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# Effects of Differently Composed Feeds and Physical Stress on Plasma Gastrin Concentration in Horses

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**Sandin A, Girma K, Sjöholm B, Lindholm A, Nilsson G: Effects of differently composed feeds and physical stress on plasma gastrin concentration in horses. Acta vet. scand. 1998, 39, 265-272.** – Plasma gastrin concentrations were determined in 6 Standardbreds (4 geldings and 2 mares) after 3 different meals consisting of unlimited amounts of hay (8-9 kg per horse), a restricted amount of hay (0.6 kg/100 kg body-weight) and grain (0.2 kg/100 kg body-weight) in combination or of grain alone (0.2 kg/100 kg body-weight). In another series of experiments the possible role of gastrin as a stress hormone was investigated. Plasma gastrin and cortisol concentrations were determined during fasting and compared with concentrations during hay feeding. In addition, gastrin and cortisol concentrations were determined before, during and after 2 kinds of physical exercise on a treadmill.

Meal stimulation significantly increased the plasma gastrin concentration, irrespective of the meal composition. An immediate and large increase in plasma gastrin concentration was found when voluminous meals were given, whereas a small meal evoked a later onset of gastrin release, suggesting that gastric distention plays an important role in inducing gastrin release during a meal. Meals consisting of grain seem to evoke a slower onset and then a more prolonged gastrin response than a hay meal, possibly due to different emptying rates of the stomach. Nervous excitation may play a minor role in the activation of gastrin release in horses. No experimental support was obtained for the idea that gastrin acts as a stress hormone in the horse.

***cortisol; exercise; feeding; fasting.***

## Introduction

Little is known about the physiological mechanisms controlling gastric acid secretion in the horse. Findings in foals (*Wilson* 1985) and adult horses (*Hammond et al.* 1986, *Murray* 1992) showing that gastric ulcer disease is common in this species motivate further studies of such mechanisms. Investigations in cats (*Uvnäs* 1942), dogs (*Nilsson et al.* 1972) and humans (*Stenquist et al.* 1979) show that the antral hormone gastrin plays an important role in stimulating gastric acid secretion, and that it exerts a trophic effect on the glandular part of the gastric mucosa (*Johnson et al.* 1969).

In association with blood sampling in man (*Nilsson,* unpublished observations), it has been found that plasma gastrin concentrations may increase considerably (400%-500%) in connection with venepuncture that causes great discomfort, suggesting that gastrin may act as a stress hormone.

The present investigation describes the postprandial plasma gastrin secretion in response to meals of different compositions of hay and grain in 6 adult Standardbreds. The study also aimed at determining plasma concentrations of gastrin and cortisol in association with supposed stress conditions. Concentrations of gastrin and cortisol were therefore determined in connection with venepuncture, strenous exercise on a treadmill, and during fasting.

## Materials and methods

### *Animals*

Six clinically healthy 6-18-year-old Standardbreds (weight 447-526 kg), comprising 4 geldings and 2 mares, were used in the experiments. Two weeks before the study the horses were taken in from pasture and put on a diet consisting of hay and grain (oats) twice daily. During the study they were kept in individual indoor boxes. Between experiments the horses were allowed indoor exercise for 30 min every day.

### *Feeding procedures*

Three differently composed meals were given. During the first meal horses were allowed to eat unlimited (approximately 8-9 kg per day) amounts of hay for 12.5 h. In the second meal, the horses were given a restricted amount of hay (0.6 kg/100 kg body-weight) in combination with oats (0.2 kg/100 kg body-weight) twice with a 6 h interval. This food was mostly consumed within an hour. In the third meal oats alone (0.2 kg/100 kg body-weight) was given twice with a 6 h interval and this food was mostly consumed within 15 min. Water and trace mineralized salt were provided freely during experiments.

### *Treadmill procedures*

Two types of trotting tests were carried out on a horizontal treadmill (Sikob, Sollentuna, Sweden).

In the first test (incremental speed test), horses started to trot at a speed of 5 m/sec after a warming up period of 2 min at a walking pace. The velocity was then gradually increased by 1 m/sec every 2 min up to 9 m/sec.

In the second test (long distance running) horses ran at an even individual speed (4-6 m/sec) for approximately 30 min. The speed was set to maintain a heart rate of 160-180 beats per min and the heart rate was monitored with an electrocardiogram (Minogram 410 System, Siemens Elema, Sweden) every 5 min.

