition (ed. MT Saastamoinen, MJ Fradinho, AS Santos and N Miraglia),
- pp. 289-304. EAAP publication No. 132, Wageningen Academic Publishers, Wageningen,
  The Netherlands.
- Jensen RB, Austbø D and Tauson AH 2012. Feeding forage before or after oats affects
  caecum pH profiles in the horse. In: Forages and grazing in horse nutrition (ed. MT
  Saastamoinen, MJ Fradinho, AS Santos and N Miraglia), pp 327-330. EAAP publication
- 560 No. 132, Wageningen Academic Publishers, Wageningen, The Netherlands.
- 561 Johnson AL, McAdams SC and Whitlock RH 2010. Type A botulism in horses in the United
- 562 States: a review of the past ten years (1998-2008). Journal of Veterinary Diagnostic

563 Investigation 22, 165-73.

Kamphues J 2013. Feed hygiene and related disorders in horses. In: Equine applied and
clinical nutrition (ed. RJ Geor, PA Harris and M Coenen), pp 367-380. Elsevier,
Amsterdam, the Netherlands.

567

Lindburg JE 2013 Feedstuffs for horses In: Equine applied and clinical nutrition (ed. RJ Geor, PA
Harris and M Coenen), pp 319 -331. Elsevier, Amsterdam, the Netherlands.Martinson K,
Coblentz, W and Sheaffer C 2011. The Effect of Harvest Moisture and Bale Wrapping on
Forage Quality, Temperature, and Mold in Orchardgrass Hay. Journal of Equine Veterinary
Science 31, 711-716.

573 McEniry J, Forristal PD and O'Kiely P 2011. Factors influencing the conservation 574 characteristics of baled and precision-chop grass silages. Irish Journal of Agricultural and 575 Food Research 50, 175-188.

576 Merritt AM and Julliand V 2013 Gastrointestinal physiology. In Equine Clinical and Applied 577 nutrition (ed. RJ Geor, PA Harris, and M Coenen), pp 3-32. Elsevier, Amsterdam, the 578 Netherlands.

579 Müller CE, Hultén C, and Gröndahl G 2011. Assessment of hygienic quality of haylage fed to
580 healthy horses. Grass and Forage Science 66, 453-463

581

582 Müller, C.E. 2012. Equine digestion of diets based on haylage harvested at different plant 583 maturities. Animal Feed Science and Technology 177, 65-74.

584 Müller CE, Johansson M, Salomonsson AC and Albihn A 2013. Effect of anaerobic digestion

- 585 residue vs. livestock manure and inorganic fertilizer on the hygienic quality of silage and
- 586 haylage in bales. Grass and Forage Science 69, 74-89
- 587 Neuhaus J, Schrödl W, Shehata AA and Krüger M 2015. Detection of Clostridium botulinum
- 588 in liquid manure and biogas plant wastes. Folia Microbiologica 60, 451-6.

- Potts L, Hinkson J, Graham B, Löest C and Turner J 2010 Nitrogen Retention and Nutrient
  Digestibility in Geldings Fed Grass Hay, Alfalfa Hay, or Alfalfa Cubes. Journal of Equine
  Veterinary Science 30, 330-334.
- Ragnarsson S and Jansson A 2011. A comparison of grass haylage digestibility and metabolic
  plasma profile in Icelandic and Standardbred horses. Journal of animal physiology and
  animal nutrition 95, 273-279.
- Ragnarsson S and Lindberg JE 2010. Nutritional value of mixed grass haylage in Icelandic
  horses. Livestock Science 131, 83-87.
- 597 Riet-Correa F, Rivero R, Odriozola E, Adrien Mde L, Medeiros RM, Schild AL 2013.
  598 Mycotoxicoses of ruminants and horses. Journal Veterinary Diagnostic Investigation 25,
  599 692-708.
- 600
- Ringmark S, Roepstorff L, Essén-Gustavsson B, Revold T, Lindholm A, Hedenström U,
  Rundgren M, Ögren G and Jansson A 2012. Growth, training response and health in
  Standardbred yearlings fed a forage-only diet. Animal 7, 746-753.
- Ringmark S, Lindholm A, Hedenstrom U, Lindinger M, Dahlborn K, Kvart C, and Jansson
  A 2015. Reduced high intensity training distance had no effect on VLa4 but attenuated
  heart rate response in 2-3-year-old Standardbred horses. Acta Veterinaria Scandinavica
  57, 1.
- Ringmark S, Jansson A 2013. Insulin response to feeding forage with varying crude protein
  and amino acid content in horses at rest and after exercise. Comparative Exercise
  Physiology 9, 209-217.
- Ringmark S 2014. A forage-only diet and reduced high intensity training distance in
  Standardbred horses. PhD thesis, Swedish University of Agricultural Sciences, Uppsala,
  Sweden.

