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Effects of total parenteral nutrition associated with glutamine, enteral fluid therapy, with or without glutamine, and fluid therapy on the lipidogram of horses subjected to starvation after laparotomy

[*Efeitos da nutrição parenteral total associada à glutamina, à fluidoterapia enteral, associada ou não à glutamina, e fluidoterapia sobre o lipidograma de equinos submetidos à inanição após laparotomia*]

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ABSTRACT

In order to evaluate the effect of total parenteral nutrition associated with glutamine and enteral fluid therapy, associated or not with glutamine, on the lipidogram of horses subjected to starvation (phase 1) and refeeding (phase 2), 16 adult healthy horses were used, mixed breed, aged between four and fourteen years and average weight of 248.40 ± 2.28kg, divided into four experimental groups, with four animals per group: Group ENTGL: enteral fluid therapy with electrolytes associated with glutamine; Group PARGL: total parenteral nutrition associated with glutamine; Group ENTFL: enteral fluid therapy with electrolytes; and Group PARFL: parenteral fluid therapy. This study was divided into two phases: phase 1 and phase 2. Phase 1 consisted of performing exploratory laparotomy and beginning of starvation, in addition to the administration of treatments, according to the group, while phase 2 consisted of re-feeding the animals. Total cholesterol values remained within the normal range for the species throughout the experimental period, but there was a slight increase during phase 1, followed by a decrease in values in phase 2. Hypertriglyceridemia in the ENFL, ENTGL and PARFL groups during phase 1 occurred because of the negative energy balance. The provision of enteral fluid therapy with electrolytes and an energy source, with or without glutamine, or parenteral fluid therapy were not effective in reversing the effects of negative energy balance. Thus, therapeutic protocols that combine enteral or parenteral fluid therapy for prolonged periods and food deprivation need additional nutritional support to avoid the deleterious effects of negative energy balance.

Keywords: hypertriglyceridemia, clinical nutrition, food deprivation

RESUMO

Com o objetivo de avaliar o efeito da nutrição parenteral total associada à glutamina e da fluidoterapia enteral, associada ou não à glutamina, sobre o lipidograma de equinos submetidos à inanição (fase 1) e realimentação (fase 2), foram utilizados 16 equinos adultos hígidos, sem raça definida, com idade variando entre quatro e 14 anos e peso médio de 248,40 ± 2,28kg, divididos em quatro grupos experimentais, quatro animais por grupo: grupo ENTGL: fluidoterapia enteral com eletrólitos associada à glutamina; grupo II PARGL: nutrição parenteral total associada à glutamina; grupo ENTFL: fluidoterapia enteral com eletrólitos; e grupo PARFL: fluidoterapia parenteral. Este estudo foi dividido em duas fases: fase 1 e fase 2. A fase 1 consistiu na realização da laparotomia exploratória e no início da inanição, além da administração dos tratamentos, de acordo com o grupo; a fase 2 consistiu na realimentação dos animais. Os valores de colesterol total se mantiveram dentro da faixa de normalidade para a espécie durante todo o período experimental, porém houve discreta elevação ao longo da fase 1, seguida de diminuição dos valores na fase 2. Hipertrigliceridemia nos grupos ENTFL, ENTGL e PARFL durante a fase 1 ocorreu em decorrência do equilíbrio energético negativo. O fornecimento de fluidoterapia enteral com eletrólitos e uma fonte de energia, associada ou não à glutamina, ou o de fluidoterapia parenteral não foram efetivos em reverter os efeitos do equilíbrio energético negativo.

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Dessa forma, protocolos terapêuticos que associem fluidoterapia enteral ou parenteral por períodos prolongados e privação alimentar necessitam de suporte nutricional adicional para evitar os efeitos deletérios do equilíbrio energético negativo

Palavras-chave: hipertrigliceridemia, nutrição clínica, privação alimentar

INTRODUCTION

Food intake is an intermittent process; however, the body consumes energy continuously. Horses adapt well to short periods of fasting using their stores of carbohydrates, fats, and proteins, in addition to activating neuroendocrine mechanisms capable of reducing energy expenditure as well as preserving body protein (Melo *et al*., 2011; Vergnano *et al*., 2017).

Decreased appetite and/or inability to feed are common findings in horses with different conditions, quickly leading to malnutrition and impaired immune function and tissue repair (Melo *et al*., 2008; Carr, 2018). Nutritional support is an auxiliary therapeutic resource in the recovery of patients with malnutrition or at risk of developing it (Barendregt *et al*., 2008; Baek *et al*., 2020), and should be considered in those animals with increased metabolic rate at example of growing animals, individuals with a previous history of malnutrition or hypophagia, patients with metabolic alteration, as well as individuals with diseases that result in increased energy demand (Carr and Holcombe, 2009; Carr, 2018; Magdesian and Bozorgmanesh, 2018).

In the clinical routine, variable periods of partial or complete food restriction are common, due to some illnesses or for the performance of some diagnostic and/or surgical procedures. However, the withdrawal of food for prolonged periods is not physiological considering the horse's dietary pattern (Di Filippo *et al*., 2021). Under natural conditions, the daily feed intake of a grass-fed horse lasts 12 to 18 hours. When fed ad libitum, fasting time does not exceed three hours (Maia *et al*., 2018).

In these situations, animals receive only maintenance fluid therapy, intravenous or enteral, in addition to daily glucose requirements in some cases (Lawson *et al*., 2021). However, no evidence was found to indicate that these routine protocols can prevent body catabolism in the postoperative period in animals kept under starvation. In this context, the use of nutritional support via total parenteral nutrition would be of fundamental importance to avoid body catabolism associated with lack of food intake, thus reducing the likelihood of occurrence of post-surgical complications associated with negative energy balance.