### *Collection of plasma samples*

Before the start of each experiment, all horses were fasted for at least 14 h. At 7 a.m. a permanent intravenous plastic catheter was inserted into a jugular vein and blood was collected in immediate association to this procedure and 30 min later. The experiments were started after a further 30 min. During hay feeding *ad lib* and fasting experiments, plasma samples were collected every 30 min for 12.5 h.

During restricted feeding, blood samples were collected every 15 min during the first hour, every 30 min for the next 3.5 h and, finally, 2 samples were taken with a one hour interval. After that, a second feeding was started and the sampling procedure was repeated. In all, 26 blood samples were collected in each experiment.

When experiments were performed on the treadmill, an intravenous plastic catheter was inserted as described above. In both trotting experiments 2 blood samples were taken when the horses were standing still on the treadmill and after one min of walking. In the long distance running experiment subsequent samples were taken every five min and the last sample 5 min after the end of the exercise. In the incremental speed test blood samples were collected during the last 15 sec at each velocity and 30 min after the end of the exercise.

20 ml of blood was taken on each sampling occasion and the blood was collected in 10 ml tubes containing 100 IU of heparin. Samples were kept on ice, centrifuged, frozen and then stored at -20 °C until assayed for gastrin and cortisol concentrations.

*Determination of plasma gastrin and cortisol concentrations*

Gastrin was determined by radioimmunoassay (*Nilsson* 1975). All samples were assayed in duplicate and some at several dilutions. Antibody no. 4562 (kindly donated by Professor Jens Rehfeld, Copenhagen, Denmark) was used in the analyses. This antibody is directed against the C-terminal end of the gastrin molecule. There is only a small cross-reactivity to the human cholecystokinin peptides, having 33 (2.6%) and 8 (0.9%) amino acids (*Rehfeld* 1981). The mean detection limit for gastrin in the different radioimmunoassays was calculated to be 8 pg/ml plasma. Intra- and inter-assay coefficients of variations were 6.5% and 8.3%, respectively. When postprandial plasma gastrin samples from the horses were diluted and the dilution curve was superimposed on the standard curve prepared of human gastrin I, the curves were fairly parallel.

Plasma cortisol concentrations were analysed with a commercial radioimmunoassay kit (TKCO5, Diagnostic Products Corp., Los Angeles, CA.) previously validated for horses (*Young & Smyth* 1988) and found to exhibit a low cross-reactivity with other steroids present in blood.

*Statistical evaluations*

Statistical calculations were performed using a computerized statistical program (JMP 3.0.2., SAS Institute Inc., Cary, NC, USA). Changes in hormone concentrations are expressed as mean ± SEM, except for the treadmill tests where changes are expressed as mean ± SEM as a percentage of the basal value before running. Statistical evaluations of plasma gastrin and cortisol results were performed by a one-way analysis of variance (ANOVA). In series A plasma concentrations of gastrin during fasting and following the 3 different meals and plasma cortisol concentrations during fasting and freely fed conditions were compared by using a Scheffe F-test. In series B, only the 2 last samples were compared with the pretest sample by using a Scheffe F-test. Differences were considered as significant at $p<0.05$.

## Results

*Plasma gastrin concentrations during feeding experiments*

After feeding with hay alone or in combination with grain, plasma gastrin concentrations increased significantly ($p<0.001$) and peaked after 30 min (Fig 1). The largest and most prolonged gastrin response was seen when horses

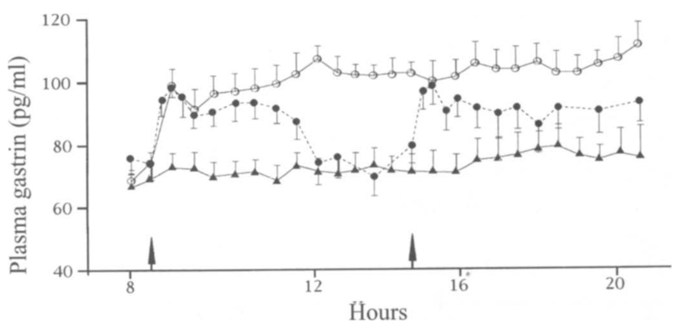


Figure 1: Mean (± SEM) plasma gastrin concentrations in 6 horses during restricted feeding with hay and grain (●), feeding hay ad lib (○) and fasting (▲). The animals were fed twice during normal feeding (arrows). Significant differences were noted between fasting and feeding hay ad lib ($p<0.001$), fasting and restricted feeding ($p<0.001$) and feeding hay ad lib and restricted feeding ($p<0.001$).

