10.21608/avmj.2025.361887.1592 Assiut University web-site: www.aun.edu.eg

THERAPEUTIC PROTOCOL AND MANAGEMENT OF EXPERIMENTALLY-INDUCED HYPERLIPIDEMIA IN DONKEYS (*EQUUS ASINUS*)

ABEER ABD EL-WARES MAHMOUD; HANAN KAMAL ELSAYED AND HUSSEIN AWAD HUSSEIN

Veterinary Internal Medicine, Department of Animal Medicine, Faculty of Veterinary Medicine, Assiut University, Assiut, Egypt.

Received: 28 February 2024; **Accepted:** 18 May 2025

ABSTRACT

Equines are more affected by lipid metabolism disturbances (hyperlipidemia), and donkeys are especially susceptible to hyperlipidemia. It is usually a primary disease process, and stress and obesity appear to be important predisposing factors or secondary diseases due to any disease that results in a negative energy balance. Treatment is critical as quickly as possible, including fluid therapy (mainly Dextrose 5%) and insulin injections. The study was set up as a prospective, observational and experimental trial including 10 donkeys. The experiment period (10 days) consisted of two stages. In the fasting stage (4 days) and the post-fasting stage (6 days), the treatment was administered for 3 days. On six occasions, the following were determined according to the effect of hyperlipidemia and its therapy: body weight, body condition score, ultrasound subcutaneous croup fat thickness (CFT), liver ultrasonography, and blood metabolites. The ultrasound CFT was significantly decreased at the fasting stage ($P<0.05$). Hepatic ultrasonography showed no changes. The portal vein (PV) diameter decreased during the 4 days of fasting. The hepatic relative echogenicity (RE) significantly increased during fasting ($P<0.05$). The lipid profile showed an increase in all parameters during fasting. The plasma glucose decreased during the fasting period ($P<0.05$) and increased in the post-fasting stage ($P<0.05$). The serum levels of FFAs and liver enzymes increased significantly ($P<0.05$). In conclusion, quick intervention with therapy and management in cases of hyperlipidemia helps easily correct any abnormalities that occur in the animal body due to a negative energy balance state.

Keywords: Treatment, Croup fat thickness, hyperlipidemia, donkeys, liver echogenicity, FFAs.

INTRODUCTION

Donkeys (Equus asinus) are economically significant in tropical and subtropical regions (Fielding and Krause, 1998; Pal *et al.,* 2002). Agriculture, tourism, transportation of commodities and the sale of crops are all aided by these working animals (Salari *et al.,* 2021). According to Costa *et al.* (2019), this significance raises their need for veterinary care. Since donkeys are neglected, some research has focused on their illnesses, medical disorders, and methods for identifying and treating their aberrant

Corresponding authors: Abeer Abd El-Wares Mahmoud and Hussein Awad Hussein
E-mail addresses: abeer199246@yahoo.com and hhussein@aun.edu.eg
Present address: Veterinary Internal Medicine, Department of Animal Medicine, Faculty of Veterinary Medicine, Assiut University, Assiut 71526, Egypt.

conditions (Gordon *et al.,* 2007; Mendoza *et al.,* 2015).
Hyperlipidemia refers to excess fatty acids rather than a problem with their elimination from the body (Watson *et al.,* 1992). According to Hughes and Dart (2004), hyperlipidemia is a pathophysiological reaction to long-term negative energy balance that causes abnormality in lipid metabolism and elevated blood levels of TG. Due to the combination of low-quality rations, stress during transit, and fasting, donkeys are more prone to hyperlipidemia (Burden and Thiemann, 2015).

The body fat reserves of various farm species can be measured using various techniques. Morphometric measures of total body fat are the primary basis for subjective body condition assessments (Pearson and Quassat, 2000). However, according to Quaresma *et al.* (2013), ultrasonographic assessment of body fat reserves is considered the gold standard and a useful instrument. The metabolism of mobilized lipidosis in donkeys with negative energy balance is carried out by the liver (Hughes and Dart, 2004), which is regarded as the second largest organ in horses (Dyce *et al.,* 2010; Divers, 2015). Hepatic lipidosis and TG build up, which result from excessive fat mobilization that exceeds the liver's capability (Mckenzie, 2011). In the diagnosis of hepatic abnormalities in horses (Durham *et al.,* 2003) and donkeys (Hussein *et al.,* 2017), liver ultrasonography has emerged as the preferred technique due to its accessibility, noninvasiveness, safety, and ease of use.

The best method for diagnosing hyperlipidemia is to analyze the serum hepatic profile and hormones that regulate energy (Watson, 1994). In ponies (Dugdale *et al.,* 2010), donkeys (Gupta *et al.,* 1999) and horses (Christensen *et al.,* 1997; Connysson *et al.,* 2010), feed deprivation causes excessive lipolysis and increases NEFA and TG concentrations. The previous prandial alterations in these metabolites, however, were not mentioned in any investigations. Furthermore, to the best of the author's knowledge, no studies have examined how feed deprivation affects donkey hepatic ultrasonography and echogenicity, as well as ultrasound assessments of subcutaneous fat and gluteal muscle thickness.

Retaining the negative energy state, restoring normal lipid content, and managing hepatic lipidosis are all important aspects of treating hyperlipidemia (Durham, 2013; Diver, 2015). To inhibit FFA mobilization and promote insulin release, nutritional support, including the consumption of high-calorie foods, should be employed (Durham and Thienmann, 2015). To reduce catabolic conditions, heparin or insulin is administered (Tarrant *et al.,* 1998; Moore *et al.,* 1994; Harold and Mckenzie, 2011). Hypoglycemia can also be treated with fluid therapy (IV Dextrose 5%) (Moore *et al.,* 1994). The present study hypothesized that hyperlipidemia affects clinical, liver ultrasonography, and biochemical parameters in donkeys. Therefore, the study evaluated the therapeutic and management of experimentally induced hyperlipidemia and its subsequent effect on clinical, hepatic ultrasonography and biochemical variations.

MATERIALS AND METHODS

1-Animals and study design

The Animal Care and Welfare Committee of the Faculty of Veterinary Medicine, Assiut University, Assiut, Egypt, ethically approved this research (06/2024/0261). All national guidelines for the care and use of animals for experimentation were followed during the research procedures, and all animals were housed and cared for according to the Egyptian Animal Welfare Act (No. 53, 1966). This work was performed in compliance with the ARRIVE guidelines and regulations. All

rules and instructions for animal care were followed in this study.

