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Inclusion of pequi (*Caryocar brasiliense* Cambess.) oil in the diet of horses subjected to physical exercise in a semiarid environment

Inclusão de óleo de pequi (*Caryocar brasiliense* Cambess.) na dieta de equinos submetidos a testes físicos no semiárido

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Highlights

Pequi oil represents viable alternative dietary supplement to equine performance
Inclusion of pequi oil did not negatively affect horses' physical performance.
Pequi oil supplementation improved nutrient digestibility.

Abstract

The inclusion of vegetable oils in equine diets aims to meet energy demands and replace grains. This study evaluated the effects of pequi oil on apparent digestibility, intake, ingestive behavior, and blood and physiological parameters of horses subjected to physical exercise. Nine male Mangalarga Marchador horses (10 ± 5 years; 390 ± 30 kg) were assigned to three dietary treatments: pequi oil, soybean oil, and no oil. Animals received Jiggs grass hay (Cynodon spp.) as roughage and a commercial concentrate supplemented with oil. The 63-day trial comprised three 21-day periods in a Latin square design. Apparent digestibility was determined by total feces collection, and ingestive behavior was recorded over 24 hours. At the end of each period, horses performed light exercise tests. Physiological parameters and blood samples were collected at rest, immediately after exercise, and 10 and 30 minutes post-exercise to assess enzymatic activity and biochemical concentrations. Pequi oil improved the

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digestibility of dry matter, crude protein, and neutral detergent fiber without causing adverse effects to the parameters assessed. Therefore, pequi oil can be safely included in the diet of Mangalarga Marchador horses subjected to physical exercises and under semiarid conditions for at least 21 days.
Key words: Animal welfare. Performance. Vegetable oil. Palatability.

Resumo

A adição de óleos vegetais à dieta de equinos visa suprir as necessidades energéticas e substituir grãos. Esta pesquisa teve como objetivo estudar o óleo de pequi na dieta de equinos submetidos a testes físicos e avaliar a digestibilidade aparente, consumo, comportamento ingestivo e parâmetros sanguíneos e fisiológicos. Foram utilizados nove cavalos machos da raça Mangalarga Marchador, com idade de 10 ± 5 anos e peso vivo de 390 ± 30 kg. Foram utilizados três tratamentos, incluindo dieta com óleo de pequi, óleo de soja e sem óleo. Os animais receberam feno de capim Jiggs (Cynodon spp.) como volumoso e concentrado comercial com adição de óleo. O período experimental teve duração de 63 dias com três períodos de 21 dias cada, e foi utilizado o delineamento quadrado em latino. A digestibilidade aparente foi avaliada pela coleta total de fezes. O comportamento ingestivo foi baseado na observação dos animais durante 24 horas. Os animais foram submetidos a testes físicos leves ao final de cada período, com medidas de parâmetros fisiológicos e coleta de amostras de sangue por punção da veia jugular para avaliação da atividade enzimática e concentrações bioquímicas, os quais foram realizados com os animais em repouso imediatamente após 10 e 30 minutos após o teste. O óleo de pequi melhorou a digestibilidade da MS, PB e FDN e não promoveu alterações prejudiciais aos parâmetros avaliados. O óleo de pequi pode ser incluído na dieta de cavalos Mangalarga Marchador submetidos a testes físicos no semiárido, pois não causa alterações prejudiciais aos animais quando fornecido por 21 dias.
Palavras-chave: Bem-estar animal. Desempenho. Óleo vegetal. Palatabilidade.

Introduction

Equines are non-ruminant herbivores with a single stomach and a highly developed large intestine that enables the use of structural carbohydrates from roughages for energy, although non-structural carbohydrates remain their primary energy source. The large and small intestines perform distinct functions in digestion. Feed is prehended through the mobility of the lips, teeth, and tongue, and chewing stimulates abundant saliva secretion, which contains amylase and other enzymes responsible for carbohydrate breakdown. In addition to being ground, feed releases compounds that can later be digested in the stomach and small intestine (Françoso, 2012).

Diets containing cereal grains, which are rich in non-structural carbohydrates, are commonly formulated to meet the energy requirements of high-performance horses. However, a proper balance between structural and non-structural carbohydrates is essential, as excessive starch intake may escape digestion in the small intestine and reach the cecum and colon. This can disrupt the intestinal microbiota, leading to gastrointestinal disorders and other health problems in horses (Cerbaro et al., 2020).