In starvation states, simple or associated with stress, the small intestine can contribute up to 20 to 25% of total endogenous glucose production. In this organ, glutamine seems to be the main gluconeogenic substrate. The reduction in glutamine concentration is directly proportional to the period of starvation or the severity of the injury, and is not readily reversible by nutritional support and, therefore, its supplementation in nutritional support protocols for critically ill patients is necessary (Biolo *et al*., 2005).

This study aimed to evaluate the effect of total parenteral nutrition associated with glutamine, enteral fluid therapy, with or without glutamine, and intravenous fluid therapy on the values of the lipidogram in horses submitted to starvation after exploratory laparotomy.

MATERIAL AND METHODS

Sixteen healthy adult mixed breed horses of both sexes, aged between four and 14 years, mean body weight of 248.40 ± 2.28kg, and body score index 3-4 (scale of 1 to 5), divided into four groups, four animals per group.

For screening and selection of animals, a complete clinical examination was performed as described by Speirs (1999) and laboratory tests (hemogram, parasitological feces). The animals underwent endo and ectoparasiticidal treatment, being initially housed in paddocks and fed daily with commercial feed (1kg/100kg live weight), tifton hay (1kg/100 kg live weight), in addition to elephant grass (Pennisetum purpureum) chopped, water and mineral salt ad libitum. After a 30-day adaptation period, the animals were randomly divided into four experimental groups:

ENTGL group: enteral fluid therapy with electrolytes (5.7g NaCl; 3.78g $NaHCO_3$; 0.37g KCl and 10g of glucose per liter of water) associated with glutamine (L-Glutamine, Ajinomoto do Brasil Indústria e Comércio de Alimentos Ltda, Laranjal Paulista/SP, Brazil). For the calculation of the total volume of fluid to be administered in the period of 24 hours, the value of 60 ml/kg of body weight was adopted as maintenance rate. The total fluid volume calculated was divided by 12 and administered, by gravity flow, every two hours via a 11x16mm nasogastric tube. Glutamine was administered at a dose of 0.5g/kg body weight. The calculated total amount of glutamine was divided by 12 and administered every two hours diluted in enteral fluid therapy. The volume of fluid to be administered, as well as the amount of glutamine were corrected daily according to the animal's weight.

PARGL group: Total parenteral nutrition (TPN) plus glutamine. TPN was prepared from solutions of amino acids (Aminoven 10%, Fresenius Kabi, Barueri/SP, Brazil), lipids (Lipovenos 20%, Fresenius Kabi) and glucose 50% (Glucose 50%, Fresenius Kabi) in equal proportions (33.33% amino acids, 33.33% lipids and 33.33% glucose 50%) to provide the energy requirement for daily maintenance. Glutamine was administered intravenously at a dose of 0.5g/kg of body weight as a sterile 1.5% solution (15 g/L).

The total amount of TPN, as well as the amount of glutamine to be administered, were corrected daily according to the animal's body weight. Both solutions were administered in continuous infusion via venous access into the right external jugular vein. The rate of administration (ml/h) was calculated by dividing the total volume to be infused by 24. Intravenous fluid therapy using lactated Ringer's solution was instituted in the amount necessary to complete the daily maintenance requirement of 60mL/kg.

ENTFL group: Enteral fluid therapy with electrolytes (5.7g NaCl; 3.78g NaHCO3; 0.37g KCl and 10g of glucose per liter of water). For the calculation of the total volume of fluid to be administered in the period of 24 hours, the value of 60 ml/kg was adopted as the maintenance rate. The total fluid volume calculated was divided by 12 and administered, by gravity flow, every two hours via a 11x16mm nasogastric tube. The volume of fluid to be administered was corrected daily according to the animal's weight.

PARFL group: parenteral fluid therapy. The volume of fluid to be administered was 60ml/kg of body weight, with half of the volume provided by lactated Ringer's solution and half by 0.9% sodium chloride saline solution. Glucose 50% was provided at a dose of 1.5g/kg of body weight diluted in saline solution. Both solutions were administered in continuous infusion via venous access into the right external jugular vein.

Venous access for TPN infusion (PARGL group) and parenteral fluid therapy (PARFL group) was obtained using a double-lumen catheter (Duocath – 12F, Intra Special Catheters, Germany), coaxial, 12-gauge, 20 cm long.

This study was divided into two phases: phase 1 and phase 2. Phase 1 consisted of performing an exploratory laparotomy and starting starvation, in addition to administering treatments, according to the group, while phase 2 consisted of refeeding the animals. During phase 1, the animals did not receive food or water beyond what was described in the group. In the first two days of phase 1, TPN administered in the PARGL group provided only 50% and 75% of maintenance energy requirements, respectively, while 100% of the requirement was provided from the third day of phase 1 until the end of phase 1. In phase 2, all animals, regardless of group, were re-fed with tifton hay, commercial concentrate, and water. Feeding was gradually reintroduced. On the first day, 4 kg of Tifton hay were provided, divided into two 2kg meals provided 12 hours apart, and 0.5kg of concentrate. On the second day, the amount of hay was increased to 8 kg of hay, provided in two meals of 4kg each, in addition to 1 kg of commercial concentrate. From the third day, the amounts of food provided were equal to the adaptation period. Each phase lasted a total of 144 hours.

To simulate a situation of surgical stress and to obtain intestinal samples, used in a parallel study carried out by Ferreira *et al*. (2022), the animals underwent two laparotomies on the flank, one at the beginning and another at the end of phase 1. After accessing the cavity, the exteriorization of the small intestine followed by the

exteriorization of the large intestine was adopted as a standard procedure, avoiding -if excessive manipulation of the handles. After the collection of intestinal biopsies and entero-anastomosis, the intestinal segment was returned to the abdominal cavity and laparotomy was performed (Melo *et al*., 2022a). Each laparotomy performed lasted an average of 60 minutes.

