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Summary

Zusammenfassung

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Impact of diet on 24-hour intragastric pH profile in healthy horses

Einfluss des Futters auf das intragastrische pH-Profil über 24 Stunden bei gesunden Pferden

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An electrode incorporated into a polyethylene hose was introduced under endoscopic control into the stomach of six fasting adult horses for long-lasting pH measurements. The intragastric pH was recorded every four seconds for a period of 24 hours. The Warmblood horses were assigned randomly to receive hay ad libitum (H group); 1.5 kg hay/100 kg BW/day and 1 kg concentrate/100 kg BW/day (C group) or protocol C plus 75 g pectin-lecithin supplement/100 kg BW/day (P group). The horses were adapted to each diet for 14 days. The 24-hour median pH value for protocol H (2.69) was significantly lower compared to protocol C (3.35) and P (3.44) ($p < 0.05$). The horses in protocol P had a significant higher percentage (40.1 %) of 24-hour intragastric pH values ≥ 4 than in protocol C (36.2 %) or in protocol H (25.3 %) ($p < 0.05$).

Keywords: horse, intragastric pH, hay, concentrate, pectin-lecithin

Unter endoskopischer Kontrolle wurde eine pH-Elektrode in den Magen bei sechs gesunden Pferden appliziert. Der intragastrale pH-Wert wurde alle vier Sekunden über 24 Stunden gemessen. Die erwachsenen Warmblutpferde erhielten drei verschiedene Diäten in randomisierter Reihenfolge: Heu ad libitum (H Gruppe); 1.5 kg Heu/100 kg KM/Tag (d) und 1 kg Kraftfutter/100 kg KM/d (C Gruppe); Protokoll C plus 75 g Pektin-Lezithin/100 kg KM/d (P Gruppe). Alle Pferde wurden über zwei Wochen an die jeweilige Diät adaptiert. Der 24-Stunden-Median-Wert des intragastralen pH-Wertes von H lag bei 2,69, bei 3,35 für C und bei 3,44 von P, wobei der von Gruppe H signifikant am niedrigsten war ($p < 0,05$). In der Gruppe H lagen 25,3 % der intragastralen pH-Messungen ≥ 4 . In den Gruppen C und P waren es dagegen 36,2 % bzw. 40,1 % ($p < 0,05$).

Schlüsselwörter: Pferd, intragastraler pH, Heu, Kraftfutter, Pektin-Lezithin

Introduction

Intragastric pH is measured either by means of gastric puncture (Sangiah et al., 1988), through the aspiration of gastric juice via a nasogastric tube (Berschneider et al., 1999), or collection of the gastric content from a cannula implanted into the stomach (Merrett, 1999). The first two methods can be used only with fasting horses, because the cannula used for puncture as well as the lumen of the tube can become clogged with undigested feed from the stomach. The pH of gastric juice from fed horses can only be collected continuously through the

surgically implanted cannula. Euthanization following feeding (Coenen, 1990) establishes the pH only at a particular point in time.

Continuous intragastric pH recording using permanently implanted esophageal electrodes is a tried-and-tested method in human patients (Emde, 1990; Pehl et al., 2003). In horses, this method is normally used to establish the efficacy of medications designed to measure the increase of intragastric pH related to the decrease of the gastric acid output, either under fasting conditions (Baker and Gerring, 1993; Murray and Schusser, 1993) or a diet of hay (Murray and Schusser, 1993).

Stomach ulcers are often caused by a diet rich in concentrated starchy feed, but poor in structure, as well as by long periods of fasting: both of these factors strongly influence 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. The therapies of choice for treatment of stomach ulcers are H_2 -blockers or proton-pump inhibitors, which serve to raise the pH level to ≥ 4 (Murray and Schusser, 1993). The dietary supplements containing pectin and lecithin can be used prophylactically to protect the mucous membranes (Clark et al., 1996; Köller et al., 2010; Murray and Grodinsky, 1992; Venner et al., 1999).

The purpose of this study was to determine the intragastric pH caused by a commercial pelleted compound feed with or without a supplement providing pectin-lecithin in addition to hay ad libitum as a control.

Material and Methods

Animals

The study used six healthy Warmblood horses (median BW: 550 kg; median age: 15 years). The horses were kept in individual box stalls with woodshavings bedding and had unlimited access to water. Prior to the commencement of the study, which was approved by the Animal Study Commission (24-9168.11-18/05), the horses were given a general, hematological and gastroscopic exam. Every horse out of six horses was adapted to each feeding regime for 14 days; after an 8-h nocturnal fasting period, the pH electrode was introduced into the stomach at 9 a. m.

