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Effects of feeding alfalfa hay in comparison to meadow hay on the gastric mucosa in adult Warmblood horses

Viola Bäuerlein[1], Christin Sabban[2], Monica Venner[1] and Ingrid Vervuert[3]

[1] Equine Clinic Destedt GmbH, Germany
[2] Equine Clinic, University of Veterinary Medicine Hanover, Germany
[3] Institute of Animal Nutrition, Nutrition Diseases and Dietetics, Faculty of Veterinary Medicine, University of Leipzig, Germany

Summary: Equine gastric ulcer syndrome (EGUS) is a common health problem in horses and foals. Beside the importance of forage feeding for the gastric mucosa, results of studies targeting the specific effect of alfalfa on different gastric mucosal regions are controversial. The objectives of this study were to determine the influence of the two forage types (1) meadow hay and (2) alfalfa hay with similar fiber length on different gastric mucosal regions in order to further clarify the effect of alfalfa hay on the equine gastric mucosa. It was hypothesized that feeding alfalfa hay will impair mucosal integrity of the pylorus and antrum pyloricum but may be a benefit for the squamous regions. In a randomized blinded prospective study with a cross-over design, 10 healthy adult warmblood horses were separated randomly into two groups each with five horses. Both groups were fed with 2 kg/100 kg BW/animal/day alfalfa hay or meadow hay in two feeding periods each 21 days long. A wash out period of 21 days was conducted between the feeding periods. Before and after feeding periods body condition score (BCS), body weight (BW), clinical health status, feces pH and particle size and gastroscopic findings were determined. Each region of the gastric mucosa was assessed and scored separately. Data such as BW, fecal pH and fecal particle size were analyzed for normal distribution by the Shapiro-Wilks test. BCS, gastroscopic findings and fecal pH were subjected to non-parametric statistical tests. Kruskal-Wallis ANOVA and Wilcoxon test were performed to compare the findings in gastric mucosal scores, BCS and fecal pH. The relative change in BW and fecal particle size were analyzed by one-factorial analysis of variance (ANOVA). Data are presented as medians, 25th and 75th percentiles. Relative BW changes and fecal particle size are given as means ± standard deviation (SD). Statistical significance was set at $p < 0.05$. Before starting the feeding trial, the prevalence of overall gastric mucosal lesions was 100% with a median severity score of 1. After the feeding period, the median lesion score of the lesser curvature of the squamous region decreased significantly from a median of 1 to a median of 0 in the alfalfa group. The median score of 0 remained constant before and after feeding the meadow hay. Findings in the pyloric region did not differ significantly between the two feeding groups. We concluded that feeding alfalfa hay seems to have no detrimental effects on the mucosal integrity at the squamous gastric region in healthy adult horses. With respect to the glandular area, feeding alfalfa hay appears to be noncritical in healthy adult horses. Further studies are needed to investigate the effects of feeding alfalfa hay in horses suffering from severe mucosal lesions at the antrum pyloricum or pylorus. Furthermore, the longer fecal particle size in horses fed alfalfa hay needs further elucidation.

Keywords: EGUS, gastric ulcer, forage, lucerne, fiber length, fecal particle size

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Correspondence: Viola Bäuerlein, Pferdeklinik Destedt GmbH, Trift 4, 38162 Destedt; viola.baeuerlein@googlemail.com

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Introduction

Equine gastric ulcer syndrome (EGUS) is a common disease in the horse (*Sykes* and *Jokisalo* 2014). More specifically it is differentiated by the European College of Equine Internal Medicine Consensus statement into Equine Squamous Gastric Disease (ESGD) and Equine Glandular Gastric Disease (EGGD), depending on the anatomical localization of the mucosal lesions (*Sykes* et al. 2015).