Vegetable fats are widely used in equine diets, particularly for athletic horses, as an energy source and supplier of essential fatty acids. These fats safely increase dietary energy density and reduce gastrointestinal disorders often associated with grain-based diets, since vegetable oils contain more energy than cereals. Moreover, equines secrete bile continuously because they lack a gallbladder, which facilitates fat digestion as bile the key fat emulsifier is constantly released into the intestinal tract (Mazzante, 2020).

Soybean oil is rich in polyunsaturated fatty acids, predominantly linoleic acid, whereas pequi oil consists mainly of oleic and palmitic fatty acids and contains high levels of antioxidants, phenolic compounds, carotenoids, and vitamins (Lindinger et al., 2017; Lisboa et al., 2020). Sá et al. (2020) reported oleic and palmitic acid contents of 51.2 ± 0.9% and 40.7 ± 1.2%, respectively.

Pequi (Caryocar brasiliense Cambess.) is a native tree of the Cerrado biome belonging to the family Caryocaraceae. It represents an important economic resource for rural communities whose livelihoods depend on fruit collection. Minas Gerais, Brazil, is the main producer and consumer, accounting for about 30% of national production through extractive organizations such as associations and cooperatives. The species is widely exploited for its high oil content, found in both pulp and seed (Lorenzo et al., 2020).

Therefore, this study aimed to evaluate the effects of including pequi oil in the diet of horses subjected to physical exercise on digestibility, intake, ingestive behavior, and blood and physiological parameters.

Material and Methods

The Ethics Committee on Animal Experimentation and Welfare of the State University of Montes Claros (CEUA/UNIMONTES) approved the experimental stage of this study, under protocol no. 02/2022. The trial was conducted from October to December 2022 at Haras ON, in Janaúba, Minas Gerais, Brazil. Laboratory analyses were performed at the Laboratories of Food and Animal Nutrition, Animal Reproduction, and Parasitology of the State University of Montes Claros, Campus I, Janaúba.

The mean annual precipitation in the region ranges from 800 to 1,200 mm, with an average temperature of 21–28 °C and relative humidity around 65%. The local climate is classified as Aw according to Köppen's classification, characterized by well-defined summer rains and dry winters (Santos et al., 2024). The municipality lies within the Cerrado–Caatinga transition zone (Borges et al., 2021).

Nine Mangalarga Marchador horses (10 ± 5 years old; 390 ± 30 kg) previously dewormed with an ivermectin-based product were used. The animals were housed in individual stalls equipped with feed and water troughs, allowing free access to water and both visual and physical contact throughout the 63-day experimental period, which consisted of three 21-day phases (18 days of adaptation and 3 days of data collection). Stalls were built with masonry walls, wooden roof framing, and clay tiles, measuring 4 × 4 m with a ceiling height of 2.8 m, and fitted with two-leaf wooden doors for external viewing and natural ventilation.

Water and commercial mineral salt were provided ad libitum. Jiggs grass hay was purchased from a single local producer and delivered periodically. The grass was harvested 35 days after regrowth for haymaking. Samples were collected at each delivery for bromatological analysis. The commercial concentrate consisted of ground corn, soybean meal, wheat bran, sugarcane molasses, and minerals, among other ingredients.

A 3 × 3 Latin square design was adopted, with three treatments, three replications, and three collection periods. Treatments were as follows: Treatment I: hay (6 kg day^{-1}) + concentrate (2 kg day^{-1}) without oil; Treatment II: hay (6 kg day^{-1}) + concentrate (2 kg day^{-1}) + 200 mL pequi oil; and Treatment III: hay (6 kg day^{-1}) + concentrate (2 kg day^{-1}) + 200 mL soybean oil.

The oil was mixed with the concentrate, which was provided twice daily morning and afternoon after roughage intake. Table 1 presents the chemical and proximate composition of the concentrate and Jiggs grass hay.

Table 1
Chemical composition of the concentrate and hay means offered to the animals during the experimental period

Nutrient (%)	Concentrate[1]	Hay[2]
Dry matter	89.00	91.09
Crude protein	12.62	17.07
Ash	8.75	7.44
Ether extract	6.72	1.42
NDF	21.01	62.02
ADF	13.48	31.24

NDF: neutral detergent fiber. ADF: acid detergent fiber.
1: Vitaequi (Hyper blend).
2: Jiggs grass (Cynodon dactylon).