614 Sarkijarvi S, Sormunen-Cristian R, Heikkila T, Rinne M and Saastamoinen M 2012. Effect
615 of grass species and cutting time on *in vivo* digestibility of silage by horses and sheep.
616 Livestock Science144, 230-239.

Séguin V, Lemauviel-Lavenant S; Garon D, Bouchart V, Gallard Y, Blanchet B, Diquelou
S, Personeni E, Gauduchon P, Ourry A 2010. Effect of agricultural and environmental
factors on the hay characteristics involved in equine respiratory disease. Agriculture,
Ecosystems and Environment 135, 206-215.

621 Virkajärvi P, Saarijärvi K, Rinne M and Saastamoinen M 2012. Grass physiology and its
622 relation to nutritive value in feeding horses. In Forages and grazing in horse nutrition (ed.
623 MT Saastamoinen, MJ Fradinho, AS Santos and N Miraglia), pp. 17-43. EAAP publication

No. 132. Wageningen Academic Publishers, Wageningen, The Netherlands.

Williams S, Horner J, Orton E, Green M, McMullen S, Mobasheri A and Freeman SL 2015,
Water intake, faecal output and intestinal motility in horses moved from pasture to a stabled
management regime with controlled exercise. Equine Veterinary Journal 47, 96–100.

628 Williams S, Tucker CA, Green M J. and Freeman SL 2011. Investigation of the effect of

629 pasture and stable management on large intestinal motility in the horse, measured using

630 transcutaneous ultrasonography. Equine Veterinary Journal, 43, 93–97.

Wolf P, Siesenop U, Verspohl J and Kamphues J 2014. Hygienic quality of feedstuffs for small
 mammals sent to the consultation service. Tierärztliche Praxis Kleintiere 42,101-106.

Farly cut Early out Late cut gy

635 Table 1. Coefficients of total tract apparent digestibility in Icelandic and Standardbred

	Early cut	Early cut	Late cut	Late cut Standardbred	SE	
	Icelandic	Standardbred	Icelandic			
						-
OM	0.536ª	0.565 <sup>b</sup>	0.431	0.427	0.006	
CP	0.636	0.660	0.478	0.479	0.012	
NDF	0.517	0.536	0.322	0.320	0.008	
ADF	-	-	-	-	-	
Energy	0.517 <sup>a</sup>	0.540 <sup>b</sup>	0.407	0.400	0.007	

636 horses fed the same early and late cut forages (after Ragnarsson and Jansson, 2011)

<sup>a, b,</sup> Values in the same row without common superscripts differ (p<0.05) and indicate breed 637

638 differences.

OM : Organic matter, CP : Crude Protein; NDF : Neutral detergent fibre; ADF : Acid Detergent 639

640 fibre

641

643

644

645 Table 2. Coefficients of total tract apparent digestibility of haylages cut (first cut) at different 646 stages of maturity (after Ragnarsson and Lindberg 2008; Ragnarsson and Lindberg, 2009) 647