To determine serum concentrations of cholesterol (HDL, LDL, VLDL and total) and triglycerides, blood samples were obtained by venipuncture in the external jugular vein in vacuum tubes without anticoagulant. Samples were collected every 24 hours during the experimental period. The first sample was collected at the beginning of phase 1 (T1), which coincided with exploratory laparotomy and interruption of food supply. During the 24 hours following the surgical procedure, the animals had access only to water. The provision of treatments began 24 hours after laparotomy and collection of biopsies.

After clot retraction, the blood was centrifuged at 3,000 rpm for five minutes, and the serum separated into 0.5 ml aliquots and frozen at -20°C until analysis processing or processed immediately according to the analyte to be analyzed. The measurement was performed in a semi-automatic biochemistry device (BA-88A semi-automatic biochemistry analyzer, Química Básica Ltda, Belo Horizonte/MG, Brazil).

The experimental design was completely randomized, in a 4x7 factorial scheme (groups x harvest time), with four replications, in phase 1, and a 4x6 factorial scheme (groups x harvest time) in phase 2, totaling thirteen times. The data were tabulated in an Excel® spreadsheet and after a normality test, and aiming at the homoscedasticity of the samples, the data were converted by Log (x + 1) and the converted values were submitted to the analysis of variance (Proc GLM) and their averages compared by the Duncan test. All values were considered significant when $p \leq 0.05$ (95% probability). Statistical analyzes were performed using the SAS program (User's..., 1999).

This experiment was approved by the Committee for Ethics in Animal Experimentation (CETEA/UFMG) under number 34/2008.

RESULTS AND DISCUSSION

This study was part of a larger experiment developed with the purpose of evaluating the metabolic and gastrointestinal mucosal response of horses subjected to starvation after exploratory laparotomy, and some results were previously published (Melo *et al*., 2021; 2022a, 2022b; Ferreira *et al*., 2022; Melo *et al*., 2022ab). Kidney and liver function parameters remained constant throughout the experimental period, with only fasting hyperbilirubinemia being observed in animals from the ENTFL, ENTGL and PARFL groups.

Regarding the surgical procedure, no complications were observed in phase 1 of the experiment, except for the development of edema or seroma/infection in the incisional line, regardless of the group, as described by Melo *et al*. (2022a). Protopapas (2000) observed that complications affecting the incision in horses treated surgically for the treatment of gastrointestinal disorders were closely associated with negative energy balance in the horse (Naylor *et al*., 1980), metabolic alteration invariably associated with absence or low food intake.

Although partial parenteral nutrition (PN) based on a glucose/amino acid mixture is used in the hospital routine for nutritional support of horses with no or low food intake, TPN was chosen in this study due to the duration of intravenous nutritional support. The inclusion of lipids in parenteral nutrition formulations allows the provision of a greater number of calories per unit volume compared to solutions containing only dextrose/amino acids. In long-term situations, as in this study, the main benefit of including lipids in the TPN formulation is that they allow a decrease in the amount of carbohydrates administered for any level of caloric support. Another advantage is that since lipid emulsions are isotonic, they moderate the hypertonicity of the TPN formulation and decrease the risk of thrombophlebitis.

Glutamine supplementation, either enterally (ENTGLU group) or parenteral (PARGLU group), was performed because it is beneficial under clinical conditions, and was based on four important aspects: a) it is an essential energy source for enterocytes and immune cells and

which cannot be replaced by other amino acids; b) during periods of metabolic stress, there is a state of relative glutamine deficiency; c) the exogenous supplementation of the amino acid allows sending glutamine to the tissues in need; d) the mucosa of the small intestine becomes atrophic when glutamine is deficient in the body, as well as during total parenteral nutrition, in the absence of digestive nutritional support (Alpers, 2006).

The choice of Ringer's isotonic crystalloid solution with Lactate in the PARGL group to complement the daily water requirement was based on the ability of TPN to induce metabolic acidosis (Melo *et al*., 2022b). The occurrence of metabolic acidosis resulting from the infusion of parenteral nutrition components is frequently observed in humans. The cause of this acidosis is uncertain, however, it is associated with the excessive formation of lactic acid induced by the administration of large amounts of glucose, as well as the administration of synthetic l-amino acids (Sugiura *et al*., 2000). Thus, the use of alkalizing solutions, such as Ringer's solution with lactate, has the potential to reduce the probability of occurrence of metabolic acidosis during TPN delivery.

Although there are no descriptions in the literature, the combination of Ringer's with lactate and 0.9% physiological saline solution as maintenance fluid therapy was commonly used in the clinical routine of the institution where this study was conducted, especially when blood gas analysis was not available to assess the acid-base status. In theory, prolonged supply of an alkalizing or acidifying solution could result in alkalosis or metabolic acidosis, respectively, particularly in patients where compensatory mechanisms fail. Thus, the use of both solutions simultaneously and interspersed could prevent the development of these acid-base disturbances in patients where the provision of fluid therapy for a prolonged period is necessary.

The use of enteral fluid therapy requires, as a basic principle of its use, the presence of normal gastrointestinal motility. However, Melo *et al*. (2021) evaluating gastrointestinal motility in a study parallel to the one described here, observed a decrease in gastrointestinal motility throughout phase 1, and its gradual return after the start of refeeding. This decrease in motility could result in a lower rate of absorption of the administered solution; however, no changes were observed in the parameters used to estimate blood volume. Other studies also report a decrease in gastrointestinal motility in animals under enteral fluid therapy, however, without apparent damage to the treatment effect, since none of the studies reported intestinal atony (Silveira *et al*., 2012; Dias *et al*., 2019).

Total cholesterol values remained within the normal range for the species throughout the experimental period (phases 1 and 2), although there was a statistical difference ($p<0.05$) between the groups within each time in both phases. phases, and between times within each group ($p<0.05$) in both phases (Table 1). There was a slight increase in serum total cholesterol concentration throughout phase 1, mainly in the ENTFL and PARFL groups, followed by a decrease in values in phase 2 in all groups.

