SCIENTIFIC ARTICLE

Effects of feed deprivation on physical and blood parameters of horses

Efeitos da privação de alimento nos parâmetros físicos e laboratoriais em cavalos

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Abstract

The objective of this study was to evaluate the effect of feed restriction on some physical and blood parameters in horses kept outdoors under natural conditions. Twenty horses were deprived of food for 48 h. They were closely monitored and examined, and blood samples were taken at the beginning (0) of the experiment and 6, 12, 18, 24, 30, 36, 42 and 48 hours afterward. During the experimental period, the control group (12 animals) had free access to water and hay, while the restricted group had free access to water only. Data were submitted to two-way analysis of variance with repeated measures, and statistical significance was $P \leq 0.05$. The horses tolerated feed restriction without complications. Feed restriction had no effect on body mass and body condition score, heart rate, respiratory rate, capillary filling time and body temperature. However, feed restriction decreased the intensity of gastrointestinal sounds ($P<0.05$) compared to the control horses. Feed restriction did not cause any changes in erythrocyte variables and gamma glutamyl transferase, creatinine, total protein, and albumin concentrations. During fasting, there was a reduction in the leukocyte response ($P<0.05$). Feed restriction significantly raised the levels of blood urea nitrogen (24 to 48 hours), aspartate aminotransferase (36 to 48 hours) and total cholesterol (42 to 48 hours). During 48 hours of fasting, there was a continuous increase in triglyceride concentration. Feed restriction for 48 h had a marked effect on the intensity of gastrointestinal sounds and was responsible for important metabolic changes in the healthy horses of our sample.

Keywords: feeding, triglycerides, animal welfare, fasting.

Resumo

O objetivo deste estudo foi avaliar o efeito da restrição alimentar sobre parâmetros físicos e sanguíneos em equinos mantidos a pasto de Tifton (Cynodon spp) e em condições naturais. Vinte cavalos foram privados de alimento por 48 h. Eles foram monitorados, examinados e amostras de sangue foram coletadas no início (0) do experimento e 6, 12, 18, 24, 30, 36, 42 e 48 horas depois. Durante o período experimental, o grupo controle (12 animais) teve livre acesso a água e feno, enquanto o grupo restrito teve livre acesso apenas a água. Os dados foram submetidos à análise de variância bidirecional com medidas repetidas e a significância estatística foi $P \leq 0,05$. Os cavalos toleraram a restrição alimentar sem complicações. A restrição alimentar por 48 h não teve efeito sobre a massa corporal e escore de condição corporal, frequência cardíaca, frequência respiratória, tempo de enchimento capilar e temperatura corporal. No entanto, o jejum alimentar diminuiu a intensidade dos sons gastrointestinais ($P <0,05$) em comparação com os cavalos do grupo controle. Não houve alteração nas variáveis eritrocitárias e nas concentrações de gama glutamil transferase, creatinina, proteína total e albumina. Durante o jejum, houve redução da resposta leucocitária ($P <0,05$) e, aumento significativo dos níveis de nitrogênio uréico no sangue (24 a 48 horas), aspartato aminotransferase (36 a 48 horas) e colesterol total (42 a 48 horas). Durante 48 horas de jejum alimentar, houve aumento contínuo da concentração de triglicerídeos. A restrição alimentar por 48 h teve um efeito marcante na intensidade dos sons gastrointestinais e foi responsável por importantes alterações metabólicas em cavalos saudáveis.

Palavras-chave: alimentação, triglicerídeos, bem-estar animal, jejum.

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Introduction

Horses are classified as monogastric grazing animals with a wide range of food sources, predominantly leaves, stems and shoots. Free-ranging horses in pastures spend 10 to 16 hours a day grazing, with each meal lasting 2 to 3 hours, separated by short intervals for rest, locomotion, and social activities (Fleurance et al., 2001; Mayes & Duncan, 1986). Unfortunately, the limitation of grazing time imposed by management practices, diseases, surgery, transportation, and athletic activities increases idle time and triggers stereotypic behavior, which affects the health of animals (Dittrich et al., 2010; Friend, 2000; Reinprecht et al., 2007). Severe ulceration of the gastric squamous epithelial mucosa, caused by excess acidity, was induced in horses by alternating 24-hour periods of feed deprivation and *ad libitum* access to hay, for a total of 96 hours of feed deprivation (Murray & Eichorn, 1996). If alimentary restriction is lengthy, the resulting negative energy and nitrogen balance will lead to weight loss, muscle loss, lipid mobilization and decreased immune system competence (Naylor et al., 1980; Naylor & Kenyon, 1981) and prolongation of recovery from illnesses due to decreased efficacy of therapeutic interventions, resulting in higher cost and poorer prognosis (Durham et al., 2004; Lopes & White, 2002).