The fatty acid profile of pequi and soybean oils was determined by gas chromatography with a flame ionization detector (GC/FID) at the Multiuser Laboratory of Biochemistry and Instrumental Analysis (Table 2). Climatological data on temperature and humidity were recorded throughout the experimental period. Mean air temperature and relative humidity were 24.09 °C and 82% in the morning, 27.58 °C and 68% in the afternoon, and 22.80 °C and 80% at night, respectively.

Table 2
Profile of fatty acids analyzed in pequi and soybean oils in mg/100 g

Fatty acid (mg/100 g)	Fatty acid profile*	
	Pequi oil	Soybean oil
Oleic acid-C18:1 (cis-9)	69.35	24.07
Palmitic Acid-C16:0	46.45	8.69
Stearic acid-C18:0	2.39	3.03
Linoleic acid-C18:2 (cis-9, cis-12)	1.71	38.89
Palmitoleic acid-C16:1 (cis-9)	0.96	0.06
Linolenic acid-C18:3(cis-9, cis-12, cis-15)	0.33	4.08
Gamma linolenic acid-C18:3(cis-6,cis-9,cis-12)	-	0.20
Myristic acid-C14:0	0.13	0.06
Arachidic acid-C20:0	0.18	-
Caproic acid-C6:0	0.20	-
Heptadecanoic acid-C17:0	0.08	0.06
Cis-10-heptadecanoic acid-C17:1	0.08	0.04
Lauric acid-C12:0	-	0.01
Elaidic acid	-	0.01
Eicosenoic acid	-	0.14
Behenic acid-C22:0	0.04	0.33

*Gas chromatography technique with flame ionization detector (CG/FID).

Water intake (L day^{-1}) was measured individually as the difference between the amount offered and the remainder over 24 hours. Body weight was estimated using thoracic circumference measured with a tape.

Apparent digestibility coefficients for dry matter and nutrients were determined by total feces collection during the final 72 hours of each experimental period. Feces were weighed, sampled, stored in labeled plastic containers, and refrigerated for subsequent analysis. Samples were pre-dried in a forced-air oven at 55 °C for 72 hours, ground in a knife mill (1-mm sieve), and stored in plastic bags until chemical analysis.

Feed and fecal samples were analyzed according to Detmann et al. (2021). Apparent digestibility coefficients were calculated based on intake, fecal output, and chemical composition of feed and feces.

Ingestive behavior was evaluated during 24-hour observation periods at the end of each experimental phase. The time spent consuming concentrate and roughage, walking, standing, lying, sleeping, showing restlessness, and drinking water was recorded by direct observation every ten minutes using a stopwatch, with one observer per animal.

At the end of each experimental period, horses performed light physical tests consisting of a 10-minute walking warm-up in a round pen followed by a 10-minute free run on a 120-meter track with light obstacles, simulating field exercise. Samples were collected at four time points: at rest, immediately after exercise, and 10 and 30 minutes post-exercise. Between exercise sessions, animals were periodically released into larger paddocks to reduce stress.

Physiological parameters measured included heart rate (HR), respiratory rate (RR), and rectal temperature (RT). Heart rate was determined with a stethoscope placed on the left thoracic region between the third and fifth thoracic vertebrae near the sternum for one minute, timed with a stopwatch. Respiratory rate was assessed by visual inspection of thoracic and nasal movements for one minute, and rectal temperature was measured with a veterinary thermometer inserted into the rectum until contact with the mucosa.

Blood samples were collected via jugular venipuncture in vacuum tubes containing appropriate additives and analyzed with a spectrophotometer. Triglycerides (TG) and total cholesterol (TC) were determined using commercial reagent kits (Doles) with heparinized plasma. Blood glucose and lactate were measured using plasma collected in vacuum tubes with sodium fluoride and glucose reagent kits (Doles). Serum enzyme activities of aspartate aminotransferase (AST), creatine kinase (CK), and lactate dehydrogenase (LDH) were determined colorimetrically using Doles reagent kits. Hematocrit was measured using EDTA-treated blood, centrifuged in capillary tubes, and read on a hematocrit card.

Mean values were subjected to analysis of variance (ANOVA), and differences among treatments were compared using Tukey's test at a 5% significance level ($P < 0.05$).

