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EVALUATION OF BLOOD BIOMARKERS CONCENTRATION IN EQUINE SUBMITTED TO EXPERIMENTAL OBSTRUCTION OF DUODENUM, ILEUM AND LARGE COLON

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ABSTRACT

This study aimed to evaluate the parameters of renal and hepatic functions in horses submitted to an experimental model of intestinal obstruction. Twenty-four animals were distributed into four different groups: instrumented control (GI), duodenum obstruction (GII), ileum obstruction (GIII) and large colon obstruction (GIV). Blood samples were collected one hour before the surgical procedure (T0); 3 hours after the obstruction/ischemia (T3i); and 1, 3, 12, 24, 72 and 120 hours after the beginning of deobstruction/reperfusion for determination of the concentration of urea, creatinine, aspartate aminotransferase, gamma-glutamyltransferase, alkaline phosphatase, albumin, glucose, fibrinogen and (total, direct and indirect) bilirubin. During obstructive period none significant alteration was observed in the biochemical parameters evaluated. After the unblocking procedure, the animals from GII and GIII presented an increase in aspartate aminotransferase, alkaline phosphatase, fibrinogen and total and direct bilirubin values. However, these changes were not associated with clinical signs of liver damage and, at the end of the observation period their values were found within normal range. Considering lack specificity of the laboratory tests for the diagnosis of hepatic lesions, it is recommended that such tests are performed concomitantly and in series.

KEYWORDS: Acute abdomen, blood biomarkers, equine.

VARIAÇÕES NAS CONCENTRAÇÕES DOS BIOMARCADORES SANGUÍNEOS DA FUNÇÃO RENAL E HEPÁTICA EM EQUINOS SUBMETIDOS À OBSTRUÇÃO EXPERIMENTAL DO DUODENO, ÍLEO E CÓLON MAIOR

RESUMO

Com o objetivo de avaliar a concentração sérica dos biomarcadores da função renal e hepática em equinos submetidos a um modelo experimental de obstrução intestinal, 24 animais foram distribuídos em quatro grupos-controle instrumentado (GI), obstrução do duodeno (GII), íleo (GIII) e cólon maior (GIV). As

amostras de sangue destinadas à dosagem de ureia, creatinina, aspartato aminotransferase, gama glutamiltransferase, fosfatase alcalina, albumina, glicose, fibrinogênio e bilirubina (total, direta e indireta) foram coletadas uma hora antes do procedimento cirúrgico (T0), ao final do período de três horas de obstrução/isquemia (T3i) e 1, 3, 12, 24, 72 e 120 horas após a desobstrução/reperfusão. Durante a obstrução não se observaram alterações nos parâmetros bioquímicos séricos avaliados. Após a desobstrução, os equinos do GII e do GIII apresentaram aumento dos valores de aspartato aminotransferase, fosfatase alcalina, fibrinogênio, bilirubina total e direta. Entretanto, tais alterações não foram associadas a sinais clínicos de lesão hepática e, ao final do período de observação, seus valores já encontravam-se entre os de normalidade. Devido à falta de especificidade dos testes laboratoriais existentes para o diagnóstico de lesões hepáticas, recomenda-se que estes sejam realizados concomitantemente e de modo seriado.

PALAVRAS-CHAVE: Abdômen agudo, biomarcadores sanguíneos, equinos.

INTRODUCTION

Colic syndrome is a very frequent emergency in equine medicine, being considered the greatest cause of death for this species. Within its etiologies, disturbances associated to ischemia and reperfusion are important, due to the high rates of incidence and death (White, 1990).

Ischemia is defined by the reduction or interruption of bloodstream, representing one of the main causes of tissue lesion (COTRAN *et al*., 1994). Cellular alterations are directly related with ischemia duration; when it lasts long enough, necrosis occurs, leading to intense and complex effects, because of endotoxins absorption and the occurrence of hydroelectrolytic and acid-base balance disorders. These disorders happen in distant organs and are more difficultly treated than ischemia disorders or intestinal resection surgery (MOORE, 1990). In such conditions, liver and kidneys are frequently damaged and severe alterations in these organs are negatively related with the animal´s recovery (SEANOR *et al*., 1984; DAVIS *et al*., 2003; DI FILIPPO & SANTANA (2007).