The present study was conducted on 10 clinically healthy adult donkeys (Equus asinus) of both sexes (3 males and 7 females). The age ranged from 2 years to 6 years. All donkeys were housed in identical paddocks (6x20cm) with an open outdoor shelter. Physical and haematological examinations were performed for each animal before the beginning of the study. Before the start of this work, all donkeys were examined for parasitic infections, and no internal parasitic eggs were detected. However, all donkeys were treated orally with piperazine citrate (as an anthelmintic drug, Stavro International co, Egypt) with a dose of 100mg/kg bw. The dose was repeated after two weeks. All the donkeys were fed green fodder, Berseem (*Trifolium alexardrnium*), and the concentrate mixture (chopped wheat straw and maize with mineral mixture).

The experimental period (10 days) consisted of two stages: the fasting stage (4 days) and the post-fasting stage (6 days). The animals had free access to water only during fasting (Fig. 1). Throughout the study, all donkeys were monitored closely and gradually refed with a small amount every 4 hours for 24 hours. They were then offered ad libitum Berseem and concentrates as pre-fasting feeding.

Therapeutic protocol and management included two primary approaches, which were nutritional and medical treatment. Nutritional treatment focused on high-quality, extremely appetizing Berseem and concentrates (Morrone *et al.,* 2024). Moreover, the medical protocol included insulin and fluid therapy. 0.1IU/KG of insulin was administrated parentally throughout the post-fasting phase (at 4 days, 5 days, and 6 days) according to Durham and Thiemann (2015) and Mendoza *et al.* (2018). Additionally, fluid therapy was employed, which included intravenous 0.9% normal saline and 5% dextrose, which are used in sufficiently high dosages (Mogg and Palmer, 1995; Divers, 2015).

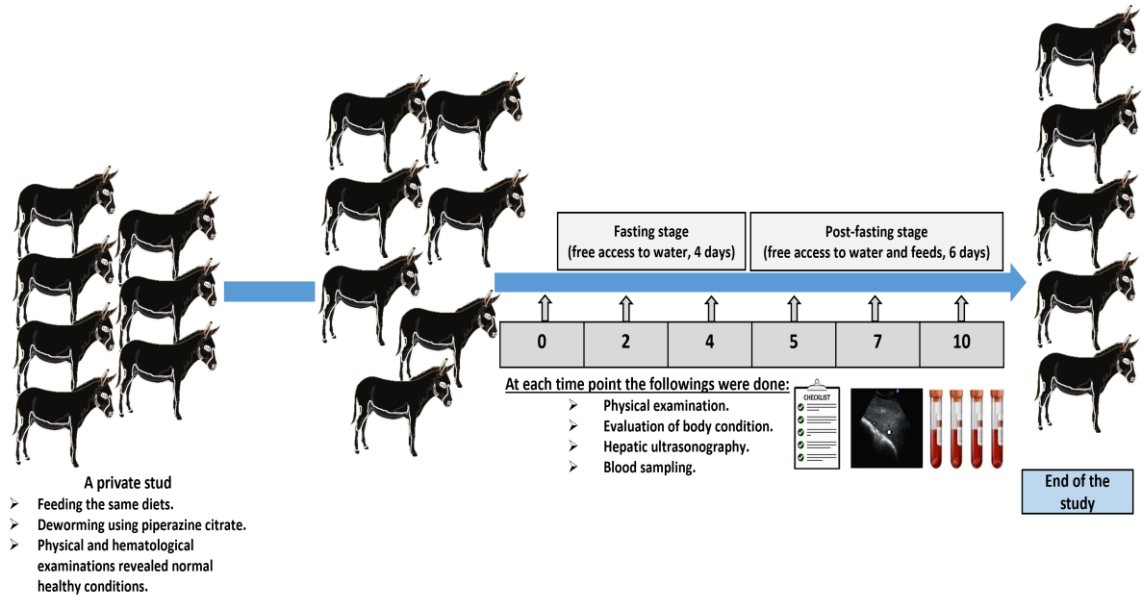

Figure 1: Schematic representation of the study design.

2-Physical examination and assessment of body condition

Physical examination, including rectal temperature, heart and respiratory rates, and capillary refilling time (CRT), was also determined for each donkey during the experimental period according to Costa and Paradis (2018). The body condition score (BCS) for all donkeys was determined using a 1-5 scale according to the chart reported by Burden (2012). Furthermore, the body weight (BW) was calculated as described previously by Pearson and Quassat (2000; Mendoza *et al.,* 2018):

Body weight (kg) = (heart circumference [cm]2.12) × (length [cm]0.688)/3801

The body weight measures included heart girth and body length. The heart girth was measured around the thorax, passing 3cm caudally to the highest point of the withers, and the body length was calculated as the distance from the end of the shoulder to the point of the buttock.

The subcutaneous croup fat (CFT) represented the subcutaneous fat depth overlying the gluteal region in donkeys and gluteal muscle thickness (GMT) was measured using ultrasonography with a *linear* probe (6-10 MHZ, My Lab One VET, Esoate, Italy). The probe was placed on the flat area anterior to the tail-head parallel to the midline (Quarsema *et al.,* 2013). Before examination, the hair was clipped close to the skin, and alcohol was used as a coupling medium; then the gel was applied. After freezing the ultrasound images, the distance from the skin to the fascia covering the gluteal muscle was defined as the CFT, while the distance from the performed fascia to the hyperechoic line from the pelvic bone was defined as GMT.

