Article

Plasma serotonin in horses undergoing surgery for small intestinal colic

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Abstract — This study compared serotonin concentrations in platelet poor plasma (PPP) from healthy horses and horses with surgical small intestinal (SI) colic, and evaluated their association with postoperative ileus, strangulation and non-survival. Plasma samples (with EDTA) from 33 horses with surgical SI colic were collected at several pre- and post-operative time points. Serotonin concentrations were determined using liquid-chromatography tandem mass spectrometry. Results were compared with those for 24 healthy control animals. The serotonin concentrations in PPP were significantly lower ($P < 0.01$) in pre- and post-operative samples from surgical SI colic horses compared to controls. However, no association with postoperative ileus or non-survival could be demonstrated at any time point. In this clinical study, plasma serotonin was not a suitable prognostic factor in horses with SI surgical colic.

Résumé — Sérotonine plasmatique chez des chevaux subissant une chirurgie pour des coliques du petit intestin. Cette étude a comparé les concentrations de sérotonine dans le plasma faible en plaquettes (PFP) de chevaux en santé et de chevaux atteints de coliques chirurgicales du petit intestin et a évalué leur association avec l'occlusion intestinale postopératoire, la strangulation et la non-survie. Des échantillons de plasma (avec EDTA) ont été prélevés auprès de 33 chevaux atteints de coliques du petit intestin à plusieurs moments préopératoires et postopératoires. Les concentrations de sérotonine ont été déterminées à l'aide d'un spectromètre de masse LC-ESI-MS/MS. Les résultats ont été comparés avec ceux de 24 animaux témoins en santé. Les concentrations de sérotonine du PFP étaient significativement inférieures ($P < 0,01$) dans les échantillons préopératoires et postopératoires provenant des chevaux atteints de coliques du petit intestin comparativement aux animaux témoins. Cependant, aucune association avec l'occlusion intestinale postopératoire ou la non-survie n'a pu être démontrée à aucun moment. Dans cette étude clinique, la sérotonine plasmatique ne s'est pas avéré un facteur de pronostic approprié chez les chevaux atteints de coliques chirurgicales du petit intestin.

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Introduction

Serotonin (5-hydroxytryptamine, 5-HT) is an important neurocrine messenger in the intestinal tract where it is produced by enterochromaffin (EC) cells and to a lesser extent by serotonergic neurons, and released upon mucosal stimulation. It interacts with serotonin receptors on afferent neurons, initiating peristaltic and secretory reflexes. Serotonin is eliminated from the interstitium by serotonin transporters on enterocytes and neurons; serotonin overflow from the gut reaches the intestinal lumen and portal circulation. In the circulation, it is quickly removed from the plasma by uptake into platelets or metabolized by monoamine oxidase in hepatic and lung endothelial cells. Platelets release serotonin upon activation. Free plasma serotonin exerts important systemic functions: it modulates platelet aggregation and is involved in vasomotor function (1–5).

As a result of normal serotonin metabolism, free plasma serotonin concentrations are influenced by multiple physiological and pathological events. In addition to reflecting platelet and vascular changes, plasma serotonin concentrations might help to assess the integrity of the gastrointestinal tract. Indeed, studies in diverse species have demonstrated an augmented serotonin release from the gut after experimental intestinal ischemia and reperfusion. In intestinal transplantation, a serotonin decrease in the intestinal wall and increase in the lumen and preservation fluid is used as a marker for ischemia-reperfusion injury (6–8). Increased serotonin concentrations after ischemia-reperfusion have been observed in the intestinal lumen (7) peritoneal fluid (9), and the mesenteric (10), portal and hepatic veins (11). In

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rats, intestinal ischemia-reperfusion caused a short-lived serotonin increase in peripheral plasma (12). In horses, results are conflicting. Whereas 1 study reported increased plasma serotonin concentrations in horses with strangulating small intestinal (SI) colic, another study showed a decreased serum serotonin concentration in horses suffering from acute abdominal pain (9,13). This discrepancy might originate from differences in methodology between studies. Various sample processing methods (centrifugation protocols, addition of platelet stabilizers) and analytical methods have been used for plasma or serum serotonin quantitation in horses (9,13–17), resulting in a high variability of reported serotonin concentrations, even in healthy horses. However, a reliable liquid chromatography-tandem mass spectrometry (LC-MS/MS) method was recently developed and validated for use in horses (18).