	Timothy haylage			Mixed grass haylage						
	Cut 1	Cut 2	Cut 3	Cut 4	SE	Cut 1 Cut 2	2 Cu	t 3 Cu	t 4	SE
DM	0.716 <sup>a</sup>	0.626 <sup>b</sup>	0.513°	0.457 <sup>d</sup>	0.011	0.691ª	0.616 <sup>b</sup>	0.619 <sup>b</sup>	0.556 <sup>c</sup>	0.010
ОМ	0.747 <sup>a</sup>	0.647 <sup>b</sup>	0.527°	0.485°	0.010	0.710 <sup>a</sup>	0.630 <sup>b</sup>	0.640 <sup>b</sup>	0.578°	0.010
CP	0.809 <sup>a</sup>	0.735 <sup>b</sup>	0.642 <sup>c</sup>	0.639°	0.014	0.765ª	0.740 <sup>ab</sup>	0.708 <sup>ab</sup>	0.688 <sup>b</sup>	0.015
NDF	0.770 <sup>a</sup>	0.646 <sup>b</sup>	0.516 <sup>c</sup>	0.440 <sup>d</sup>	0.009	0.717ª	0.584 <sup>bc</sup>	0.594 <sup>b</sup>	0.520 <sup>c</sup>	0.013
ADF	0.746 <sup>a</sup>	0.629 <sup>b</sup>	0.480 <sup>c</sup>	0.400 <sup>d</sup>	0.008	0.685ª	0.545 <sup>b</sup>	0.555 <sup>b</sup>	0.485 <sup>b</sup>	0.017
Energy	0.733ª	0.633 <sup>b</sup>	0.515°	0.468 <sup>c</sup>	0.006	0.670 <sup>a</sup>	0.600 <sup>b</sup>	0.595 <sup>b</sup>	0.560 <sup>b</sup>	0.011

648 <sup>a, b, c,</sup> Values in the same row without common superscript differ (p<0.05).

649 DM : Dry matter; OM : Organic matter, CP : Crude Protein; NDF : Neutral detergent fibre; ADF

650 : Acid Detergent fibre

651

652

	Microorganism	Classification	Category	Species examples
				Flavobacterium
	Aerobic bacteria	Fairly die	1	Pseudomonas
		Epiphytic	I	Xanthomonas
				Erwinia
				Bacillus spp
		Spoilage	2	Staphylococcus
				Micrococcus
			3	Streptomyces spp.
				Verticillium
		Epiphytic	4	Acremonium
			4	Fusarium
				Aurebasidium
	Aerobic moulds			Aspergillus
		Spoilage	5	Penicillium
			5	Scopulariopsis
				Wallemia
			6	<i>Mucor</i> spp.
	Yeasts	Spoilage	7	All species
655				
656				

# Table 3: Classification of microorganisms in feedstuffs according to Kamphues (2013)

# 660 Table 4: Benchmark for microorganisms in feedstuffs (cfu per g feed) according to Kamphues661 (2013)

	Aerob	ic bacteria x	10 <sup>6</sup> cfu, g	I	Moulds x 10 <sup>3</sup> o	cfu, g	10 <sup>3</sup> , g
Category <sup>1</sup>	1	2	3	4	5	6	7
Hay	30	2	0.15	200	100	3	150
Straw	100	2	0.15	200	100	5	400
Grass silage	0.2	0.2	0.01	5	5	5	200
Corn silage	0.4	0.2	0.03	5	5	5	1000

662 <sup>1</sup>Categories; 1: Epiphytic bacteria; 2 and 3: Spoilage indicating bacteria; 4: Epiphytic moulds; 5

663 and 6: Spoilage indicating moulds; 7: yeasts all species

667 Table 5 Some suggested forage related risk factors for gastric ulcers and colic (see also Andrews

668 et al., 2015, Durham, 2013)