Specific biochemistry tests used to evaluate hepatic function can be classified in four groups: indicative of hepatocellular lesion, represented by aspartate aminotransferase (AST); indicative of cholestasis, represented by alkaline phosphatase (AP) and gamma-glutamyltransferase (GGT); bilirubin measurement to evaluate hepatic storage, conjunction and secretion; and albumin, glucose and serum nitrogen urea to evaluate hepatic synthesis (DIAL, 1995).

In renal function evaluation, urea and creatinine can be taken into account as markers of possible alterations in glomerular filtration rates, working as an evolution parameter, monitoring treatment and the disease progression (DUNCAN *et al*., 1994). Urea, the final product of protein metabolism, is excreted by the kidneys; however, at least 40% of it is reabsorbed by renal tubules. Consequently, urea blood levels are an idicative of renal function and can work as an index of glomerular filtration rate, although creatinine is more appropriate in this case, because the amount of creatinine present in the kidney is constant and it is not reabsorbed by the renal tubules (STEVEN & SCOTT, 2002). Creatinine, derived from creatine and phosphocreatine during muscle metabolism, is also excreted by renal glomerulus. Serum creatinine level is influenced by muscle mass and physical training (DUNCAN *et al*., 1994) and, contrary to urea, it is not altered by hyperproteic diets or gastrointestinal hemorrhage (MEYER & HARVEY, 1998).

Considering the previous observations, the purpose of the current study was to evaluate and compare the alterations in the levels of blood biomarkers of renal and hepatic functions related with colic syndrome repercussion. The beginning and the behavior of such alterations were determined in function of time and whether they can be used in renal and hepatic lesions diagnostic as well as in the prognosis of colic in equine.

MATERIAL AND METHODS

A total of 24 undefined breed equine were used: eight non-pregnant females, and 12 castrated males and four non-castrated males. The animals

were at mean age of 6.2 ± 3.0 years, presented body score from three to four (SPEIRS, 1997) and mean body weight of 295.9 ± 32.7 kg. One week before the experiment, right after clinical and sanitary status assessment, endoparasites (mebendazole, 50 mg kg^{-1} - Platelmin Equino – UCB S.A) and ectoparasites (deltametrine at 0.025% - Butox P – Intervet S.A.) control was carried out. The animals were kept in paddocks and were fed coast-cross (*Cynodon dactylon*) hay-based diets and water at will. Commercial concentrate diet (Tec Horse – Purina) was offered twice a day, in equivalent amount to 1% body weight (2.5 a 3.4 kg), added 50 g/day of mineral supplement (Omolen Ephos – Purina).

The animals were divided into four groups of six (two females, three castrated male and one non-castrated male): one control group (GI), which was submitted to the same anesthesia and surgical procedures, however, not to the intestinal obstruction; and three obstructed groups. Intestinal obstructions were carried out in three different segments: duodenum (GII), ileum (GIII), and large colon – pelvic flexure (GIV).

The animals were kept in squeeze chute and after trichotomy and anti-sepsis of the paralumbar fossa, the animals were sedated with acepromazine 1% (0.025 mg kg-1, IV - Acepran 1% – Univet S.A), xylasine hydrochloride 2% (0.5 mg kg^{-1}, IV - Virbaxil 2% – Virbac.) and meperidine (4 mg kg^{-1}, IM - Dolosal – Cristália). Right after that, local infiltration anesthesia was carried out by the (1:1) association of lidocaine 2% (Lidovet – Bravet) with bupivacaine 0.75% (Neocaine 0.75% – Cristália), both without vasoconstrictor. In order to imitate natural conditions, the animals were submitted to previous water and food restriction.

Intestine fragments were identified by laparotomy, with animals standing, from right flank to duodenum and ileum, and from left flank to large colon. After that, a (number-3) penrose drain was positioned around the intestinal loop and, after its closure, intestinal obstruction was initiated, according to the model described by DATT & USENIK (1974). At this point, the animals received 1.5 mg kg^{-1} tramadol hydrochloride (Tramal – Cristália), IV (NATALINI & ROBINSON, 2000). Afterwards, simple continuous sutures of transverse abdominal muscles and of skin were performed by the use of 0-vicryl and 4-nylon, respectively. Obstructions were maintained for three hours and, after this period, they were reversed by means of the same surgical access and protocols used to perform them. Drains were removed and abdominal cavities were closed according to the technique described by TURNER & MCILWRAITH (2002).