Despite continuing improvements in the surgical and postoperative care for horses with colic, postoperative complications including ileus, endotoxemia, and laminitis, are still a substantial problem. Recent publications report up to 33% of horses developing ileus after SI colic surgery (19–21). Estimating the odds of developing postoperative ileus (POI) for an individual horse is difficult. Both local lesions at the level of the intestinal wall and the associated systemic inflammatory and endotoxemic reactions contribute to the development of ileus. The free plasma serotonin concentration is a potential predictive parameter for POI, since it may reflect intestinal integrity as well as the circulatory effects associated with inflammation or endotoxemia. Therefore, serotonin quantitation might be an aid in prognosticating the outcome in horses with SI colic. Moreover, knowledge of plasma serotonin changes in colic horses is also important in the quest for an effective treatment for ileus, since certain classes of prokinetic drugs target serotonin receptors (1,22). A risk of receptor desensitization (23) might exist when these drugs are used in patients with already elevated serotonin levels.

The primary objective of this study was to investigate changes in plasma serotonin concentrations in horses that underwent SI colic surgery in a single university hospital, compared to healthy horses. In addition, associations of perioperative plasma serotonin levels with the presence of a strangulating lesion, the development of POI and non-survival were evaluated.

Materials and methods

Study population of small intestine surgical colic horses and healthy controls

The colic group consisted of 33 horses (14 mares, 16 geldings, and 3 stallions, with a mean ± SD age of 11 ± 6 y) that underwent SI colic surgery at the Faculty of Veterinary Medicine, Ghent University, Belgium between July 2009 and September 2010. The control group consisted of 24 horses (13 mares, 9 geldings, and 2 stallions, with a mean ± SD age of 12 ± 4 y). All control horses were clinically healthy; none had hirsutism or other signs of Cushing's disease.

Collection and preparation of samples

Blood samples of the horses with colic were collected at admission and immediately after surgery. This was followed by daily sampling until intestinal transit resumed, or until euthanasia or natural death of the horse. To avoid possible circadian variations, as previously found for serotonin in serum of athletic horses (24), the daily follow-up samples and all samples of control horses were always collected in the morning. Since horses with colic were presented for surgery at different times of the day, the interval between the postoperative sample and the first morning sample varied from 4 to 22 h (mean 13 h; SD 5h).

In accordance with previously published work (9), samples were collected into ethylenediamine tetraacetic acid (EDTA) tubes and immediately put on ice. Directly after sample collection, clomipramine hydrochloride (Sigma-Aldrich, Bornem, Belgium), final concentration of 1 μM, phenelzine sulphate (Sigma-Aldrich), 10 μM, and acetyl salicylate (Sanofi-Aventis, Diegem, Belgium), 1 mM, were added to inhibit platelet serotonin uptake, metabolism, and release, respectively (9,14). Samples were centrifuged at 300 × *g* for 10 min at 4°C, followed by 5000 × *g* for 20 min at 4°C for the supernatant, to obtain platelet poor plasma (PPP). Samples for the activation protocol were prepared by adding only clomipramine hydrochloride, 1 μM, and phenelzine sulphate, 10 μM, to whole blood in EDTA, thus inhibiting platelet serotonin uptake and metabolism but not release. Subsequently, platelet rich plasma (PRP) was obtained by centrifuging at 300 × *g* for 10 min at 4°C. All samples were stored at −80°C until assayed.

Measurement of plasma serotonin concentrations

Plasma serotonin concentrations were analyzed by LC-MS/MS as previously described (18). In brief, to an aliquot of 500 μL PPP, a solution of deuterated serotonin (CDN Isotopes, Nieuwegein, The Netherlands) was added as an internal standard. A liquid extraction into ethyl acetate was performed, followed by chromatographic separation on a C18 column (Eclipse Plus C18 column; Agilent, Diegem, Belgium) with an acetic acid-acetonitrile mobile phase gradient elution. The linearity of the LC-MS/MS method has been assessed between 3 and 100 ng/mL, the method had a limit of quantification of 3 ng/mL and its detection limit was 0.1 ng/mL (18).