During feed deprivation, there is intense catabolism of proteins, which leads to muscle loss and negative nitrogen balance (Dugdale et al., 2010). Plasma triglycerides increased up to 1000 mg/dL (12.5 mmol/L) in horses subjected to complete feed deprivation for 5–8 days (Naylor et al., 1980). Other studies have found an increase in levels of serum triglycerides, aspartate aminotransferase and alanine aminotransferase and decrease of glucose concentrations (Seifi et al., 2002). The effect of food restriction in horses has been evaluated in many studies (Connysson et al., 2010; Dugdale et al., 2010; Tóth et al., 2018). However, to our knowledge there are no reports of the effects of controlled feed restriction on horses kept outdoors under natural conditions in a tropical country. The aim of our study was to investigate the impacts of feed deprivation on the health of horses kept solely in pastures. Physical and blood variables were measured to identify parameters capable of early detection of health and welfare problems of horses kept under feed restriction, a frequent condition during sporting activities, transportation and veterinary treatment.

Material and methods

Animals and housing

This study was in accordance with the Brazilian animal ethics regulations and was approved by the ethics committee of Norte Fluminense Darcy Ribeiro State University, Rio de Janeiro, Brazil (protocol number 384). Thirty-two mixed-breed stallions were used, aged 6.4 ± 2.0 years, with initial body weight of 404.19 ± 46.93 kg. The initial body condition score (BCS) ranged from 3.0 to 4.0 points, where the BCS scale varies from 0 = emaciated to 5 = obese (Carroll & Huntington, 1988). Horses underwent a physical examination prior to inclusion in the study and were considered clinically normal. All horses had been regularly dewormed and none had been receiving any other medication for at least the past 4 weeks. The horses' diet consisted of *ad libitum* Tifton grass hay (*Cynodon* spp.) at the start of the study.

Experimental design

The animals were allocated into control (12 animals) and treatment groups (20 animals), with similar mean BCS, body weight and age of both groups. The two groups were housed separately in two identical paddocks under natural light in an open outdoor shelter. The paddock had a concrete floor with no vegetation. The study was conducted in the summer, during which the mean minimum temperature was 24.2 ± 0.4 ^{0}C and maximum was 32.6 ± 0.6 ^{0}C, with average relative humidity of 75.0 ± 1.8%. The animals were normally kept in this paddock year around with hay and trace mineralized salt *ad libitum*. During the experimental period, the control group had free access to water and Tifton 85 hay (*Cynodon* spp.), while the restricted group only had free access to water.

Clinical examination and sample collection

Blood was first collected (T0) from the right external jugular vein with a 20G 4 cm needle after a 7-day acclimatization period, in the morning about 4 hours after receiving hay. Additional blood samples were collected 6, 12, 18, 24, 30, 36, 42 and 48 hours later. The samples were drawn

into a 10 ml syringe and then transferred to three different collection tubes (one containing no anticoagulant, the second with 10% EDTA and the third containing sodium fluoride). The samples were centrifuged, and the serum was placed in Eppendorf tubes, which were stored at -20 °C for 7 days until biochemical analysis. The glucose was measured immediately after collection. The physical parameters, including heart rate (HR, bpm), respiratory rate (RR, mpm), capillary filling time (CPT, seconds), body temperature (BT, °C) and gut motility and sound (1 = normal, 2 = moderate, 3 = no motility or sound), were evaluated at the same times. Following the fasting period, the animals were monitored closely for an additional 48 h and were fed with small quantities of hay every 4 h for 24 h, then offered hay *ad libitum*.

Laboratory analysis

Routine hematological parameters (hemoglobin, red blood cell count, mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), total white blood cells and total leukocytes) were determined with an MS4 Auto Hematology Analyzer. Leukocyte differential counting was carried out by optical microscopy based on stained blood smears (New Prov®). Hematocrit levels were determined by the microhematocrit method. The biochemical parameters (total protein, albumin, aspartate aminotransferase, gamma glutamyl transferase, total cholesterol, triglycerides, glucose, creatinine, and blood urea nitrogen) were measured with an automated biochemical analyzer (E-225-D, Labquest, CELM).

Statistical analysis

The experimental design was completely randomized. The assumption of normality of errors was verified by the Kolmogorov-Smirnov test (α=0.05). Initially, the data were submitted to analysis of variance with the model that included the time and feeding status as factors. We performed analysis of variance with the General Linear Models Procedure (PROC GLM of SAS) to test hypotheses regarding unbalanced data.

Data collected without fasting (n=12) were compared with data generated during feed deprivation in different hours (n=20). The Tukey test was applied for post hoc comparison at 5% probability. All the analyses and statistical procedures were performed using the SAS program (Statistical Analysis System, 1999).

Data of physical and hematological parameters and total protein, albumin, aspartate transaminase and gamma glutamyl transferase concentrations were expressed as mean ± SD and statistical significance was set at $P < 0.05$ for all analyses. The nonparametric trait gut motility was expressed as median.

Results

No serious behavioral changes were observed when the horses were deprived of feed, and although the feed-restricted horses were more lethargic, they remained alert and interested in their surroundings. Feed restriction did not cause any changes in body condition score, heart rate, respiratory rate, capillary filling time and body temperature (Table 1). However, the feed restriction led to a decrease in the intensity of gastrointestinal sounds compared to control horses (12 to 48 hours). In this period, there was a reduction of intestinal motility of 90% in the animals in the RT group. When compared to the baseline moment (0), the animals of both groups showed decreased heart rate, respiratory rate and body temperature, but except for body temperature, the values remained within the normal range for horses.