3-Hepatic ultrasonography

Each donkey was prepared for liver ultrasonography. First, hair covering the right side of the abdomen was clipped and shaved, then alcohol was applied, followed by the application of coupling gel. Ultrasound examination was performed using a real-time B-mode scanner with a multifrequency micro convex probe (My Lab One VET, Esoate, Italy) (Williams *et al.,* 2014; Hussein *et al.,* 2017). The liver was identified on the right side of the abdomen (Williams *et al.,* 2014). The left lobes are located ventrally near the 7^{th} to 10^{th} intercostal spaces, and the right lobes are positioned more dorsally and opposite the 9^{th} to 16^{th} intercostal spaces (Divers, 2015). Ultrasound images were performed by placing the probe in each intercostal space dorsally and then moving it ventrally parallel to the ribs. The hepatic dimensions, including size, depth, and thickness, were measured. The hepatic size was calculated as the distance between the dorsal and ventral margins of the liver. The location and diameter of the caudal vena cava and portal vein were also measured. Moreover, all hepatic ultrasound images were stored to assess liver echogenicity throughout the study. Stored images were converted from 8-bit RGB images to 8-bit grayscale images, subsequently, the photos were digitilized, and the mean echogenicity (ME) of the region of interest (ROI) (defined as the mean pixel brightness or mean grey level of the liver) was calculated using image processing software (ImageJ, National Institutes of Health, Bethesda, Maryland, USA) with a scale of 255 grey levels (0 = black; 255 = white). The grey distribution appears as a histogram, and the software system automatically calculates the mean value (echogenicity). Values of probe frequency, gain, depth, and place at the same intercostal space on the animal were kept the same throughout the study for accurate measurements of liver echogenicity.

4-Blood sampling and laboratory analysis

Two blood samples were collected by jugular venipuncture from each donkey. The first sample was collected in tubes with sodium fluoride as an anticoagulant for glucose determination (Mendoza *et al.,* 2018) and the second sample without an

anticoagulant. Blood samples were collected before morning feeding. Further blood samples were taken at 2,4,5,7, and 10 days of the study. All collected blood samples were chilled on ice, centrifuged at 1500 RPM for 10 min., aliquoted and stored at -20 ℃ until measurements. The serum samples were assayed for the levels of TG, total cholesterol, high-density lipoprotein (HDL), low-density lipoproteins (LDL), activities of gamma-glutamyl transferase (GGT), alkaline phosphatase (ALP), and total bilirubin, urea, and creatinine. Detection of insulin concentration has been measured by using a commercial radioimmunoassay previously validated for donkeys (Gorden and McKeever, 2005; Mendoza *et al.,* 2015). ELISA kit supplied by Sun Long (Sun Long BiotechCo, Ltd, China). The other biochemical indices were analyzed using commercial test kits according to the instructions of the manufacturers (Spectrum Diagnostics, Egypt), and a UV spectrophotometer (Optizen 322o UV, Mecasys Co, Ltd, Korea) was used for this purpose.

5-Statistical analysis

The data are presented as the means ± standard errors of the mean (SE). For statistical evaluation, the data were analyzed using IBM SPSS Statistics (Version 25, Munich, Germany). First, the normality of all the variables was tested using the Kolmogorov-Smirnov test. All tested variables were normally distributed. Moreover, a repeated measures analysis of variance was calculated, where the donkeys' numbers were the subjects and the time relative to sampling was set as a repeated variable. The variations within the same group at different time points were assessed using the least significant difference (LSD)

RESULTS

1-Effect of fasting-induced hyperlipidemia on physical parameters and body condition

All feed-restricted donkeys showed dullness and depression during the fasting period. Table 1 illustrates the effect of fasting-induced hyperlipidemia on temperature, heart and respiratory rates, and CFT. All vital and clinical parameters were still within normal ranges, and there were no significant variations among time points. CRT revealed an increase during the fasting period in all donkeys compared with the zero-day before fasting ($P<0.05$). Table (2) summarizes the different methods for evaluating the body condition and body weight in the experiment. The fasting period showed no significant variations in BW, BCS, and ultrasound-related GMT ($P>0.005$). However, there was a significant change in the croup fat thickness measurements during the fasting stage in comparison with the first day of the study ($P<0.05$). The ultrasound CFT decreased gradually after fasting till the end of the study (Fig. 2).

Table 1: The effect of fasting on the physical parameters of donkeys (*Equus asinus*)

Parameters	Days relative to fasting†						F-values
	Fasting stage			Post-fasting stage			
	0	2	4	5	7	10	
Temperature (° C)	37.1±0.1	37.5±0.3	37.0±0.3	37.2±0.2	37.1±0.3	37.2±0.1	0.366
Heart rate (beats/min)	46.0±0.2	43.0±0.2	40.0±0.1	41.0±0 .2	42.0±0.3	44.0±0.3	0.086
Respiratory rate (cycles/min)	14±0.2	13±0.2	12±0.4	13±0.4	14±0.3	14±0.1	0.299
CRT (sec)	1.5±0.3^{b}	2.3±0.1^{a}	2.2±0.2^{a}	1.9±0.4^{b}	1.8±0.2^{b}	1.6±0.3^{b}	0.004

Abbreviations: CRT, Capillary refill time. †Values are mean ± standard error of the mean.
‡F-values of times relative to fasting.
^{ab}means different superscript letters in the same row of the same group, differ significantly ($p< 0.05$).

Table 2: The effect of fasting on the body condition of donkeys (*Equus asinus*)

Parameters	Days relative to fasting†						F-values
	Fasting stage			Post-fasting stage			
	0	2	4	5	7	10	
BW (Kg)	206±2.3	197±2.1	192±2.5	189±2.6	193±1.5	192±1.2	0.810
BCS (unit)	2.9±0.4	2.8±0.2	2.7±0.1	2.6±0.4	2.6±0.3	2.6±0.2	0.386
Ultrasound CFT (mm)	9.9±0.5[a]	9.2 ±0.4[a]	7.7±0.5[b]	7.4±0.3[b]	7.9±0.4[b]	8.2± 0.5[b]	0.005
Ultrasound GMT (cm)	4.2±0.2	4.0±0.4	3.8± 0.1	3.7±0.4	3.7±0.3	3.6±0.2	0.910

Abbreviations: BW, body weight; BCS, body condition score; CFT, croup fat thickness; GMT, gluteal muscle thickness. † Values are mean ± standard error of the mean.
‡ F-values times relative to fasting. [ab]Means in the same row differ significantly ($p<0.05$).