Results and Discussion

Relative air humidity ranged from 68% to 82%, and air temperature from 24.80 °C to 27.58 °C during the experimental period. Mean water intake was not affected by diet ($P > 0.05$); horses fed pequi oil, soybean oil, and no oil consumed 32.11, 31.67, and 31.33 L day^{-1}, respectively (Table 3). According to Silva et al. (2022), the ideal relative air humidity for equines ranges from 50% to 70%. Therefore, animals were within the ideal range during the afternoon, but higher morning and evening values placed them outside the comfort zone, potentially triggering physiological adjustments to maintain homeostasis, especially during exercise. This variation likely resulted from rainfall during the experimental period.

Equine thermoneutral zone is 5–25 °C (Ramalho et al., 2012). Temperatures in the morning and evening remained within this range, but afternoon values exceeded it, reducing thermal comfort. Despite these fluctuations, water consumption remained stable among treatments, likely due to mild temperatures and the rainy season. Climatic conditions thus contributed to consistent water intake. All diets supplied with oil were well accepted, and no feed refusals, changes in fecal consistency, or gastrointestinal disorders were observed.

No significant differences were found in average daily gain (ADG), initial live weight, or final live weight ($P > 0.05$) among treatments. Similarly, Magalhães (2011) reported no changes in body mass in horses fed different ether extract levels. Dry matter intake, expressed both in kg day^{-1} and as a percentage of body weight, also did not differ among treatments, except for ether extract intake, which increased due to oil supplementation. Vinhedo et al. (2019) found comparable results when evaluating Quarter Horses fed concentrates containing 125 mL of coconut or corn oil.

Table 3

Intake in kg/day and % of live weight and digestibility of DM, CP, EE, NDF, NFC of diets with no oil or with soybean or pequi oils offered to Mangalarga Marchador equines in the semi-arid region

Variable*	Fatty acid profile*			CV (%)	*P*
	Soybean oil	Pequi oil	No oil		
Initial live weight (kg)	368.72 a	365.20 a	361.12 a	5.66	0.765^{ns}
Final live weight (kg)	382.78 a	379.67 a	375.44 a	9.73	0.914^{ns}
ADG (g day^{-1})	223 a	229 a	227 a	3.11	0.654^{ns}
DM intake (kg day^{-1})	6.48 a	6.53 a	6.79 a	9.51	0.874^{ns}
CP intake (kg day^{-1})	1.09 a	1.07 a	1.06 a	11.17	0.898^{ns}
EE intake (kg day^{-1})	0.439 a	0.410 a	0.200 b	5.2	0.006<0.05
NDF intake (kg day^{-1})	3.56 a	3.51 a	3.47 a	12.4	0.898^{ns}
NFC intake (kg day^{-1})	1.60 a	1.61 a	1.59 a	5.78	0.677^{ns}
DM intake (%)	1.80 a	1.76 a	1.81 a	1.18	0.541^{ns}
CP intake (%)	0.28 a	0.28 a	0.28 a	1.74	0.802^{ns}
EE intake (%)	0.112 a	0.102 a	0.0535 b	4.94	<0.05
NDF intake (%)	0.93 a	0.92 a	0.92 a	2.87	0.842^{ns}
NFC intake (%)	0.411 a	0.415 a	0.420 a	3.83	0.857^{ns}
DIGDM (%)	66.14 ab	70.32 a	64.17 b	7.42	<0.05
DIGCP (%)	67.83 b	73.45 a	65.09 b	6.7	<0.05
DIGEE (%)	73.21 a	71.4 a	69.88 a	7.22	0.571^{ns}
DIGNDF (%)	62.86 b	66.76 a	64.39 ab	5.32	<0.05
DIGADF (%)	58.55 a	58.31 a	59.12 a	4.3	0.784^{ns}
DIGNFC (%)	77.81 a	78.65 a	80.04 a	4.29	0.831^{ns}

ADG: average daily gain. DM: dry matter. CP: crude protein. EE: ether extract. NDF: neutral detergent fiber. ADF: acid detergent fiber. NFC: non-fibrous carbohydrate. DIGDM: dry matter digestibility. DIGCP: crude protein digestibility. DIGEE: ether extract digestibility. DIGNDF: neutral detergent fiber digestibility. DIGADF: acid detergent fiber digestibility. DIGNFC: non-fibrous carbohydrate digestibility. CV: coefficient of variation.
*ns: not significant
Means followed by different letters in the row differ from each other by the Tukey test (P<0.05).