Feed restriction had no effect on red blood cells, hematocrit, hemoglobin, MCV, and MCHC (Table 2). During fasting (12 to 42 hours), there was a reduction in the leukocyte response ($P<0.05$). The mean values of eosinophils (42 and 48 hours) and lymphocytes (36 to 48 hours) decreased during feed restriction (Table 3). The segmented neutrophils (24 to 36 hours) increased during feed restriction (Table 3). No changes were observed in the responses of band neutrophils and monocytes.

No changes were observed in the concentrations of creatinine (Table 4), gamma glutamyl transferase, total protein, and albumin (Table 5) during feed restriction. However, the restriction led to an increase (36 to 48 hours) in the activity of aspartate aminotransferase ($P<0.05$) compared to control horses (Table 5). The levels of blood urea nitrogen (BUN), glucose and total cholesterol were significantly elevated by feed restriction at 24 to 48 hours (Table 4) compared with the control horses. However, the mean values of glucose were within reference ranges for equines

Table 1. Mean ± SD of physical parameters of horses during feed restriction (RT) for 48 h and control horses (CT).

		Hours of feed restriction								
Parameter		**0**	**6**	**12**	**18**	**24**	**30**	**36**	**42**	**48**
Heart rate (bpm)	CT	46.0^{Aa}	42.1^{Aab}	37.6^{Aabc}	34.3^{Abc}	36.3^{Aabc}	34.3^{Abc}	36.0^{Aabc}	29.6^{Ac}	30.3^{Ac}
		±9.26	±9.93	±8.94	±7.13	±13.48	±6.31	±9.34	±6.71	±6.20
	RT	43.8^{Aa}	42.3^{Aab}	37.6^{Aabc}	35.5^{Abc}	35.5^{Abc}	35.9^{Abc}	33.3^{Ac}	32.6^{Ac}	35.5^{Ac}
		±9.45	±8.67	±6.51	±4.99	±5.54	±6.44	±6.81	±7.2	±6.71
Respiratory rate (mpm)	CT	27.2^{Aa}	29.6^{Aa}	19.0^{Aab}	14.0^{Ab}	20.6^{Aab}	21.0^{Aab}	15.3^{Ab}	14.0^{Ab}	19.8^{Aab}
		±7.85	±9.71	±5.15	±5.25	±13.08	±7.65	±7.00	±5.53	±10.03
	RT	25.0^{Aa}	24.0^{Aa}	18.4^{Aabc}	17.3^{Abc}	18.9^{Aabc}	21.2^{Aab}	14.2^{Ac}	13.0^{Ac}	19.0^{Aabc}
		±11.00	±7.28	±4.75	±5.78	±7.18	±7.44	±3.60	±4.78	±5.09
Body temperature (^{0}C)	CT	37.6^{Aab}	37.9^{Aa}	37.6^{Aab}	36.9^{Ac}	37.3^{Abc}	37.6^{Abc}	37.4^{Aabc}	37.2^{Abc}	37.3^{Aabc}
		±0.56	±0.54	±0.41	±0.47	±0.35	±0.38	±0.35	±0.25	±0.32
	RT	37.2^{Aa}	37.6^{Aa}	37.4^{Aab}	37.3^{Aab}	37.2^{Aab}	37.3^{Aab}	37.3^{Aab}	37.0^{Ab}	37.0^{Ab}
		±0.55	±0.34	0.58	±0.30	±0.42	±0.46	±0.53	0.45	0.41
Capillary filling time (sec)	CT	2.1^{Aa}	2.0^{Aa}	2.0^{Aa}	2.1^{Aa}	2.0^{Aa}	2.1^{Aa}	2.0^{Aa}	2.1^{Aa}	2.27^{Aa}
		±0.40	±0.43	±0.51	±0.39	±0.45	±0.40	±0.00	±0.40	0.47
	RT	2.15^{Aa}	2.05^{Aa}	2.15^{Aa}	2.10^{Aa}	2.10^{Aa}	2.15^{Aa}	2.15^{Aa}	2.10^{Aa}	2.1^{Aa}
		±0.37	±0.22	±0.37	±0.31	±0.31	±0.37	±0.37	±0.31	±0.31

Means followed by different uppercase letters in the same column indicate significant differences between groups by the Tukey test at 5% probability; Means followed by different lowercase letters in the same row indicate significant differences between times by the Tukey test at 5% probability.

Table 2. Mean ± SD of hematological parameters of horses during feed restriction (RT) for 48 h and in control horses (CT).