Assessment of *in vitro* platelet activation

Platelets store serotonin in their dense granules and release it upon activation (4). Therefore, inadvertent platelet activation during sample collection and preparation can increase the plasma serotonin concentration in the sample. Two additional internal quality control tests were performed to assess inadvertent serotonin release, caused by platelet activation during sample handling and pretreatment.

First, the risk of *in vitro* serotonin release was evaluated. To assess the sensitivity of equine platelets for *in vitro* platelet activation, serotonin concentrations were assayed after the application of 3 different activation protocols on PRP obtained from healthy control horses. In activation group 1, PRP samples of 11 horses (3 mares and 8 geldings, with a mean ± SD age of 12 ± 5 y) were subjected to 2 freeze-thaw cycles. Samples for activation groups 2 and 3 and serum samples were collected from 6 horses (3 mares and 3 geldings, with a mean ± SD ages of 11 ± 3 y). Activation group 2 consisted of 6 samples that

Table 1. Mean (SEM) and median (SD) serotonin concentrations in platelet poor plasma measured by LC-MS/MS in healthy control horses and in surgical small intestinal colic horses at admission, directly after surgery, and the next morning

Sample	Mean (SEM) serotonin (ng/mL)	Median (SD) serotonin (ng/mL)
Healthy control (n = 24)	19.5 (2.4)	17.8 (12.0)
Colic admission (n = 30; 20 S + 10 NS)	8.0 (1.1)	6.4 (5.8)
Colic postoperative (n = 30; 20 S + 10 NS)	11.2 (3.6)	6.2 (19.5)
Colic morning 1 (n = 27; 17 S + 10 NS)	7.3 (1.3)	6.5 (6.9)

A log transformation was performed before statistical analysis.
S — Strangulating colic; NS — Non-strangulating colic; SEM — standard error of the mean; SD — standard deviation.

underwent sonication (Vibra-Cell sonicator; Sonics & Materials, Newtown, Connecticut, USA) for 30 s. For activation group 3, platelets were activated by adding collagen (Equine collagen type 1; American Biochemical and Pharmaceutical, Epsom, UK), 15 μg/mL to 6 PRP samples. Finally, a group of 6 serum samples, allowed to clot completely during 1 h at 37°C and centrifuged for 10 min at 1500 × *g*, served as positive controls with maximal platelet activation.

Second, the platelet activation status of all samples and controls was evaluated by measuring platelet activation parameters β-thromboglobulin (β-TG) and platelet factor 4 (PF4) (24,25). These chemokines are released into the plasma by activated platelets, together with serotonin. In human platelets, β-TG and PF4 are more easily released than serotonin by *in vitro* procedures (24). In case of *in vivo* activation, β-TG immediately binds to the endothelium, causing the β-TG/PF4 ratio to decrease. Therefore, augmented β-TG and PF4 concentrations together with an increased β-TG/PF4 ratio are considered an indication of *in vitro* platelet activation (25). The analyses of β-TG and PF4 were performed according to a sandwich ELISA protocol that was previously described and validated for horses (9). The β-TG/PF4 ratios were determined by comparing optical density (OD) values.

Statistical analysis

After preliminary testing on a subset of samples to determine the standard deviation, sample size calculations were done (Win Episcope 2.0; University of Edinburgh, Edinburgh, UK). For demonstrating a difference in serotonin concentrations of 6 ng/mL between horses with colic and controls at different time points, with a confidence interval (CI) of 95% and a statistical power of 80%, a minimal sample size n = 22 in each group was needed. Serotonin plasma data needed to be log transformed to obtain a normal distribution. To determine whether pre- and post-operative serotonin levels in SI colic horses differed from those in the control group, a one-way analysis of variance (ANOVA) was used, with equal variances assumed and a Dunnett *post hoc* test.