During the postoperatory period, antimicrobian therapy was perfomed with bezanthine penincillin (Pentabiótico Veterinário Reforçado – Fort Dodge), at the dosage of 30,000 UI kg^{1}, IM, each 48 hours, totalling three applications. Flunixin meglumin was used as analgesic and anti-inflammatory (injectable flunixin – UCB S. A) at the dosage of 0.5 mg kg^{-1}, IV, every 24 hours, for two days. Surgical wound dressing was carried out with topic polyvinylpyrrolidone-iodine at 1% twice a day, until the stitches were withdrawn on the tenth postoperatory day. Blood samples were obtained by jugular vein puncture, one hour before the begining of the surgical procedure (T0), at the end of the 3-hour obstrucion/ischemia period (T3i) and one, three, 24, 72, 120 and 168 hours after disobstruction, i.e., during reperfusion period (T1r-T168r). During the obstruction or ischemia phase (Ti), samples were obtained at 30-minute regular intervals until three hours of obstruction. Nevertheless, in order to make the tables, only the results obtained three hours after the begining of the obstruction were used, the other moments were excluded. Such procedure enabled the elaboration of clearer and more objective tables, without jeopardizing the general analysis.

For the evaluation of serum-biochemistry parameters, kinetics UV urea (Diacetyl monoxime method), colorimetric creatinine (Lustosa-Basques method), aspartate aminotransferase (kinetic UV method), kinetic PNP alkaline phosphatase (modified Roy method), albumin (bromocresol green method), and gama-glutamiltransferase (modified Szasz method) blood samples, collected in flasks without anticoagulant, were used. Samples were centrifuged at 2,000 rpm for five minutes and, after syneresis, the serum obtained by this process was placed in eppendorf tubes, identified and stored at -20ºC until assessment. Then, samples were analysed with the aid of a reagent set for diagnosis and posterior spectrophotometric readings. Globular volume (GV) were obtained in microhematocrit tubes, centrifuged

at 14,000G, for five minutes, with subsequent reading in a special scale.

Glucose and fibrinogen were assessed immediately after blood collection. Glucose was assessed in plasma fluoride (end-point kinetic method), with the aid of a reagent set for diagnosis (Labtest – Sistema de Diagnósticos Ltda. – Lagoa Santa, Brazil) and a biochemistry analyser (Labquest – Labtest); fibrinogen was assessed in citrated plasma (tubes with 3.8% sodium citrate), according to the chronometric procedure described by CLAUSS (1957), with the aid of a reagent set for diagnosis (Wiener commercial kit – Rosário, Argentina) and posterior readind in a specific analyser (Quick timer DRAKE.). A totally random experimental design was used, with four groups and eight collections. When significance was observed among groups within each period, Tukey test ($p<0.05$) was applied for mean comparison (SAMPAIO, 2002), by SAS statistical software (Statistical Analysis of System – version 8).

RESULTS AND DISCUSSION

Urea and creatinine values are presented in Table 1 along with respective means, standard deviation and statistics.

TABLE 1. Mean and standard error of urea (mg/dL) and creatinine (mg/dL) of equine in the control group (GI) and equines submitted to ischemia in duodenum (GII), ileum (GIII) and large colon (GIV), one hour after the surgical procedure (T0), at the end of ischemia (T3i) and 1, 3, 24, 72, 120 and 168 hours after reperfusion (T1r-T168r)

Groups	Time (h)							
	T0	T3i	T1r	T3r	T24r	T72r	T120r	T168r
				Urea				
I	20.4±5.2a	26.7±6.4a	23.3±10.6a	25.2±7.6a	36.4±9.1a	23.3±3.9a	21.3±4.1a	19.9±5.2a
II	24.3±6.7a	30.6±6.7a	26.7±7.7a	29.2±9.7a	38.5±12.5a	25.7±8.8a	27.9±8.9a	25.3±11.9a
III	32.4±12.7a	34.6±8.4a	34.4±8.4a	37.1±8.1a	37.5±10.5a	28.8±12.2a	25.6±7.7a	29.9±18.1a
IV	30.6±6.8a	32.5±10.7a	33.2±6.8a	36.9±9.6a	36.5±11.2a	25.5±9.2a	25.2±5.8a	25.7±6.5a
				Creatinine				
I	1.1±0.3a	1.4±0.2a	1.3±0.2a	1.4±0.2a	1.30±0.30a	1.1±0.0a	1.2±0.1a	1.0±0.2a
II	1.3±0.1a	1.5±0.1a	1.5±0.3a	1.5±0.2a	1.41±0.11a	1.3±0.3a	1.3±0.2a	1.1±0.3a
III	1.3±0.2a	1.6±0.2a	1.6±0.8a	1.5±0.2a	1.38±0.23a	1.3±0.1a	1.1±0.2a	1.1±0.1a
IV	1.4±0.2a	1.5±0.1a	1.4±0.1a	1.5±0.2a	1.43±0.19a	1.2±0.2a	1.2±0.2a	1.1±0.1a

Different capital letters, in the colunms, indicate significant differences among the groups ($P<0.05$).
Different small letters, in the lines, indicate significant differences among the moments ($P<0.05$).