Clearly there is a role of nutrition in induction and maintenance of squamous gastric ulcers (*Andrews* et al. 2015). In general, for prevention of EGUS/EGSD, a minimum dry matter (DM) intake of ≥ 1.5% – 2% BW (body weight) (*Andrews* et al. 2015, *Harris* et al. 2017) with an absolute minimum recommendation of 1.25% DM intake of BW (*Harris* et al. 2017) and avoiding long periods of fasting are recommended (*Vervuert* and *Coenen* 2004, *Andrews* et al. 2015). High forage diets increase chewing activity which is linked to the production of more saliva (*Nadeau* and *Andrews* 2009). However little evidence exists for the exact role of diet (*Andrews* et al. 2015, *Sykes* et. al 2015) as well as for the nutritional management in glandular gastric disease (*Andrews* et al. 2015, *Sykes* et al. 2015). Nevertheless, it has been suggested that diet might influence the risk for EGGD in a manner similar to EGUS (*Sykes* and *Jokisalo* 2015) and that the principles for feeding might be the same for horses with squamous and/or glandular disease (*Andrews* et al. 2015).

Besides the importance of forage feeding, there are different studies targeting the specific role of alfalfa in equine gastric

health. Alfalfa contains large amounts of beneficial buffering components like calcium and protein (*Cuddeford* 1994). Previous studies concluded that alfalfa may be useful for control and prevention of squamous gastric ulcers (*Nadeau* et al. 2000, *Lybbert* et al. 2007, *Vondran* et al. 2017). In contrast, alfalfa chaff has been shown to induce gastric mucosal lesions at the pylorus and/or antrum pyloricum in different age groups probably due to mechanical injury (*Fedtke* et al. 2015, *Vondran* et al. 2017) and particle size (*Vondran* et al. 2016).

The aim of the present study was to evaluate the effects of feeding alfalfa hay compared to meadow hay on the gastric mucosa in ten healthy adult horses in order to clarify the controversial role of alfalfa in equine gastric mucosal health. For that reason, the gastroscopic findings of each gastric region were scored separately.

We hypothesize that feeding alfalfa hay compared to meadow hay has a different influence on each gastric region. In particular, we hypothesize that feeding alfalfa hay will potentially impair mucosal integrity of the pylorus and/or antrum pyloricum as shown before with alfalfa chaff. The results could help to optimize future feeding regimes and to prepare specific feeding recommendations.

Material and methods

Animals

Between October and December 2018, ten Warmblood geldings aged 8 to 24 years with a mean age of 13.7 ± 4.1 years and a mean body weight (BW) of 625 ± 59 kg were included in the study.

Before the study all horses were kept on pasture. 24 hours before study begin, the geldings were stabled under similar stabling and feeding conditions in single stalls on straw. Each stall contained self-drinking troughs and closed feeders out of shatter-proof plastic for the forage feeding. The horses were turned out on sand paddocks for four hours daily.

The horses had been regularly dewormed and vaccinated. None of the horses showed signs of systemic illness in repeated clinical examinations.

Study Protocol

All examinations took place on the teaching farm of the Faculty of Veterinary Medicine, University of Leipzig. All horses were owned by the Institute of Animal Nutrition, Nutrition Diseases and Dietetics of the Faculty of Veterinary Medicine, University of Leipzig, Germany.

The study was divided into two feeding periods of 21 days each, with a wash out period of 21 days in between. In the wash out period, all subjects were fed meadow hay.

The horses were separated randomly into two groups (1) meadow hay and (2) alfalfa hay with five horses in each group. Both groups were fed daily with a forage amount of 2 kg/100 kg BW/animal/day in the same way and in identical amounts in both parts of the study. During the washout period they received meadow hay ad libitum. The amounts of forage were weighed using the electronic balance Mettler Toledo ID1® (Mettler-Toledo GmbH, Albstadt) with a weighing range of 50 kg and a measurement accuracy of 10 g.

The first part of the study took place from October to November 2018. Five horses were fed with alfalfa hay and five horses were fed with meadow hay. On days 0 and 21 determination of body condition score (BCS) and BW was done and clinical and gastroscopic examination were performed as described below.

The second part was performed from November to December 2018 in a cross over under the same protocol.

The gastroscopic examinations were done after twelve hours of starvation and two hours of water withdrawal. In that period, the straw was removed from the stalls and the horses housed on rubber mats.

