# The impact of feeding a high-fibre and high-fat concentrated diet on the recovery of horses suffering from gastric ulcers

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Summary: The objective of this study was to find out whether a change from a grain-rich concentrated feed to a grain-free, fibre- and fatenriched concentrated diet (FFD), leads to an improvement in gastric ulcers in horses. The experiment was conducted as a prospective experimental study. Thirty-four adult horses with gastric lesions – six mares and 28 geldings – fulfilled the inclusion criteria of this study. The average age was  $11\pm 4$  (mean  $\pm$  SD) years and the horses weighed between 358 kg and 697 kg. Twenty horses were fed the FFD, while 14 horses were controls (C) and received their usual concentrated feed. Each horse underwent gastroscopy twice, at an interval of eight weeks. An independent evaluation of the examination and the scoring of the lesions, using the Equine Gastric Ulcer Council 0-4 scoring system for equine gastric ulcer syndrome (EGUS), was performed by an experienced gastroscopist. The horses with EGUS scores ≥3 received additional omeprazole at the recommended dosage for 14 days. The horses of the FFD group were adapted to the new diet whereas the control group received their usual concentrate. In the control group, only the amounts of hay and concentrate were adjusted to the requirement of the respective horse. After the change of the ration, the starch intake (FFD  $0.09 \pm 0.05$  g/kg BW; C  $1.38 \pm 0.62$  g/kg BW) was significantly different. However, there was no difference concerning the hay intake (FFD  $21.2 \pm 3.5$  g/kg BW/day; C  $23.3 \pm 5.1$  g/kg BW/day), the Ca intake (FFD 0.129±0.034g/kg BW/day; C 0.112±0.019g/kg BW/day), the Mg intake (FFD 0.042±0.009g/kg BW/day; C  $0.042 \pm 0.011$  g/kg BW/day) or the intake of the praecaecal digestible protein (FFD  $1.42 \pm 0.38$  g/kg BW/day; C 1.58  $\pm$  0.39 g/kg BW/day). The prevalence and severity of the mucosal lesions significantly decreased in the FFD group (p < 0.001). No significant correlation between the EGUS and the other risk factors, such as workload, behaviour or typical symptoms was found in this study. The change of the concentrated feed to FFD improved the gastric ulcerations in both squamous and glandular mucosa.

Keywords: horse, EGUS, gastric, ulcer, concentrate, feeding, nutrition

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#### Introduction

The gastric mucosal lesions, also known as equine gastric ulcer syndrome (EGUS), occur with a prevalence of 53–93% in horses (*Hammond* et al. 1986, *Vatistas* et al. 1994, *Murray* et al. 1996) and often lead to poor performance by the animal. *Tamzali* et al. (2011) found gastric lesions in 93% of the highlevel endurance horses during the competition season. In a group of 80 military horses in Brazil, 45% suffered from gastric ulcers (*Aranzales* et al. 2012). Another researcher found different degrees of gastric ulceration in 40% of 60 examined horses which did not show any clinical symptoms and in 59% of 48 horses with mild symptoms, like weight loss, poor body condition or reduced appetite (*Niedzwiedz* et al. 2013).

In Canada, a study of 94 thoroughbred horses revealed EGUS in 74.5% of the examined horses (*Marques* et al. 2011). The prevalence of EGUS in foals ranges between 30 and 50% (*Andrews* et al. 1999) particularly in weanlings (*Dahlkamp* et al. 2012). Before weaning, *Dahlkamp* et al. (2012) found gastric ulcers in 38 out of 79 foals they examined. After weaning, the number of affected foals, as well as the severity, increased significantly. A lot of studies have demonstrated the risk factors for ulceration. Equine gastric ulcer syndrome is often related to non-steroidal anti-inflammatory drug administration; but it is also considered to be stress-related, triggered by transport (*McClure* et al. 2005) or increased workload (*Bell* et al. 2007, *Dionne* et al. 2003, *Jonsson* and *Egenvall* 2006, *Tamzali* et al. 2011) and high starch diet (*Nadeau* and *Andrews* 2009, *Luthersson* et al. 2009b).

The management of feeding and diet play important roles in ulcer development. In a population of 201 Danish horses from 23 different stables, *Luthersson* et al. (2009b) diagnosed gastric ulceration in 53% (number of lesions  $\geq 2$  [0-4], severity  $\geq 2$  [0-5]). The described risk factors included the intake of more than 2g of starch per kg body weight (BW)/day or more than 1g of starch per kg BW/meal, straw as a single source of roughage, no water on the pasture, and periods of roughage deprivation of more than six hours (*Luthersson* et al. 2009b).