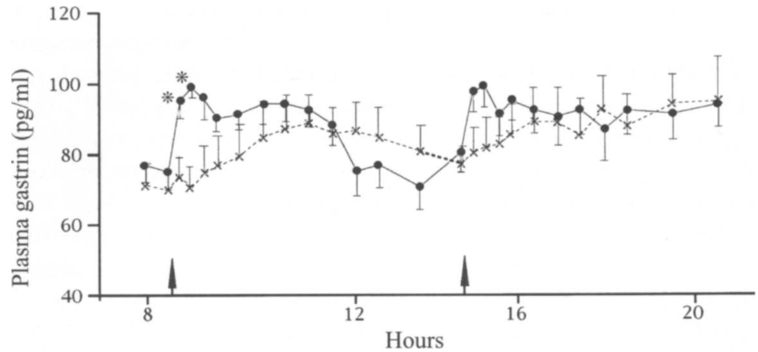


Figure 2: Mean (± SEM) plasma gastrin concentrations in 6 horses during restricted feedings with hay and grain (●) or grain only (×). The animals were fed twice (arrows). Values indicated by ✻ are significantly ($p<0.05$) elevated compared with the corresponding values during grain feeding. Significant changes ($p<0.05$) were also found between plasma gastrin concentrations during restricted feeding with grain and fasting.

were allowed to eat hay freely, whereas plasma gastrin concentrations during the combined meal returned to fasting levels within 4 h. When only the small amount of grain was given, the onset of gastrin release was delayed in comparison with that after the hay meals. Peak concentrations, which were similar to those during the combined feeding, were not reached until 2.5 h after the start of the meal (Fig 2). When only grain was given, the increase in gastrin secretion was also somewhat but not significantly more prolonged than after the combined meal. In all cases the gastrin concentrations after meals rose to levels that were significantly higher than those found during fasting ($p<0.01$) and the secretory patterns following afternoon feedings resembled those observed in the morning.

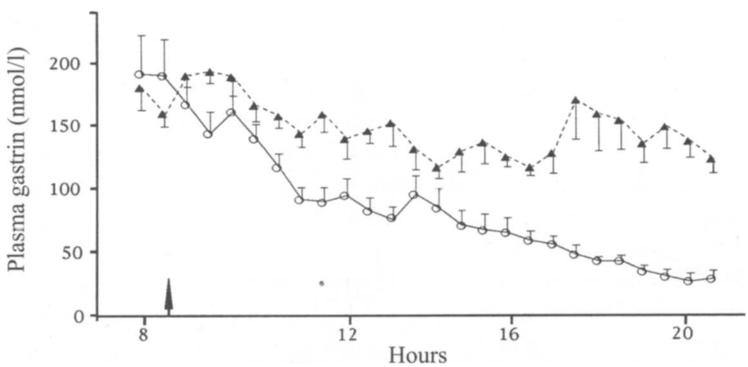


Figure 3: Mean (± SEM) plasma cortisol concentrations in 6 horses during feeding hay ad lib (O) and fasting (▲). Arrow indicates the start of hay feeding.

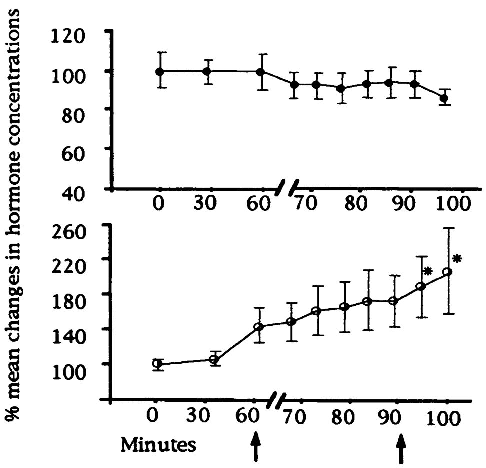


Figure 4 a and b: Mean (± SEM) changes (as percentages of initial values) in plasma gastrin (●, 100 % ± 9.4 is equal to 86.8 ± 8.2 pg/ml) and cortisol (O, 100% ± 6.4% is equal to 121.8 ± 7.8 nmol/l) concentrations during 30 min of trotting in 6 horses. Values indicated by ✻ are significantly ($p<0.05$) elevated compared with the basal values. Arrows indicate the start and end of the running period.