Likewise, Creole horses fed hay and concentrate supplemented with ricinoleic acid derived from castor oil also showed no differences in intake (Godoi et al., 2009). According to Godoi et al. (2009), incorporating lipid sources in equine diets may reduce dry matter intake by partially replacing non-structural carbohydrates with fats. This shift is beneficial for athletic horses, as it reduces digestive tract fill, meets energy requirements, and lowers the risk of colic or diarrhea while improving performance.

Treatments influenced dry matter (DM) digestibility, with higher values in the pequi oil diet compared with soybean oil and control diets. Similarly, Delobel et al. (2008) observed greater DM digestibility (66.5%) in high-oil diets than in low-oil diets (64.01%) when supplementing horses with 80 g of flaxseed oil. Conversely, Jansen et al. (2002) found no DM digestibility differences when including 366 g day^{-1} of soybean oil in equine diets.

Pequi oil supplementation also affected crude protein (CP) digestibility ($P < 0.05$), yielding a coefficient of 73.45%. Jansen et al. (2002) reported similar results, suggesting that higher fat content may reduce bacterial growth in the large intestine, thereby decreasing fecal microbial protein.

Ether extract (EE) digestibility did not differ among treatments. In contrast, Godoi et al. (2009) found higher EE digestibility ($P < 0.05$) in oil-supplemented diets compared with oil-free diets in equines fed soybean oil. No significant differences were detected in acid detergent fiber (ADF) digestibility, consistent with Williams et al. (2017), who reported no effects of vegetable oil supplementation on ADF digestion in cannulated ponies. Pombo et al. (2021) also observed no changes in ADF digestibility when providing 0-300 ppm of carvacrol essential oil to castrated ponies.

However, differences were observed in neutral detergent fiber (NDF) digestibility, which was higher in horses fed pequi oil (66.76%). Since hemicellulose represents the most digestible fraction of fiber present in NDF but absent in ADF this finding indicates that pequi oil enhanced hemicellulose digestion, improving overall fiber utilization.

Pequi oil exhibits high intestinal digestibility, with oleic and palmitic acids as its main fatty acids. It also demonstrates strong antioxidant activity due to the presence of carotenoids, particularly β-carotene, a vitamin A precursor (Cruvinel, 2021; Pinto et al., 2018). No effect ($P \geq 0.05$) was observed on non-fibrous carbohydrate (NFC) digestibility. The inclusion of concentrate generally increases the digestibility of non-fibrous carbohydrates and their fractions in mixed diets without impairing fiber digestion. However, in hyperlipidemic diets containing up to 13% ether extract, the digestibility of fibrous and non-fibrous carbohydrate fractions remains unaffected. Diets with 21% ether extract, on the other hand, reduce cellulose and non-fibrous carbohydrate digestibility and their hydrolysable and rapidly fermentable fractions (Magalhães et al., 2017).

Regarding ingestive behavior, diets affected ($P < 0.05$) only the time animals spent restless (Table 4). Horses fed the oil-free diet showed longer periods of restlessness, whereas those receiving oil-supplemented diets were calmer, likely due to greater satiety induced by dietary fat. Time spent eating did not differ among treatments (Table 4), and other behavioral parameters remained unchanged.

Table 4
Ingestive behavior (in hours and % of the time) of Mangalarga Marchador equines receiving diets with soybean or pequi oils or without oil in the semi-arid region

Variable (hour and percentage)	Diet			CV (%)	*P*
	Soybean oil	Pequi oil	No oil		
Concentrate intake	1.03(4.3) a	0.87(3.6) a	0.98(4.0) a	21.03	0.500^{ns}
Roughage intake	4.92(20.5) a	4.94(20.5) a	5(20.8) a	20.23	0.994^{ns}
Walking	1.39(5.7) a	2.39(9.9) a	1.67(6.9) a	23.12	0.100^{ns}
Standing	10.33(43) a	9.22(38.4) a	9.59(39.9) a	16.74	0.355^{ns}
Lying down	3.54(14.7) a	4(16.6) a	3.68(15.3) a	27.69	0.778^{ns}
Sleeping	1.63(6.7) a	1.37(5.7) a	1.17(4.87) a	25.45	0.320^{ns}
Restless	0.074(0.3) b	0.147(0.6) b	0.462(1.9) a	25.13	0.0067*
Ingesting water	1.61(6.7) a	1.72(7.1) a	1.72(7.1) a	22.99	0.785^{ns}

Means followed by different letters in the row differ from each other by the Tukey test (p<0.05). CV: coefficient of variation.