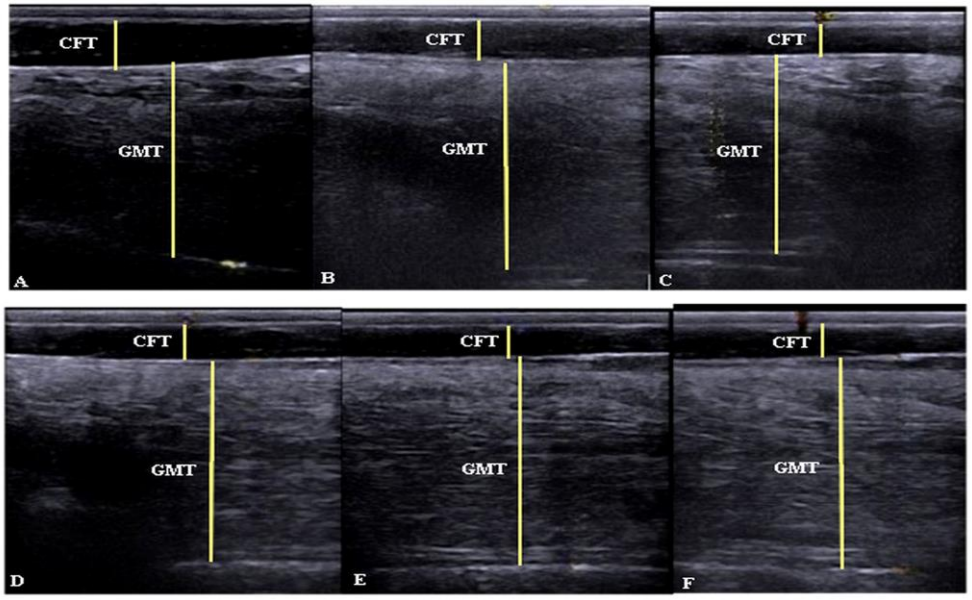

Figure 2: Ultrasound images of a donkey show the impact of fasting on the measurements of croup fat (CFT) and gluteal muscle thicknesses (GMT) throughout the study. Image (A), just before fasting, reveals 11.23mm of CFT and 4.60cm of GMT. Image (B), after 2 days of fasting, shows 10.89mm of CFT and 4.57cm of GMT. Image (C), after 4 days of fasting, represents 8.02mm of CFT and 4.58cm of GMT. Image (D), after 5 days of study, indicates 8.08mm of CFT and 4.59cm of GMT. Image (E), after 7 days of the study, reveals 8.12mm of CFT and 4.56cm of GMT. Image (F), after 10 days of study, shows 8.10mm of CFT and 4.58 mm of GMT.

2-Effect of fasting-induced hyperlipidemia on hepatic ultrasonography

Table (3) shows different ultrasound measurements of the donkey liver throughout the study. Ultrasound imaging of the liver showed no significant variations in size, thickness, and depth among different time points. However, the mean values of portal vein diameter decreased gradually during the fasting period and then increased at the end of the study ($P<0.05$). By contrast, CVC showed a decrease in diameter through the fasting stage, but there were no significant variations among the different time points of the study ($P>0.05$). The ultrasound determination of liver echogenicity revealed a substantial increase in the 2,4,5 days ($P<0.001$) shown in Fig. (3). It peaked at 4 days in all fasted donkeys. Furthermore, hepatic ultrasonography revealed that the liver parenchyma was brighter in colour during the fasting time, indicating increased liver echogenicity (Fig.4), however, this brightness was slight but significant.

Table 3: Effect of fasting on the hepatic ultrasound measurements of donkeys (*Equus asinus*)

Parameters	Days relative to fasting†						F-values
	Fasting stage			Post-fasting stage			
	0	2	4	5	7	10	
Size (cm)	12±0.6	12.2±0.4	12.1±0.8	12.4 ±0.5	12.0±0.6	11.9±0.5	0.941
Depth (cm)	2.2±0.3	2.1±0.5	2.2±0.4	2.3±0.6	2.2±0.1	2.1± 0.1	0.281
Thickness (cm)	6.9±0.3	6.8±0.6	6.8±0.5	6.7 ±0.8	6.8± 0.4	6.9±0.3	0.414
PVD (cm)	1.8±0.3[a]	1.6±0.4[b]	1.5±0.2[b]	1.6±0.3[b]	1.7±0.5[a]	1.8±0.4[a]	0.022
CVC (cm)	2.7±0.6	2.5±0.3	2.4±0.3	2.5±0.3	2.6±0.5	2.7±0.4	0.140

Abbreviations: PVD, portal vein diameter; CVC, caudal vena cava diameter.
† Values are mean ± standard error of the mean. ‡ F-values of times relative to fasting.
[ab] Means in the same row differ significantly ($p<0.05$).

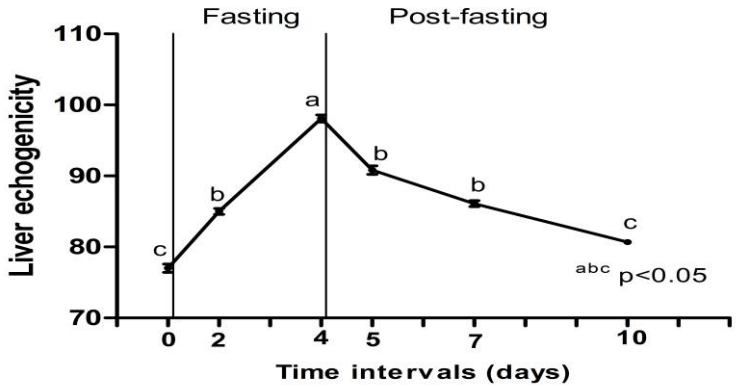

Figure 3: Diagram illustrates the significant variations of liver echogenicity during the study periods. Data represent mean ± SE.