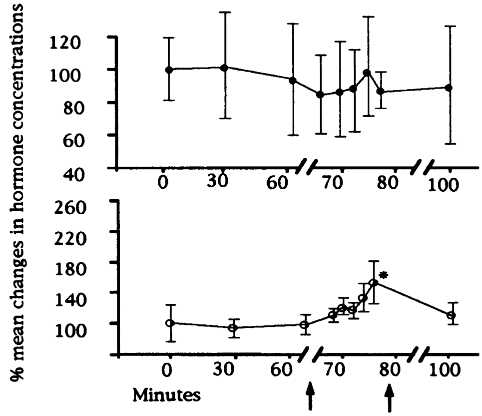


Figure 5 a and b: Mean (± SEM) changes (as percentages of initial values) in plasma gastrin (●, 100 % ± 24.4 is equal to 78.0 ± 15.1 pg/ml) and cortisol (O, 100% ± 19.2% is equal to 170.7 ± 35.8 nmol/l) concentrations during running at different speeds in 3 horses. Values indicated by ✻ are significantly ($p<0.05$) elevated compared with the basal values. Arrows indicate the start and end of the running period.

*Plasma gastrin and cortisol concentrations during venepuncture, fasting and treadmill tests*

Venepuncture. No changes in plasma gastrin (72.4 ± 2.4 pg/ml and 71.8 ± 1.8 pg/ml ) or plasma cortisol (185.1 ± 25.3 nmol/l and 174.5 ± 20.6 nmol/l) concentrations were noted when comparing concentrations in association with venepuncture and 30 min later.

Fasting. After fasting overnight, average plasma gastrin concentrations remained at a fairly constant level (range 67-77 pg/ml) throughout the 12.5 daytime hours when plasma samples were collected. Cortisol concentrations, on the other hand, decreased during both fasting and hay feeding experiments (Fig 3). However, levels during fasting stayed significantly higher than after feeding and with higher significance at the end of the day ($p<0.05$-$0.001$).

Treadmill tests. Plasma gastrin concentrations were unchanged during both the treadmill tests, whereas cortisol levels increased (Figs 4 and 5). Plasma cortisol concentrations observed at the end of the 30 min treadmill test were significantly ($p<0.05$) higher than pretest levels (n=6). Plasma cortisol concentrations during the short treadmill test also increased significantly ($p<0.05$).

## Discussion

*Feeding studies*

Plasma gastrin concentrations after feeding were significantly higher than those during fasting, and the gastrin responses after feeding varied with the composition of the meals. A rapid onset of gastrin release was found when hay was given alone or in combination with grain, whereas the small grain meal caused a slow onset of gastrin release. Furthermore, the duration of gastrin release varied with the composition of the meals. The highest peak concentrations of gastrin were found when the horses had free access to hay, and the plasma level remained at an even and high level throughout the 12.5 h observation period. When instead horses were given a limited ration of hay in combination with grain, plasma gastrin concentrations returned to fasting levels after a few hours. In comparison, grain alone caused a more prolonged but not statistically significant gastrin release. Other studies have demonstrated that gastric emptying is faster when horses are fed with hay than with grain (*Meyer et al.* 1980). Thus, the more prolonged gastrin response following grain feeding in our study might be due to a prolonged stimulation of gastrin release from the antral mucosa by grain remaining in the stomach.

Studies in the dog show that gastrin is released by nervous stimulation at the beginning of a meal and then by gastric stimulation, which is responsible for the main release of gastrin during the meal (*Nilsson et al.* 1972). The gastric release of gastrin is due to both mechanical (*Debas et al.* 1974) and chemical (*Elwin* 1974) stimulation of the gastric antrum. These mechanisms seem to operate in most monogastric animals. It is not known to what extent similar mechanisms exist in the horse antrum.

However, in view of the quick gastrin responses following the voluminous hay meals and the slow onset of gastrin release after the small amounts of grain, it seems reasonable to assume that mechanical distention is a principal factor causing gastrin release in the horse. This view is also supported by recent observations of *Schusser & Obermayer-Pietsch* (1992), who found raised plasma gastrin concentrations in colic horses with gastric distention and a correlation between the degree of distention and the plasma level of gastrin.

In other species, nervous excitation by sham feeding causes an immediate release of gastrin into the blood (*Nilsson et al.* 1972, *Stenquist et al.* 1979). The nervous stimulation is induced by the sight, smell and taste of food which is drained by an esophageal cannula and not permitted to reach the stomach. In the present studies the food reached the stomach in all experiments. The delayed onset of gastrin release when horses were given the small amount of grain might therefore mean that nervous gastrin release plays a minor role during meal stimulation in this animal.