		Hours of feed restriction								
Parameter		**0**	**6**	**12**	**18**	**24**	**30**	**36**	**42**	**48**
Red blood cells (x10^6/µl)	CT	7.14^{Aa}	7.15^{Aa}	7.21^{Aa}	7.18^{Aa}	6.74^{Aa}	6.71^{Aa}	6.91^{Aa}	7.08^{Aa}	6.81^{Aa}
		±1.02	±1.14	±1.08	±0.85	±0.76	±0.74	±0.94	±0.97	±0.85
	RT	6.71^{Aa}	6.62^{Aa}	6.56^{Aa}	6.55^{Aa}	6.55^{Aa}	6.55^{Aa}	6.55^{Aa}	6.55^{Aa}	6.55^{Aa}
		±0.97	±0.42	±1.15	±1.25	±1.38	±1.25	±1.20	±1.32	±1.36
Hematocrit (%)	CT	35.09^{Aa}	34.87^{Aa}	35.10^{Aa}	34.99^{Aa}	33.49^{Aa}	32.73^{Aa}	33.43^{Aa}	34.40^{Aa}	32.83^{Aa}
		±4.09	±4.87	±4.39	±3.43	±3.50	±3.10	±3.92	±4.10	±3.62
	RT	34.09^{Aa}	33.20^{Aa}	33.27^{Aa}	33.16^{Aa}	33.56^{Aa}	33.20^{Aa}	33.07^{Aa}	33.25^{Aa}	34.07^{Aa}
		±3.64	±3.85	±4.70	±5.30	±5.85	±5.12	±5.00	±5.08	±5.49
Hemoglobin (g/dl)	CT	11.07^{Aa}	11.08^{Aa}	11.02^{Aa}	11.03^{Aa}	10.34^{Aa}	10.21^{Aa}	10.60^{Aa}	10.72^{Aa}	10.23^{Aa}
		±1.60	±1.90	±1.67	±1.19	±1.16	±1.09	±1.42	±1.51	±1.26
	RT	11.13^{Aa}	10.55^{Aa}	10.45^{Aa}	10.50^{Aa}	10.77^{Aa}	10.74^{Aa}	10.66^{Aa}	10.66^{Aa}	10.01^{Aa}
		±1.55	±1.42	±1.70	±1.98	±2.12	±1.96	±1.88	±1.96	±2.25
MCV (fl)	CT	49.24^{Aa}	48.92^{Aa}	48.83^{Aa}	48.83^{Aa}	48.83^{Aa}	49.00^{Aa}	48.58^{Aa}	48.75^{Aa}	48.25^{Aa}
		±2.27	±2.57	±2.59	±2.41	±2.40	±2.37	±2.50	±2.45	±2.63
	RT	51.05^{Aa}	51.10^{Aa}	51.10^{Aa}	50.90^{Aa}	51.20^{Aa}	51.30^{Aa}	50.95^{Aa}	51.00^{Aa}	50.80^{Aa}
		±3.33	±3.40	±3.45	±3.39	±3.52	±3.57	±3.50	±3.85	±3.46
MCHC (%)	CT	31.46^{Aa}	31.66^{Aa}	31.36^{Aa}	31.52^{Aa}	31.52^{Aa}	31.19^{Aa}	31.73^{Aa}	31.09^{Aa}	31.10^{Aa}
		±1.26	±1.32	±1.09	±0.84	±1.12	±0.61	±1.15	±0.85	±0.78
	RT	32.53^{Aa}	31.71^{Aa}	31.29^{Aa}	31.55^{Aa}	31.93^{Aa}	32.23^{Aa}	32.12^{Aa}	31.95^{Aa}	32.14^{Aa}
		±1.33	±1.07	±1.18	±1.25	±1.29	±1.32	±1.40	±1.59	±1.54

Means followed by different uppercase letters in the same column indicate significant differences between groups by the Tukey test at 5% probability; Means followed by different lowercase letters in the same row indicate significant differences between times by the Tukey test at 5% probability.

Table 3. Mean ± SD of leukocyte counts in horses during feed restriction (RT) for 48 h and in control horses (CT).