Equine gastric ulcer syndrome is not always associated with typical symptoms. In previous publications (*Murray* et al. 1989, The Equine Gastric Ulcer Council 1999, *Venner* et al. 2001, *Jonsson* and *Egenvall* 2006, *Murray* 2009, *Hepburn* 2014), a broad spectrum of symptoms was described, including reduced appetite, poor coat, bruxism, yawning, flehmen, recurrent colic, poor performance and body condition, changes in temperament due to pain and discomfort, and a decline in performance.

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Depending on the location of the lesions, Equine Squamous Gastric Disease (ESGD), affecting the squamous mucosa is distinguished from the Equine Glandular Gastric Disease (EGGD), where the lesions are found in the glandular mucosa and the pylorus (*Merritt* 2009). Equine Glandular Gastric Disease is not as well-described (*Sykes* and *Jokisalo* 2015) and has a worse response to medical treatment (*Sykes* et al. 2014a,c, *Sykes* et al. 2015b).

The drug of choice for the treatment of EGUS is omeprazole at a dosage of 4 mg/kg bodyweight once daily for at least 28 days per os (*Andrews* et al. 1999); but also, lower doses of 2 mg/kg bodyweight and combinations with sucralfate have been found to be effective in the recent studies (*Sykes* et al, 2015a,b, *Hepburn* 2014). Scientific work on this subject is also focused on additives such as Saccharomyces cerevisiae and magnesium hydroxide (*Sykes* et al. 2014b), sea buckthorn berries and pulp (*Huff* et al. 2012), feed additives containing salts of organic acids in combination with B-vitamins (*Hellings* and *Larsen* 2014) or a pectin-lecithin complex (*Sanz* et al. 2014) for the treatment of gastric ulcers. The aim of the present study was to find out whether a grain-free diet with low starch and high fibre and fat content enriched with certain herbs can support the healing process of gastric ulcers in horses.

## Animals, material, and methods

Feed

The tested diet (Mucovital prebiotic<sup>®</sup> by Mühldorfer Nutrition AG, Germany) is a grain-free, fibre- and fat-enriched concentrated diet (FFD), which was developed to maintain the digestive health, including gastric health, especially of sensitive horses. According to the producer's claim, it is a complete, premixed feed, which can regenerate the inflamed gastric mucosa through its unique combination of herbal ingredients. Table 1 shows the results of the sieve analysis.

# Composition of the FFD

41.1% alfalfa meal, 10.4% fruit (apple) pomace (dried),10.2% rice bran, 10% alfalfa hay (gently dried), 5.5% lignocellulose, 5.0% sunflower seed kernels, 3.9% linseed extract powder, 2.6% milk thistle oil, 2.6% locust bean (chopped), 2.0% Jerusalem artichoke concentrate, 1.5% cane syrup, 1.0% calcium carbonate, 1.0% beetroot chips, 1.0% apple chips, 0.9% carrots (dried), 0.5% sodium chloride, 0.1% ginkgo leaves, 0.1% haw thorn leaves, 0.1% milk thistle, 0.1% artichoke, 0.1% dandelion. The difference to 100% is the proportion of premixes in the recipe (0.3%).

# Nutritional additives (per kg)

Vitamin A 14.000 I.U., vitamin D3 (E671) 800 I.U., vitamin E ( $\beta$ -tocopheryl-acetate) 205.00 mg, iron (E1, iron (II) sulphate) 140.00 mg, zinc (E6, zinc oxide) 120.00 mg, manganese (E5, manganese-(II)-oxide) 70.00 mg, copper (E4, cupric-(II)-sulphate) 20.00 mg, selenium (E8, sodium selenite) 0.35 mg, iodine (3b202, calcium iodate) 1.40 mg, cobalt (3b304) 0.15 mg, molybdenum (E7, sodium molybdat) 0.28 mg.

Analytical components of the FFD (% of original substance; Futtermittellabor Rosenau, Wieselburg, Austria; values in brackets are according to the label)

Dry matter 91.4%, crude protein 12.5 (12)%, crude fat 8.2 (7.8)%, crude fibre 25 (21.7)%, crude ash 10.1 (8.8)%, N-free extracts 35.6%, starch 4.6%, neutral detergent fibre 55.0%(calculated), calcium 1.97 (1.5)%, phosphorus 0.37 (0.5)%, magnesium 0.24 (0.2)%, potassium 1.3%, sodium 0.09%.

### Digestible energy

9.6 MJ DE/kg (according to the label); Metabolisable energy: 8.58 MJ ME/kg (calculated by the formula: + g crude protein  $\times$  0.0129 + g crude fat  $\times$  0.0420 + g crude fibre  $\times$  0.0019 + g N-free extracts  $\times$  0.0185 -3.54; *Meyer* and *Coenen* 2014)

# Digestible protein

8.70% (according to the label). Praecaecal digestible protein (pcdCP): 8.1% (calculated by the formula: pcdCP (g/kg) =  $16.19 + 0.746 \times g$  crude protein -  $0.051 \times g$  NDF; *Meyer* and *Coenen* 2014)

Table values (*Meyer* and *Coenen* 2014) were used to calculate the energy and nutrient content of hay and oats and for mixed concentrates the values provided by the producers were taken. The concentrates used in the control group during the study period were either grain (mainly oats; only one horse received oats and corn flakes and one horse received oats and oil), or muesli or mash. No pelleted concentrates were used.