According to Fonseca et al. (2015), equine feeding behavior depends on multiple factors, including feed quantity and nutritional quality, housing conditions, and physical or visual contact with other horses, in addition to individual behavioral traits. Longland et al. (2017) also reported no behavioral differences in adult ponies fed hay and complementary feeds containing added oil. Costa et al. (2021) further highlighted that climatic conditions can affect feeding behavior, as environmental discomfort alters heat exchange mechanisms and the animal–environment thermal balance.

Table 5 presents the physiological parameters of heart rate (HR), respiratory rate (RR), and rectal temperature (RT).

Table 5

Physiological parameters of Mangalarga Marchador equines at different collection times receiving diets with pequi and soybean oils and no oil undergoing physical tests in the semi-arid region

Heart rate (HR) bpm					
Collection time	Soybean oil	Pequi oil	No oil	Mean	*P*
Rest	34.68 Ac	36.88 Ac	35.56 Ac	35.70	0.341
Immediately after	106.24 Aa	96 Aa	99.12 Aa	100.45	0.546
10 minutes after	60.88 Ab	60.44 Ab	59.12 Ab	60.14	0.247
30 minutes after	41.76 Ac	38.2 Ac	40 Ac	39.98	0.312
P	0.054*	0.049*	0.066*	-	-
Respiratory rate (RR) mpm					
Collection time	Soybean oil	Pequi oil	No oil	Mean	*P*
Rest	26.24 Ab	26.68 Ac	26.24 Abc	26.38	0.416
Immediately after	66.24 Aa	68 Aa	62.24 Aa	65.49	0.238
10 minutes after	54.24 Aa	47.12 Abb	42.24 Bb	-	0.062*
30 minutes after	39.56 Ac	34.68 Ab	31.56 Ab	35.26	0.349
P	0.067*	0.072*	0.064*	-	-
Rectal temperature (RT) °C					
Collection time	Soybean oil	Pequi oil	No oil	Mean	*P*
Rest	37.36 Aa	37.19 Ab	37.26 Aa	37.27	0.592
Immediately after	38.82 Aa	39.09 Aa	38.70 Aa	38.87	0.472
10 minutes after	38.47 Aa	38.49 Ab	38.23 Aa	38.39	0.653
30 minutes after	37.95 Aa	38.01 Ab	38.03 Aa	38.00	0.201
Mean	38.15	-	38.05	-	-
P	0.418	0.043*	0.344	-	-

Means followed by different uppercase letters in the row and different lowercase letters in the column differ from each other by the Tukey test at a 5% significance.

Heart rate (HR) values were similar among diets but increased immediately after exercise in all treatments, followed by a gradual decline, returning to baseline after 30 minutes. Respiratory rate (RR) showed a similar pattern, except at 10 and 30 minutes post-exercise, when horses fed the oil-free diet exhibited faster recovery. No interaction between treatments and collection times was detected for rectal temperature.

The immediate post-exercise increase in HR is a physiological response. Lindinger et al. (2017) also observed increased HR and RR after physical exercise regardless of oil supplementation. These parameters rise temporarily to maintain homeostasis, returning to baseline approximately 30–40 minutes after exercise (Cerbaro et al., 2020). In this study, horses reached baseline HR after 30 minutes. RR values at 30 minutes remained higher ($P < 0.05$) than at rest but were within the normal resting range of 20–40 breaths per minute for adult horses (Lindinger et al., 2017). This faster respiratory recovery in oil-fed animals can be explained by the lower carbon dioxide yield from fatty acid oxidation compared with glucose oxidation, reducing respiratory effort (Mazzante, 2020). Similarly, Gomes et al. (2019) found no significant changes (P < 0.05) in RT when evaluating vegetable oil diets in Arabian horses during endurance tests. These authors suggest that such increase in rectal temperature results from the intense metabolic heat produced during the endurance test.

Table 6 shows the mean serum concentrations of creatine kinase (CK), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), and lactate. No differences were observed in CK activity among diets at rest or immediately after exercise. However, vegetable oil supplementation induced a CK peak immediately post-exercise, which subsequently declined. Horses fed pequi oil exhibited a faster CK reduction than those fed soybean oil, returning more rapidly to baseline levels. This quicker recovery may be attributed to the presence of vitamin E and carotenoids in pequi oil, compounds with antioxidant properties that prevent oxidative damage (Weigel, 2014).