		Hours of feed restriction								
Parameter		**0**	**6**	**12**	**18**	**24**	**30**	**36**	**42**	**48**
Leukocytes (x10³/μL)	CT	10.92^{Aa}	10.05^{Aa}	9.67^{Aa}	9.04^{Aa}	9.89^{Aa}	9.86^{Aa}	9.14^{Aa}	9.42^{Aa}	9.16^{Aa}
		±2.51	±2.04	±1.51	±1.86	±2.20	±2.19	±3.10	±1.52	±1.30
	RT	10.16 ^{Aa}	9.09^{Aab}	8.42^{Bb}	7.97^{Bb}	8.06^{Bb}	7.90^{Bb}	7.71^{Bb}	7.70^{Bb}	8.14^{Ab}
		±2.22	±2.22	±1.64	±1.73	±1.77	±2.73	±1.94	±1.82	±1.76
Eosinophils (x10³/μL)	CT	0.77^{Aa}	0.61^{Aab}	0.59^{Aab}	0.61^{Aab}	0.49^{Aab}	0.34^{Ab}	0.52^{Aab}	0.51^{Aab}	0.57^{Aab}
		±0.40	±0.15	±0.21	±0.24	±0.35	±0.22	±0.26	±0.35	±0.43
	RT	0.69^{Aa}	0.65^{Aa}	0.48^{Aa}	0.55^{Aa}	0.64^{Aa}	0.48^{Aa}	0.35^{Aa}	0.27^{Ba}	0.31^{Ba}
		±0.65	±0.57	±0.42	±0.52	±0.75	±0.26	±0.34	±0.21	±0.23
Band Neutrophils (x10³/μL)	CT	0.19^{Aa}	0.00^{Aa}	0.11^{Aa}	0.25^{Aa}	0.00^{Aa}	0.00^{Aa}	0.83^{Aa}	0.00^{Aa}	0.00^{Aa}
		±0.53	±0.00	±0.34	±0.46	±0.00	±0.00	±0.29	±0.00	±0.00
	RT	0.41^{Aa}	0.36^{Aa}	0.36 ^{Aa}	0.17^{Aa}	0.20^{Aa}	0.34^{Aa}	0.10^{Aa}	0.00^{Aa}	0.51^{Aa}
		±0.19	±0.23	±0.16	±0.35	±0.36	±0.23	±0.25	0.00	±0.23
Segmented Neutrophils (x10³/μL)	CT	5.95^{Aa}	5.78^{Aa}	5.47^{Aa}	5.59^{Aa}	6.33^{Ba}	6.20^{Ba}	6.62^{Ba}	6.34^{Aa}	5.91^{Aa}
		±1.85	±1.73	±1.10	±1.70	±2.14	±2.26	±2.12	1.59	±1.15
	RT	5.49^{Aa}	5.99^{Aa}	5.61^{Aa}	5.60^{Aa}	8.56^{Ab}	9.60^{Ab}	8.89^{Ab}	5.14^{Aa}	5.32^{Aa}
		±1.51	±2.10	±1.24	±1.30	±1.60	±1.31	±1.86	±1.85	±1.71
Lymphocytes (x10³/μL)	CT	3.35^{Aa}	3.22^{Aa}	3.16 ^{Aa}	2.46^{Aa}	2.68^{Aa}	3.03^{Aa}	2.51^{Aa}	2.39^{Aa}	2.17^{Aa}
		±0.95	±1.44	±0.83	±0.68	±0.83	±1.04	±1.05	±0.75	±0.94
	RT	3.60 ^{Aa}	3.15^{Aab}	2.89^{Aab}	2.67^{Aab}	2.78^{Aab}	2.85^{Aab}	2.22^{Ab}	2.10 ^{Ab}	2.23^{Ab}
		±1.49	±1.33	±1.26	±1.6	±1.09	±1.02	±0.91	±0.72	±0.95
Monocytes (x10³/μL)	CO	0.32^{Aa}	0.29^{Aa}	0.27^{Aa}	0.36^{Aa}	0.32 ^{Aa}	0.26^{Aa}	0.28^{Aa}	0.23^{Aa}	0.20^{Aa}
		±0.16	±0.16	±0.17	±0.24	±0.18	±0.19	0.24	±0.16	0.18
	RT	0.34^{Aa}	0.25^{Aab}	0.21^{Ab}	0.27^{Aab}	0.24^{Aab}	0.23^{Ab}	0.21^{Ab}	0.15^{Ab}	0.19^{Ab}
		±0.25	±0.17	±0.11	±0.10	±0.13	±0.13	±0.10	±0.11	±0.12

Means followed by different uppercase letters in the same column indicate significant differences between groups by the Tukey test at 5% probability; Means followed by different lowercase letters in the same row indicate significant differences between times by the Tukey test at 5% probability.

Table 4. Mean ± SD of levels of serum triglycerides (TG), total cholesterol (BT), glucose, blood urea nitrogen (BUN) and creatinine in horses during feed restriction (RT) for 48 h and in control horses (CT).

		Hours of feed restriction								
Parameter		**0**	**6**	**12**	**18**	**24**	**30**	**36**	**42**	**48**
TG (mmol/L)	CT	16.42^{Aa}	16.75^{Aa}	13.33^{Aa}	15.67^{Aa}	15.83^{Aa}	17.67^{Aa}	17.42^{Aa}	17.75^{Aa}	17.67^{Aa}
		±8.67	±4.81	±8.03	±7.36	±5.29	±5.21	±5.50	±5.12	±6.83
	RT	19.45^{Ae}	25.2^{Be}	28.65^{Be}	45.00^{Bc}	69.05^{Bcd}	105.8^{Bd}	159.05^{Bc}	197.30^{Bb}	245.80^{Ba}
		±5.51	±11.00	±13.22	±24.41	±32.78	±53.96	±66.19	±96.67	±116.13
BT (mmol/L)	CT	79.75^{Aa}	87.4^{Aa}	84.41^{Aa}	81.91^{Aa}	83.83^{Aa}	85.25^{Aa}	92.83^{Aa}	93.83^{Ba}	93.00^{Ba}
		±14.74	±11.70	±9.44	±13.21	±11.00	±14.60	±14.74	±13.76	±14.42
	RT	86.80^{Aa}	92.80^{Aa}	93.75^{Aa}	95.95^{Aa}	100.95^{Aa}	103.3 ^{Aa}	112.16^{Aa}	116.8^{Aa}	117.3^{Aa}
		±23.88	±25.69	±38.32	±30.02	±27.93	±29.50	±32.49	±36.74	±35.69
Glucose (mmol/L)	CT	73.66^{Aa}	71.00^{Aa}	67.33^{Aa}	60.75^{Aa}	77.27^{Aa}	64.00^{Aa}	70.58^{Aa}	70.25^{Ba}	63.08^{Aa}
		±10.38	±14.48	±9.08	±15.97	±14.66	±15.50	±14.60	±14.60	±12.55
	RT	78.75^{Aab}	72.05^{Aa}	69.60^{Ab}	76.00^{Aab}	78.10^{Aab}	71.05^{Aab}	68.63^{Ab}	84.95^{Aab}	80.05^{Bab}
		±8.27	±5.51	±13.33	±7.53	±8.93	±14.83	±12.53	±16.59	±14.81
BUN (mmol/L)	CT	35.5^{Aabcd}	38.2^{Aabc}	39.9^{Aa}	39.0^{Aab}	37.4^{Aabc}	31.8^{Acd}	32.5^{Abcd}	32.2^{Acd}	30.7^{Ad}
		±8.15	±8.24	±9.02	±9.65	±9.65	±7.09	±6.02	±8.86	±5.94
	RT	34.3^{Ae}	36.9 ^{Acde}	36.5^{Acde}	35.1^{Ade}	50.2^{Ba}	40.5^{Bbcd}	42.2^{Bbc}	43.5^{Bb}	45.6^{Bab}
		±10.14	±10.61	±10.87	±9.43	±6.76	±7.90	±9.83	±8.46	±11.51
Creatinine (mmol/L)	CT	0.98^{Aa}	1.04^{Aa}	1.03^{Aa}	0.98^{Aa}	1.01^{Aa}	0.90^{Aa}	0.99^{Aa}	0.90^{Aa}	0.89^{Aa}
		±0.09	±0.10	±0.13	±0.11	±0.16	±0.11	±0.12	±0.11	±0.17
	RT	1.06^{Aab}	1.11^{Aa}	1.12^{Aa}	1.07^{Aab}	1.07^{Aab}	0.94^{Aabc}	0.97^{Aabc}	0.84^{Ac}	0.89^{Abc}
		±0.15	±0.15	±0.15	±0.17	±0.18	±0.24	±0.25	±0.27	±0.24