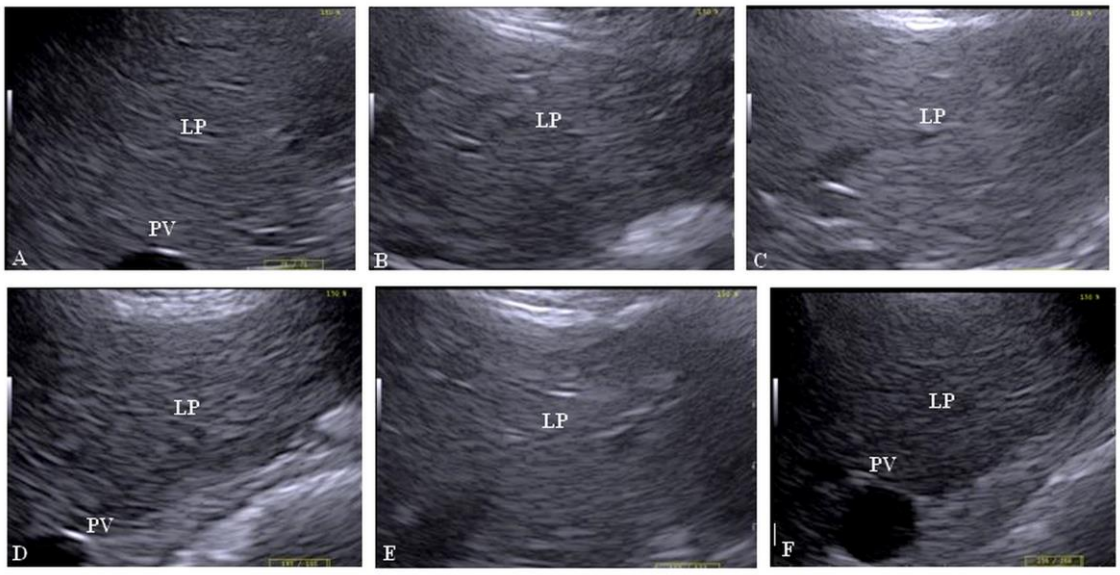

Figure 4: Ultrasound images of the liver, at the mid-way of the 14th intercostal space of the right side, of a donkey show variation in hepatic echogenicity during the study. Image (A), just before fasting, reveals 72 of echogenicity. Image (B), after 2 days of fasting, shows 80 of echogenicity. Image (C), after 4 days of fasting, represents 90 of echogenicity. Image (D), after 5 days of studying, indicates 88 of echogenicity. Image (E), after 7 days of study, reveals 80 of echogenicity. Image (F), after 10 days of the study, shows 77 of echogenicity.

3-Effect of fasting-induced hyperlipidemia on blood metabolites

Figure (5) illustrates the variations in the lipid profile of donkeys during the study, where the serum levels of TG, TC, VLDL, HDL, and LDL significantly varied among time points. After 4 days of fasting, the serum metabolites peaked and gradually decreased after the fasting period. The duration of fasting had significant effects on the mean TG ($P<0.001$), total cholesterol ($P<0.001$), VLDL ($P<0.001$), HDL ($P<0.05$), and LDL ($P<0.001$) levels. It was noted that it was quickly decreasing in all metabolites, especially TG, during the therapy period, which reflects the action of the insulin hormone as an anticatabolic hormone for decreasing body lipolysis.

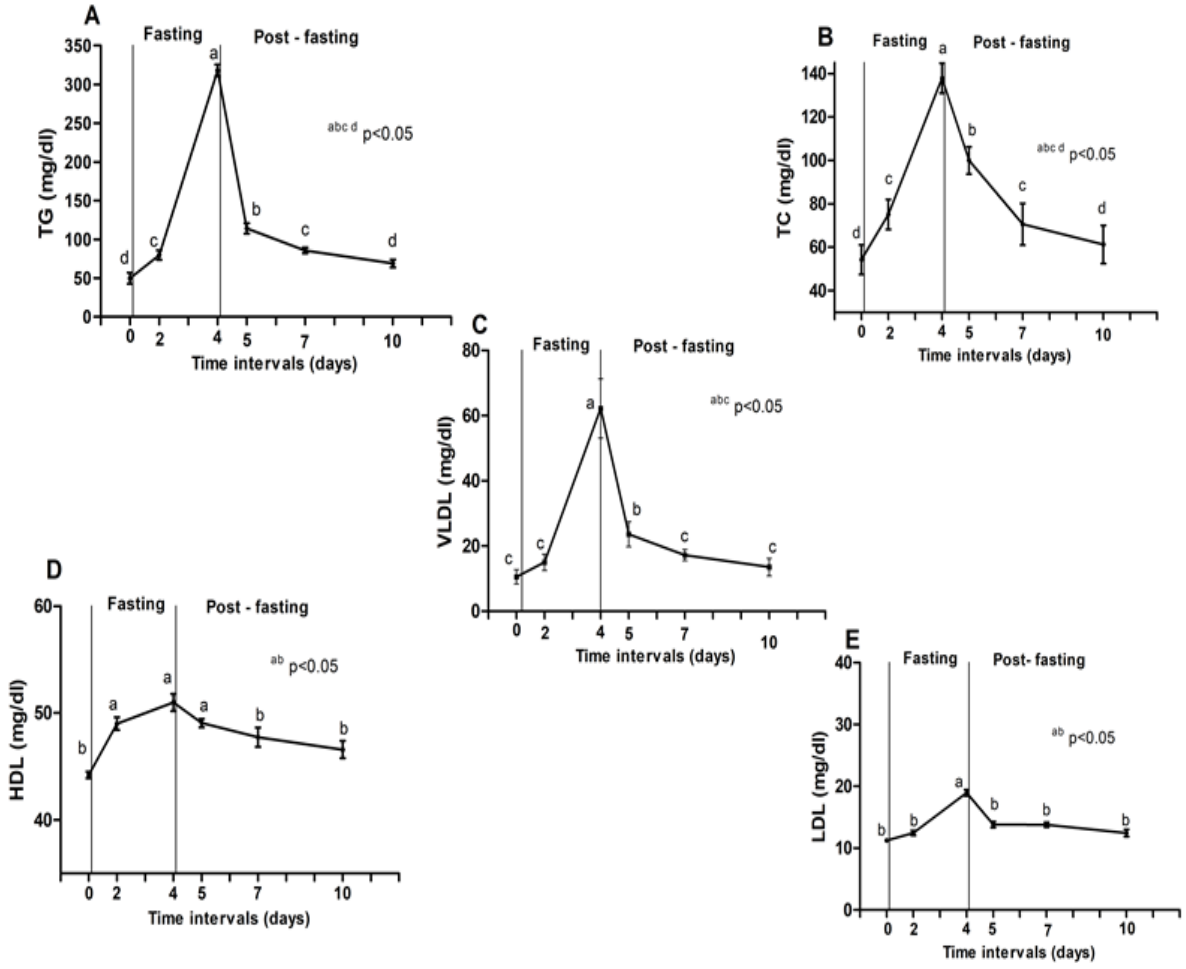

Figure ٥: Concentrations of triglycerides (TG, A), total cholesterol (B), high-density lipoproteins (HDL, C), and low-density lipoproteins (LDL, D) in donkeys of the fasting group during the study period. Data represent mean ± SE.