Intragastric pH measurement

A glass electrode with an internal reference electrode (type K14088-pH-0544, Medtronic GmbH, Duesseldorf, Germany) was fixed to the end of a flexible polyethylene hose, 4 m long, with a diameter of 14 F (3 mm). The hose was marked with a cm scale and, thus, a 20 g metal weight could be attached 10 cm before the end of the hose, thereby assuring the individual positioning of the glass electrode in the ventral stomach. Electrode calibration was carried out prior to each protocol at room temperature using buffer solutions with a pH of 1.07 and 7.01, respectively. With the help of a gastroscope (Fritz GmbH, Tuttlingen, Germany), the glass electrode was then guided through the nasal cavity and esophagus into the stomach of the sedated horse (0.02 mg detomidine hydrochloride/kg BW i. v.). The electrode was anchored 10–20 cm away from the cardia in the ventral stomach, where gastric secretions accumulate.

The pH recordings began 2 h following sedation, corresponding to feed provision. Values were recorded every 4 s for a period of 24 h, using a battery-powered recording device (Digitrapper® 100, 300 g, Medtronic GmbH, Duesseldorf, Germany), which was fastened in the pocket of a mane protector (Boett GmbH, Germany).

Feeding regimes

The horses were assigned randomly to receive ration H, C or P. These three different rations were used: Ration H (H) consisted of hay ad libitum. Average daily consumption was 2.4 kg hay/100 kg BW. Ration C (C) limited the hay to 1.5 kg/100 BW/day and included 1 kg of concen-

trate/100 kg BW/day, which was divided into maximal 2.8 kg pelleted concentrate (“Energie Pellets”, Eggersmann GmbH, Germany) and the second part was completed with rolled oats (e. g. The daily quantity of a horse with 500 kg BW is 2.8 kg pelleted concentrate plus 2.2 kg rolled oats which was divided into four feeding amounts of 1.25 kg each). The daily quantity of concentrate and oats was divided between four feedings (every 6 h): the hay was fed three times a day (every 8 h). Hay was fed 30 min after the concentrate. Ration P (P) consisted of ration C plus 75 g of a pectin-lecithin supplement/100 kg BW/day (Pronutrin®, Boehringer Ingelheim GmbH, Germany). The supplement was divided equally between the first, third and fourth feedings of concentrated feed. The actual time it took the horse to consume the ration was recorded. The data on the chemical composition of the feeds, assayed by using the Weender feed analyzing method (Naumann and Bassler, 2012), are summarized in Table 1.

Statistics

Polygram Net™ (Medtronic, Inc.) and Microsoft Excel® were used to record the individual measurements. SPSS 11.5 for Windows was employed for data analysis.

The pH values were recorded every 4 s, so that 21 600 values could be established per horse over 24 h. In view of the plethora of data, the 4-s values per minute were bundled into a median. Based on this, 1 440 values could be established per horse and 8 640 per feed protocol. The Kolmogorov-Smirnov test was used to determine normal distribution.

Statistical proof was then applied using the Friedman test in accordance with the recommendations of Emde (1990) in order to compare the repeated measurements at various points in time within a given feed regime. The Wilcoxon test served to compare the various regimes with each other at various points in time. Pearson’s chi-square test was used for frequency comparisons. Test results of $p < 0.05$ were considered significant.

TABLE 1: Chemical compositions of feeds

Nutrients	Unit	Hay	Oats	Pelleted concentrate	Pectin-lecithin supplement
Dry matter (DM)	% as fed	94.12	88.06	89.95	93.41
Ash	% DM	6.36	2.67	11.20	3.43
Crude protein	% DM	9.93	11.13	13.73	7.47
Crude fiber	% DM	34.67	8.81	7.75	15.81
Crude fat	% DM	1.02	5.97	4.00	15.63
NDF	% DM	68.23	25.58	27.14	29.89
ADF	% DM	37.86	10.96	10.59	21.97
Starch	% DM	0.49	35.46	23.72	3.64
Sugar	% DM	1.13	0.81	4.26	12.35
Calcium	% DM	0.32	0.08	1.97	0.63
Phosphor	% DM	0.25	0.45	0.79	0.60
Magnesium	% DM	0.17	0.11	0.62	0.19
Digestible energy	MJ/kg	7.88	14.52	12.73	15.27
Metabolizable Energy	MJ/kg	5.86	13.5	11.5	14.4
Buffering capacity against alkaline	mmol NaOH/g	19.40	9.42	15.14	29.71