The energy requirement (ME) was calculated using the following equations: ME (MJ/d) = 0.52 MJ ME/kg BW<sup>0.75</sup> and 0.4 MJ ME/kg BW<sup>0.75</sup> only for the Friesian horse and the Riding Pony according to the GfE recommendations (Gesell-schaft für Ernährungsphysiologie 2014). The energy requirement calculation included an adjustment for the activity level (no, light, and medium work). The assignment of the ME increments above maintenance (0%, 25% and 50%) to the classifications "no", "light", and "medium" was used.

#### Animals

Thirty-four leisure horses with gastric lesions were selected for the study. They were presented as patients at the Tierklinik Mitterndorf between December 2014 and January 2017, with signs of colic. The horses showed abdominal pain or weight loss. A gastroscopy was performed on each horse for

Table1	Table1         Percentages of different particle sizes of the FFD					
(Futterm	(Futtermittellabor Rosenau, Wieselburg, Austria).   <i>Verteilung der</i>					
Partikelgrößen der fett- und faserreichen Diät (FFD) angegeben in						
Prozent (Futtermittellabor Rosenau, Wieselburg, Austria).						
Diet >5 >4 >3,15 >2 >0.5 <0.5						
FFD	82.2	7.8	4.7	3.5	1.7	0.1

diagnostic reasons. Only mares and geldings between five and 20 years of age were enrolled in this study. During the fasting period before the gastroscopy, the horses were stabled in 3.3 x 4 m boxes with wood shavings as bedding. The horses had an average age of 11 years and an average body weight of 523 kg. There were six mares and 28 geldings of different breeds represented as follows: 23 Warmblood horses, five Quarter Horses, two Lipizzans, one Trotter, one Noriker, one Friesian horse and one German Riding Pony.

## Feeding and housing

A precise survey including the anamnesis, in particularly of feeding and keeping, was carried out by using standardized questionnaires. Only horses which received hay of adequate quality and quantity (at least 1.5 kg/100 kg bodyweight) as a source of roughage were recorded. The owners had to weigh the amount of hay given per day. They also had to bring a sample and the colour, the smell, the structure, and any contamination of the hay were evaluated and classified following a scoring scale (Kamphues et al. 2014). The horses also had to be fed a concentrated feed before the start of the study. All participating horses stayed in boxes with straw (n = 21) or wood shavings (n = 13) as bedding and had access to a paddock. Horses housed in open stables or with access to grazing were excluded, since, usually, the feeding could not be adequately monitored, and a correct trial could not be guaranteed. For the calculation of the rations, the energy supply was calculated and adjusted individually for each horse with a computer program specially designed for the study. The concentrated feed was replaced by the FFD in the FFD group. In the control group, the usual concentrated feed was still used; only the required quantities were calculated and adjusted. All owners or horse keepers received a feeding plan designed for their respective horses with instructions on feeding management (quantity/meal, the order of feeding, feed). The horse owners undertook to feed nothing else than what was recommended in the feeding plan over the entire period of the study and to adhere strictly to the quantity specifications. No changes were made to the ration and the environment during the study period.

#### Behaviour, stress and workload

In the anamnesis, the focus was also on the nature of the horses, behavioural disorders, and possible stress situations. Symptoms, associated with gastric problems, described in existing literature, including discomfort at saddling or grooming, yawning, flehmen, poor appetite, or poor body condition were documented. The usual training and management conditions were exactly documented and had to be maintained over the entire study period without major deviations. Based on the owner's information about the use of the horse, a classification of the performance level into three categories (no, light, and medium work) followed. None of the horses worked hard. The behaviour of the horse was judged as calm and attentive, nervous, or lethargic. Only animals without other serious diseases were included in the study. Cribbing horses were excluded. The horses were not allowed to receive long-term therapies with drugs, which cause the development or worsening of the gastric ulcers (e.g. NSAID's), and anthelmintics throughout the study period.