The stomach was examined with a 3 m flexible video endoscope 60130 PKS/NKS (Karl Storz GmbH & Co. KG, Tuttlingen). For gastric air insufflation a hand pump and for removing the air the New Aspiret® pump (CA-MI srl, PILASTRO, ITALY) were used. The horses were sedated IV with detomidine hydrochloride (Cepesedan®, CP Pharma, Burgdorf) in a dosage of 0.01 to 0.02 mg/kg BW.

Prior to each gastroscopy horses' feces were sampled to determine pH and particle size. Measurement of fecal pH was done in fresh feces by an electronic device (Dostmann electronic GmbH®, Wertheim-Reicholzheim, Germany). A sample size of 10 g of feces was mixed in a ratio of 1:2 with distilled water. The pH was read after 2 min of equilibration. For the analyses of fecal particle sizes, samples were stored at –20 °C. The fecal particle sizes were determined by wet sieve analysis with sizes between < 1 mm up to 8 mm.

Methods

BW was measured with an electronic scale (Texas Trading GmbH, Windach).

BCS was determined according to the system of *Kienzle* and *Schramme* (2004). There were six body regions (neck, shoulder, chest wall, back and croup, hip and the tail base) assessed by a scale of 1 (cachectic) to 9 (obese). From the values of the six body regions, the arithmetic mean was then calculated and the BCS expressed as a score.

The gastroscopic findings were evaluated during all four gastroscopic examinations by the same two experienced persons who were blinded to the allocation of the horses to the different feeding-groups. The scoring of the squamous mucosa was based on the rating key of *Andrews* et al. (1999) (Table 1) and the evaluation of the glandular mucosal regions according to a score system described by *Vondran* et al. (2016) (Table 2).

Feedstuff analyses

Dry matter (DM) was determined after oven-drying (105 °C) to constant mass. Crude ash was obtained by ashing the feeds in the muffle furnace (6 h, 600 °C). Crude nutrients were assayed by the Weende system. Neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) were analyzed by Fibertec® (Tectator, Rellingen, Germany) (Table 3).

Data analyses

Statistical analysis was performed by a statistical software program STATISTICA® 7.1 (StatSoft GmbH, Hamburg).

Data such as BW, fecal pH and fecal particle size were analyzed for normal distribution by the Shapiro-Wilks test. Data for BW and fecal particle size were normally distributed. BCS, stomach scores and fecal pH were subjected to non-parametric statistical tests.

Kruskal-Wallis ANOVA and Wilcoxon test were performed to compare the findings in gastric mucosa scores, BCS and fecal pH. The relative change in BW and fecal particle size were analyzed by one-factorial analysis of variance (ANOVA). Except for relative BW changes and fecal particle size, data are presented as medians, 25th and 75th percentiles.

Relative BW changes and fecal particle size are given as means ± standard deviation (SD). Statistical significance was set at $p < 0.05$.

Table 1 Scoring of the squamous gastric regions according to the European College of Equine Internal Medicine, based on *Andrews* et al. (1999). | *Bewertung der squamösen Schleimhautregionen gemäß des European College of Equine Internal Medicine, auf Basis des Systems von Andrews et al. (1999).*

Grade	Characteristics
0	Epithelium intact and no appearance of hyperkeratosis
1	Mucosa intact, but areas of hyperkeratosis
2	Small, single or multifocal lesions
3	Large, single or extensive superficial lesions
4	Extensive lesions with areas of apparent deep ulceration

Table 2 Scoring of glandular gastric region designed by *Vondran* et al. (2016). | *Bewertung der glandulären Schleimhaut mithilfe des modifizierten Scores nach Vondran et al. (2016).*

Grade	Characteristics
0	Epithelium intact and no appearance of hyperemia (reddening) or fibrinosuppurative areas
1	Intact flat mucosa, but with small single or multifocal areas of reddening
2	Raised mucosa with large single or multifocal areas of reddening or fibrinosuppurative areas, no signs of bleeding
3	Raised mucosa with hemorrhagic and fibrinosuppurative areas
4	Ridged or depressed mucosa with severe signs of bleeding or with large and distinct fibrinosuppurative areas

Results

Feed intake: There were no feed leftovers throughout the study for both feeding groups.