Results

The horses tolerated the inserted tube well for the entire 24-h period and showed neither lack of appetite nor other unusual behavior. Occasional observations were coughing at the beginning of feeding and limited amounts of nasal discharge. Pulse/respiratory frequency and cell blood count were consistent within the normal range, both before and during the trial. When the electrode was introduced, 4/6 horses had a normal squamous mucosa and glandular mucosa (= grade 0/4) and 1/6 had a grade 2/4 (single lesion in the squamous mucosa of lesser curvature = Equine Squamous Gastric Disease = ESGD) in all protocols. One horse had only a grade 2/4 ESGD (single lesion in greater curvature) in protocol H (Sykes et al., 2014).

Feed intake and consumed, and time spent on feed ingestion are listed in Table 2. Horses in protocol H needed an average of 10 h and 42 min during the day to consume hay.

The gastric pH data were not normally distributed. The 24-h median value of the intragastric pH measurements in ration H was significantly lower than in ration C and P (Tab. 3).

The 24-h pH profile of intragastric pH during hay provision ad libitum (H) showed values > 3 up to a maximum of 4.7 between hour 4 and 9 of the recording period (Fig. 1). The lowest and highest intragastric pH values were 1.26 and 4.71, respectively, during the first, and 1.49 and 3.42, respectively, during the second 12-h period

TABLE 2: Duration of feed ingestion and amount of feed intake of six horses which were randomly assigned fed with ration H (hay), C (concentrate) or P (pectin-lecithin supplement); values in mean ± SD.

	Time spent on feed ingestion, min/day		Feed intake, kg/day	
	hay	concentrate	hay	concentrate
Ration H	642 ± 107	-	13.1 ± 1.7	-
Ration C	552 ± 85	53 ± 10	7.8 ± 1.7	5.5 ± 0.7
Ration P	610 ± 66	64 ± 11	8.15 ± 1.0	5.9 ± 0.8

TABLE 3: 24-median intragastric pH values of ration H (hay), C (concentrate) and P (pectin-lecithin supplement) of 8 640 one-minute medians per protocol. Value with a superscript is significantly higher than value of ration H ($p \leq 0.05$).

	Ration H	Ration C	Ration P
Median (1 st -3 rd quartile)	2.69 (1.57-4.02)	3.35 ^a (1.93-4.5)	3.44 ^a (1.88-4.55)

TABLE 4: Analysis of frequency (% and 95 % confidence interval) of intragastric pH measurements (Pearson's chi-square test: significant differences in comparisons between dietary treatment H (hay ad libitum), C (concentrate), P (pectin-lecithin supplement) ($p \leq 0.05$).

	pH ≤ 1.99	pH 2.00-3.99	pH ≥ 4.00
Ration H*	33.4 (32.4-34.4)	41.2 (40.3-42.3)	25.3 (24.4-26.2)
Ration C*	26.2 (25.3-27.2)	37.6 (36.6-38.8)	36.2 (35.0-37.0)
Ration P*	27.7 (26.8-28.8)	32.2 (31.4-33.4)	40.1 (38.8-40.9)

*8 640 items of data per feeding protocol, see methods of data processing.

(Fig. 1). The majority of pH values scattered below 3.99 (Tab. 4). During treatments C and P, a significantly lower number of pH measured values fell below 3.99, relative to treatment H (Tab. 4). The lowest intragastric pH values in protocol C were observed at hour 6.5 (pH 1.27) and the highest at hour 13 (pH 4.89) after the beginning of the measurements (Fig. 2). The lowest intragastric pH values in protocol P were observed at hour 12 (pH 1.88) and the highest at hour 19 (pH 4.52) (Fig. 2).

Discussion

Intragastric pH can be influenced by animal- and feed-related factors: e. g. salivary and gastric secretions, structure of feed and rate of feed intake, dry matter of feed, dry matter of gastric contents, layering of the stomach content, buffering capacity of the feed, gastric emptying rate, reflux from the duodenum; the position of the electrode based on stomach motility results in a variety of data obtained, independent from the criteria mentioned above (Coenen, 1990; Murray and Grodinsky, 1992; Murray and Schusser, 1993).

The position of the electrode in the stomach after safe deposition under endoscopic control was not proven in this study, but, based on the pH levels measured in comparison to the results of Husted et al. (2008), the pH electrode is likely within the ventral stomach (oral region of Antrum pyloricum), within the stomach contents or between the glandular mucosa surface and the gastric contents (Damke, 2008).

