Effects of feeding different roughage-based diets on gastric mucosa after weaning in warmblood foals

Alexandra Fedtke1, Matthias Pfaff2, Jan Volquardsen3, Monica Venner1 and Ingrid Vervuert4

1 Equine Clinic, Destedt, Germany
2 Equine Clinic Ankum, Germany
3 Equine Clinic Bönsen, Germany
4 Institute of Animal Nutrition, Nutrition Diseases and Dieterics, Faculty of Veterinary Medicine, University of Leipzig, Germany

Summary: Equine gastric mucosal ulcer syndrome (EGUS) is a well-known disease entity in equine medicine. Many studies have evaluated this disorder, by investigating prevalence, etiology and treatment. The effect of feeding different roughage-based diets to weanling foals has not been examined to date. In this study, 89 weanlings aged between 172–174 days were randomly allocated to two feeding trials. Trial 1 consisted of three groups: Group 1 alfalfa chaff (group 1), hay (Group 2) and Group 3: a total mixed ration (TMR). There were 20 foals in each group. Trial 2 consisted of two groups: Group 4: molassed alfalfa chaff and Group 5: hay. There were 19 foals in each group. Gastroscopic examination was performed immediately before weaning and after a feeding period of 15–16 days. In the first part of the study (or trial 1) the prevalence of gastric mucosal lesion was 44% with a low severity score (median 0; 25/75% percentile: 0/0) before weaning. There was a significant increase in the number of lesions in the Pars nonglandularis of the Curvatura minor in all groups of trial 1 post weaning. The group 1 (alfalfa chaff) exhibited significantly more mucosal lesions at the pylorus compared to group 2 (hay) and 3 (TMR). Significant healing of gastric ulcers was seen in the hay and TMR group. Results of the second part of the study (trial 2) indicated a prevalence of 86% before weaning increasing to 97% after the feeding period. There was an increase in the number of lesions at the Curvatura major and minor of both Pars nonglandularis and Pars glandularis in both groups (hay and alfalfa chaff). A significantly higher number of lesions at the pylorus were seen in the molassed alfalfa chaff group only. Despite the high number and severity score of the lesions none of the weanlings showed clinical signs associated with a gastric disorder.

Keywords: EGUS / feeding / alfalfa chaff / weaning / foal / nutrition

Introduction

Gastric mucosal ulcers are a common disease entity in race, sport and leisure horses as well as in weanlings. The combination of clinical signs like colic, slow eating, poor body condition, bruxism or depressed behavior and gastric mucosal lesions found at gastroscopy are summarized as equine gastric mucosal ulcer syndrome (EGUS) (Schusser et al. 2006, May et al. 2012). A prevalence of 78% was detected in Thoroughbred horses in training, showing no clinical signs. However, horses showing clinical signs had up to 100% gastric mucosal ulceration (Murray et al. 1989). Andrews et al. (1999) found a prevalence of EGUS in foals between 30 and 50% showing clinical signs of gastrointestinal discomfort. Dahlkamp et al. (2012) obtained similar results of prevalence but in clinically normal weanlings.

The following factors are implicated in the development of superficial or ulcerative gastric mucosal lesions: nonsteroidal anti-inflammatory drugs, diets containing high amounts of grain, strenuous exercise and stress due to transport or starvation periods of 24 h as well as stall confinement (Murray 1994, Murray and Eichorn 1996, Andrews et al. 1999, Murray and Grady 2002). Horses have a continuous gastric acid secretion of 1.5 l containing 40 to 60 mmol hydrochloric acid (HCl) per hour (Picavet 2002) but buffering saliva production starts only with feed intake (Alexander 1966). Due to the lack of a protective mucus layer, the non-glandular portion is vulnerable to a prolonged exposure to HCl. The membrane potential of the Pars nonglandularis cells decreases which results in an impairment of the sodium transport system. Consequently an osmotic influx of water into the cells leads to swelling and degradation (Carney et al. 1981, Argenzio et al. 1999). Furthermore, periods of starvation result in an increased concentration of bile acids through gastrooduodenal reflux. Within this acid milieu in the stomach, bile acids are not dissociated and due to their lipophilic property they penetrate the gastric mucosa and damage ion transport systems of the epithelium (Argenzio 1999). As a consequence the mucosa becomes more permeable for hydrogen ions (Bernscheider et al. 1999). In addition, short chain fatty acids (SCFA) such as butyrate, acetate and propionic acid are produced by bacterial fermentation after the intake of high starch diets. SCFA act in a synergistic way with hydrochloric acid (HCl) by producing mucosal injuries as well as by interacting with the sodium transport system and therefore weaken the mucosal barrier (Nadeau et al. 2003).