Body weight/BCS: Mean ± (SD) BW was 631 ± 51.5 kg before feeding meadow hay and 630 ± 55.5 kg before feeding alfalfa hay. BW did not differ significantly between the two feeding groups before study begin ($p = 0.81$). During the feeding trial, the BW of the horses of the alfalfa group increased by 0.6 % (635 ± 55.6 kg) whereas in the meadow hay group the BW decreased by 0.5 % (627 ± 53.4 kg).

There was no significant change in BCS in both groups before and after the feeding trial (Table 4).

Gastroscopic findings

Lesser curvature of the squamous region: Before the feeding trial mucosal lesions were observed with a median score of 1 in the alfalfa group, which decreased to a median score of 0 at the end of the trial ($p < 0.05$). The meadow hay group had a median of 0 in the beginning and at the end of the feeding period (Fig. 1).

Saccus caecus ventriculi: No lesions were seen before or after the feeding period for both forage types.

Table 3 Nutritive values meadow and alfalfa hay per kg/DM in %. *Nutritive Werte von Wiesenheu und Luzerneheu per kg/TM in %.*

Nutritive values	Meadow hay	Alfalfa hay
Dry matter (DM) in %	92.92	92.4
Crude ash	5.08	7.23
Crude protein	9.63	12.4
Crude fiber	31.1	34.0
Crude fat	1.89	1.18
Nitrogen free extracts (NfE)	30.2	24.5
Neutral detergent fiber	64.31	51.79
Acid detergent fiber	33.18	41.36
Acid detergent lignin	3.42	7.61
Calcium	0.33	0.60
Potassium	0.13	2.78
Magnesium	0.15	0.14
Phosphorus	0.00	0.26
Metabolisable energy (MJ/kg DM)	7.61	6.64

Table 4 BCS (n = 10) before and after the feeding of alfalfa hay or meadow hay (median [Min / Max]). | *BCS (n = 10) vor und nach der Fütterung von Luzerneheu und Wiesenheu (median [Min / Max]).*

Ration	Before feeding trial	After feeding trial
Alfalfa hay	5 (3/7)	5 (3/7)
Meadow hay	5 (3/7)	5 (3/7)

Greater curvature of the squamous region: Before starting the first feeding period, one horse in each group showed a lesion of score 1. The other horses showed no gastric findings in this region. There were no changes in both groups and no significant increases in lesion scores during the feeding period.

Greater curvature of the glandular region: Before the first feeding period, two horses in the alfalfa group and one horse in the meadow group presented lesions of a score of 1 with an initial median score of 0 in both groups.

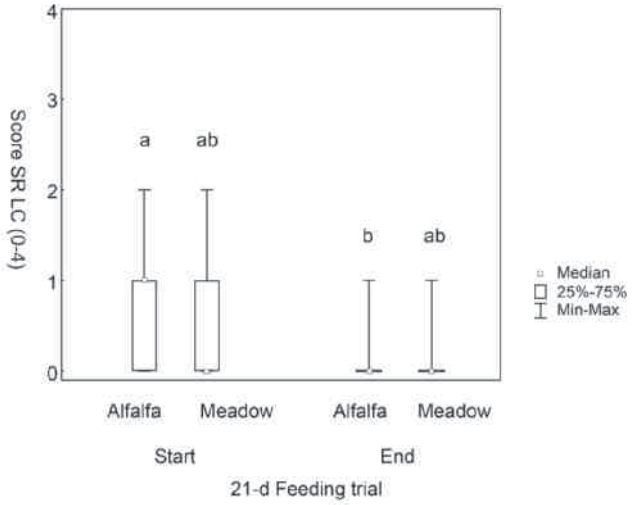

Fig. 1 *Gastroscopic findings of the lesser curvature of the squamous region (median, 25% and 75% percentile) at the beginning and end of the feeding trial. Unequal letters indicate significant effects (n = 10). (SRLC: Squamous Region Lesser Curvature) | Gastroskopische Befunde der squamösen Schleimhaut im Bereich der kleinen Kurvatur (Median, 25% und 75% Perzentil). Vergleich zu Beginn und am Ende der 21-tägigen Fütterung der unterschiedlichen Futterrationen. Ungleiche Kleinbuchstaben kennzeichnen signifikante Effekte mit p < 0,05 innerhalb der Gruppe (n = 10). (SRLC: Squamous Region Lesser Curvature)*