The results obtained are similar to those observed by Naylor *et al*. (1980) and Frank *et al*. (2002) when observing a slight increase in the serum concentration of total cholesterol in horses during an experimental starvation protocol of 136 hours or 36 hours, respectively. Slight elevations in serum cholesterol concentration in starved horses suggest that most cholesterol is synthesized locally and is not involved in serum transport processes (Naylor *et al*., 1980). Thus, this metabolite is not as reliable as the triglyceride concentration in the assessment of nutritional status (Frank *et al*., 2002).

Regarding the concentration of triglycerides, a statistical difference ($p<0.05$) was observed between the groups within each time in both phases, and between the times within each group ($p<0.05$) in both phases (Table 2). There was an increase in the concentration of triglycerides in all groups during phase 1, with the most pronounced increase in the ENTFL group (6.7 times) when compared to the other groups. In phase 2, a decrease in triglyceride concentration was observed over time ($p<0.05$) in all groups, except for the PARFL group, as the energy balance was gradually restored through voluntary feeding.

Table 1. Mean ± standard error of total cholesterol concentration (mg/dl) of horses under total parenteral nutrition associated with glutamine (PARGL), enteral fluid therapy with (ENTGL) or without glutamine (ENTFL), and fluid therapy (PARF) submitted to starvation after exploratory laparotomy

Times	Experimental groups				
	ENTFL	ENTGL	PARFL	PARGL	Total
Phase 1 – Starvation					
T_1	83.50±7.07aA	68.25±12.98aA	86.75±15.45aA	78.50±16.50aA	73.75±6.81a
T_2	88.00±10.08aA	62.25±9.21aB	94.75±5.72aA	93.75±21.31aA	82.68±6.60a
T_3	89.50±11.89aA	79.75±10.96aA	102.50±25.16aA	87.25±20.17aA	91.06±9.56a
T_4	93.25±9.05aA	75.75±7.79aA	78.50±8.27aA	92.50±13.10aA	86.75±4.65a
T_5	92.50±9.26aB	65.75±10.57aA	77.50±4.76aA	91.50±6.19aB	81.18±4.73a
T_6	105.00±12.53bB	69.50±11.63aA	75.00±5.36aA	86.50±10.56aA	83.62±3.37a
T_7	107.00±18.56bB	67.00±11.01aA	72.50±8.89aA	74.50±19.76aA	79.93±8.40a
Total	93.39±4.25A	69.75±3.90B	83.92±9.65A	86.32±5.64AB	
Phase 2 – Refeeding					
T_8	107.50±12.61aA	72.00±7.54aB	121.25±11.27aB	108.00±17.96aB	104.20±14.14a
T_9	88.75±14.30bA	63.66±9.35bB	65.00±3.89bB	94.50±24.91abA	78.93±8.00ab
T_{10}	85.75±14.04bA	46.66±8.96bC	67.50±7.23bB	58.33±3.52bB	66.28±5.93b
T_{11}	83.50±12.55bA	99.66±49.93bAB	61.50±4.83bB	62.00±1.73bB	76.07±10.61b
T_{12}	83.50±23.84bA	61.66±8.21bA	61.50±4.71bA	47.00±14.52bA	64.71±7.83b
T_{13}	79.66±9.61bA	52.00±6.11bB	58.25±2.78bB	50.33±13.67bA	59.92±4.82b
Total	88.47±5.94A	65.94±8.52B	72.50±8.98AB	73.15±8.13B	

Reference value: 75 – 150mg/dl.
Means followed by different lowercase letters in the column and uppercase in the row differ ($p<0.05$ – Duncan's test).

Table 2. Mean ± standard error of triglyceride concentration (mg/dl) of horses under total parenteral nutrition associated with glutamine (PARGL), enteral fluid therapy with (ENTGL) or without glutamine (ENTFL), and fluid therapy (PARF) submitted to starvation after exploratory laparotomy

Times	Experimental groups				
	ENTFL	ENTGL	PARFL	PARGL	Total
Phase 1 – Starvation					
T_1	35.50±4.27aA	19.50±5.31aB	45.75±23.31aAB	48.50±20.66aAB	37.31±7.71a
T_2	77.25±13.02bA	27.50±4.51aB	61.50±15.85aA	58.50±16.64aA	56.18±7.58a
T_3	143.75±29.47cA	43.50±9.26bB	81.00±18.98bA	137.00±48.94bA	101.31±17.30b
T_4	173.00±28.42cA	36.50±5.31aB	55.00±7.38aB	66.00±18.14aB	82.62±15.80b
T_5	217.75±47.83cA	105.75±61.04bA	39.25±6.04aB	117.50±32.11bA	120.06±25.01b
T_6	258.25±59.29cA	86.25±39.49bB	33.25±6.34aC	51.00±13.69aB	107.18±28.22b
T_7	222.50±46.31cA	81.00±21.65bB	37.25±12.61aC	76.00±22.74aB	105.73±23.43b
Total	161.14±19.12A	56.25±11.59B	50.42±5.64B	79.21±10.94B	
Phase 2 – Refeeding					
T_8	217.50±37.98aA	137.66±44.59aA	56.25±17.50aB	190.50±60.19aA	151.33±25.52a
T_9	158.50±70.04ªA	49.00±13.57bB	42.50±1.19aB	314.50±166.32aA	147.26±52.42a
T_{10}	68.50±13.87bA	27.66±10.39bA	36.75±3.56aA	40.66±18.47bA	44.71±6.87b
T_{11}	49.75±9.09bA	27.33±7.31bB	41.25±4.75aA	44.00±9.53bA	41.28±4.07b
T_{12}	74.50±26.38bA	24.66±5.81bB	41.50±2.84aA	38.33±7.21bB	46.64±8.71b
T_{13}	59.66±26.66bA	24.00±6.65bB	42.25±5.97aA	36.00±10.40bA	40.61±6.98b
Total	106.69±18.94A	48.38±12.05B	43.41±3.16B	124.85±40.62A	

Reference value: 4-44 mg/dl.
Means followed by different lowercase letters in the column and uppercase in the row differ ($p<0.05$ – Duncan's test).