Means followed by different uppercase letters in the same column indicate significant differences between groups by the Tukey test at 5% probability. Means followed by different lowercase letters in the same row indicate significant differences between times by the Tukey test at 5% probability.

Table 5. Mean ± SD of total protein, albumin, aspartate transaminase (AST) and gamma glutamyl transferase concentrations in horses during feed restriction (RT) for 48 h and in control horses (CT).

		Hours of feed restriction								
Parameter		**0**	**6**	**12**	**18**	**24**	**30**	**36**	**42**	**48**
Total Protein ($g\ L^{-1}$)	CT	7.54^{Aa}	7.61^{Aa}	7.70^{Aa}	7.75^{Aa}	7.82^{Aa}	7.87^{Aa}	7.88^{Aa}	8.13^{Aa}	8.20^{Aa}
		±0.45	±0.47	±0.59	±0.56	±0.80	±1.25	±0.89	±0.77	±0.93
	RT	7.32^{Aa}	7.4^{Aa}	7.60^{Aa}	7.53^{Aa}	7.60^{Aa}	7.73^{Aa}	7.88^{Aa}	8.26^{Aa}	8.06^{Aa}
		±0.5	±0.93	±0.95	±1.07	±0.70	±0.88	±1.16	±1.28	±1.31
Albumin ($g\ L^{-1}$)	CT	2.46^{Aa}	2.55^{Aa}	2.56^{Aa}	2.58^{Aa}	2.58^{Aa}	2.62^{Aa}	2.66^{Aa}	2.63^{Aa}	2.60^{Aa}
		±0.27	±0.21	±0.31	±0.37	±0.24	±0.18	±0.31	±0.27	±0.27
	RT	2.42^{Ab}	2.48^{Aab}	2.58^{Aab}	2.60^{Aab}	2.65^{Aab}	2.66^{Aab}	2.66^{Aab}	2.74^{Aa}	2.69^{Aa}
		±0.52	±0.22	±0.25	±0.28	±0.21	±0.28	±0.21	±0.22	±0.27
AST ($U\ L^{-1}$)	CT	213^{Aa}	213^{Aa}	221^{Aa}	212^{Aa}	214^{Aa}	218^{Aa}	206^{Ba}	184^{Ba}	190^{Ba}
		±51.8	±56.3	±57.0	±65.7	±58.7	±62.4	±32.7	±66.8	±57.2
	RT	238^{Aa}	249^{Aa}	247^{Aa}	235^{Aa}	240^{Aa}	235^{Aa}	259^{Aa}	261^{Aa}	264^{Aa}
		±52.7	±44.5	±40.6	±45.4	±53.6	±52.1	±70.9	±59.5	±62.4
GGT ($U\ L^{-1}$)	CT	15.2^{Aa}	16.7^{Aa}	17.2^{Aa}	17.1^{Aa}	16.1^{Aa}	15.0^{Aa}	16.1^{Aa}	17.2^{Aa}	17.8^{Aa}
		±5.9	±4.8	±5.4	±5.8	±4.5	±6.1	±6.7	±5.9	±6.7
	RT	17.9^{Aa}	19.5^{Aa}	18.8^{Aa}	16.0^{Aa}	15.8^{Aa}	15.9^{Aa}	16.9^{Aa}	17.9^{Aa}	17.1^{Aa}
		±5.1	±5.1	±5.2	±6.3	±7.9	±5.9	±6.7	±6.6	±8.3

Means followed by different uppercase letters in the same column indicate significant differences between groups by the Tukey test at 5% probability; Means followed by different lowercase letters in the same row indicate significant differences between times by the Tukey test at 5% probability.