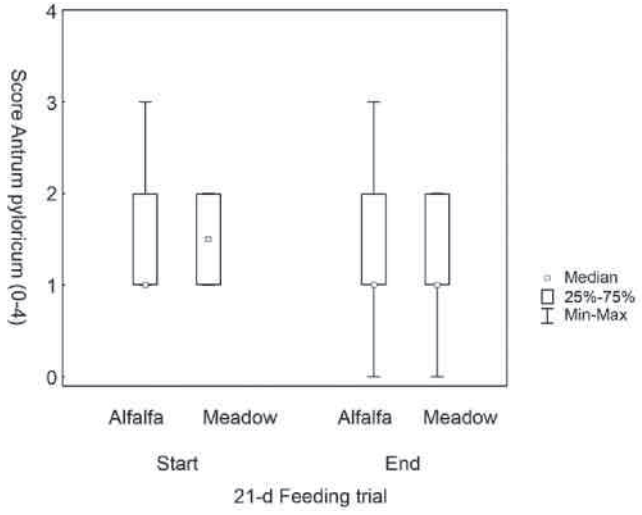

Fig. 2 *Gastroscopic findings of the antrum pyloricum before and after the feeding period (scores: median, 25% and 75% percentile) (n = 10). | Gastroskopische Befunde (Median, 25 % und 75 % Perzentil) im Bereich des Antrum pyloricum. Vergleich zu Beginn und am Ende der 21-tägigen Fütterung der unterschiedlichen Futterrationen (n = 10).*

After the feeding period, no horse in the alfalfa group and only one horse in the meadow hay group still showed lesions with a score of 1. The median lesion score of 0 did not change ($p > 0.05$).

Antrum pyloricum: No change of the median score was observed in the alfalfa group comparing beginning and end of the feeding period with a median score of 1. At the beginning of the feeding period, the meadow hay group had a median score of 1.5. This value decreased to 1 at the end of 21 days of feeding. However, there was no significant difference in lesion score ($p > 0.05$) (Fig. 2).

Pylorus: The median lesion score at the pylorus was 0 in both groups at the beginning of the study. It numerically increased in both groups by 0.5 during the feeding period ($p > 0.05$) (Fig. 3).

Feces: In terms of the fecal particle sizes there were no significant differences between the two feeding groups before the feeding trial. After the feeding period, there was a higher percentage of fecal particles in the 8 mm fraction and a lower percentage of fecal particles in the < 1 mm fraction in the alfalfa group and a higher percentage of fecal particles in the 8 mm fraction in the meadow hay group (Table 5).

The fecal pH of horses fed with meadow hay was 7.0 before and 6.65 after the feeding period ($p > 0.05$). Horses provided alfalfa hay showed a significant increase in pH in the feces from 6.85 to 7.75 after the feeding trial ($p < 0.05$).

Discussion

In the present study we evaluated the effect of different forage types (alfalfa hay and meadow hay) with comparable length on gastric mucosal integrity in ten adult warmblood horses.