Table (4) summarizes the fasting effects on energy-related metabolites, including glucose, insulin and FFAs ($P<0.05$, $P<0.05$, $P<0.001$, respectively). The glucose level had been recorded at a lower level at 4 days of fasting than at zero days (before fasting), then it was noted that there was a high increase in glucose levels at 5, 7 days after fasting, reaching approximately the pre-fasting level. By contrast, FFA serum levels were greater during the fasting time compared with before fasting; however, there were no significant variations in serum insulin levels among different time points.

Hepatic enzymes, including GGT (P<0.001) and ALP (P<0.05), showed significant differences during the fasting period. They peaked at 4 days of fasting, then rapidly decreased during the post-fasting period. Furthermore, the fasted donkeys showed a significant increase in the urea levels (P<0.001), then decreased gradually after fasting. By contrast, serum total bilirubin and creatinine concentrations showed no significant differences during the fasting stage compared with zero days.

Table 4: The effect of fasting on the blood metabolites in donkeys (*Equus asinus*)

Metabolites			Days relative to fasting†				F-values
	0	Fasting stage		Post-fasting stage			
		2	4	5	7	10	
Glucose (mg/dl)	96±3.1[a]	78±4.3[b]	48±2.9[c]	92±4.7[a]	94±4.5[a]	95±3.8[a]	0.001
Insulin (mU/L)	3.4±0.4	N/A	4.2±0.2	N/A	N/A	4.1±0.2	0.920
FFA (mmol/l)	0.43±0.07[b]	N/A	1.2±0.05[a]	N/A	N/A	0.61±0.07[c]	0.000
GGT (IU/l)	64±3.1[c]	110±2.8[b]	130±6.1[a]	108±6.1[a]	100±5.6[b]	90±6.4[c]	0.000
ALP (IU/L)	116±4.5[c]	180±5.7[b]	250 ±5.6[a]	200±5.6[b]	115 ±5.4[c]	114±4.5[c]	0.010
Total bilirubin(mg/dl)	0.97±0.1	0.96±0.09	0.98±0.05	0.96±0.2	0.97±0.1	0.95±0.1	0.031
Urea (mmol/l)	6.6±0.6[b]	7.9±0.7[b]	9.1±0.5[a]	7.8±0.4[b]	6.9±0.5[b]	6.7±0.3[b]	0.001
Creatinine (mg/dl)	0.97±0.1	0.98±0.2	0.99±0.2	0.99±0.1	0.97±0.1	0.90±0.2	0.082

Abbreviations: FFA, free fatty acids; GGT, gamma-glutamyl transferase; ALP, alkaline phosphatase
† Values are mean ± standard error of the mean. ‡ F-values of times relative to fasting.
[abc]Means in the same row differ significantly (p<0.05).
N/A means not applicable.

DISCUSSION

Feed restriction, either accidentally or relative to a negative energy balance state, is commonly noted in equine (Durham *et al.,* 2013; Divers, 2015). This condition leads to hyperlipidemia, which is characterized by disturbances of lipid metabolism and abnormalities in hepatic and renal functions (Hughes and Dart, 2004). To our knowledge, no studies have reported the effect of fasting on ultrasonographic imaging of CFT, GMT and the liver in donkeys suffering from hyperlipidemia, and the effect of treatment on biochemical parameters was also not previously reported. Therefore, this study was designed to investigate the impact of therapeutic protocol and management on body condition, liver ultrasonography results, and blood metabolites in fasting-induced hyperlipidemia in donkeys.

In the present study, the most common signs observed were depression, dullness, and weakness during the fasting stage. Similar findings were reported in ponies (Baetz and Pearson, 1972; Brinkmann *et al.,* 2013), in donkeys and mules (Gupta *et al.,* 1999), and in horses (Toth *et al.,* 2018). Clinical parameters were unremarkable, and there were no significant variations between different time points. However, CRT increased during the fasting period in all donkeys, which may be due to decreased water intake (Horton *et al.,* 1996). It was reported that feed restriction may lead to low water consumption in ponies and horses (Tasker, 1967; Naylor, 1977; Freeman *et al.,* 2021). In the present study, the therapeutic protocol, including fluid therapy, enhanced and improved CRT to return to the pre-fasting level rapidly.

In the present research, the evaluation of body fat reserves with body weight and body condition score system exhibited no significant changes throughout the study. These results could be attributed to both BCS and BW methods that are considered subjective tools for the estimation of animal body fat reserves (Ferguson 1996; Mottet *et al.,* 2009). Dugdale *et al.* (2010) suggested that BCS and BW are not good indicators for the estimation of weight loss in horses.

In the current study, the mean values of croup fat thickness (CFT) were significantly decreased during the fasting stage and improved post-therapy. The drop in the CFT during fasting could be attributed to fat mobilization as a result of negative energy balance. The little improvement in the croup fat thickness after therapy may indicate the cessation of fat mobilization following the therapeutic protocol, including IV dextrose. Dugdale *et al.* (2010) reported decreased subcutaneous fat depth on ultrasound measurements in pony mares suffering from feed restriction. By contrast, in this study, there were no changes observed in the ultrasound measurements of GMT in all fasted donkeys, which indicated the absence of muscular protein breakdown or disturbance in protein metabolism. Conversely, Dugdale *et al.* (2010) reported that negative energy balance is associated with muscle loss due to the intense breakdown and catabolism of proteins.

Ultrasonography is a good noninvasive tool for the assessment of the equine liver and the detection of any abnormalities (Rantanen, 1986; Durham *et al.,* 2003). Ultrasound imaging of the liver measurements (size, thickness, and depth) showed no significant variations among time intervals in the present study, indicating no hepatomegaly despite fasting. In the current study, portal vein diameter decreased during fasting, which may be attributed to decreased portal blood flow during this stage. Moreover, the liver echogenicity increased during fasting in all donkeys compared to the first day of the study. Increasing liver echogenicity may be due to hepatic fatty infiltration as a result of negative energy balance. Durham *et al.* (2003) reported increased ultrasound hepatic echogenicity in some cases of hepatic lipidosis in donkeys.