During the experimental period, it was observed that the occurrence of hypertriglyceridemia varied according to the group, occurring earlier in the ENTFL group. In phase 2, the ENTFL and PARFL groups showed a progressive decrease in triglyceride values over the days after the start of refeeding, while in the ENTGL and PARGL groups there were peaks of elevation in triglyceride concentration at T8 and T9, respectively. These spikes in elevation were followed by a sharp decrease in triglyceride values.

Hyperlipemic, hyperlipidemic and hypertriglyceridemic syndromes have been described in horses and all refer to elevations in the serum concentration of triglycerides. As observed in this study, hypertriglyceridemia has been described in horses subjected to situations of stress or starvation for varying periods of time (Frank *et al*., 2002; Tóth *et al*., 2018; Freeman *et al*., 2021).

Hypertriglyceridemia is associated with periods of negative energy balance and is a physiological response to mobilize the energy reserve present in fat stores. Elevated concentrations of triglycerides can interfere with various physiological functions in the body, especially insulin sensitivity. This interference can result in exacerbation of the clinical picture by decreasing the body's ability to limit fat mobilization (McKenzie III, 2011).

The occurrence of hypertriglyceridemia in the ENFL, ENTGL and PARFL groups was expected because of the negative energy balance that the animals were subjected to (DeNotta and Divers, 2020). When the average daily energy provided in each group during phase 1 was calculated, it was observed that the animals in the ENTFL group received on average 6.41% of the maintenance energy requirement (MER), those in the ENTGL group 10.97% of MER, PARFL animals received 15.76% of MER, while PARGL animals received an average of 53.82%, 79.32%, 104.32%, 104.32%, 104.31% and 104.30% of MER on the first, second, third, fourth, fifth and sixth days, respectively. It is noteworthy that the MER above 100% in the PARGL group was due to the energy provided by the glutamine solution.

The occurrence of hypertriglyceridemia in the PARGL group can be interpreted as a metabolic complication of TPN, since 100% of the MER was being supplied and, therefore, a negative energy balance would not be occurring, or as a physiological response of the organism to the supply of the lipid emulsion. Once the energy balance was restored, via refeeding, a decrease in triglyceride values was observed in all groups, however, a peak in triglyceride concentration in the PARGL group between T7 and T9 was observed.

The hypertriglyceridemic response in the horse results from the low capacity of the liver to use free fatty acids for energy production, as well as the low efficiency of the pathway to produce ketone bodies in the horse. Excessive fat mobilization can overwhelm the liver's ability to process triglycerides to VLDL, leading to triglyceride accumulation and hepatic lipidosis (McKenzie III, 2011).

Hypertriglyceridemia is a common metabolic complication in humans under TPN (Denton *et al*., 2018). Its occurrence may be related to the exaggerated synthesis of fatty acids from excessive glucose infusion or decreased lipid clearance (Boscarino *et al*., 2021).

Krause and McKenzie III (2007) observed five cases of hypertriglyceridemia in a study evaluating the use of TPN in 49 newborn foals in an intensive care unit, results corroborated by Myers *et al*. (2009) in another study with 53 foals. Hypertriglyceridemia occurred in the group of animals receiving TPN. Durham *et al*. (2004) using the same proportion for the TPN formulation to be used in horses in the postoperative period, did not observe the occurrence of hypertriglyceridemia in the animals that received it, contrasting with the results of the present study.

Interpreting the elevation of triglyceride concentration as a complication of TPN in the absence of specific clinical symptoms can be erroneous, since the infusion of the lipid emulsion alone can result in an elevation of serum triglyceride concentration. Unlike the ENTFL, ENTGL and PARFL groups, which showed an increase in triglycerides over time, in the PARGL group, the increase occurred more quickly after supplying 100% of the daily energy needs via TPN.

In phase 2, even after the animals underwent a new surgical procedure, triglyceride values returned to initial values during the refeeding process. These results underscore that starvation may have a more preponderant role in raising triglyceride concentrations than surgical stress itself.

Once in the bloodstream, lipid particles bind to apoproteins that promote their hydrolysis by lipoprotein lipase, releasing fatty acids. Free fatty acids are either oxidized for energy or stored as triglycerides in adipose tissue. The remainder is metabolized in the liver by hepatic lipase

generating VLDL and LDL, thus elevations in triglyceride concentration as well as in VLDL activity can be expected during the infusion of lipid emulsions (Btaiche and Khalidi, 2004). This response was also observed in horses receiving increasing doses of oil in the diet, as demonstrated by Pastori (2007).

Regardless of elevation of triglyceride concentration in this study, lipemic samples were identified only in the PARGL group. Dunkel and McKEnzie III (2003) suggested that the prevalence of hypertriglyceridemia may be underestimated in horses, as they present serum/plasma with normal macroscopic appearance in the face of elevations in triglyceride concentration.

Regarding very low-density lipoprotein (VLDL), an increase in serum activity was observed over time in all groups during phase 1, being more pronounced in the ENTFL and ENTGL groups. In phase 2, a decrease ($p<0.05$) of its serum activity was observed over time (Table 3). Regarding the groups, there was a significant difference ($p<0.05$) between the groups with higher values being observed in the ENTFL and PARGL groups.