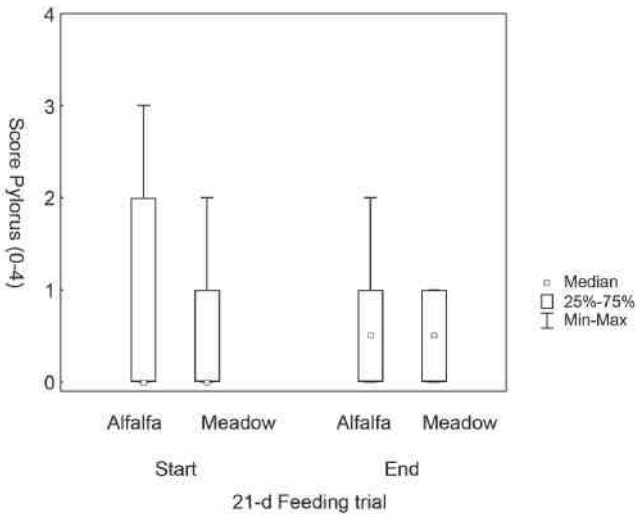

Fig. 3 *Gastroscopic findings of the pylorus (median, 25% and 75% percentile). Comparison at the beginning and at the end of the 21-day feeding (n = 10). | Gastroskopische Befunde (Median, 25 % und 75 % Perzentil) im Bereich des Pylorus. Vergleich zu Beginn und am Ende der 21-tägigen Fütterung der unterschiedlichen Futterrationen (n = 10).*

To ensure standardized experimental conditions, it was necessary to change the feeding and stabling management 24 hours before starting the trial from 24-hours pasture to individual stalls. Horses are evolutionary considered as "creatures of habit", who get fixed on their routines and are disturbed by changes in their daily life (*Mönki* et al. 2016). The management change immediately before our study may have induced the initial prevalence of 100 percent of gastric lesions although only low lesion scores were detected. However, gastric mucosal lesions may have already been present during 24-hour turnout. Even if changes in stabling did not affect acid exposure within either region of the equine stomach (*Husted* et al. 2008), stall confinement alone compared to pasture turnout appears to be an important factor in the development of gastric ulcers, probably as a result of altered eating behavior (*Murray* and *Eichorn* 1996). In future studies a longer adaptation period between changing the stabling conditions and beginning of the study should be considered in order to reduce stressful situations and thus eventually the induction of gastric ulcerations. In the present study, the stressful event of changing the housing status has at least not led to an obviously reduced feed intake. There were no feed leftovers throughout the study period for both feeding groups.

Alfalfa hay has been recommended as a source of forage in previous studies with the intention to prevent gastric mucosal ulcers in horses (*Nadeau* et al. 2000, *Lybbert* et al. 2007). One suspected beneficial component of alfalfa is a recently found alfalfa specific pectic polysaccharide (APPS). It was purified from the stem of alfalfa and was characterized as a rhamnogalacturonan I type pectin. APPS showed significant anti-inflammatory effects against pro-inflammatory cytokine genes, especially IL-1β in lipopolysaccharide-stimulated mouse macrophage cells (*Chen* et al. 2015). The relationship between cytokines expressed by gastric mucosa compared to the histopathology demonstrated that the IL-1β gene expression was detected only in 2/16 foals with evidence of gastritis and gastric ulcerations compared to 0/6 foals without evidence of these gastric diseases (*Mariella* et al. 2013). In adult horses no significant differences were observed for IL-1β mRNA expression with IL-1β found in 3/7 samples of healthy horses and in 9/11 samples of horses with gastritis (*Pietra* et al. 2010). With regard to those findings, further studies are needed to clarify if APPS has anti-ulcerative effects on the equine gastric mucosa due to or besides IL-1ß suppression or whether the suspected beneficial effects of alfalfa are due to other ingredients.