Besides optimizing feeding and housing management, the treatment with pharmaceutical drugs such as omeprazole reduces gastric acid production, resulting in gastric pH values above 4 (Birkmann et al. 2014). Other drugs like sucralfate improve the prostaglandin E (PGE) synthesis, by stimulating the bicarbonate and mucus secretion and impeding hydrochloric acid release (Bell et al. 2007). With regard to feeding practices, a high forage intake and a low starch intake are
strongly recommended. For example, hay or grass intake during 12 to 16 hours per day may improve gastric mucosa health as constant saliva flow will buffer gastric pH up to 4 as reviewed by Vervuert and Coenen (2002). In order to offset the problems associated with the rapid consumption and episodic feeding of concentrate it is common practice to recommend that chopped roughage be mixed with the concentrate or, that roughage (grass meal or hay) be fed before the concentrate. In several studies a protective effect of feeding alfalfa hay to horses has been reported. The beneficial effect of feeding alfalfa hay appeared to be related to an increase in gastric pH above 4. This is due to the buffering properties of calcium and protein as well as the higher production of acetate, propionate and isovalerate in the stomach content (Haaland et al. 1982, Nadeau et al. 2000, Lybbert 2007).

The aim of the present study was to investigate the effects of feeding alfalfa chaff on gastric mucosa in weanlings. We hypothesized that feeding a high proportion of alfalfa chaff would improve gastric mucosa in foals similarly to the results obtained in adult horses when fed with alfalfa hay.

Materials and Methods

Study design

89 warmblood weanlings were included in a blinded control study consisting of two consecutive feed trials. The first trial (October to November 2011) consisted of three groups, with 20 weanlings in each group. The second trial (November to December 2011) consisted of two groups with 19 weanlings in each group. In both trials a gastroscopic examination was performed before weaning and again after the feeding period of 15–16 days.

Animals

On one horse breeding farm, 89 warmblood foals (41 females, 48 males) were included in this study. All foals were born there and kept in the same environment until after weaning. In Trial 1, foals were aged 174 ± 4.75 days (alfalfa group), 172 ± 5.99 days (TMR group) and 173 ± 4.65 days (hay group) at weaning and in the second trial between 181 ± 7.93 (molassed alfalfa group) and 186 ± 9.25 (hay group) days without any significant differences in the age of the foals between groups and trials. One week prior to the study, the foals were maintained in groups of 10 to 30 mares and foals in barns (27 × 9 m²) with free access to a paddock (17 × 9 m²). In the barns, the animals were bedded on straw. Weaning was performed on the same day for all foals of each trial respectively. Mares and foals were separated from each other and housed in different locations on the stud farm. Before and after the weaning procedure the foals remained within the same barns until the study was finished.

Feeding

Prior to the start of the trials, each mare and foal received a daily total mixed ration (TMR) of ~9 kg (as fed) offered in long feeding trough hereby providing one feeding place per mare and foal. Hay was provided ad libitum in hayracks. The TMR was divided into a morning and evening feed. The rations were formulated to meet or to exceed the mares' energy and nutrient requirement. The feed consisted of 3 kg of corn silage, 3 kg of grass silage, 2 kg of oat, 0.5 kg of straw, 0.3 kg of soybean meal, 0.05 kg of a commercial mineral vitamin supplement and 0.04 kg of CaCO₃. Water was provided ad libitum. Nutrient analyses of the feedstuffs are shown in Table 2.