In the present study, the serum concentrations of glucose decreased significantly during the fasting stage, because of negative energy balance. This condition could be attributed to the depletion of carbohydrate reserves and the induction of hyperlipidemia (Naylor *et al.,* 1980; Hughes and Dart, 2004). All donkeys showed high glucose levels during the post-fasting stage, which could be explained by the use of dextrose in the therapeutic protocol. By contrast, Blackmore and Brobst (1981) reported hyperglycemia in hyperlipidemic donkeys. Increased FFA levels during the fasting stage can be explained by excessive lipid mobilization because of negative energy balance. Feed deprivation increases NEFA levels in horses (Depew *et al.,* 1994; Nadal *et al.,* 1997; Connysson *et al.,* 2010), and in ponies (Dugdale *et al.,* 2001). However, insulin levels showed no changes during the fasting stage in all donkeys. Insulin inhibits hormone-sensitive lipase, so it is considered an anticatabolic hormone (Glade and Reimers, 1985). Gluco-corticoids released through negative energy balance states lead to the reduction of insulin secretion as a stress response of the body (Toth *et al.,* 2018). However, Bertin *et al.* (2016) reported no significant changes in insulin during fasting in horses. The same results were recorded in feed-restricted mares by Sticker *et al.* (1995).

In the current study, lipid profiles including TG, TC, HDL, LDL, and VLDL increased significantly during the fasting stage, which could be explained by the mobilization of peripheral fat stores during

the catabolic state due to negative energy balance (Naylor *et al.,* 1980; DeFilippo *et al.,* 2021). Hypercholesterolemia was noted in fasted donkeys and mules (Gupta *et al.,* 1999), and in horses (Sticker, 1995; Seifi *et al.,* 2002). Gupta *et al.* (1999) reported increasing concentrations of LDL during fasting in fasted donkeys and mules.

In the present study, increased activities of GGT and ALP during the fasting stage suggest impaired liver functions, as a result of subclinical hepatic lipidosis. Similar observations were reported in donkeys and mules (Gupta *et al.,* 1999). Moreover, in the current research. Increased blood urea concentrations during fasting may be due to either the mild dehydration state of the animal (Freeman *et al.,* 2021) or impairment of renal function due to fatty infiltration in the kidneys. During negative energy balance, subsequent lipomobilization and fat accumulate in the liver and kidneys (Harold and Mckenzie, 2011). However, in the present work, it was suggested that increased urea concentrations may be due to mild dehydration as confirmed clinically by increased CRT. Connysson *et al.* (2001) observed increasing urea levels in feed-deprived horses. In the current study, there were no significant variations in total bilirubin and creatinine levels during the fasting stage, indicating the fasting has no drastic effect on total bilirubin and creatinine. Similar findings were reported elsewhere by Difilippo *et al.* (2021) and Toth *et al.* (2018).

The therapeutic and nutritional plan includes insulin injections and dextrose 5% with sufficient feeding. Dextrose is fuel for the body because of hypoglycemia and fluid therapy to improve animal dehydration (Mogg and Palmer, 1995). The gradual re-feeding procedures of fasted donkeys aimed to re-establish the voluntary feed intake in sufficient amounts to progressively cover energy needs (Bookbinder and Schott, 2021). In the present study, Insulin was injected to minimize lipomobilization. Tarrant *et al.* (1998) concluded that insulin injection is essential for decreasing the catabolism of the stored fat and activating lipogenesis. Moreover, Durham and Thiemann (2015) mentioned that insulin promotes VLDL uptake into adipocytes and decreases hepatic lipidosis. In addition, Insulin also favours glucose uptake from the bloodstream into the cells (Morrone *et al.,* 2024).

CONCLUSION

In donkeys, fasting results in a reduction of CFT and ultrasound hepatic echogenicity. Both of them are considered valuable indicators for the diagnosis of hyperlipidemia. Based on these findings, the therapeutic protocol and management procedures applied in the present study may provide an effective treatment plan for hyperlipidemia in donkeys.

List of abbreviations
CFT: Croup fat thickness
PV: Portal vein
CVC: Caudal vena cava
RE: Relative echogenicity
FFA: Free fatty acids
TG: Triglycerides
TC: Total cholesterol
VLDL: Very low-density lipoprotein
HDL: High-density lipoprotein
LDL: Low-density lipoprotein
GGT: ɣ-glutamyl transferase
ALP: Alkaline phosphatase
BCS: Body condition score

REFERENCES

Baetz, A.L. and Pearson, J.E. (1972): Blood constituent changes in fasted ponies. Animal Journal Veterinary Research, 33(10), 1941–1946.

Bertin, F.; Taylor, S.; Bianco, A. and Sojka-Kritchevsky, J. (2016): The Effect of Fasting Duration on

Baseline Blood Glucose Concentration, Blood Insulin Concentration, Glucose/Insulin Ratio, Oral Sugar Test, and Insulin Response Test Results in Horses. Journal Veterinary Internal Medicine, 30(5), 1726–1731.

Blackmore, D.J. and Brobst, D. (eds.). (1981): Biochemical Values in Equine Medicine. The Animal Health Trust, UK.

Bookbinder, L. and Schott, H.C. 2nd (2021): Refeeding syndrome in a miniature donkey. Journal Veterinary Emergency Critical Care, 31(5), 668–673.

Brinkmann, L.; Gerken, M. and Riek, A. (2013): Effect of long-term feed restriction on the health status and the welfare of a robust horse breed, the Shetland pony (Equus ferus caballus). Research Veterinary Science, 94(3), 826-31.

Burden, F. (2012): Practical feeding and condition scoring for donkeys and mules. Equine Veterinary Education, 24(11), 589–596.

Burden, F. and Thiemann, A. (2015): Donkeys are different. Journal Equine Veterinary Science, 35(5):376-382.

Christensen, R.A.; Malinowski, K.; Massenzio, A.M.; Hafs, H.D. and Scans, C.G. (1997): Acute effects of short-term feed deprivation and refeeding on circulating concentrations of metabolites, insulin-like growth factor I, insulin-like growth factor binding proteins, somatropin, and thyroid hormones in adult geldings. Journal Animal Science, 75(5), 1351-1358.

Connysson, M.; Essén-Gustavsson, B.; Lindberg, J.E. and Jansson, A. (2010): Effects of feed deprivation on Standardbred horses fed a forage-only diet and a 50:50 forage-oats diet. Equine Veterinary Journal, 42(38), 335-340.

Costa, L. (2017): Chapter 3: history and physical examination of the horse. In: Costa L, Paradis M, editors. Manual of clinical procedures in the horse. 1st ed. Philadelphia: Wiley & Sons; p. 230–438.