In that context nutrients such as calcium, magnesium and protein may contribute to an increase in gastric pH in horses (*Cuddeford* 1994). In rats, *Fisher* et al. (1990) have shown that hypercalcemia affects basal and histamine-stimulated acid secretion differently, depending on the amount of dietary calcium. In general the buffering effect is not likely due to intragastric pH neutralization but rather due to a higher calcium influx into cells that are involved in gastric acid secretion (parietal, mast, and gastrin G cells). In the parietal cells, for example, calcium is able to reduce cAMP levels and thereby inhibit acid secretion (*Fisher* et al. 1990). In addition hydrogen chloride (HCl) decreases the cellular sodium transport in the equine stomach. Sodium is supposed to play a role in causing cell damage which can be reversed by the addition of calcium carbonate (*Andrews* et al. 2006). On the basis of these studies, it has been hypothesized that calcium may have protective anti-ulcerative effects in the stomach of horses. Other nutrients such as water-soluble carbohydrates (e.g. starch) may impact gastric mucosa by their influence on bacterial fermentation processes and the production of short chain fatty acids (SCFA). High concentrations of SCFA like acetic-, butyric- and propionic acid in combination with an acidic stomach pH of approximately 4 may have a detrimental impact on the squamous gastric mucosa. It has been shown that SCFA decreases the cellular sodium transport (*Nadeau* et al. 2003, *Andrews* et al. 2006) as well as the cellular membrane potential which leads to a restriction of the cellular sodium-potassium-ATPase and mucosal protective barrier (*Nadeau* et al. 2003).

Furthermore next to the buffering properties alfalfa has some beneficial nutritional effects (*Cuddeford* 1994). The apparent digestibility of the organic matter and crude protein of alfalfa was significantly higher than in timothy hay (*Cuddeford* et al. 1992). In our study, the calculated metabolizable energy content was lower for alfalfa hay than for meadow hay. On the other hand the BW increased in horses fed with alfalfa hay, but decreased in horses fed with meadow hay. As the German energy system considers the metabolizable energy of feedstuffs, it can be speculated that the energy evaluation of alfalfa seemed to be underestimated (*Flachowsky* et al. 2014). Under practical feeding conditions in horses there are controversial findings regarding various alfalfa preparations and their impact on the gastric mucosa in different regions of the stomach. After feeding an alfalfa hay-grain diet in adult horses, a significantly higher gastric fluid pH was found during the first five hours after feeding. In addition, lower numbers and lower severity scores of squamous gastric lesions were found

Table 5 Particle distribution (in percent) for the different sieve sizes (in Mw ± SD). (Different lower case letters indicate significant differences with $p < 0.05$ within a row). | *Partikelverteilung (in Prozent) für die unterschiedlichen Siebgrößen (in Mw ± SD). (Unterschiedliche Kleinbuchstaben kennzeichnen signifikante Unterschiede mit $p < 0,05$ innerhalb einer Zeile).*

Sieve size	Meadow hay Before feeding trial	Alfalfa hay After feeding trial	Meadow hay	Alfalfa hay
8 mm	3.41 ± 1.87^{a}	5.65 ± 5.43^{ac}	5.85 ± 3.64^{c}	15.77 ± 7.08^{b}
4 mm	20.56 ± 3.86^{a}	19.30 ± 4.87^{a}	22.38 ± 5.08^{a}	22.80 ± 2.92^{a}
2 mm	20.31 ± 2.54^{a}	21.74 ± 3.99^{a}	18.72 ± 3.02^{a}	21.12 ± 4.29^{a}
1 mm	15.47 ± 2.82^{a}	14.36 ± 2.89^{a}	13.52 ± 2.36^{a}	14.20 ± 3.30^{a}
< 1 mm	40.25 ± 4.05^{a}	38.96 ± 8.73^{a}	39.53 ± 4.84^{a}	26.11 ± 5.33^{b}

Different lower case letters indicate significant differences with $p < 0.05$ within a row

in horses fed alfalfa hay compared to horses fed a bromegrass hay diet (*Nadeau* et al. 2000). The authors concluded that the buffering properties of calcium as well as the protein might play an anti-ulcerative role (*Nadeau* et al. 2000). In yearlings alfalfa hay reduced ulcer severity scores in the squamous region and prevented the development of gastric ulcers in 92% of the foals in comparison to foals fed with coastal bermuda grass hay (*Lybbert* et al. 2007). In the present study, with regard to the squamous region of the lesser curvature, the median lesion score decreased from 1 (0/1) to 0 (0/0) after the feeding trial of alfalfa hay. These beneficial changes might be related to the described buffering ingredients. The meadow hay cannot be evaluated in this context, as neither lesions were present at the beginning nor did they develop in the course of the study.