After weaning for both trials, the foals were randomly and blindly allocated to one of the following groups:

- **Group 1:** Alfalfa chaff I (n=21): alfalfa chaff (5.5 kg/foal/day) was divided into two meals and fed in the morning and evening. 2.7 kg oats/foal/day mixed with 0.05 kg/foal/day mineral vitamin supplement were provided in three meals. Each kg of the alfalfa chaff was humidified with 0.5 l of water and alfalfa chaff was mixed by hand with the oats. The particle size of the alfalfa chaff is shown in Table 1.
- **Group 2:** TMR (control) (n=16): total mixed ration (TMR) twice daily (9 kg/foal/day) and hay ad libitum. The TMR consisted of 3 kg corn silage, 3 kg of grass silage, 2 kg of oat, 0.5 kg of straw, 0.3 kg of soybean meal, 0.05 kg of a commercial mineral vitamin supplement.
- **Group 3:** Hay I (control) (n=16): hay ad libitum and 2.7 kg oats/foal/day mixed with 0.05 kg/foal/day mineral vitamin supplement were provided into three meals.

The second trial consisted of two groups (n=19 in each group):

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**Table 1**  Percentage (%) of different particle sizes for alfalfa chaff

<table>
<thead>
<tr>
<th>Forage</th>
<th>&gt;4 mm</th>
<th>&gt;2 mm</th>
<th>&gt;1 mm</th>
<th>&gt;0.5 mm</th>
<th>&lt;0.5 mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alfalfa chaff</td>
<td>33.6</td>
<td>11.9</td>
<td>10.6</td>
<td>12.6</td>
<td>36.6</td>
</tr>
<tr>
<td>Molassed alfalfa chaff</td>
<td>40.0</td>
<td>10.4</td>
<td>4.8</td>
<td>4.3</td>
<td>40.1</td>
</tr>
</tbody>
</table>

**Table 2**  Chemical composition of the feeds (g/kg DM)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Hay</th>
<th>Alfalfa chaff</th>
<th>TMR</th>
<th>Oats</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude ash</td>
<td>53</td>
<td>71</td>
<td>59</td>
<td>25</td>
</tr>
<tr>
<td>Crude protein</td>
<td>93</td>
<td>150</td>
<td>120</td>
<td>142</td>
</tr>
<tr>
<td>Crude fiber</td>
<td>290</td>
<td>412</td>
<td>231</td>
<td>115</td>
</tr>
<tr>
<td>Crude fat</td>
<td>24</td>
<td>21</td>
<td>33</td>
<td>47</td>
</tr>
</tbody>
</table>
• Group 4: Molassed alfalfa chaff (n=18): molassed alfalfa chaff (5.5 kg/weanling/day) twice daily. 0.05 kg/weanling/day mineral vitamin supplement was combined with 2.7 kg oats/weanling/day and provided three times a day.
• Group 5: Hay II (control) (n=18): hay ad libitum as well as 2.7 kg oats/weanling/day mixed with 0.05 kg/weanling/day mineral vitamin supplement provided three times a day.

The foals in each group and in each trial were housed together in the same barns (27 x 9 m²) prior to weaning until the end of the study with free access to a paddock (17 x 9 m²) and bedded on straw. All weanlings had free access to water at all times and received the respective rations for 16 days. Feed intake was monitored by one trained person and any leftovers were collected and re-weighed to measure the daily feed intake. Feedstuffs were weighed using an electronic scale (MAUL parcel 50, Jakob Maul GmbH, Bad König, Germany) with a precision of measurements of 10 g.

Gastroscopy

Gastroscopy was performed 3 to 4 days prior to weaning and after 15 to 16 days of the feeding period in the first trial and after 15 days in the second trial respectively. Before gastrosopic examination, milk and feed were withheld for 10 hours. Before gastroscopy all foals underwent a clinical examination. The foals were sedated with 0.02 mg per kg BW iv detomidinhydrochloride (Domosedan®, Elanco Animal Health, Bad Homburg, Germany). A 3.25 m flexible video endoscope (Karl Storz GmbH & Co. KG, Tuttingen) was connected to a monitor (Gastro Pack® Karl Storz GmbH & Co. KG, Tuttingen) and a light source was used.