Animals from GI, GII, GII and GIV did not show alterations ($P<0.05$) on urea and creatinine levels during all the experimental period. These results differ from the ones obtained by DI FILIPPO & SANTANA (2007), who evaluated equine with natural-origin colic and observed that animals that did not survive presented higher urea and creatinine levels than the animals that survived. According to these authors, alterations were attributed to the decrease of tissue perfusion, septicemy and the use of anesthetic drugs, especially α2-agonist. According to COLES (1984), increased urea and creatinine levels are solely derived from hypotension. Hypotension, common in colic animals, decreases tissue perfusion and the speed of glomerular filtration, with consequent increase of these biomarkers in bloodstream.

In the present study, mantaining intestinal obstruction for three hours was not enough to cause significant hydric alterations (Table 2). DATT & USENIK (1974), using the same intestinal obstruction model, observed severe hydric alterations

in animals submitted to duodenum and ileum obstruction; however, only between six to twelve hours of obstrucion. Except the animals submitted to obstrucion of the smaller colon, all the other animals evaluated by DATT & USENIK (1974) died.

TABLE 2. Mean and standard error of globular volume (%)of equine in the control group (GI) and equines submitted to ischemia in duodenum (GII), ileum (GIII) and large colon (GIV), one hour after the surgical procedure (T0), at the end of ischemia (T3i) and 1, 3, 24, 72, 120 and 168 hours after reperfusion (T1r-T168r)

	Time (h)							
Groups	T0	T3i	T1r	T3r	T24r	T72r	T120r	T168r
	Globular volume							
I	26.06±3.22a	23.76±3.29a	24.43±3.35a	23.70±3.17a	25.80±2.66a	26.50±1.63a	26.30±0.46a	27.03±0.58a
II	30.61±3.96a	24.26±3.12a	25.68±3.34a	28.30±5.90a	26.50±2.65a	28.76±3.11a	27.81±4.49a	26.01±2.22a
III	28.30±3.46a	25.25±4.10a	26.40±4.03a	26.71±2.90a	26.58±3.68a	27.83±3.13a	26.90±2.26a	26.65±2.76a
IV	27.73±7.33a	24.45±6.74a	25.36±6.10a	25.80±5.94a	27.80±7.23a	26.78±4.86a	27.70±5.75a	27.10±5.79a

Different capitall letters, in the colunms, indicate significant differences among the groups (P<0.05).
Different small letters, in the lines, indicate significant differences among the moments (P<0.05).

Values regarding aspartate aminotransferase, gamma-glutamyltransferase and alkalin phosphatase with the respective means, standard deviations and statistics are presented in Table 3.

TABLE 3. Mean and standard error of aspartate aminotransferase (U/L), gamma-glutamyltransferase (U/L), alkaline phosphatase (U/L) of equine in the control group (GI) and equines submitted to ischemia in duodenum (GII), ileum (GIII) and large colon (GIV), one hour after the surgical procedure (T0), at the end of ischemia (T3i) and 1, 3, 24, 72, 120 and 168 hours after reperfusion (T1r-T168r)