# Experimental design

Each of the 34 horses underwent gastroscopy twice. They were transported to the clinic one day before the examination. In preparation for the aastroscopy, the horses were deprived of food 12 hours before the examination. Free access to water was ensured by providing both, automatic waterers and water buckets. For the endoscopic examination, the horses were placed in an examination stand and were intravenously sedated with detomidine hydrochloride (Domidine® 0.01-0.02 mg/kg body weight) in combination with butorphanol (Alvegesic<sup>®</sup> 0.02–0.05 mg/kg body weight). The gastroscopic examinations were performed using a flexible 3m videoendoscope (EVIS Olympus CF Type 100HI, OLYMPUS AUSTRIA Ges.m.b.H., Vienna). The stomach was inflated with air until its mucosal lining was smooth. The gastric contents were removed from the mucosa by flushing water through the endoscope biopsy channel. The endoscope was advanced systematically along the great curvature to the antrum pyloricum and into the proximal small intestine section to visualize the entire mucosa during each gastroscopy. All visible findings were determined according to the method described by Sykes and Jokisalo (2014). The EGUS scoring system of the Equine Gastric Ulcer Council 1999, from grades 0 to 4 (adapted by Sykes et al. 2015a), was assessed and documented. The squamous and glandular mucosa were evaluated separately. Pictures and videos were continuously taken during the evaluation. The scoring system used is shown in Table 2.

The gastroscopy was repeated, eight weeks after the first one, to check the effect of the feeding. Once enrolled in the study, the horses were randomly allocated to the FFD or control groups. To obtain as objective a result as possible, the study was performed as a blind study. The investigator who undertook all of the gastroscopic examinations and scoring remained blind to the group allocation until the scoring was com-

 Table 2
 The grading system for equine gastric ulcer disease adapted from EGUS Council 1999 (Sykes et al. 2015a). | Das angepasste

 Bewertungssystem für Magengeschwüre beim Pferd des EGUS Council 1999 (Sykes et al. 2015a).

Grade	Gastric mucosa	
0	The epithelium is intact and there is no appearance of hyperkeratosis	
I	The mucosa is intact, but there are areas of hyperkeratosis	
II	Small single or multifocal lesions	
III	Large single or extensive superficial lesions	
IV	Extensive lesions with areas of apparent deep ulceration	

pleted and recorded. Another researcher was responsible for the randomization and medication.

### Groups

The 34 horses were divided into an FFD group (20/34) and a control group (14/34), after the first gastroscopy, at random by pulling slips of paper with an "F" for FFD or a "C" for control out of a hat. All horses with EGUS  $\geq$  3 (n = 16 FFD group, n = 9 control group) received omeprazole at a dosage of 4 mg/kg SID for 14 days due to the severity of the disease. Omeprazole was used in this study, as a commercially formulated paste (Equinor<sup>®</sup>, Norbrook Laboratories Limited, Newry, UK). It was administered each day before the first feeding in the morning since *Andrews* et al. (1999) demonstrated that the administration of omeprazole in the fasting state increases its bioavailability. Each horse was weighed at the beginning and at the end of the experiment and the body condition score was documented.

## Statistical Analysis

All data were collected by hand and registered using the Microsoft<sup>®</sup> Excel<sup>®</sup> program from Windows 10<sup>®</sup>. Videos and pictures were recorded and stored. To determine if there was a significant difference between the two examined groups, the Pearson's chi-squared test, the Wilcoxon-test and the Mann-Whitney-U-test were used. An independent t-test was performed to calculate nutrient intake (all these data were parametric; Kolmogorov-Smirnov-Test). The differences in the nutrients intake and improvement of gastric lesions between the groups as well as the correlation between EGUS and other factors such as workload, symptoms, and behaviour were established using the IBM SPSS-Software (version 23). A p-value of less than 5% (p<0.05) was considered statistically significant.

#### Results

#### Feed and feeding

Before the start of the study, the horses received between 0.5 and 5.25 kg concentrated feed/day. The calculated amount

of starch intake per meal and per kg body weight exceeded 1 g in 18 horses, the daily ingested amount of starch was higher than 2 g in 19 horses and seven horses ingested almost 5 g starch/kg body weight/day.

Before treatment, we found significant differences in the workload of the horses (median [25th /75th percentiles] FFD group 2[1/2]; control group 2[2/2]; p=0.02) and starch intake (g/kg BW; FFD  $1.52 \pm 0.91$ ; C  $2.34 \pm 1.28$ ; p=0.04). The energy intake per kg BW<sup>0.75</sup> was significantly higher in the control group (p=0.014) according to the higher workload. The intake of the praecaecal digestible protein (pcdCP)/kg BW) was lower in the FFD group (FFD  $1.31 \pm 0.28$ ; C  $1.70 \pm 0.49$ ).

After the changeover of the ration, we had differences in the starch intake (FFD  $0.09 \pm 0.05$  and C  $1.38 \pm 0.62$  g/kg BW; p = 0.00), calcium content/MJ ME (FFD  $0.79 \pm 0.06$ ; C  $0.60 \pm 0.08$ ; p = 0.00) and Ca:P-ratio (FFD  $1.94 \pm 0.17$ ; C  $1.55 \pm 0.17$ ; p = 0.00). We found no significant difference concerning the hay intake between the groups; after the start of the study, the FFD group consumed 21.2 g/kg BW/day and the control group 23.3 g/kg BW/day.