Eight separate regions of the stomach were evaluated: Saccus caecus ventriculi, Pars nonglandularis and Pars glandularis of the Curvatura major, Pars nonglandularis and Pars glandularis of the Curvatura minor, the pylorus in combination with the antrum pyloricum and the proximal part of the duodenum. Gastric mucosa was graded blindly by an experienced investigator (M.V.). The scoring system (Score 0 to 4) validated by Andrews et al. (1999) for each gastric region was used.

Body weight

An electronic weigh scale was used to measure body weight (Firma Müller, Jagstzell, Germany) before the first and second gastroscopic examination.

Statistics

Body weight (BW) was expressed as the mean ± SD or as a percentage of BW. The stomach scores were presented as the median, 25/75% percentile, minimum and maximum. Data analysis was performed using a statistical software program (STATISTIKA, StatSoft). For BW was normally distributed using Shapiro-Wilk W test. The Wilcoxon signed rank test was used to compare the differences within a group before and after weaning. Kruskal-Wallis ANOVA was performed to compare the different treatment groups. Statistical significance was established at p < 0.05.

Results Trial 1

Body weight

The mean body weight prior to weaning was 252 ± 26.5 kg in the hay group, 252 ± 21.9 kg in the TMR group and 251 ± 19.2 kg in the alfalfa chaff group. At the end of the trial no significant changes in body weight were measured (hay 248 ± 26.9 kg; TMR 258 ± 21.3 kg; alfalfa chaff 254 ± 20.3 kg, feeding p > 0.05). The mean daily dry matter intake (DMI) in each weanling was 7.2 ± 0.02 kg of TMR, 5.0 ± 0.03 kg feeding hay I and 4.87 ± 0.98 kg feeding alfalfa chaff, respectively. The DMI feeding TMR was significantly higher than feeding hay or alfalfa chaff (p < 0.05).

Gastric lesion scores

Before weaning, the prevalence of gastric mucosa lesions (≥ one lesion considering all locations in the stomach) was 44%, but with a very low severity score (median 0, 25/75% percentile: 0/0). None of the foals showed any clinical signs associated with gastric mucosa lesions, such as poor body condition, colic, slow eating or depression. After weaning, the prevalence of gastric mucosa lesions (≥ one lesion considering all locations in the stomach) increased significantly up to 93%. Weaning and feeding did not significantly affect gastric mucosa of the Curvatura major of the Pars nonglandularis and Pars glandularis (Figure 1). Gastric mucosa lesions increased significantly in the Pars nonglandularis of the Curvatura minor from a median score of 0 (25/75 percentile: 0/0) before weaning to a median score of 2 (25/75 percentile: 0–1/2) after weaning for feeding hay or TMR (Figure 2) or to a median score of 3 (25/75 percentile: 2/4) for feeding alfalfa chaff I (Figure 2, weaning and feeding p < 0.05). In the group of foals fed with alfalfa chaff the pylorus showed a median score of 0 (25/75 percentile: 0/0.5) before weaning and a median score of 2 (25/75 percentile: 2/2) after weaning (weaning p < 0.05, Figure 3). Significant lower gastric mucosa lesions around the pylorus were found in the group fed with hay (median score of 0, 25/75% percentile: 0/1, Figure 3) and the TMR-group (median score of 0, 25/75% percentile: 0/0, Figure 3) after weaning compared to the group fed with alfalfa chaff (feeding p < 0.05). No gastric mucosa lesions were found in the Saccus caecus or in the proximal duodenum before and after weaning (data not shown) in the different feeding regimes.

Trial 2

Body weight

Before weaning, the mean body weight of the foals in the group fed with molassed alfalfa was 240 ± 22.9 kg and at the end of the study 254 ± 24.2 kg. Weanlings of the hay group had a body weight of 257 ± 25.8 kg before weaning and of 260 ± 25.1 kg at the end of the study (treatment p > 0.05). The mean daily dry matter intake (DMI) in each weanling was
6.3 kg ± 1.17 kg in the molassed alfalfa chaff group and 5.2 ± 0.03 kg in the hay group respectively.