(Table 3). During 48 hours of fasting, there was a continuous increase in triglyceride concentration compared to the control horses (Table 4).

Discussion

In this study, the horses were exposed to stress lasting 48 hours caused by feed deprivation. Although severe behavioral changes were not observed during this restriction, the animals became more lethargic (reduction of ambulation and interaction) compared to the control animals, similar to the results described for ponies (Brinkmann et al., 2013), donkeys and mules (Gupta et al., 1999), Akhal Teke horses (Tóth et al., 2018) and sheep (Naqvi & Rai, 1988). These behavioral changes can be associated with normal adaptive behavior to conserve energy (Gupta et al., 1999) and may also be interpreted as an indicator of reduced welfare (Tóth et al., 2018). To our knowledge, our study is the first to analyze the effects of food restriction on blood and clinical parameters in horses maintained before the study in pasture in the summer. As expected, the horses tolerated the two days of fasting without notable clinical signs. Similar results were described in Caspian miniature horses (Seifi et al., 2002), Akhal Teke horses (Tóth et al., 2018), Thoroughbred horses (Freeman et al., 2021), and mules and donkeys (Gupta et al., 1999) during fasting for 2 to 10 days.

The reduction in mean intensity of gastrointestinal noise during fasting (12 to 48 hours) was associated with a decrease in myoelectric activity (Ross et al., 1990). In a previous study, a similar decrease in the intensity of gastrointestinal sounds was noticed after 24 hours of fasting (Naylor et al., 2006). Gastrointestinal sounds originate from the movement of the intestinal content in response to contractions (Jones & Blikslager, 2004). However, it has been suggested that the nature of the intestinal content, particularly the amount of gas present, also affects the intensity of these sounds. Normal intestinal motility is controlled by myoelectric, neural and humoral factors. The extrinsic and intrinsic portions of the neural control system are interconnected, and in turn are controlled by hormones, intestinal distention and/or feeding state (Davies, 1989). Thus, the findings of this study confirm that feed deprivation reduces the movement of intestinal contents (Table 1), which can have significant implications for horses that are fasting during sporting activities (Connysson et al., 2010), transportation (Friend, 2000), medical treatment or general anesthesia (Andersen et al., 2006; Bailey et al., 2016; Nelson et al., 2013), and in horses that cannot eat because of anorexia or disease (Dunkel & McKenzie, 2003). There is interdependence between the water of the intestinal tract and the extracellular water

aimed at digestion while preserving the water balance, maintaining the enterosystemic cycle. Horses drink less when they are not eating, and this fact should be considered when assessing animals under food restriction (Freeman, 2021).

Unlimited access to water prevented dehydration and azotemia in this study (Tables 2, 4 and 5). Similar results were observed in 8 healthy adult Thoroughbred geldings during 4 days of feed deprivation (Freeman et al., 2021). Although the feed restriction caused an increase in the values of the total plasma protein and creatinine in these Thoroughbreds geldings, the mean values of these metrics were within reference ranges for equines. The concentrations of creatinine, albumin and total protein also did not differ significantly in healthy Caspian miniature horses where food but not water was withheld for 48 hours (Seifi et al., 2002). The maintenance of total protein in adult Standardbred horses in training fed only on forage before feed restriction for 12 h was associated with the use of internal fluid compartments to maintain plasma volume. This strategy can be beneficial for the maintenance of the pre-race water balance of exercising horses (Connysson et al., 2010). However, total protein, albumin and creatinine increased in donkeys and mules during feed restriction for 10 days (Gupta et al., 1999). The results were not associated with stress on renal or liver function but were associated with less glomerular filtration (Naqvi & Rai, 1988). Finally, the reduction of plasma TP concentrations in 10 female Shetland ponies during 4 months of feed restriction was associated with malnutrition (Brinkmann et al., 2013).

The leukocyte response observed in animals submitted to feed restriction was associated with stress (Table 3). Stress promotes the release of epinephrine and corticosteroids, which promote the mobilization of neutrophils from the marginal compartment to the circulation, as well as reducing the diapedesis of cells to tissues, increasing the concentration of neutrophils in the bloodstream. On the other hand, the action of cortisol causes lymphopenia, mainly by the destruction of steroid-sensitive T lymphocytes in the blood, together with marginalization and sequestration of lymphocytes in the extravascular space and the inhibition of lymphopoiesis (Marques et al., 2019). In this study, we noted neutrophilia (24 to 36 hours) and lymphopenia (36 to 48 hours of food restriction). Similar results were described in horses subjected to feed restriction for 5 days (Naylor & Kenyon, 1981). These changes indicated that food deprivation increased the susceptibility to bacterial infections, leading to the conclusion that persistent anorexia in chronic bacterial infections may limit horses' ability to clear the infection (Naylor & Kenyon, 1981). However, in chronically starved horses, greater numbers of immature and mature neutrophils, monocytes and eosinophils were observed, associated with a lower number of lymphocytes (Muñoz et al., 2010). According to the authors, knowledge of laboratory findings in emaciated horses can be useful for scoring the intensity of emaciation and for establishing a prognosis and plan for recovery of health.