In the FFD group, the starch intake differed highly before and after the treatment  $(1.52 \pm 0.91)$  and  $0.09 \pm 1.05 \text{ g/kg}$  BW/day, respectively). Also, the amounts of calcium and magnesium intake and the Ca-P-ratio were significantly different in the FFD group before and after the changeover (Table 3). But even in the control group, the adaptation of the rations decreased the starch intake significantly (2.34  $\pm$  1.28 mg/kg BW before; 1.38  $\pm$  0.68 after; p = 0.02).

#### Endoscopic findings

A complete examination of the squamous mucosa (fundus ventriculi) was possible in all 34 horses. The margo plicatus and the glandular mucosa (corpus ventriculi) could also be evaluated down to the gastric juice sea. The pylorus region could also be seen in all horses. The gastroscopic examinations were conducted and scored in real time by an experienced veterinarian. Two horses had alterations of the glandular mucosa (EGGD) only, 11 horses had alterations of the squa-

 Table 3
 Mean intake of energy and nutrients of both groups before and after treatment. | Durchschnittliche Energie- und Nährstoffaufnahme in beiden Gruppen vor und nach der Behandlung.

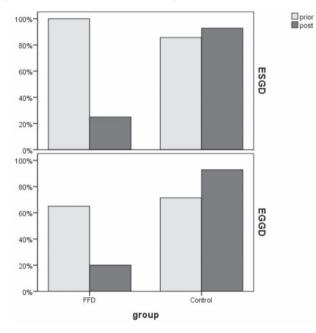
Parameter	FFD group prior	FFD group post	Control group prior	Control group post
Work load <sup>1</sup>	1.40±0.75 <sup>A</sup>	1.40±0.75 <sup>A</sup>	$2.07 \pm 0.48^{B}$	2.07±0.48 <sup>B</sup>
MJ ME/kg BW <sup>0.75</sup>	$0.81 \pm 0.16^{A}$	0.78±0.15 <sup>A</sup>	$0.97 \pm 0.19^{B}$	$0.88 {\pm} 0.13^{\text{B}}$
Hay g/kg BW	19.89±3.76	21.2±3.54	22.27±4.94	23.27±5.10
Fiber g/kg BW	5.89±1.07	6.44 ±1.16	6.78±1.62	6.71±1.37
Starch g/kg BW	$1.52 \pm 0.91^{\alpha A}$	$0.09\ \pm 0.05^{\text{bA}}$	$2.34\!\pm\!1.28^{\circ B}$	$1.38 \pm 0.62^{\text{bB}}$
Protein <sup>2</sup> g/kg BW	$1.31 \pm 0.28^{\text{A}}$	1.42 ±0.38	1.70±0.41 <sup>B</sup>	1.58±0.39
Protein <sup>2</sup> g/MJ ME	7.74±0.73°	$8.75 \pm 1.05^{b}$	$8.32 \pm 1.07$	8.37±1.15
Ca g/kg BW	0.106±0.024°	$0.129 \pm 0.034^{b}$	0.119±0.027	0.112±0.019
Mg g/kg BW	0.038±0.012	$0.042 \pm 0.009$	0.045±0.012	$0.042 \pm 0.011$
Ca:P	1.62±0.24°	$1.94 \pm 0.17^{bA}$	1.52±0.24	1.55±0.17 <sup>B</sup>

Data expressed as mean±SD; <sup>1</sup>1 no work, 2 light work, 3 medium work; <sup>ab</sup> difference before-after treatment; <sup>AB</sup> difference between the two groups; BW body weight; <sup>2</sup>precaecal digestible protein

mous mucosa (ESGD), only and 21 horses had lesions in both regions. All 20 horses in the FFD group had lesions of the squamous mucosa. In the control group, 12 out of the 14 horses (85.7%) had lesions and two horses (14.3%) showed no lesions in this region. Thirteen horses (65%) in the FFD group and 10 horses (71.4%) in the control group showed lesions in the glandular mucosa. Figure 1 shows the number of horses with lesions in the two regions in a bar chart.

Table 4 shows the number of horses with lesions in the different regions of the stomach (dorsal squamous fundus, margo plicatus, ventral glandular fundus, antrum and pylorus) in detail.

Table 5 shows the median lesion scores of the squamous and glandular region for both groups at the first and second gastroscopy. In the squamous region, the median (25th/75th