To determine possible associations between the categorical predictor variables (POI, strangulation and non-survival) and serotonin concentration in the SI colic horses, taking into account the repeated sampling, a maximum likelihood mixed model was built with repeated measurements and a compound symmetry repeated covariance type. A stepwise multivariable model building procedure was used. First, all predictors were tested univariably for their association with the serotonin concentration. Variables with $P < 0.20$ were withheld for the multivariable model. This model was built stepwise-backwards, gradually excluding non-significant variables. Biologically possible interactions between main effects were tested. Model fit was evaluated through analysis of the residuals.

Logistic regression models were built to determine the possible value of pre- and post-operative serotonin levels for the prediction of POI, strangulation, or non-survival. For this purpose survival was defined as discharge from the clinic and POI was only determined in horses that survived for at least 24 h after surgery, and defined as > 20 L of reflux within 24 h or > 8 L on a single occasion. Univariable associations between predictor variables were determined by the Chi-square test. An identical multivariable model building strategy, as described for the mixed model, was applied. Model fit was evaluated with the Hosmer-Lemeshow test.

Finally, a one-tailed paired *t*-test was used for comparison of serotonin concentrations in the various activated samples with negative controls. For all calculations, significance was set at $P < 0.05$. *P*-values < 0.10 were considered a trend. All analyses were done with SPSS v. 22 software (IBM, New York, New York, USA).

Results

Intraoperative findings

Strangulating obstructions were found in 67% (22/33) of the SI colic horses, 17 of which had severe lesions that required intestinal resection. The most frequent strangulating obstructions were entrapments into the epiploic foramen (n = 6) or the gastrosplenic ligament (n = 5) and strangulations around pedunculated lipomas (n = 6). Inguinal hernias and strangulations in other rents were also observed. None of the 11 horses with non-strangulating obstructions, which were most frequently ileal impactions (n = 6), needed a resection. Out of 20 horses that survived at least the first 24 h after surgery, 8 (40%) developed POI. Of all horses, 36% (12/33) did not survive to discharge, 4 of which were euthanized during surgery. Non-survivors were 9 out of 22 horses (41%) with strangulating lesions and 3 out of 11 (27%) with non-strangulating lesions.

Plasma serotonin in horses with small intestine surgical colic

Serotonin concentrations in PPP of SI colic horses that were measured at admission, immediately after surgery, and the next morning (morning 1) were significantly lower ($P < 0.01$) than the baseline values in healthy controls (Table 1). The difference between horses with colic and controls remained significant after omission of an outlier (control horse 6; serotonin concentration 63.3 ng/mL). The distribution of PPP serotonin concentrations in control horses and in the colic group at admission, according to strangulations, is shown in Figure 1.

There was no significant effect of sampling time on serotonin concentration within the SI colic group. The pre- and post-operative serotonin levels were lower at all time points in horses that developed POI compared with those that did not (Figure 2)