**Gastric lesion scores**

Before weaning, the prevalence of gastric mucosal lesions (zone lesion considering all locations in the stomach) was 86%, but with a very low severity score (median 0, 25/75 percentile: 1/0). None of the foals showed any clinical signs that are commonly related to the presence of gastric mucosal lesions, such as poor body condition, colic, slow eating or depression.

After weaning, the prevalence of gastric mucosal lesions (zone lesion considering all locations in the stomach) increased significantly up to 97%. After weaning gastric mucosal lesions of the Curvatura major of the Pars nonglandularis and Pars glandularis increased numerically in the molassed alfalfa chaff group (median 1, 25/75% percentile 0/2, Figure 1, p = 0.06), whereas similar gastric mucosal lesions scores were recorded for hay II before (median 1, 25/75% percentile 0/1) and after weaning (median 1, 25/75% percentile 0/2, Figure 1). Gastric mucosal lesions increased slightly in the Pars nonglandularis of the Curvatura minor after weaning in both diet groups (Figure 2, weaning p = 0.06). Feeding molassed alfalfa chaff significantly induced gastric mucosal lesions in the pylorus from a median score of 0 (25/75 percentile: 0/0) before weaning to a median score of 1 (25/75 percentile: 1/1) after weaning (weaning p < 0.05). No pylorus findings were monitored in hay group before and after weaning (feeding p < 0.05). No gastric mucosa lesions were found in the Saccus caecus or in the proximal duodenum before and after weaning (data not shown) for both diets.

**Discussion**

In both trials there was a high prevalence of gastric mucosal lesions (first trial: 44%, second trial: 86%) but with a low severity score already before weaning. Gastric mucosal lesions increased significantly after weaning up to 93% or 97% in the first trial and in the second trial respectively. In earlier studies a lower prevalence of gastric mucosal lesions had been reported. Murray (1989) showed a prevalence of 9% (foals aged 3–10 months), 30% in foals aged 70–85 days (Murray 1999) and 41–55% in foals with an age of 148–237 days (Dahlkamp et al. 2012). After weaning most gastric mucosal lesions were found in the Pars glandularis and the pylorus especially in the group fed alfalfa chaff.

In the first trial, one limitation of the study was the reduced feed intake of the alfalfa chaff during the first three days after weaning compared to hay or TMR group. In consequence the ratio between roughage and concentrate was different between the various feeding groups. Hay intake is linked to liquid gastric chyme content due to a high saliva production and the liquid chyme enters the stomach in a rapid manner (Meyer et al. 1980). In contrast dry chyme content after concentrate intake is associated with a longer retention time in the stomach (Meyer et al. 1980, Coenen 1990, Murray and Schusser 1993). A prolonged gastric emptying seemed to be related to a higher secretion of hydrochloric acid and a more intensive bacterial fermentation (Argenzio 1974, de Fombelle et al. 2003). Bacterial fermentation results in the production of short chain fatty acids (SFCA) which are able to penetrate the lipophilic mucosa resulting in cell swelling, blockage of intracellular sodium transport system and damage of these epithelial cells (Nadeau et al. 2003), especially under the conditions of a low gastric pH. Furthermore, a decreased saliva production by a low forage intake is associated with a lowered buffering function in the stomach (review Vervuert
and Coenen 2004). However, the weanlings of the second trial had an increased dry matter intake when fed the molassed alfalfa chaff than feeding hay, which attenuates the limitation of the first trial.

Alfalfa hay is reported to have a protective effect on gastric mucosa by buffering gastric pH due to its high contents of calcium, magnesium and protein. Feeding alfalfa hay combined with grain to six adult horses over a period of 14 days increased pH in gastric juice over 2 to 5 hours after feeding (Nadeau et al. 2000). Furthermore the number and severity of gastric mucosal lesions located in the Pars nonglandularis were significantly lower compared to horses fed bromegrass hay (Nadeau et al. 2000). It is speculated that the protective effect of alfalfa hay on the stomach mucosa is caused by the higher calcium and protein content, which may have a buffering effect (Nadeau et al. 2000). Lybbert (2007) published similar results in 24 yearlings.