	Time (h)							
Groups	T0	T3i	T1r	T3r	T24r	T72r	T120r	T168r
	Aspartate aminotransferase							
I	241±60abc	234±24bc	217±47c	237±26bc	325±63Babc	350±50Ba	340±77ab	256±52abc
II	235±36c	234±26c	238±23c	261±22c	458±61Aab	564±74Aa	313±47bc	282±50c
III	239±61c	245±41c	246±72c	270±86c	465±157Aab	518±97Aa	356±73bc	321±79bc
IV	258±26b	266±36ab	253±51b	281±30ab	371±46Ba	333±59Bab	316±37ab	275±43ab
	Gamma-glutamyltransferase							
I	33.9±3.2a	21.2±6.5a	19.0±5.6a	19.0±5.6a	23.3±3.2a	23.3±3.2a	19.0±0.0a	21.2±3.8a
II	24.2±8.6a	21.0±6.7a	18.9±4.2a	18.9±8.2a	23.0±10.6a	24.2±9.5a	23.1±6.8a	20.9±8.0a
III	25.1±12.1a	22.0±9.9a	20.9±8.9a	20.0±5.0a	23.1±8.8a	22.1±8.9a	19.9±10.4a	20.0±6.4a
IV	31.8±8.9a	27.5±8.6a	24.3±8.4a	28.6±10.4a	27.5±9.5a	25.4±10.6a	24.3±10.1a	27.5±9.5a
	Alkaline phosphatase							
I	304.0±82.6a	267.9±48.0a	262.4±59.7a	251.3±27.8a	267.9±51.9Ba	248.7±50.8Ba	278.8±73.9a	284.4±34.0a
II	258.4±46.4a	266.7±62.8a	243.2±52.3a	269.4±58.5a	299.4±81.7Aa	309.3±112.9Aa	252.9±56.6a	244.6±51.5a
III	312.2±93.7a	287.3±106.1a	287.4±93.3a	279.0±99.8a	295.6±109.4Aa	319.1±75.5Aa	321.9±67.6a	342.6±96.5a
IV	317.8±81.6ab	295.7±85.6ab	283.3±58.2b	443.6±228.7a	261.6±76.0Bb	269.4±50.5ABb	268.8±75.1b	284.7±85.3b

Different capital letters, in the colunms, indicate significant differences among the groups (P<0.05).
Different small letters, in the lines, indicate significant differences among the moments (P<0.05).

In T24r and T72r, there was an increase in the activity of serum enzymes aspartate aminotransferase and alkaline phosphatase in animals from GII and GIII. However, no significant changes in serum activity of gamma-glutamyltransferase. According to DAVIS *et al*. (2003) and DI FILIPPO & SANTANA (2007), increased AST and AP in the serum of horses with colic is due to possible infection ascending from the organ through the bile duct; the absorption of endotoxin or inflammatory mediators by portal circulation; blocking of bile ducts; hepatic hipoxia associated with systemic inflammatory response symdrome (SIRS) and with the shock. Although AST and AP are found in different tissues, the serum activity of such enzymes is not specific to any tissue, but muscles and liver may be considered their main source (STEVEN & SCOTT, 2002; CÂMARA & SILVA *et al*., 2007; DI FILIPPO & SANTANA, 2008).

The origin of increased serum AST and AP could only be determined when its isoenzymes were measured simultaneously (DUNCAN *et al*., 1994; MOORE *et al*., 1990). As the clinical use of these tests has not become routine yet, the differentiation must be made by the history and the physical examination of the animal, as it was explained by DIAL (1995). However, the appearance of clinical symptoms in cases of liver disease depends on the involvement of more than 70% of hepatocellular mass, and the lesion, regardless its cause, is usually associated with some degree of cholestasis, because the hepatocytes dilate and obstruct bile tubules (DUNN, 1992). Furthermore, the lack of specificity and the high variability of clinical manifestations of liver disease often cause them to be overlooked or confused with acute abdomen cases (AMORY *et al*., 2005; DI FILIPPO & SANTANA, 2007).

According to RYU *et al*. (2004), hyperthemia, icteric mucosa and colic intermitent episodes were the only signs observed in a 13-year-old Thoroughbred mare with hepatic lesion. Because of the similarities between such symptoms and the ones presented by equine with colic originated in the gastroenteric system, laboratory tests are recommended in order to have a differential diagnosis (WEST, 1996).

Table 4 presents values of fibrinogen, albumin and glucose with the respective means, standard deviations and calculated statistics.