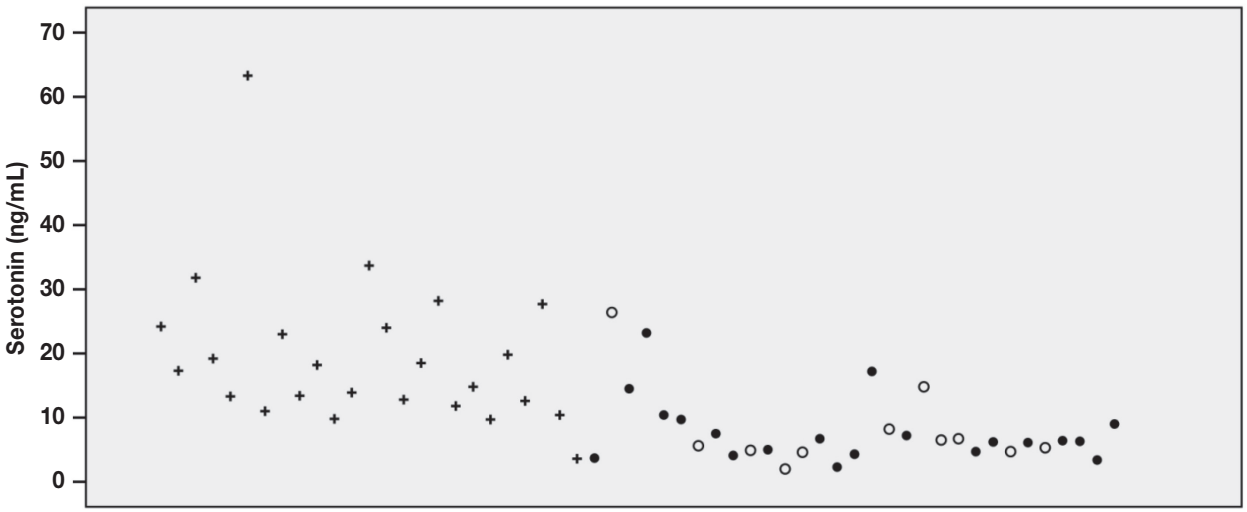

Figure 1. Platelet poor plasma serotonin concentrations in healthy control horses and in admission samples from small intestinal (SI) surgical colic horses.
Subjects ($n = 58$; + = healthy control, o = non-strangulating SI surgical colic, ● = strangulating small intestine surgical colic)

and in non-survivors compared with survivors (Figure 3). However, this difference was never significant.

Association of plasma serotonin with development of postoperative ileus and non-survival

Univariable analysis already showed that plasma serotonin levels were neither associated with the development of POI nor with non-survival, at any time point. No significant associations were found in the multivariable models for POI or strangulation. The final model for non-survival only consisted of POI, with significantly higher odds (OR = 15; 95% CI: 1.9 to 116) for non-survival in horses that developed POI compared with horses that did not. In the study sample 63% of the horses with POI died, compared with 10% in the non-POI group ($P < 0.01$).

Assessment of *in vitro* platelet activation

Platelet rich plasma activation (Table 2) by freeze-thaw cycles did not produce increased serotonin concentrations compared to controls ($P = 0.97$). Moderate increases in free serotonin were observed after both sonication ($P < 0.01$) and addition of collagen ($P < 0.01$). In serum, markedly increased serotonin concentrations (Table 2) were observed ($P < 0.01$) compared to PPP.

The OD values for the platelet activation parameters β-TG and PF4 were very low for most horses. For β-TG, mean OD was 0.08 (SD: 0.16) and for PF4, mean OD was 0.07 (SD: 0.22). There was no effect of any applied activation protocol (activation groups 1–4) on either β-TG or PF4. However, a number of individual horses (3 healthy controls and 2 colic horses) had consistently higher OD values ranging from 0.40 to 0.72 for β-TG and from 0.45 to 1.06 for PF4. These values remained constant in repeated sampling and were not influenced by applying activation protocols. The β-TG/PF4 ratio remained unchanged in these 5 horses, indicating that the elevated levels reflected an *in vivo* situation and not *in vitro* platelet activation (25).

Discussion

Even for healthy horses, reported normal values for plasma serotonin range from 2.5 to 90 ng/mL (9,14–17). In diseased horses, both increased and decreased plasma or serum serotonin values have been observed (9,13,26). Part of the observed differences may be related to technical difficulties in serotonin analysis and the use of different sample pretreatment protocols. Therefore, in the current study much attention was paid to using highly reliable methods for both sample handling and analysis.

All samples were handled according to a protocol that limits the risk of inadvertent *in vitro* platelet activation (9,14). Additionally, several tests were performed to assess the possibility of inadvertent changes to PPP serotonin levels in either the colic or control group. Unfortunately, β-TG and PF4 were found to be unsuitable parameters for platelet activation in the horse. In contrast with human platelets (24), horse platelets released serotonin, but no β-TG or PF4 during *in vitro* activation. Freeze-thawing of samples was not sufficient to augment free serotonin levels and there was no serotonin release into plasma, unless platelets underwent lysis by sonication or activation by the addition of collagen. Therefore it can be concluded that, during normal sample handling, the risk of inadvertent serotonin changes by *in vitro* platelet activation is very low in horses. This risk was reduced even further in the current study by the immediate cooling of samples, the addition of stabilizing agents and by continuous cooling of samples during centrifugation. The latter can, however, lead to somewhat higher serotonin values compared to centrifugation at room temperature, probably because the reuptake of serotonin into platelets is suppressed (24).