Our results demonstrating greater gastrin release when horses were fed with hay than with grain seem to be in contrast to those obtained by *Smyth et al.* (1988). They found no gastrin release when horses were given hay, but significant increases when they were fed with pellets. This discrepancy is difficult to explain, but methodological problems with the gastrin radioimmunoassay they used may have contributed. When their assay was evaluated for use in horses, basal concentrations of gastrin could only be detected in about 10% of the plasma samples examined (*Young & Smyth* 1988).

*Studies during supposedly stressful conditions*

In association with clinical determinations of plasma gastrin concentrations in humans it has been observed (*Nilsson,* unpublished observations) that several patients have had very high plasma gastrin concentrations in connection with painful and complicated venepuncture. Concentrations in blood samples collected through the same catheter 10-15 min later have been considerably lower. This observation has led us to consider gastrin as a possible stress hormone. However, when gastrin and cortisol concentrations were determined in blood samples from horses in connection with venepuncture and later, neither gastrin nor cortisol concentrations increased, even when the venepuncture was experienced as painful by the horse.

High cortisol concentrations during fasting experiments have previously been demonstrated in pigs (*Dantzer et al.* 1980). In our study cortisol concentrations were also higher during fasting than in feeding experiments. However, no notable change in gastrin concentrations was found.

The possible role of gastrin as a stress hormone in horses was also investigated when horses trotted on a treadmill. No effect on plasma gastrin levels was observed during the 2 types of treadmill experiment. However, during both experiments plasma cortisol concentrations increased. One interpretation of the higher cortisol and unchanged gastrin concentrations during treadmill and fasting experiments may be that under these conditions horses experienced stress and that gastrin does not operate as a stress hormone in the horse. However, increased concentrations of cortisol need not necessarily indicate stress in the sense that the animals feel uncomfortable. During both the fasting and the physical exercise experiments, the increase in cortisol concentrations might instead be part of the physiological events by which catabolic metabolism is activated.

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## Sammanfattning

*Effekter av olika utfodringstyper och fysisk stress på plasmagastrinkoncentrationen hos häst.*

Plasmagastrinkoncentrationen bestämdes hos sex varmblodiga travare (4 valackar och 2 ston) efter 3 olika utfodringar bestående av obegränsad mängd hö (8-9 kg per häst), en begränsad mängd hö (0.6 kg/100 kg kroppsvikt) och havre (0.2 kg/100 kg kroppsvikt) i kombination eller av enbart havre (0.2 kg/100 kg kroppsvikt). I en annan serie försök undersöktes gastrinets eventuella roll som stresshormon. Plasmagastrin och kortisol koncentrationerna bestämdes under fasta och jämfördes med motsvarande tidpunkter efter höutfodring. Gastrin och kortisol bestämdes även under 2 olika former av fysiskt arbete på en rullande matta.

Plasmagastrinkoncentrationerna ökade signifikant ($p<0.01$) efter utfodring, oberoende av utfodringstyp. Den högsta och mest långvariga gastrinsekretionen sågs när hästarna hade fri tillgång till hö. Den kombinerade utfodringen med havre och hö gav ungefär lika höga koncentrationer av gastrin i plasma som enbart hö, men durationen av gastrinsekretionen var kortare. När enbart havre gavs sågs däremot en fördröjd igångsättning, men förlängd frisättning av gastrin.

Resultaten ger stöd för följande slutsatser och tolkningar.

Måltidsstimulering frisätter gastrin hos häst, oberoende av om kraft- eller grovfoder ges. Distention av magsäcken är viktig som gastrinfrisättande faktor under den gastriska fasen av stimuleringen. Det fördröjda gastrinsvaret efter det att en relativt liten mängd havre hade givits kan tyda på att distentionen under denna typ av utfodring inte räckte till för att omedelbart frisätta gastrin. Den initialt uteblivna gastrinstegringen i detta experiment kan även indikera att nervös stimulering spelar en mindre roll vid aktiveringen av gastrinfrisättningen hos häst. Det förlängda svaret av gastrinsekretionen efter havreutfodring är förmodligen orsakat av en långsammare magsäckstömning.

Gastrin synes inte fungera som ett stresshormon hos häst. De högre kortisolkoncentrationerna under fasta och fysisk aktivitet kan innebära att kortisol deltar i aktiveringen av den katabola metabolismen i samband med fasta och fysiskt arbete, snarare än att hästen upplever något obehagligt.

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