Table 6
Serum activity of the enzymes creatine kinase, aspartate aminotransferase, and lactate dehydrogenase of Mangalarga Marchador equines subjected to physical tests at different collection times receiving diets including pequi oil, soybean oil, and no oil in the semi-arid region

Creatine kinase CK (U L^{-1})					
Collection time	Soybean oil	Pequi oil	No oil	Mean	*P*
Rest	190.89 Ad	189.25 Ac	199 Ad	193.04	0.574
Immediately after	320.51 Aa	299.48 Aa	311.36 Aa	310.45	0.686
10 minutes after	277.95 Ab	242.97 Bb	272.2 Ab	-	0.023*
30 minutes after	242.81 Ac	188.87 Bc	236.02 Ac	-	0.042*
P	0.063*	0.059*	0.068*	-	-
Aspartate aminotransferase AST (U L^{-1})					
Collection time	Soybean oil	Pequi oil	No oil	Mean	*P*
Rest	197.76 Ad	201.70 Ac	203.93 Ac	201.13	0.126
Immediately after	284.52 Aa	273.36 Aa	282.8 Aa	280.22	0.391
10 minutes after	257.9 Ab	245.45 Bb	256.68 Ab	-	0.054*
30 minutes after	232.69 Ac	228.21 Ac	237.06 Ab	232.65	0.201
P	0.0051*	0.0043*	0.0055*	-	-
Rectal temperature (RT) °C					
Collection time	Soybean oil	Pequi oil	No oil	Mean	*P*
Rest	187.74 Bc	207.26 Ac	207.51 Ad	-	0.045*
Immediately after	295 Aa	318.35 Aa	287.82 Aa	300.39	0.612
10 minutes after	244.43 Ab	247.09 Ab	250.89 Ab	247.47	0.323
30 minutes after	220.28 Ab	223.89 Ac	233.05 Ac	225.74	0.161
P	0.069*	0.058*	0.037*	-	-

Means followed by different uppercase letters in the row and different lowercase letters in the column differ from each other by the Tukey test at a 5% significance.

Lindinger et al. (2017) evaluated an energy supplement rich in omega-3 fatty acids in crossbred horses subjected to exercise and reported mild to moderate muscle damage after testing, evidenced by increased serum concentrations of CK and AST. The authors concluded that prolonged exercise promotes oxidative damage to muscle cell membranes, leading to leakage of these muscle-specific enzymes into the bloodstream, where elevated levels may persist for up to 24 hours post-exercise.

No significant difference ($P > 0.05$) was observed in serum aspartate aminotransferase (AST) among treatments. However, serum AST increased immediately after exercise in all groups, followed by a similar decline in horses receiving oil-supplemented diets and a slower decrease in those fed the oil-free diet, which had not reached baseline values after 30 minutes. Each diet produced a peak in AST activity immediately after the test, followed by a progressive reduction until 30 minutes post-exercise.

In contrast, M. Oliveira et al. (2019) found no effect of soybean and rice oil supplementation on serum AST concentrations in Arabian horses subjected to physical tests. Although AST activity increased immediately after exercise in the present study, these values remained within the normal physiological range, indicating no confirmed muscle injury.

Lactate dehydrogenase (LDH) concentration was lower ($P < 0.05$) at rest in horses fed the diet containing soybean oil. Across treatments, LDH peaked immediately and 10 minutes after exercise, regardless of diet. In horses receiving pequi oil, LDH values returned to resting levels 30 minutes post-test, which was not observed in horses fed soybean or oil-free diets. Brandi et al. (2010) also reported increased serum LDH activity with rising exercise intensity in horses fed soybean oil–supplemented diets. The rise in LDH likely reflects mild muscle damage. Typically, LDH increases more slowly than CK but remains elevated longer; both enzymes are evaluated jointly to assess exercise intensity. LDH concentrations in all treatments (187.74–318.35 U L^{-1}) remained below reference limits, suggesting no clinically relevant muscle damage.

Table 7 presents the results for blood glucose, triglycerides, cholesterol, and hematocrit. Glucose concentrations did not differ ($P > 0.05$) among diets or sampling times. Similarly, Mazzante (2020) reported no significant effect of increasing dietary soybean oil on blood glucose in horses before or after exercise. The glucose values observed here (75–115 mg dL^{-1}) fall within the normal reference range for horses (Rodrigues et al., 2025), possibly due to the low-intensity exercise applied. The mild anaerobic demand may not have been sufficient to elicit changes in glucose metabolism. Typically, plasma insulin decreases and plasma glucose increases transiently during exercise (T. M. Oliveira et al., 2016), but the timing between sampling points in this study may have masked these fluctuations.