Disease	Risk factor	Possible (non-exclusive)	Ref
		explanation	
Gastric	Frequency of forage	Decreased production of	Luthersson et al., 2009
ulcers -	feeding. Intervals >	saliva, slower passage rate	
squamous	6h may increase risk	and reduced buffering	
	of ulcers	capacity in the stomach	
Gastric	Straw as the main	Straw may provide low	Luthersson et al., 2009
ulcers -	forage source in	levels of additional	Wichert <i>et al.,</i> 2008
squamous	horses/ponies may	buffering support (low in	
	increase risk of	protein, low in calcium).	
	ulcers	Straw may create mucosal	
		irritation, and may affect	
		the nature of the fibrous	
		mat within the stomach. In	
		addition, the potential for	
		poor hygienic quality	
		(including increased risk of	
		mycotoxins) may play a	
		role.	
Gastric ulcer	Feeding alfalfa chaff	The glandular lesions could	Fedtke <i>et al.,</i> 2015
- glandular	increased the	be a result of mechanical	
	number of glandular	injury caused by the very	
	ulcers compared to	small particles/physical	

hay or alfalfa pellets properties of the alfalfa in weanlings or adult chaff horses

Colic Reduced intake of Any change in forage Cohen et al., 2000 in \_ general grass\*, limited intake intake will cause changes Hassel et al., 2004 in the microflora in the Hillyer et al., 2002 of forage. Importantly a change in the type hindgut. This may cause Hudson et al., 2001 of forage fed. \* NB increased risk Eq. Change of hay within of dysfermentation, change in Horses with two weeks without motility, adaptation (Tinker et al., duodenitis-proximal and several physiological changes in 1997; Cohen et al.. jejunitis were were the GI tract. A change in 1999). Change between significantly more likely within previous 2 two silages with different hav to have grazed pasture weeks may increase risk of crude protein content control than the colic between 4.9 to 10 (Muhonen et al., 2008). population (Cohen et times. Change from hay to al., 2006) haylage or silage from the botanical origin same (Muhonen et al., 2009).

Little and Blikslager 2002

Colic Feeding costal Indigestible fibers have Bermuda hay reduced water holding and impaction capacity, releasing can changes in cause the microflora and can cause altered motility

#### 670 Figures

Figure 1. A very schematic overview giving some definitions and characteristics of hay, haylage 671 672 and silage (WSC=water soluble carbohydrates) made without additives. It is important to note 673 that DM content (and water activity) will determine the potential for fermentation so are linked with 674 lactate production. In general as lactate starts to be produced the WSC and pH will start to 675 reduce, although other factors can influence pH (including buffer capacity). The WSC of any 676 preserved crop will be lower than the fresh forage it originated from due to respiration (plant and 677 microbial) post harvesting but the WSC content of haylage compared with the hay from the same 678 fresh pasture will depend on whether there have been any lactic acid (or VFA production) If there 679 is virtually no lactic acid (or VFA) production, then there will be no decrease in total WSC in 680 haylage compared to hay (Muhonen et al 2009).

681

Figure 2. A schematic figure on how minimum required air humidity (relative humidity, RH) for
mould growth varies with ambient temperature (adapted from Lehmann, 1971).

684 Figure 3. The relationship between airtight stored grass forage dry matter (DM) and lactate 685 content. At DM contents higher than 50 % (dotted line) the lactate content is very low. Data from 686 Wilkinson et al., 1976 (3 observations, 16- 45%DM); Müller, 2007 (10 observations 29-68%DM); 687 Muhonen, 2008 (8 observations, 36-81%DM); Ragnarsson, 2009 (8 observations, 36-81%) and 688 Sarkijärvi et al., 2012 (6 observations, 36-55%DM). All observations are from grass forage 689 preserved in wrapped bales except for Wilkinson et al., 1976 which were in silos. Forages were 690 preserved without additives except 6 observations, with lactic acid inoculant, from Muhonen, 2008 691 and formic acid inoculant in the Sarkijärvi et al., 2012 observations. Data fits (R<sup>2</sup>=0.84) the 692 equation:  $y = 0.0406x^2 - 5.4306x + 179.3$  and the equation is relevant in the interval 16-70 % DM.

Figure 4. A schematic illustration (not to scale) of the maturation of plants and the effect on dry matter content (NB in Southern European countries the increase in DM is more dramatic than for Northern Europe as illustrated by the two lines), digestible plus metabolizable (ME) and Net (NE) energy plus digestible protein content on a wet matter (WM) basis. The extent of such changes may be affected by environmental conditions as well as management practices.