Besides the sample handling protocol and the additional internal quality controls described, another strength of the current study is the use of a reliable and validated LC-MS/MS method (18) for serotonin quantification.

The main finding of this study was that plasma serotonin concentrations were significantly lower in SI surgical colic

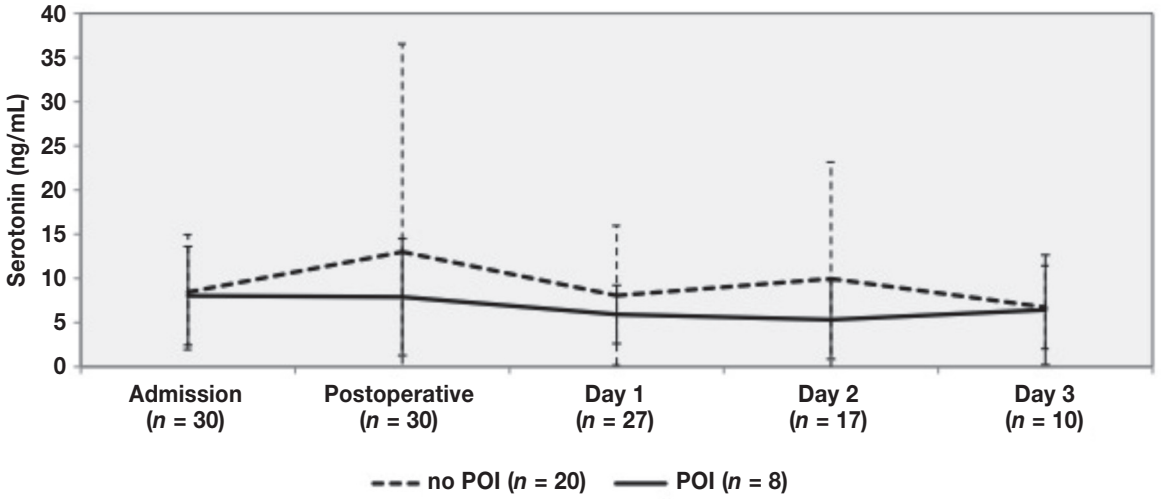

Figure 2. Platelet poor plasma serotonin concentrations over time in small intestinal surgical colic horses with or without postoperative ileus.

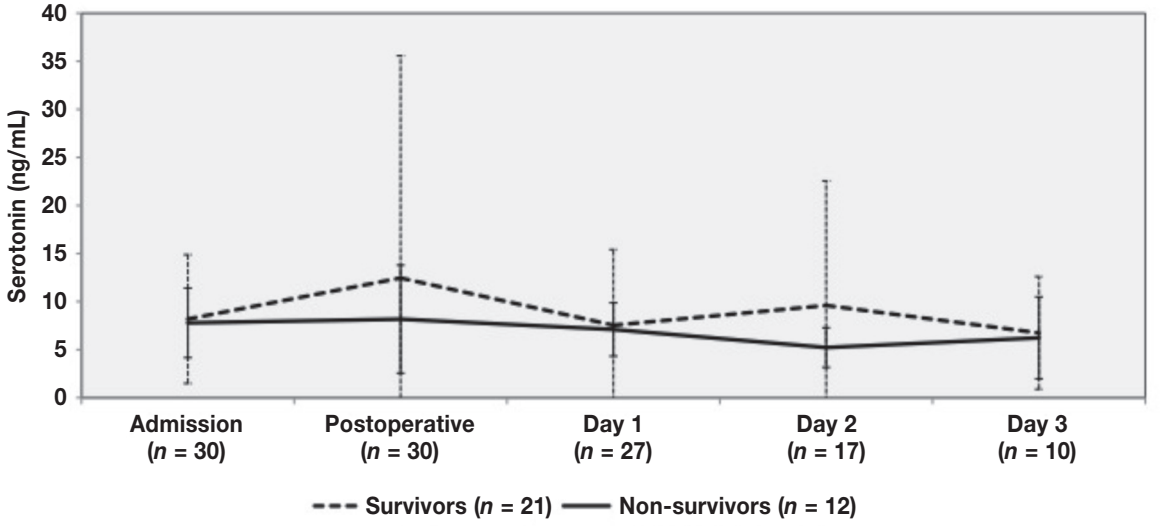

Figure 3. Platelet poor plasma serotonin concentrations over time in small intestinal surgical colic horses according to short-term survival.