Plasma triglyceride concentrations were generally unaffected by diet or collection time, except immediately after exercise, when horses fed the pequi oil diet showed higher values ($P < 0.05$) than those on the oil-free diet. However, triglyceride concentrations immediately after testing were similar between pequi and soybean oil diets. Ramalho et al. (2012) also observed increased triglyceride concentrations after exercise, followed by a rapid decline within 5 minutes, attributing this pattern to mobilization of adipose tissue during exertion.

Serum cholesterol concentrations did not differ significantly ($P > 0.05$) among diets. However, temporal variation occurred only in oil-supplemented groups. Horses fed the pequi oil diet exhibited elevated cholesterol levels immediately after exercise, returning to baseline within 10 minutes. Conversely, horses receiving soybean oil showed cholesterol concentrations similar to baseline immediately after exercise but lower values at 10 and 30 minutes post-test. Cintra (2016) also reported increased plasma cholesterol in horses fed vegetable oil during exercise. The magnitude of these changes may relate to the oil inclusion level and the low exercise intensity, which likely did not induce substantial lipid mobilization.

Packed cell volume (PCV) typically increases proportionally with exercise intensity, as higher workload promotes hemoconcentration and redistribution of plasma volume during physical effort.

Table 7
Glucose, triglycerides, cholesterol, and hematocrit of Mangalarga Marchador equines subjected to physical tests at different collection times receiving diets including soybean or pequi oils or no oil in the semi-arid region

Glucose (mg dL^{-1})					
Collection time	Soybean oil	Pequi oil	No oil	Mean	*P*
Rest	88.56 Aa	87.44 Aa	85.38 Aa	87.12	0.357
Immediately after	97.38 Aa	95.01 Aa	93.37 Aa	95.25	0.128
10 minutes after	88.72 Aa	89.13 Aa	89.57 Aa	89.14	0.327
30 minutes after	84.69 Aa	84.38 Aa	83.94 Aa	84.33	0.569
Mean	89.83	88.99	88.06	-	-
P	0.641	0.778	0.567	-	-
Triglycerides (mg dL^{-1})					
Collection time	Soybean oil	Pequi oil	No oil	Mean	*P*
Rest	28.88 Ab	26.84 Ab	28.39 Ab	28.03	0.214
Immediately after	50.86 Aba	54.26 Ba	46.66 Aa	-	0.048*
10 minutes after	35 Ab	34.85 Ab	36.82 Ab	35.55	0.433
30 minutes after	28.59 Ab	29.38 Ab	28.85 Ab	28.94	0.311
P	0.063*	0.041*	0.068*	-	-
Cholesterol (mg dL^{-1})					
Collection time	Soybean oil	Pequi oil	No oil	Mean	*P*
Rest	118.49 Aa	112.44 Ab	110.22 Aa	113.71	0.149
Immediately after	127.34 Aa	126.49 Aa	120.66 Aa	124.83	0.528
10 minutes after	115.46 Aab	112.06 Ab	111.35 Aa	112.95	0.321
30 minutes after	109.32 Ab	111.98 Ab	108.19 Aa	109.83	0.413
Mean	-	-	112.60	-	-
P	0.024*	0.062*	0.412	-	-
Hematocrit (%)					
Collection time	Soybean oil	Pequi oil	No oil	Mean	*P*
Rest	39.89 Ab	40 Ab	41 Ab	40.29	0.335
Immediately after	49.78 Aa	49.67 Aa	51.33 Aa	50.26	0.198
10 minutes after	45.78 Ab	44.56 Ab	44.56 Ab	44.96	0.219
30 minutes after	40.89 Ab	40.89 Ab	41.67 Ab	41.15	0.314
P	0.056*	0.044*	0.038*	-	-

Means followed by different uppercase letters in the row and different lowercase letters in the column differ from each by the Tukey test at a 5% significance.

Conclusion

The inclusion of pequi or soybean oils in the diet did not affect dry matter intake or ingestive behavior, nor did it alter the physiological or serum parameters of exercising horses. However, pequi oil improved the digestibility of dry matter, crude protein, and neutral detergent fiber in equine diets.

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