TABLE 4. Mean and standard error of fibrinogen (mg/dL), albumin (g/dL) and glucose (mg/dL) of equine in the control group (GI) and equines submitted to ischemia in duodenum (GII), ileum (GIII) and large colon (GIV), one hour after the surgical procedure (T0), at the end of ischemia (T3i) and 1, 3, 24, 72, 120 and 168 hours after reperfusion (T1r-T168r)

Groups	Time(h)							
	T0	T3i	T1r	T3r	T24r	T72r	T120r	T168r
				Fibrinogen				
I	300±154a	200±89a	166±51a	300±236a	200±154a	233±206Ba	133±51Ba	300±154a
II	216±98b	283±132ab	233±81ab	233±103ab	250±122ab	330±89Aa	300±109Aa	200±109b
III	283±160ab	233±136b	233±136b	250±122ab	216±98b	333±136Aa	266±150ABab	250±122ab
IV	166±51a	250±104a	266±136a	200±109a	200±109a	233±103Ba	250±122Ba	250±122a
				Albumin				
I	1.8±0.3a	1.6±0.1a	1.8±0.1a	2.0±0.6a	2.2±0.4a	2.1±0.4a	2.0±0.5a	2.0±0.1a
II	2.0±0.2a	2.0±0.1a	2.2±0.1a	2.2±0.2a	2.2±0.2a	2.1±0.3a	2.1±0.2a	2.0±0.2a
III	1.9±0.3a	1.5±0.4b	1.4±0.4b	1.3±0.6b	2.0±0.6a	1.8±0.5a	1.7±0.6ab	1.7±0.4ab
IV	1.9±0.2a	1.8±0.2a	1.9±0.2a	1.9±0.a3	2.0±0.4a	1.9±0.2a	2.1±0.1a	2.0±0.2a
				Glucose				
I	75.0±6.4a	86.0±10.6a	84.8±13.2a	92.0±5.1a	87.1±29.7a	75.2±3.0a	86.3±23.8a	81.8±6.5a
II	84.8±10.0bc	132.6±60.3a	189.7±43.7a	117.6±42.4ab	100.8±10.7abc	80.4±10.1c	86.0±8.9bc	83.0±8.8bc
III	81.4±11.8a	88.2±12.8a	102.3±23.2a	90.8±11.7a	90.5±17.6a	79.7±7.1a	81.8±9.2a	81.3±6.8a
IV	88.2±12.5a	90.8±9.8a	103.4±23.6a	110.9±20.9a	110.8±20.1a	81.6±10.0a	89.5±15.6a	92.9±18.8a

Different capital letters, in the colunms, indicate significant differences among the groups (P<0.05).
Different small letters, in the lines, indicate significant differences among the moments (P<0.05).

In T72r there was an increase in fibrinogen values in equine of GII and GIII, which remained in T120r in animals from GII. This increase was either caused by the existence of the inflammatory response

stimulated by the surgical procedure or it was due to the intestinal disorder, as it was explained by FAGLIARI & SILVA (2002). Fibrinogen provides a substrate for fibrin formation and tissue repair, forming a matrix for the migration of inflammatory cells (TAMZALI *et al*., 2001). Similar results were obtained by DATT & USENIK (1974); however, for these authors, hyperfibrinogenemia was due solely to dehydration with consequent increase of total protein in the bloodstream. As it was mentioned earlier, water imbalance was not observed in the tested animals at any moment of this study.

There were no changes in albumin values among the animals tested; however, these values decreased in GIII in T3i, T1r and T3r, comparing with the beginning of the assessment (T0). This reduction could be misinterpreted as liver injury, but, in this case, the reduction would only occur after extensive and chronic liver lesion, and not after an acute case, such as those reported in equine with colic (AMORY *et al*., 2005; THRALL, 2007). Similarly to the statements by McGORUM *et al*. (1999), it is believed that the decrease was due to the intestinal inflammatory process, the mobilization of protein to the focus of injury and anorexia.

After maintaining the intestinal obstructions for three hours (T3i) and one hour after disobstruction, increase in glucose values in equine from GII was observed. These results confirm the ones found by DI FILIPPO & SANTANA (2007). These authors believe that hyperglycemia at the initial phases of the process is due to the increase of glycogenolysis, stimulated by the increase of circulating catecholamines. However, in animals from GII, hyperglycemia remained Constant until the seventh post-operatory day, differing from what was observed in this study, in which glucose plasma levels return to normality or subnormality values when energy resources were over DI FILIPPO & SANTANA (2007).

According to GRULKE et al. (2003), persistent hyperglycemia is a common finding only in animals with acute pancreatic injury, triggered during the colic episode by hypovolemia, septicemia and also by mechanical compression of the body due to the accentuated distention of the intestinal loop. The pancreatic injury intereferes with the production and release of insulin, besides releasing trypsin to the peritonial space and plasma. Trypsin, by activating the inflammatory cascade and the leukocytes, can lead to multiple organ failure and thus jeopardize the recovery of animals with colic.

The values of total, direct and indirect bilirubin, with means, standard deviations and calculated statistics are given in Table 5.