In contrast to the study of *Lybbert* et al. (2007), where the authors merely referred to the squamous area, other studies found detrimental effects of different alfalfa formulations on the glandular regions. *Nadeau* et al. (2000) detected glandular lesions in only 1/6 of the horses but these authors did not differentiate the exact part of the glandular region. Former studies have demonstrated the impact of diet's particle size on the gastric mucosa in horses and foals. Warmblood weanlings fed with alfalfa chaff and molassed alfalfa chaff exhibited significantly more mucosal lesions at the pylorus compared to a hay-grain diet or a total mixed ratio fed group (*Fedtke* et al. 2015). Mechanical injury due to alfalfa chaff was suspected as an initiator of gastric glandular lesions in young horses (*Fedtke* et al. 2015). *Vondran* et al. (2016) observed higher pyloric ulcer scores in weanlings during the weaning process after feeding more coarse alfalfa chaff compared to alfalfa pellets with a smaller particle size. Because both alfalfa preparations contained similar protein and calcium values, these authors also concluded that the harsh stem might cause mechanical injury (*Vondran* et al. 2016). In consequence the pyloric region might be more susceptible to acidic and other chemical insults. In adult horses fed alfalfa chaff without any stressful events more lesions were found at the antrum pyloricum than in horses fed meadow hay. The median score at the antrum pyloricum raised from 0 to 2 (*Vondran* et al. 2017). These findings emphasize the importance of scoring the glandular and squamous gastric regions separately. In the present study, the meadow hay group showed a slight improvement in the median lesion score in contrast to the alfalfa group with a consistent median lesion score. However, there was no significant difference in lesion score.

One aim of this study was to determine whether feeding the whole alfalfa plant in form of dried hay may result in similar, beneficial findings in the squamous region and detrimental effects at the glandular areas as seen before. However, in the present study, findings on the pylorus and antrum pyloricum did not differ significantly between the two different forage diets meadow hay and alfalfa hay. Therefore our hypothesis, that alfalfa hay might result in more lesions in the pyloric area, was rejected. Interestingly, in our present investigation we found a lower percentage of small fecal particles (< 1 mm) and a higher percentage of fecal particles > 8 mm in horses fed alfalfa hay when compared to meadow hay. It is important to note that the chewing process is exclusively responsible for the degree of grinding of feedstuff, and the fecal particle sizes reflect the grinding process in the oral cavity. A future comparison of the chewing process and the fecal particle sizes between alfalfa hay, meadow hay and alfalfa chaff compared to mucosal lesion findings would be interesting in order to investigate the mechanical effects of the three different forage types on the gastric mucosa. Furthermore, the impact of alfalfa hay on already existing mucosal lesions at the pylorus and antrum pyloricum remains unclear and needs further elucidation.

Conclusion

As alfalfa is a plant which grows under hot conditions, alfalfa might be a feedstuff of interest in equine nutrition. Feeding alfalfa hay seems to have beneficial effects on the mucosal integrity at the squamous gastric region in horses and foals which has been previously reported by others. With respect to the glandular region, feeding alfalfa hay appears to be non-critical in healthy adult horses. These results stand in contrast to feeding alfalfa chaff, as alfalfa chaff seems to have detrimental effects on the antrum pyloricum and pylorus which has been recently published in adult horses and weaned foals. Further studies are necessary to investigate the effects of feeding alfalfa hay in horses suffering of severe mucosal lesions at the antrum pyloricum or pylorus. Furthermore, the longer fecal particle size in horses fed alfalfa hay needs further elucidation. The swallowing of these particles may increase the risk of esophageal obstruction especially in older horses with dental problems.

Statement of conflict of interests

There is no conflict of interest. The authors of the article follow neither financial nor personal interests that may affect the results of the study and the article.

Animal welfare statement

This study was performed at the Institute of Animal Nutrition, Nutrition Diseases and Dietetics of the Faculty of Veterinary Medicine, University of Leipzig in Germany.

All experimental procedures were officially approved by the Leipzig District Government (No. TVV 45/18).

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