Table 3. Mean ± standard error of very low-density lipoprotein concentration (mg/dl) of horses under total parenteral nutrition associated with glutamine (PARGL), enteral fluid therapy with (ENTGL) or without glutamine (ENTFL), and fluid therapy (PARF) submitted to starvation after exploratory laparotomy

	Experimental groups				
Times	ENTFL	ENTGL	PARFL	PARGL	Total
Phase 1 – Starvation					
T_1	10.57±2.64aA	3.90±1.06aB	9.15±4.66aA	12.15±3.96aA	8.94±1.70a
T_2	15.45±2.60aA	5.50±0.90aB	12.30±3.17aA	11.70±3.32aA	11.23±1.51a
T_3	28.75±5.89bA	8.70±1.85aB	16.20±3.79bA	27.40±9.78bA	20.26±3.46b
T_4	34.60±5.68bA	7.30±1.06aB	11.00±1.47bB	13.20±3.62aB	16.52+3.16b
T_5	43.55±9.56bA	21.15±12.20bA	7.85±1.20aB	23.50±6.42bA	24.01±5.00b
T_6	51.65±11.85bA	17.25±7.89bB	6.65±1.26aB	10.20±2.73aB	21.43±5.64b
T_7	44.50±9.26bA	16.20±4.33bB	7.45±2.52aB	15.20±4.54aB	21.14±4.68b
Total	32.72±3.72a	11.25±2.31b	10.08±1.12b	16.19±2.15b	
Phase 2 – Refeeding					
T_8	43.50±7.59bA	27.53±8.91bB	11.25±3.50bB	38.10±12.03bA	30.26±5.10a
T_9	12.29±2.69aB	9.80±2.71aB	8.50±0.23aB	62.90±33.26bA	29.45±10.48a
T_{10}	13.70±2.77aA	5.53±2.07aB	7.35±0.71aB	8.13±3.69aB	8.94±1.37b
T_{11}	9.95±1.81aA	5.46±1.46aA	8.25±0.95aA	8.80±1.90aA	8.25±0.81b
T_{12}	14.90±5.27aA	4.93±1.16aB	8.30±0.56aB	7.66±1.44aB	9.32±1.74b
T_{13}	11.93±5.33aA	4.80±1.33aB	8.45±1.19aB	7.20±2.08aB	8.12±1.39b
Total	21.33±3.78A	9.67±2.41B	8.68±0.63B	24.97±8.12A	

Reference value: 0.70 – 10.00 mg/dl.
Means followed by different lowercase letters in the column and uppercase in the row differ ($p<0.05$ – Duncan's test).

Between-group variations in plasma VLDL concentration in response to starvation were observed during this study and are in agreement with results from other studies evaluating the concentration of this metabolite in horses under negative energy balance (Naylor *et al.*, 1980; Frank *et al.*, 2002). The occurrence of considerable increases in the activity of this metabolite in the present study, mainly in the ENTFL, ENTGL and PARFL groups, probably resulted from the ineffectiveness of supplying part of the NEM in reversing or decreasing the physiological response to surgical stress and starvation. In the PARGL group, in addition to the effect of surgical stress and starvation, it should be considered that the infusion of lipid emulsions results in a significant increase in VLDL activity, as previously discussed.

During starvation states, hormone-sensitive lipase activity is increased resulting in the release of fatty acids from adipose tissue into the circulation. Most fatty acids are transported to the liver where they can be completely oxidized via β-oxidation to provide energy, partially oxidized to produce ketone bodies, or re-esterified to form triglycerides. Triglycerides produced in the liver are then released into the

circulation in the form of VLDL, resulting in elevated VLDL concentrations (Hughes *et al*., 2004).

In horses, VLDL is the major transporter of triglycerides and contains about 57% triglycerides and 15% phospholipids (Watson *et al*., 1993). Thus, the elevation of VLDL occurs concomitantly with that observed with triglycerides, corroborating the data obtained in this study, since VLDL is the main transporter of triglycerides in horses.

In addition, the results of triglycerides and VLDL indicate that the horse may have a less pronounced metabolic response in the use of triglycerides as an energy source in situations of starvation and stress. Results from Geelen *et al*. (2001) indicate that triglycerides conducted within the VLDL molecule are hydrolyzed more rapidly as tissues adapt to use fatty acids for energy production.

The decrease in serum VLDL activity in phase 2 is consistent with results from other researchers (Naylor *et al*., 1980; Frank *et al*., 2002; Hughes *et al*., 2004), indicating restoration of energy balance and lower rate of lipolysis. The peak of serum VLDL activity observed between T7 and T9 in the PARGL group resulted from the abrupt interruption of NEM supply to the animals in this group, resulting in exaggerated mobilization of fat stores to replace the ready-to-use energy source that the animals were receiving.

HDL activity remained stable throughout phase 1, with no statistical difference between times within each group ($p>0.05$), although there was a difference between groups within T7 and T13 ($p<0.05$). Higher activities of this metabolite were observed in the ENTFL group, resulting in a statistical difference between this group and the other experimental groups when analyzing the group as a whole. The same behavior was observed in phase 2 (Table 4).

Table 4. Mean ± standard error of high-density lipoprotein concentration (mg/dl) of horses under total parenteral nutrition associated with glutamine (PARGL), enteral fluid therapy with (ENTGL) or without glutamine (ENTFL), and fluid therapy (PARF) submitted to starvation after exploratory laparotomy