TABLE 5. Mean and standard error of total bilirubin (mg/dL), direct bilirubin (mg/dL) and indirect bilirubin (mg/dL) of equine in the control group (GI) and equines submitted to ischemia in duodenum (GII), ileum (GIII) and large colon (GIV), one hour after the surgical procedure (T0), at the end of ischemia (T3i) and 1, 3, 24, 72, 120 and 168 hours after reperfusion (T1r-T168r)

	Time (h)							
Groups								
	T0	T3i	T1r	T3r	T24r	T72r	T120r	T168r
					Total Bilirubin			
I	0.43±0.00a	1.22±0.41a	0.86±0.22a	1.26±0.35a	0.82±0.33Ba	0.93±0.40Ba	1.05±0.35a	0.94±0.30a
II	1.27±0.59a	1.33±0.59a	1.42±0.51a	1.42±0.53a	1.75±0.65Aa	1.42±0.52ABa	1.23±0.36a	1.36±0.30a
III	0.86±1.09a	0.94±0.24a	0.94±0.39a	0.91±0.23a	1.45±0.68ABa	1.21±0.43ABa	1.04±0.30a	0.96±0.35a
IV	1.18±0.99ab	0.89±0.23b	0.76±0.17b	0.97±0.29ab	1.10±0.09ABab	1.82±1.13Aa	1.02±0.35ab	0.97±0.31ab
					Direct Bilirubin			
I	0.00±0.00a	0.16±0.24a	0.00±0.00Ba	0.16±0.24Ba	0.05±0.08Ba	0.03±0.02Ba	0.45±0.12a	0.46±0.02a
II	0.33±0.37a	0.42±0.37a	0.49±0.36Aa	0.71±0.27Aa	0.79±0.40Aa	0.68±0.49Aa	0.59±0.41a	0.56±0.43a
III	0.26±0.14a	0.23±0.24a	0.23±0.25ABa	0.24±0.15Aa	0.33±0.21Ba	0.20±0.13Ba	0.20±0.21a	0.17±0.19a
IV	0.04±0.05a	0.34±0.31a	0.31±0.25ABa	0.32±0.27ABa	0.40±0.23ABa	0.36±0.18ABa	0.50±0.42a	0.27±0.23a
					Indirect Bilirubin			
I	0.43±0.01a	1.06±0.24a	0.86±0.22a	1.10±0.17a	0.76±0.36a	0.89±0.43a	0.60±0.23a	0.47±0.28a
II	0.93±0.77a	0.90±0.68a	0.93±0.67a	0.71±0.59a	0.96±0.59a	0.74±0.47a	0.64±0.35a	0.80±0.57a
III	0.60±1.06a	0.70±0.38a	0.71±0.47a	0.67±0.26a	1.12±0.57a	1.00±0.31a	0.83±0.09a	0.79±0.21a
IV	1.13±0.95ab	0.55±0.19b	0.44±0.17b	0.64±0.23ab	0.70±0.23ab	1.46±1.10a	0.52±0.32b	0.69±0.21ab

Different capital letters, in the colunms, indicate significant differences among the groups (P<0.05).
Different small letters, in the lines, indicate significant differences among the moments (P<0.05).

An increase of total bilirubin was observed in animals from GII and GIII at T24r and T72r. An increase of direct bilirubin was verified in the blood of animals from the same groups at T1r, T3r T24r and T72. These increases were caused by either the interruption of the bile flow or the inability of excretion by the liver as a result of a portal-systemic venous shunt, frequent in cases of equine colic, as described by RYU et AL. (2004).

CONCLUSION

Equine submitted to extraluminal intestinal obstruction model for three hours did not present alterations in the levels of blood biomarkers of renal and hepatic lesion. Only after the obstruction/reperfusion na increase in aminotransferase aspartate, alkalin phosphate, fibrinogen, total and direct bilirubin values was observed in animals from GII and GIII. However, such alterations were not associated with clinical signs of hepatic lesions and, at the end of the observation period, the values were within normality rates.

Due to lack of especificity of the existent laboratorial tests for the diagnosis of hepatic lesions, it is recommended that such tests are performed concomitantly and in series. Depending on the tissue repair activity, cellular location, enzyme removal from plasma rate, as well as the kind, severity and duration of injury or stimulus, and also the number of affected hepatocytes, the magnitude and duration of the enzyme activity in plasma are different.

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