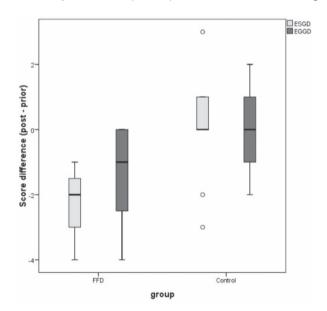


**Fig. 1** Bar chart of the number of horses (in %) with lesions (EGUS-Grade 1–4) in the squamous and glandular region of the stomach before and after treatment. Squamous region: FFD group 20/5\* (100%/25%); Control group 12/13\* (85,7%/92,9%). Glandular region: FFD group 13/4\* (65%/ 20%); Control group 10/13\* (71,4%/92,9%). \*Number of horses (before treatment (prior)/after treatment(post)). | Säulendiagramm mit Angabe der Anzahl der Pferde (in %) mit Läsionen (EGUS-Grad 1–4) im Bereich der kutanen und glandulären Magenschleimhaut vor und nach der Behandlung. Kutane Schleimhaut: FFD Gruppe 20/5\* (100%/25%); Kontrollgruppe 12/13\* (85,7%/92,9%). Glanduläre Schleimhaut: FFD Gruppe 10/13\* (71,4%/92,9%). \*Anzahl der Pferde (vor der Behandlung (prior)/nach der Behandlung (post).

percentiles) lesion score was 3 (2/3.8) before and 0 (0/0.8) after the treatment in the FFD group (p = 0.000), and 2 (2/3.3) before and 3 (1.8/3) after the treatment in the control group (p = 0.832). In the glandular region, the median lesion score was 1.5 (0/3) before and 0 (0/0) after the treatment in the FFD group (p = 0.001), and 2 (0/3) before and 1 (1/2) after the treatment in the control group (p = 0.642).

Figure 2 shows the change in the median lesion scores for both groups in a box and whisker plot. It shows that the grade of gastric ulcers in the FFD group significantly improved in both areas of the stomach. In the FFD group, the squamous mucosa improved in five (25%) horses and 15 (75%) horses had a total healing. The glandular mucosa improved in three (15%) horses while a total healing was seen in 10 (50%) horses, while seven (35%) horses had no lesions in this area.

In the control group, the squamous mucosa improved in two (14.3%) and worsened in four (28.6%) horses, and one (7.1%) horse had no lesions in this area. The glandular mucosa improved in six (42.9%) horses, there was no change



**Fig. 2** Box and whisker plot demonstrating median (continuous line), interquartile ranges (represented by outer edges of box) and ranges (error bars) of the differences in the scores of the squamous region (ESGD) and the glandular region (EGGD) of the stomach (post-prior treatment) in both groups (FFD and control). | *Boxplot Diagramm mit Median (durchgezogene Linie), Interquartilsabstand (äußere Kanten der Box) und Spannweite (Whiskers) zur Änderung im EGUS-Score der kutanen (ESGD) und der glandulären (EGGD) Magenschleimhaut von Therapie- und Kontrollgruppe im Vergleich vor und nach der Therapie.* 

 Table 4
 Number of horses with lesions in the different stomach regions in detail in both groups before and after treatment. / Anzahl der Pferde mit Läsionen in den unterschiedlichen Magenregionen im Detail in beiden Gruppen vor und nach der Behandlung.

Group	Squamous	Squamous region		Glandular region	
	Dorsal squamous fundus	Margo plicatus	Ventral glandular fundus	Antrum and Pylorus	
FFD group	14/2	18/3	2/0	13/4	
(N=20)					
Controlgroup	3/5	12/13	1/0	10/13	
(N = 14)					

Table 5	EGUS-Grading of the squamous region (ESGD) and the glandular region (EGGD) in both groups before and after treatment.   EGUS-
Grading de	r kutanen Magenschleimhaut (ESGD) und der glandulären Magenschleimhaut (EGGD) in beiden Gruppen vor und nach der Behandlung.

ESGD				EGGD	
Group	Before treatment	after treatment	Before treatment	after treatment	
FFD	3 (2/3.75)	0(0/0.75)	1.5(0/3)	0(0/0)	
(N=20)					
Control	2(2/3.25)	3(1.75/3)	2(0/3)	1(1/2)	
(N=14)					

Data expressed as median (25<sup>th</sup> /75<sup>th</sup> percentiles)

in two (14.3%) horses, and worsened in five (35.7%) horses, while one (7.1%) horse had no lesions in this area.

#### Body weight

The average body weight ( $\pm$  SD) of the 34 horses was 526  $\pm$ 77 kg. The lightest horse weighed 358 kg at the beginning; the heaviest horse weighed 697 kg. The horses in the FFD group gained weight during the study, in contrast to the horses in the control group. The horses in the control group even lost weight. At the time of the first examination, the horses in the FFD group had an average body weight of 536 kg; the horses in the control group weighed 512 kg on the average. However, at the time of the second gastroscopy, the horses in the FFD group had an average body weight of 557 kg, while the horses in the control group weighed 510 kg.