The GGT activity remained similar to the pre-fast values, which corroborates the observations in mules and donkeys submitted to feed restriction for 10 days (Gupta et al., 1999). During feed deprivation, horses (Tóth et al., 2018), miniature horses and miniature donkeys (Moore et al., 1994) had significantly increased activity of serum GGT. Since GGT is concentrated in the liver, kidneys and pancreas, changes in its serum activity during a period of feed restriction could reflect an injury to one of these organs or intra- and extra-hepatic cholestasis (Moore et al., 1994).

During the period of food deprivation (36 to 48 hours), activity of serum AST increased significantly ($P < 0.05$). Similar results were observed in Caspian miniature horses, in which food but not water was withheld for 48 hours, where the results were associated with fatty infiltration of the liver. AST is an enzyme sensitive to liver damage, but it can also indicate hyperlipidemia and therefore can be considered in the diagnostic protocol for hyperlipidemic horses (Seifi et al., 2002). Is this study, hyperlipidemia was observed in the animals during feed restriction (Table 4). AST activity decreased significantly on the 9th and 10th days of feed restriction in mules and donkeys (Naqvi & Rai, 1988). Decreased activity of AST and other enzymes during feed deprivation stress indicated that overall body metabolism slowed down in relation to the normal requirements (Brinkmann et al., 2013; Gupta et al., 1999). We believe that this did not occur in the present study because the animals consumed only grasses and were not supplemented with diets rich in carbohydrates prior to the study.

Hyperlipemia (42 to 48 hours), hyperlipidemia (6 to 48 hours) and increased BUN (24 to 48 hours) were observed in the animals during feed restriction (Table 4). These results can be associated with anorexia (Seifi et al., 2002). During anorexia/hypophagia, the mobilization of adipose lipids and catabolism of body proteins for energy production are responsible for these metabolic changes

(Connysson et al., 2010). Hypovolemia can also cause an increase in serum BUN concentration in horses, but it was not detected in this study (Table 2). Similar metabolic alterations were described in horses during 96 hours of starvation (Tóth et al., 2018), in Caspian miniature horses during 48 hours of starvation (Seifi et al., 2002) and in mules and donkeys after feed restriction for 10 days (Gupta et al., 1999). Triglycerides are esterified fatty acids mainly produced in the liver from their precursors. During catabolic states, peripheral fat stores are mobilized, so the serum concentration of esterified fatty acids increases. The abundance of fatty acids and TG in the liver and peripheral tissues may alter cell functions due to their physicochemical properties (Naylor et al., 1980), and hepatic lipidosis can occur if this process is exhausted (McKenzie, 2011). Given the above, the presence of hypertriglyceridemia can be a good indicator of pre-existing hypophagia, but hypophagia may exist without concomitant hypertriglyceridemia (Naylor et al., 1980).

Cholesterol concentration also increased after feed deprivation in horses (Seifi et al., 2002), mules and donkeys (Gupta et al., 1999). However, Watson and Love (1994) suggested that levels of plasma lipids (except triglycerides, such as cholesterol), phospholipids and free acids rise, but such increases are less marked and are therefore less useful for diagnosis of hyperlipidemia in horses. The maintenance of glucose levels during feed restriction in our study (Table 5) suggests that hepatic gluconeogenesis and/or glycogenolysis plays an important role in glucose homeostasis. The central nervous system depends on the energy supply via glucose, and physiological mechanisms have been developed to keep blood glucose concentration constant. Similar results were observed in female Shetland ponies after 4 months of feed restriction (Brinkmann et al., 2013), and in adult horses after 12 hours of fasting (Bertin et al., 2016; Vervuert et al., 2009). Although the results obtained from healthy horses cannot be extrapolated directly to ill horses, the results can help to better understand the metabolic processes during long periods of feed restriction (Tóth et al., 2018). It may be that in sick horses, complex metabolic processes and high energy and protein requirements can prolong recovery and negatively influence the effectiveness of therapeutic interventions (Naylor & Kenyon, 1981; Salman et al., 1998).

Conclusions

In conclusion, the horses tolerated food restriction for 48 hours without serious clinical complications, but they developed important metabolic changes that can negatively influence treatment and prolong recovery of sick, injured and/or inappetent horses.

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Ethics statement

Ethical approval for the study was granted by the ethics committee of Norte Fluminense Darcy Ribeiro State University (protocol number 384). All procediments were consented by the animal owner.

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Conflicts of interest

No conflict of interest.

Authors' contributions

PADF - Has written the manuscript. PADF, BRD, APA and CRQ - reviewed the manuscript. PADF, BRD, APA and CRQ - Were involved in the execution of the study. BRD and APA - Responsible for the laboratory analyses. CRQ - Responsible for the statistical analysis of the data. PADF and BRD - designed the study.

Availability of complementary results

The study was carried out at Laboratório de Clínicas e Cirurgia Animal, Universidade Estadual do Norte Fluminense Darcy Ribeiro – (UENF), Campos dos Goytacazes, RJ, Brazil.

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