	Experimental groups				
Times	ENTFL	ENTGL	PARFL	PARGL	Total
Phase 1 – Starvation					
T_1	62.25±10.62aA	51.75±3.17aA	58.25±7.66aA	58.00±6.46aA	59.87±6.23a
T_2	59.00±22.42aA	50.25±2.95aA	59.00±7.42aA	62.50±5.97aA	62.68±6.11a
T_3	69.50±13.30aA	41.25±3.75bA	57.00±15.23aA	52.00±6.80aA	54.93±5.50a
T_4	60.25±4.15aA	53.50±3.66aA	54.50±6.84aA	56.75±6.62aA	54.37±2.68a
T_5	65.25±8.98aA	49.25±11.22aA	55.25±5.20aA	57.50±7.42aA	56.81±4.07a
T_6	62.75±5.89aA	49.75±11.35aA	53.75±4.71aA	50.75±4.53aA	53.62±3.52a
T_7	81.00±19.61aA	54.66±2.66aB	52.25±3.09aB	48.00±8.49aB	58.73±6.15a
Total	71.14±5.31A	49.14±2.39B	54.17±2.75B	54.35±2.43B	
Phase 2 – Refeeding					
T_8	57.00±10.37aA	48.33±3.71aA	40.75±7.07aA	45.00±2.82aA	47.73±3.54a
T_9	48.75±8.54aA	45.66±2.33aA	36.00±6.27aA	36.50±6.99aA	41.46±3.41a
T_{10}	50.50±13.13aA	46.66±4.91aA	42.25±4.47aA	43.33±12.19aA	45.78±4.38a
T_{11}	49.00±13.34aA	41.00±5.29aA	36.50±2.53aA	47.00±13.27aA	43.28±4.57a
T_{12}	54.00±12.99aA	44.66±7.83aA	36.50±6.03aA	42.66±12.00aA	44.57±4.87a
T_{13}	60.66±12.83aA	40.00±6.08aB	31.00±4.91aB	40.33±12.33aB	42.07±5.04a
Total	53.00±4.39A	44.38±1.96B	37.16±2.09B	42.30±3.50B	

Reference value: 52.00 – 65.00 mg/dl.
Means followed by different lowercase letters in the column and uppercase in the row differ ($p<0.05$ – Duncan's test).

The observed HDL activity agrees with the results of Bauer *et al*. (1990) in a group of ponies subjected to food deprivation. Watson *et al*. (1993) demonstrated that HDL corresponds to 61%, VLDL 24% and LDL 15% of the lipoproteins in healthy horses that are not deprived of food, not being influenced by food restriction in their serum activity. In horses, most plasma cholesterol is in HDL, unlike in humans where most of it is found in the VLDL and LDL fractions (Ileri-Büyükoglu and Güldür, 2005). Thus, the behavior of total cholesterol and HDL are similar in relation to starvation in the horse, as observed in this study.

LDL activity did not show group x time interaction or difference (p>0.05) over time within groups throughout phase 1. However, differences (p<0.05) were observed between groups within groups. each time (Table 5). In phase 2, no difference (p>0.05) was observed over time within groups, except for the PARGL group. Despite the differences observed in both phases, mean values of LDL activity remained within the reference values for the species, except for ENTFL. The results are similar to those of Bauer *et al*. (1990) when not observing elevation of LDL activity in ponies under starvation.

LDL serves as a source of cholesterol to meet the needs of extrahepatic tissues for the synthesis of membranes and steroid hormones. The uptake of LDL particles by different tissues through interaction with specific receptors is intensely regulated and plays a central role in regulating plasma cholesterol concentration. When there is a decrease in the concentration of intracellular cholesterol, there is an increase in the synthesis of receptors and, consequently, in the uptake of this lipoprotein in the plasma, resulting in a decrease in its concentration, the reciprocal also being true (Pastori, 2007). Thus, the non-occurrence of changes in LDL activity observed in this study is explained by the absence of changes in total cholesterol concentration.

Table 5. Mean ± standard error of low-density lipoprotein concentration (mg/dl) of horses under total parenteral nutrition associated with glutamine (PARGL), enteral fluid therapy with (ENTGL) or without glutamine (ENTFL), and fluid therapy (PARF) submitted to starvation after exploratory laparotomy

	Experimental groups				
Times	ENTFL	ENTGL	PARFL	PARGL	Total
Phase 1 – Starvation					
T_1	23.74±3.67aA	11.83±2.03aB	18.89±2.72aB	13.87±5.23aB	22.08±4.70a
T_2	22.80±5.56aA	14.70±7.39aA	18.36±3.65aA	14.29±3.98aA	19.29±2.54a
T_3	22.74±4.33aA	13.34±2.65aB	19.23±8.93aAB	16.34±4.95aAB	17.91±2.70a
T_4	20.40±2.78aA	10.52±1.20aB	18.75±4.80aA	14.28±4.01aAB	15.27±1.80a
T_5	26.75±4.00aA	11.69±0.86aB	25.99±9.31aA	17.09±4.46aA	18.54±2.81a
T_6	31.84±11.72aA	11.00±3.73aB	17.60±2.24aAB	11.56±3.66aA	18.00±3.63a
T_7	34.79±10.20aA	12.03±4.05aB	25.43±12.79aA	27.94±18.57aA	27.78±6.32a
Total	27.53±3.22A	13.20±1.43C	20.91±2.62AB	16.48±2.99BC	
Phase 2 – Refeeding					
T_8	22.93±5.76aA	8.88±3.35aB	15.84±2.45bAB	39.00±26.24aA	22.52 ± 7.11a
T_9	12.29±2.69bB	7.36±2.02aB	36.00±6.27aA	16.99±11.23abB	12.44 ± 2.96a
T_{10}	21.71±8.22abA	8.22±2.03aB	11.02±2.88bB	9.51±2.62bB	13.15 ± 2.77a
T_{11}	16.40±7.45abA	8.62±3.35aA	11.18±2.17bA	12.48±2.22bA	8.25 ± 0.81b
T_{12}	16.34±5.97abA	7.72±1.94aA	13.13±3.19bA	10.74±2.99bA	9.32 ± 1.74b
T_{13}	30.42±11.43aA	5.85±1.94aB	7.96±1.51cB	11.80±3.82bAB	13.54 ± 3.65a
Total	19.56±2.77A	7.77±0.90B	11.83±1.02AB	17.88±5.69A	

Reference value: 18.00 – 38.00 mg/dl.
Means followed by different lowercase letters in the column and uppercase in the row differ (p<0.05 – Duncan's test).

Horses that receive TPN show less weight loss than animals that do not (Durham *et al*., 2004), as observed in this study (Table 6). However, the assessment of weight loss can be subjective, especially when the animals' fecal production during the period of nutritional support is not considered. Therefore, the estimate of weight loss should be considered in conjunction with fecal output, as continuous fecal output can result in considerable weight loss without corresponding loss of lean mass and/or fat.