A certain characteristic of the equine stomach is the continuous secretion of acidic juices, which is independent of feed intake (Cambell-Thompson and Merritt, 1987; Merritt, 1999). Intragastric pH values in fasting horses are measured consistently < 2 (median 1.55: Murray and Schusser, 1993; 1.88 ± 0.18: Murray and Grodinsky, 1992; 1.63 ± 0.06 to 1.97 ± 0.11: Sangiah et al., 1989).

Literature data show that feed ingestion induces significant changes. Hay intake corresponded to a 24-h average pH at 3.1 (Murray and Schusser, 1993). Daurio et al. (1999), also feeding hay, observed a pH at 2.73 ± 0.33. Differences between those values and the recordings in the present study may result from the varying amounts of hay consumed, which is associated with different volumes of saliva. The horses in our study consumed an average of 2.4 kg hay/100 kg BW in protocol H. The actual amounts of hay consumed were not given in the Daurio study. Berschneider et al. (1999) demonstrated a pH of 1.9 in gastric juice aspirate following hay feeding in comparison to 4.88 following consumption of concentrate.

According to recent studies, the consumption of hay compared to concentrate results in a higher and longer lasting rise in plasma gastrin in eight-month-old foals (Wilson et al., 2007). The cause of gastrin release is related to stomach distention based on the amount of feed consumed, as well as mastication and the parasympathetic pathway, which stimulate the gastrin cells in the antrum through polypeptides releasing gastrin (Schmidt-Wilcke, 2001; Schusser and Obermayer-Pietsch, 1992). The horses in our study required 49 ± 8 min to consume 1 kg of hay compared to 10 ± 2 min/kg of ration C. These results were similar to the study of Brøkner et al. (2006) and Smyth et al. (1989). The amount of feed consumed per day, rate of intake (g feed per min), energy content of feed and chemical composition of feed could increase

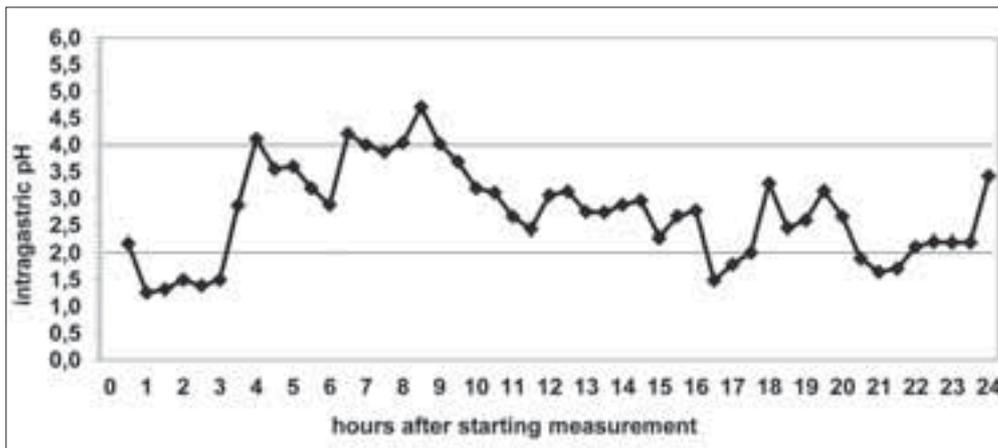


FIGURE 1: Median intragastric pH curve measured in six horses fed with ration H (hay ad libitum) over 24 h (based on individual medians per 30 min; measurements started at 11 a. m. = h 0 on the x-axis).