horses compared to healthy controls. The serotonin concentrations remained low at least until the first morning after surgery. Significantly lower serotonin concentrations in horses with signs of acute colic were previously observed in a study (13) comparing serotonin concentrations in healthy horses and horses suffering from various conditions. However, the previous study focused on serum rather than plasma serotonin concentrations and used an enzyme-linked immunosorbent assay (ELISA) method for analysis, complicating the comparison with the current results. In contrast to these results, another study (9) demonstrated increased plasma serotonin concentrations, measured by HPLC, in a small group of horses that underwent SI colic surgery. Besides the analytical differences between these studies, multiple possible physiological and pathological explanations for these varying results exist; they are addressed in the following paragraphs.

Serotonin concentrations in PPP are thought to reflect the amount of recently synthesized and secreted serotonin in EC cells. However, this association is confounded by many variables. Decreased PPP serotonin values, as observed in the surgical colic group, could be associated with an increased clearance of serotonin from the plasma, a decreased amount of enterally released serotonin reaching the plasma, or a combination of both events. On a molecular level, serotonin clearance by uptake into platelets, enterocytes and hepatic cells is affected by serotonin transporter (SERT) expression, activation and desensitization (2). Besides SERT, backup transport mechanisms with a low affinity, but high capacity, play a role (23). After uptake into the cells, serotonin is degraded predominantly to 5-hydroxyindoleacetic acid, with urine as its primary excretion route (3).

Platelet stabilization by the previous administration of nonsteroidal anti-inflammatory drugs (NSAIDs) could contribute to lower PPP serotonin concentrations in horses with colic. Indeed NSAIDs were administered to 22 out of 33 colic horses before admission to the clinic, and to all horses in the peri-operative period. Serum glucocorticoid levels are also elevated in horses

Table 2. Mean (SD) activated and non-activated plasma serotonin concentrations and serum concentrations, measured by LC-MS/MS in healthy control horses. Group means before and after activation were compared by paired *t*-tests

Sample	Mean (SD) serotonin (ng/mL) in PPP sample	Mean (SD) serotonin (ng/mL) in activated PRP or serum sample	*P*-value
Freeze-thawed PRP (n = 11)	14.8 (7.9)	14.7 (6.5)	0.97
Sonicated PRP (n = 6)	18.4 (6.2)	59.0 (12.6)	< 0.01
Collagen PRP (n = 6)	18.4 (6.2)	57.2 (15.9)	< 0.01
Serum (n = 6)	18.4 (6.2)	221.9 (71.7)	< 0.01

PPP — Platelet poor plasma; PRP — Platelet rich plasma.

with colic (13,27) and could be associated with enhanced platelet stabilization (28) and decreased free serotonin concentrations. Indeed, lower plasma serotonin values have been observed in horses with Cushing's disease compared with controls (15). However, it is questionable if the effects of NSAIDs on platelets or endogenous glucocorticoid upregulation can outweigh endotoxemic events leading to increased platelet activation in surgical colic. Unfortunately it was not possible to evaluate the degree of platelet activation by assaying β-TG and PF4 in horses.

Intestinal ischemia and reperfusion is associated with a massive release of serotonin towards the intestinal lumen and with a decreased number of EC cells in the mucosa (6,7,29). In our study population, the SI contents were removed during colic surgery through enterotomy or by emptying the intestine towards the cecum. A temporary depletion of intestinal serotonin stores could therefore account for the prolonged decrease of PPP serotonin concentrations. Evaluating the duration of the serotonin deficit, we found that PPP serotonin values were significantly lower until at least the morning after surgery. A limited number of horses were also sampled the 2nd (n = 17) and 3rd (n = 10) morning after surgery. Serotonin concentrations appeared to remain lower than in controls, but the number of samples was too small to show a significant difference.

Many internal and external factors have reportedly influenced plasma and serum serotonin concentrations in horses. Factors such as diet (16,30), seasonal (14) or circadian (24) variations, physical exercise (31) or the presence of certain behavioral problems (32) might therefore have affected the currents results. This might also explain the presence of a number of outliers and the relatively large SD values in the postoperative samples and also, to a lesser extent, in the control group.