#### Body condition score

The body condition score from Henneke et al. (1983), ranging from one to nine, was used in this study. The participating horses had a body condition score (BCS) between 3/9 and 5/9. The horses in the FFD aroup showed a slight improvement in the average nutritional status from a median (25th/75th percentiles) BCS of 4 (4/5) to a BCS of 5 (4/5) (p = 0.004). The horses in the control group changed from a median BCS of 4.5 (4/5) to 4 (4/5) (p = 0.56). From a total of 13 horses with a poor body condition (BCS<5) in the FFD group, the BCS of eight horses improved by one degree. In the control group, only one horse from a total of seven horses (BCS < 5) had improved by one degree, five horses remained unchanged, and the score even deteriorated in one horse (BCS 4 to BCS 3). Neither at the beginning of the study a difference due to the body condition scores could be found between the groups (p = 0.69), nor after the treatment (p = s0.10), although a slight trend could be observed.

#### Discussion

The aetiology and therapy of EGUS have already been discussed in many studies. But there are few studies on the preventive measures, especially the influence of feeding. The research for the methods to treat or prevent gastric ulcers effectively, without using expensive pharmaceutical agents is a new trend in veterinary medicine (*Bonelli* et al. 2016, *Sutton* 2016). To meet this growing need, this study aimed to evaluate the effectiveness of a change in the feeding regime as a part of the treatment of EGUS in adult horses. The positive effects of the ingredients of the tested feed on the gastrointestinal tract are well known. Alfalfa is rich in protein and calcium and is, therefore, often recommended for its buffering properties, to prevent gastric lesions (*Vondran* et al. 2017). In rats, the basal secretion of gastric acid was inhibited when a high-calcium diet was fed (*Fisher* et al. 1990). The same effect was seen in horses fed an alfalfa hay-grain diet (*Nadeau* et al. 2000). Also, high amounts of protein can act as a buffer against acidity and therefore plays a major role in ulcer formation (*Cleave* 1974), as previously seen on other studies in horses (*Nadeau* et al. 2000) and cattle (*Haaland* et al. 1982).

Milk thistle has an anti-inflammatory and antiulcerogenic effect. Dandelion has an appetizing and a secretory effect on the upper gastrointestinal tract and helps with dyspeptic disorders. Linseed has anti-inflammatory effects due to the high content of n-3-fatty acids and protects the mucous membrane of the stomach (*Aichberger* et al. 2006).

Caraile et al. (2004) described a positive effect for the treatment and prevention of gastric ulceration by the addition of corn oil (linoleic acid) to decrease the gastric acid output and increase the prostaglandin E2 and sodium production. The tested FFD contains linolenic acid in the linseed oil and linoleic acid in the milk thistle oil. Fat increases the secretion of bicarbonate-rich mucus (Cargile et al., 2004). The horses that were fed the FFD had a longer ingestion time (mean feeding time 10:29 min for 500 g), due to the rich structure of the feed because of the addition of lianocellulose. To inaest 1 kg oats, horses need only around 10 minutes (Meyer and Coenen 2014). A positive effect is the increased saliva production while chewing, which buffers the gastric acid. Saliva contains bicarbonate, which acts to neutralize the acidity of the gastric juices and so, helps to protect the gastric mucosa from its corrosive effects (Hepburn 2016). Saliva production is related to the particle structure of the diet; for example, feeding of 1 kg of hay results in 3 to 6L of saliva production, while feeding 1 kg of grain results in 1 to 1.7 L of saliva production in adult horses (Meyer et al. 1986, Vondran et al. 2016). The content of starch in the FFD is very low (4.6%). Gastric ulcers are often caused by a diet rich in concentrated starchy feed but poor in structure, which leads to an acid intragastric pH. Unphysiological long-lasting pH values of ≤2 and prolonged exposure of the pars glandularis and the pars nonglandularis to gastric acid are thought to be ulcerogenic (Reese and Andrews 2009, Damke et al. 2015).

The results of *Fedtke* et al. (2015) show that the particle size of the feed may also play a role in ulcer development. They found that foals fed alfalfa chaff had a significantly higher lesion score especially in the pyloric region, compared to foals fed hay or a total mixed ration. They assumed that alfalfa chaff induced lesions probably due to mechanical injury to the mucosa, as the pyloric region is an area of the stomach with high motility. These findings were similar to those of other authors studying weanlings and adult horses (*Vondran* et al. 2016, *Vondran* et al. 2017). As the horses of the control group received either oats or muesli it is not likely that the grounding of the concentrate or the FFD influenced the outcome. The particle size of the FFD was lower than of the oats and possibly, similar to that of the muesli or mash.

Our results showed a significant ulcer improvement in the horses in the FFD group. 35% of the treated horses showed an improvement and 65% showed a total healing of the ulcers. There was no horse without a change or with deterioration in the EGUS score. In contrast, 14.3% horses of the control group showed an improvement of the ulcers. No horse in this group showed a total healing. 71.4% showed no change of score and 14.3% even got worse.