In contrast to these studies a superior effect by feeding alfalfa chaff on stomach mucosa in weanlings was not observed in our study. In fact, a significantly higher lesion score in the pyloric region was detected in the alfalfa chaff and molassed alfalfa chaff groups when compared to the other feeding groups. A possible explanation for the changes at the pylorus might be the particle size of the alfalfa chaff. Studies in pigs showed that feeding diets with a small particle size are associated with esophagogastric ulcers (Mahan et al. 1966) and gastric ulcers (Mößeler et al. 2012). In pigs Mößeler et al. (2010) fed four different diets with different grinding intensities and different physical forms (pelleted against meal). Feeding a finely ground and pelleted diet resulted in a liquid gastric chyme with similar pH values for the different regions of the stomach. In contrast, a coarsely ground diet caused a stratification of the gastric chyme with large pH differences for the different regions. However, finely ground diets were associated with more severe stomach ulcers in the Pars nonglandularis than feeding coarsely ground unpelleted diets (Mößeler et al. 2012). From the results obtained in pigs we speculate that particle size is also an important issue in gastric mucosa health in the horse, but from our study it is still not clear whether the fine or the harsh, acanthous structure of alfalfa chaff may have an impact on gastric mucosa. In an ongoing experiment, foals fed alfalfa chaff had significantly higher gastric mucosa lesions especially at the pylorus, than feeding finely ground alfalfa pellets (Vondran et al., under revision). Therefore we assume that alfalfa chaff induced gastric mucosa lesions probably due to mechanical injury to the mucosa as the pyloric region is an area of the stomach with high motility.

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Animal welfare statement

The project was approved by the Ethics Committee for the Animal Rights Protection of the District Government, in accordance with the German legislation for animal rights and welfare (A07/10).

References

Lybbert T. C. (2007) Gastric ulcer syndrome in exercising horses fed different types of hay. Texas A & M University, Master Thesis
Erweiterte Zusammenfassung

Effekte verschiedener Raufutterzulagen auf die Magenschleimhaut nach dem Absetzen bei Warmblutfohlen


Im ersten und zweiten Studienabschnitt kam es zu keiner statistisch signifikanten Änderung der Körpermasse der Absetzer in Abhängigkeit der Fütterung. Im Mittel nahmen die Absetzer der TMR Gruppe um 2,8% und die der Luzerne Gruppe um 1,1% an Gewicht zu. Die Heugruppe hingegen verzeichnete eine Körpergewichtsabnahme im Mittel um 1,3%. Die mittlere Trockenmassenaufnahme lag mit 7,2 ± 0,02 kg pro Absetzer in der TMR Gruppe statistisch signifikant höher im Vergleich zu 5,0 ± 0,03 kg in der Heugruppe und 4,87 ± 1,0 kg in der Luzernehälckselgruppe. Die Prävalenz der Magenulzera im
ersten Teil lag bereits vor dem Absetzen bei 44%, und stieg nach dem Absetzen auf 93%. Besonders die Pars nonglandularis der Curvatura minor zeigte bei der TMR- und Heugruppe nach dem Absetzen Läsionen der Magenschleimhaut. Der Pylorus wies in der Luzernehäckselgruppe bei signifikant mehr Fohlen Läsionen (Score Median: 0, 25/75% Perzentil: 2/2) auf im Vergleich zur TMR- (Score Median: 0, 25/75% Perzentil: 0/0) oder Heufütterung (Score Median: 0, 25/75% Perzentil: 0/1).

Im zweiten Studienabschnitt war eine mittlere Trockenmasseaufnahme pro Absetzer mit 5,2 kg ± 0,03 kg in der Heugruppe und mit 6,3 ± 1,17 kg in der melassierten Luzernehäckselgruppe zu beobachten. Es wurden bei 86% der Fohlen vor dem Absetzen Magenläsionen festgestellt. 15 Tage nach dem Absetzen zeigte sich ein Anstieg auf 97%. Die Beurteilung des Pylorus ergab statistisch signifikant mehr Läsionen in der melassierten Luzernehäckselgruppe (Score Median: 1, 25/75% Perzentil: 1/1) verglichen mit einer befundfreien Heugruppe.


Schlüsselwörter: EGUS / Fütterung / Luzerne-Häcksel / Absetzen / Fohlen