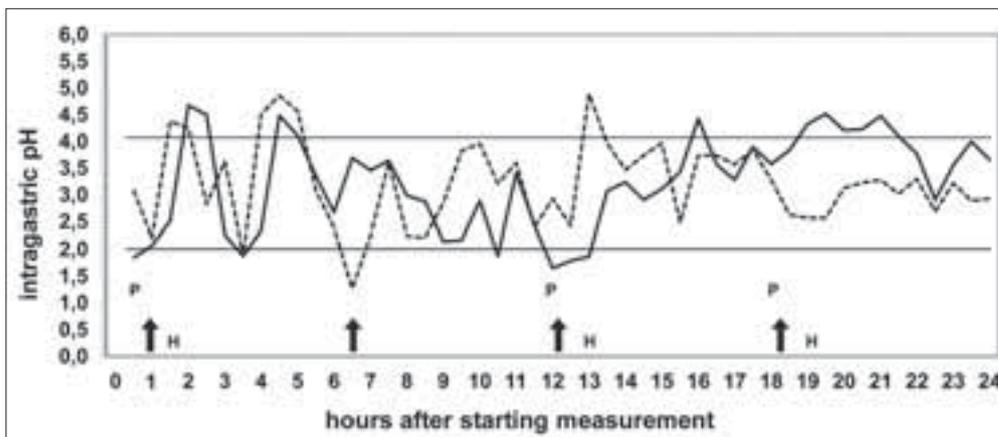


FIGURE 2: Median intragastric pH curve measured in six horses fed with ration C (concentrate, dotted line) or P (pectin-lecithin supplement, lined) over 24 h (data processing, see Fig. 1; indicate provision of feed: concentrate ↑, hay (H), and pectin-lecithin supplement (P); measurement started at 11 a. m. = h 0 on the x-axis).

gastrin secretion. Gastric emptying is influenced by the dry matter in the gastric contents (Coenen, 1992; Meyer et al., 1980). In summary, the inclusion of any type of concentrate in the meal speeds up feed intake, reduces the volume of saliva per unit of ingested feed, elevates dry matter in the stomach and reduces gastric emptying. These responses are reflected by differences in the intragastral environment that, in part, is reflected by pH.

The buffering capacity of feed may have a decisive influence on intragastric pH. The buffering capacity of the concentrate in our study (9.42 mmol NaOH/g oats; 15.14 mmol NaOH/g concentrate) against alkaline is distinctly lower than that of hay (19.40 mmol NaOH/g hay), whereas the pectin-lecithin supplement has the highest buffering capacity (29.71 mmol NaOH/g).

The physical characteristics of the gastric contents may create a further impact on intragastric pH. Compared with hay, feed concentrate stimulates less production of saliva (mastication time is 10 ± 2 min per kg concentrate compared to 72 ± 10 min regarding hay in ration C, Tab. 2).

An increase in dry matter and decrease in the particle size of gastric contents promotes the formation of a solid mass which becomes less easily acidified by the corresponding acid secretion (Coenen, 1990).

Intragastric pH variations during the course of the day can be evened out by feeding hay ad libitum. A bactericidal effect and the activation of digestive enzymes occur only with acidic pH, e. g. the transformation of pepsinogen into pepsin at pH 1 (Sayegh et al., 1999). The decreased intragastric pH < 3 induced by hay ad libitum

inhibits the gastrin receptor of the parietal and gastrin cells and stimulates gastric emptying.

Prolonged mastication of hay compared with feed concentrate produces more saliva, which contains epidermal growth factor, thereby resulting in a greater regenerative effect on the epithelial cells in the pars non glandularis, which, in turn, reduces the occurrence of gastric ulcers, favors stomach emptying and continually stimulates the feed-forward reflexes.

Pectin-lecithin supplement (ration P) caused significantly higher 24-h pH values than ration H or C. Only the first and third feeding resulted in a rise of intragastric pH levels to ≥ 4 , which lasted only a short time (Köller et al., 2010). The pectines contained in the used supplement form thermoreversible gels at a pH value of ≤ 3 . These pectines react with and stabilize mucus (Keiser-Nielsen, 1947). Bile acids can be bound by pectines in stomach and duodenal juices (Rydning and Berstad, 1984). The lecithins of the supplement stabilize the mucin layer on the stomach mucous membrane (Wassef et al., 1978) and promote its ability to repulse acids in the pars glandularis and the phospholipid bilayer in the pars non glandularis (Ethell et al., 2000). Based on Köller et al. (2010) pectin-lecithin supplement raises the total mucus concentration in the gastric juice over a period of 6 h. Fat, which is high in ration P, inhibits the secretion of acids in the stomach and increases the concentration of PGE2 and the secretion of bicarbonate-rich mucus (Cargile et al., 2004). The pH-raising effect, however, is very limited compared with H₂-antagonists and Omeprazole (Murray and Schusser,

1993). However, the inclusion of the supplement into the diet consisting of hay plus concentrate did not meliorate the impact of the concentrate on gastric pH.

In conclusion, a hay ad libitum ration causes lower intragastric pH, less intra-gastric pH variations compared with feed concentrate four times a day, increases the duration of mastication and produces more saliva. The increased amount of saliva with the epidermal growth factor could induce the regeneration of squamous epithelium, and the reduced intragastric pH could have a bactericidal effect, catalyse pepsinogen into pepsin and reduce gastric emptying time in a physiological manner (Sykes et al., 2014).

Conflict of interest

The authors declare that there is no protected, financial, occupational or other personal interest in a product, service and/or a company which could influence the contents or opinions presented in the manuscript.

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