The greater weight loss demonstrated by the ENTFL and PARFL groups, both in absolute terms and in percentage terms (Table 7), indicates that these animals entered a state of catabolism, justifying the weight loss that occurred. Prolonged reduction in food consumption basically leads to energy deficiency. As a result, body weight is lost while the body's energy stores are used (muscle fats and proteins). Thus, despite the hydroelectrolytic maintenance provided by enteral and parenteral fluid therapy, this method did not provide adequate nutritional support for maintaining body weight.

Table 6. Mean ± standard error of body weight (kg) of horses under total parenteral nutrition associated with glutamine (PARGL), enteral fluid therapy with (ENTGL) or without glutamine (ENTFL), and fluid therapy (PARF) submitted to starvation after exploratory laparotomy

	Experimental groups				
Times	ENTFL	ENTGL	PARFL	PARGL	Total
Phase 1 - Starvation					
T_1	276.25±20.14aA	279.00±10.59aA	253.33±6.00aAB	225.75±14.87aB	258.93±8.83[a]
T_2	272.25±17.93aA	275.50±9.68aA	240.50±6.91aAB	224.50±14.72aB	253.18±8.04[a]
T_3	270.00±21.05aA	275.50±9.86aA	234.75±6.60aB	225.00±14.43aB	251.31±8.44[a]
T_4	263.75±19.29aA	275.50±9.86aA	231.75±8.35bB	225.00±14.43aB	249.00±8.20[a]
T_5	260.00±19.68aA	274.25±10.96aA	230.50±7.85bB	223.25±13.49aB	247.00±8.16[a]
T_6	255.75±20.11bA	273.00±11.00aA	227.50±6.13bB	222.75±13.26aB	244.75±8.08[a]
T_7	254.50±19.98bA	273.33±15.40aA	224.25±5.35bB	219.50±11.26aB	240.86±8.42[b]
Total	264.64±6.75A	275.22±3.62A	233.96±2.82B	223.67±4.62B	
Phase 2 - Refeeding					
T_8	255.25±20.00aB	273.33±14.74aA	227.00±6.17aC	219.75±11.40aD	241.86±8.35[a]
T_9	259.00±18.80aB	277.00±12.42aA	235.50±8.38aC	215.00±15.00aD	246.71±8.84[a]
T_{10}	260.50±19.28aB	278.33±11.86aA	237.50±7.21aC	218.33±15.89aD	248.71±8.74[a]
T_{11}	262.00±18.96aB	279.33±11.62aA	239.00±6.09aC	218.00±15.56aD	249.71±8.68[a]
T_{12}	262.75±19.09aB	280.00±11.01aA	240.00±6.37bC	220.00±15.27aD	250.78±8.59[a]
T_{13}	252.66±21.07aB	281.33±10.97aA	239.75±5.51aC	218.33±15.89aD	247.38±8.61[a]
Total	258.95±7.04B	278.22±4.22A	236.45±2.59C	218.31±5.08D	

Means followed by different lowercase letters in the column and uppercase in the row differ ($p<0.05$ – Duncan's test).

Table 7. Mean ± standard error of weight differences (kg) between surgeries and the end of the refeeding period of starved horses after exploratory laparotomy submitted to parenteral fluid therapy (PARFL), enteral fluid therapy associated or not with glutamine (ENTFL) and glutamine-associated total parenteral nutrition (PARGL)

	Experimental groups			
Variable	ENTFL	ENTGL	PARFL	PARGL
PinPf (kg)	11.75±1.25[A]	0.50±6.06[B]	7.75±5.66[A]	2.50±4.33[B]
Pc1Pc2 (kg)	17.75±4.32[A]	3.00±1.58[B]	16.25±4.25[A]	5.00±3.51[B]
PfrPc2 (kg)	10.00±1.73[A]	8.00±4.58[A]	15.50±2.39[A]	5.00±2.88[B]
PPinPf (%)	4.23±0.20[A]	0.01±2.22[AB]	3.00±2.22[A]	0.54±0.31[B]
PPc1Pc2 (%)	6.73±1.71[A]	1.13±0.58[B]	6.67±1.66[A]	1.94±1.44[B]
PPfrPc2 (%)	3.91±0.79[B]	2.97±1.78[A]	6.45±1.00[B]	2.14±1.16[A]

PinPf: initial weight minus final weight; Pc1Pc2: weight of the first surgery minus weight of the second surgery; PfrPc2: final weight of the refeeding period minus weight of the second surgery; PinPf: percentage of initial weight minus final weight; Pc1Pc2: percentage of the weight of the first surgery minus the weight of the second surgery; PfrPc2: percentage of the final weight of the refeeding period minus the weight of the second surgery.
Means followed by different capital letters in the line differ ($p<0.05$ – Duncan Test).

The assessment of weight loss is more reliable when evaluating the percentage loss than the absolute one, as the difference in weight between different animals may underestimate the actual loss. Diet and hydration status can change body weight by 5% to 10%. Consequently, changes in body weight need to be considered as a function of hydration status, food intake and other procedures that may have occurred during case management. In this sense, Dias *et al*. (2019), when evaluating different hydroelectrolytic replacement modalities in experimentally dehydrated horses through a dehydration protocol associating water and food fasting for 36 hours, observed a significant reduction in body weight in the evaluated animals. Differently from the aforementioned study, in the present study, only food restriction occurred, and thus, the results obtained here are exclusively associated with weight loss associated with the absence of food intake.

CONCLUSION

Enteral fluid therapy with electrolytes and an energy source, associated or not with glutamine, was not effective in reversing the effects of

negative energy balance. Thus, therapeutic protocols that associate enteral fluid therapy for prolonged periods and food deprivation require additional nutritional support to avoid the deleterious effects of negative energy balance. Although hypertriglyceridemia was identified in the total parenteral nutrition group, this was due to the infusion of lipids directly into the circulation.

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