In this study, the healing rate of the FFD group horses was higher than in similar studies (Bonelli et al. 2016). We only used horses with EGUS grade 2 or higher because of the higher clinical relevance of the disease at a severity score  $\geq 2$ . In this study, no correlation was found between age or sex and the EGUS severity score. Further, the score severity was not significantly related to workload, behaviour or stabling (straw or wood shavings as a bedding) and no significant correlation was found between the gastric score severity and the typical symptoms described previously in the existing literature (Ehlers et al. 2016, Andrews and Nadeau 1999). These results are in line with the findings reported by Hepburn (2014) in a large study of sport and leisure horses in the UK. In contrast, a study on thoroughbred racehorses identified sex, trainer, no grass turnout, direct contact to each other, and heavy work as the risk factors for squamous ulceration (Habershon-Butcher et al. 2012).

During diet calculation, we found out that 12 horses in our study had received too high amounts of grain before the study period (>2g starch/kg bodyweight/day). The results obtained by *Luthersson* et al. (2009a,b) in Denmark clearly show that the amount of starch fed per day or per meal is a very important factor in ulcer development. This type of feed increases the acidity of the gastric content due to the volatile fatty acids derived from the intragastric fermentation of the ingested soluble carbohydrates. It may also reduce hay uptake, which limits the production of the buffering saliva (*Tamzali* et al. 2011, *Nadeau* et al., 2000, 2003a,b).

In the present study, the other 22 horses developed gastric ulcers although the starch content in the ration was lower than 2 g/kg body weight/day. As there was no correlation with the workload, other environmental factors may have played a role. According to *Luthersson* et al. (2009b), water deprivation at the paddock or more than 1g starch intake/meal increase the risk of gastric ulcers as well. After changing the ration, the starch content in the FFD group was very low and given the protective substances in the FFD and the high amount of hay the horses received, a positive outcome was as expected. However, the fact that the horses of the control group did not or hardly improve was not expected, because the starch in the adapted rations was lower than 2 g/kg body weight/day and the amount of hay was even higher than in the FFD group and twice as much as calculated for racehorses in Turkey by *Kaya* et al. (2018). Obviously, there may be factors which we could not observe in this study like humananimal interactions which may influence the outcome of gastric ulcers in horses. Nevertheless, the intake of starch or grain seems to be the most important parameter concerning the outcome for horses suffering from EGUS.

The fasting procedure in this study was sufficient to enable the gastric mucosa and the pylorus to be evaluated fully in all horses. However, it is not possible to guarantee that all small lesions were seen (*Andrews* et al. 2002). As has been found in the previous studies, most of the lesions were found adjacent to the margo plicatus (*Luthersson* et al. 2009a). Out of the 34 horses in this study, 30 (88.2%) had lesions in this region and 23 (67.6%) had lesions around the pylorus. In our study, the incidence of lesions at the pylorus is higher than described in the literature but maybe, that is because a lot of other studies did not evaluate this region of the stomach (*Chameroy* et al. 2006, *Luthersson* et al. 2009a, *Marqueset* al. 2011).

One limitation when comparing the studies on EGUS in horses, is the classification of ulcers, in particularly, the severity level used to define the presence or absence of ulceration (*Luthersson* et al. 2009a). Different studies use different scoring systems to describe the ulcers, which makes a comparison difficult. In order to enable a comparison with future studies, in the current study, the EGUS scoring system of the Equine Gastric Ulcer Council (1999), from grades 0 to 4, was used for describing the gastric lesions as recommended by *Sykes* et al. (2015a). We analysed the squamous and glandular region independently to enable the identification of the risk factors for each anatomical type of lesion, as recommended by *Luthersson* et al. (2009a).

In our study, the horses in the control group hardly improved and even got worse EGUS severity scores, although they had received omeprazole in the recommended dosage over the first 14 days after the first gastroscopy. An administration for 14 days has been proven effective at the Tierklinik Mitterndorf for the treatment of EGUS in previous cases. It was found that the gastric lesions of most of the horses were cured by that time. However, the manufacturer's recommendation is the administration of it for at least 21 days. In order to check the success of the medication with omeprazole in the current study, another gastroscopy had to be carried out after the first two weeks since we didn't know whether the ulcers did not improve or if they became worse when the medication was stopped.

The poor results only in the control group are another argument for the possibility that full grain may play an important role in ulcer development and healing since there was no other difference between the two groups. This is also confirmed by the significant difference in the EGUS score severity between the groups.

# Conclusion

Feeding a high-fibre and high-fat diet seems to be highly effective for the improvement of gastric lesions. Thus, further

studies are needed to verify whether a prolonged administration will help to prevent a recurrence of gastric lesions in the horses at risk. The equine veterinarians may take into consideration full grain diets as a potential cause of EGUS in horses and thus, change the diet to a grain-free, fibre- and fatenriched diet.

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## Animal Welfare Statement

The experimental protocol was discussed and approved by the institutional ethics and animal welfare committee of the University of Veterinary Medicine Vienna in accordance with the Good Scientific Practice guidelines and the national legislation.

## Conflict of interest statement

The authors disclose no conflict of interest.

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