Both the diet and the time after feeding can influence plasma serotonin concentrations. Lower concentrations are measured in horses fed a high starch diet compared to a high fiber diet (30) and in fasted horses compared to fed horses (16). An increased serotonin release from EC cells after food intake, with increased plasma levels persisting for several hours, is also observed in humans (33). In the current study, control horses had been fed less than 5 h before sampling or were grazing at pasture. Horses in the colic group, however, received little, if any, food in the immediate postoperative period, which could account for at least part of the observed decrease in PPP serotonin concentrations. Preoperative diets in the colic group were variable in starch/fiber ratio while horses in the control group all received a balanced diet containing adequate amounts of fiber. This may have contributed to lower preoperative serotonin values in some horses of the colic group.

Seasonal variations of plasma serotonin might have affected the results of this study, since all control samples were collected in May, whereas colic samples were collected throughout the year. Although Bailey et al (14) did observe slightly higher plasma serotonin values in the spring, the observed differences were not significant. In another study (15), no seasonal differences in plasma serotonin concentrations could be demonstrated, making significant effects on the current results unlikely.

Circadian variations of serum serotonin have previously been demonstrated (34). Therefore all samples of control horses and the follow-up samples of colic horses were taken in the morning. A disadvantage of this method was the variable interval between the first postoperative sample and the "morning 1" sample. If the surgical procedure by itself affected plasma serotonin levels, there might be a variable effect on "morning 1" serotonin levels depending on the interval between surgery and sampling. However, no significant effects of elective abdominal surgery (castration of cryptorchid stallions) on plasma serotonin levels could be demonstrated in a previous study (9).

The effect of physical exercise on the observed serotonin values is unclear. One study demonstrated increased serotonin concentrations in equine plasma and whole blood shortly after exercise (31). None of the horses in the current study had been exercised before sampling, but the physical activity during a colic episode, the transport to the clinic, and postanesthetic recovery might have also affected serotonin levels, similarly to a bout of exercise. This effect, however, if it occurred, would only affect preoperative values. Surgery and post-anesthetic recovery do not seem to affect plasma serotonin concentrations (9).

A trend for lower basal serotonin concentrations was found in cribbing horses (32). No clear stereotypic behavior was noted in any of the colic or control horses.

Serotonin is an important mediator of intestinal motility, and decreased intestinal motility in human patients with constipation-predominant irritable bowel disease has been associated with impaired mucosal serotonin release and decreased plasma serotonin concentrations (33,35). The current results on equine colic cases might therefore be due to a colic and/or surgery associated phase of decreased motility. This is, to the authors' knowledge, the first study that evaluated, albeit in a limited population, the potential association of equine PPP serotonin concentrations with POI and non-survival. Plasma serotonin concentrations in this study could not predict the development

of clinical POI or non-survival — the latter strongly associated with POI. In this limited group of horses, POI was not associated with intestinal strangulation either. Indeed, multivariable analyses have shown that intestinal resection, rather than mere strangulation, is an important risk factor for POI (20,21). Therefore it would be interesting to evaluate, in a larger population of horses with and without small intestinal resections, the time to normalization of serotonin values during a longer follow-up period.

The final effects of serotonin on the intestine will depend on not only plasma serotonin concentrations, but also on the balance between receptor activation and desensitization. Local serotonin concentrations at the level of the intestinal mucosa, and their association with postoperative intestinal recovery, might therefore more adequately reflect the net effects of serotonin on intestinal motility. Possibly, these local measurements could also be used to estimate the level of intestinal injury, as described in other species (6–8).

Despite the finding that serotonin levels in SI surgical colic horses are significantly lower than in healthy horses, it can be concluded that the broad range of reported normal values, the multiple internal and external factors influencing plasma serotonin concentrations, and the apparent lack of association with either ileus or non-survival, do not support the use of PPP serotonin as a routine prognostic factor in clinical cases. Future research on serotonin changes in colic horses should probably aim at measuring mucosal rather than plasma concentrations.

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