DePew, C.L.; Thompson, D.L.; Fernandez, J.M.; Sticker, L.S. and Burleigh, D.W. (1994): Changes in concentrations of hormones, metabolites, and amino acids in plasma of adult horses relative to overnight feed deprivation followed by a pellet-hay meal fed at noon. Journal of Animal Science, 72 (6), 1530–1539.

Divers, T.I. (2015): The equine liver in health and disease. Am Association Equine Practice, 6, 66-103.

Di Filippo, P.A.; Duarte, B.R.; Albernaz, A.P. and Quirino, C.R. (2021): Effects of feed deprivation on physical and blood parameters of horses. Brazilian Journal Veterinary Medicine, 43(1),1-10.

Dugdale, A.H.A.; Curtis, G.C.; Cripps, P.; Harris, P.A. and Argo, C.M.G. (2010): Effect of dietary restriction on body condition, composition and welfare of overweight and obese pony mares. Equine Veterinary Journal, 42(7), 600-610.

Durham, A.E. (2013): Hyperlipemia. In: Geor RJ, Harris PA, *et al.,* eds. Equine Applied and Clinical Nutrition. London, UK: Elsevier-Saunders, 512–520.

Durham, A.E.; Newton, J.R.; Smith, K.C.; Hillyer, M.H.; Hillyer, L.L.; Smith, M.R.W. and Marr, C.M. (2003): Retrospective analysis of historical, clinical, ultrasonographic, serum biochemical and haematological data in prognostic evaluation of equine liver disease. Equine Veterinary Journal, 35(6), 542- 547.

Durham, E. and Thiemann, A.K. (2015): Nutritional management of hyperlipidemia. Equine Veterinary Education, 27(9), 482–488.

Dyce, K.K.M.; Sack, W.O. and Wensing, C.J.G. (2010): Textbook of Veterinary Anatomy, 4th ed. Philadelphia, PA: Saunders, 551–560.

Ferguson, J.D. (1996): Implementation of a body condition scoring program in dairy herds. Feeding and managing the transition cow. Proceedings of the Pennsylvania Annual Conference, University of Pennsylvania, Center for Animal Health and Productivity, Kennett Square, PA.

Fielding, D. and Krause, P. (1998): Donkeys. In: The Tropical Agriculturist. Macmillan Education Ltd., London and Basingstoke.

Freeman, D.E.; Mooney, A.; Giguère, S.; Claire, J.; Evetts, C. and Diskant, P. (2021): Effect of feed deprivation on daily water consumption in healthy horses. Equine Veterinary Journal, 53 (1), 117–24.

Glade, M.J. and Reimers, T.J. (1985): Effects of dietary energy supply on serum thyroxine, triiodothyronine and insulin concentrations in young horses. Journal Endocrinol, 104, 93-98.

Gordon, M.E.; McKeever, K.H.; Betros, C.L. and Manso, H.C. (2007): Plasma leptin, ghrelin and adiponectin concentrations in young fit racehorses versus mature unfit Standardbreds. The Veterinary Journal, 173, 91–100

Gupta, A.K.; Pal, Y. and Yadav, M.P. (1999): Effect of feed deprivation on biochemical indices in equids. Journal Equine Veterinary Science, 10(2), 33-38.

Harold, C. and Mckenzie, I.I.I. (2011): Equine hyperlipidemias. Veterinary Clinic Equine. 27: 59-72.

Horton, G.M.J.; Baldwin, J.A.; Emanuele, S.M.; Wohlt, J.E. and McDowell, L.R. (1996): Performance and blood chemistry in lambs following fasting and transport. Animal Science, 62 (1), 49–56

Hughes, K.J. and Dart, A.J. (2004): Equine hyperlipemia: a review. Australian Veterinary Journal, 82(3), 136-142.

Hussein, H.A.; Ibrahim, A.; Ali, M.F. and Abdel-Maksoud, F.M. (2017): Development and Complications of Blind and Ultrasound-Guided Percutaneous Liver Biopsy Techniques in Donkeys (Equus asinus). Journal Equine Veterinary Science, 58, 24-33

Mckenzie, III H.C. (2011): Equine hyperlipidemias. Veterinary Clinics of North America: Equine Practice, 27(1),59-72.

Mendoza, F.J.; Aguilera-Aguilera, R.; Gonzalez- DeCara, C.A.; Toribio, R.E.; Estepa, J.C. and Perez-Ecjia, A. (2015): Characterization of the intravenous glucose-tolerance test and the combined glucose-insulin test in donkeys. The Veterinary Journal, 206, 371- 376.

Mendoza, F.J.; Toribio, R.E. and Perez-Ecija, A. (2018): Donkey Internal Medicine—Part I: Metabolic, Endocrine, and Alimentary Tract Disturbances. Journal Equine Veterinary Science, 65,66–74.

Moore, B.R.; Abood, S.K. and Hinchcliff, K.W. (1994): Hyperlipemia in 9 miniature horses and miniature donkeys. Journal Veterinary Internal Medicine, 8(5), 376-381

Mottet, R.; Onan, G. and Hiney, K. (2009): Revisiting the Henneke body condition scoring system: 25 years later. Journal of Equine Veterinary Science, 29, 417–418.

Mogg, T.D. and Palmer, J.E. (1995): Hyperlipidemia, hyperlipemia, and hepatic lipidosis in American miniature horses: 23 cases (1990–1994). Journal Animal Veterinary Medicine Association, 207(5), 604–7.

Morrone, S.; Sechi, S.; Carta, C.; Senes, A.; Rocco, R.; Pinna Parpaglia, M.L.; Sanna Passino, E. and Cappa, M.G. (2024): Nutritional management of hyperlipidemia in jenny: a brief report. Veterinary Research Communications, 48, 3323-3329.

Naylor, J.M. (1977): The nutrition of the sick horse. Journal Equine Medicine Surgery, 1,64–70.

Nadal, M.R.; Thompson, D.L.; Kincaid, L.A. (1997): Effect of feeding and feed deprivation on plasma concentrations of prolactin, insulin, growth hormone, and metabolites in horses. Journal of Animal Science, 75 (3), 736–744.

Naylor, J.M.; Kronfeld, D.S. and Acland, H. (1980): Hyperlipemia in horses: effects of undernutrition and disease. Animal Journal Veterinary Research, 41 (6),899-905.

Pal, Y.; Kumar, S. and Gupta, A.K. (2002): Blood gases, acid-base and physiological indices in donkeys as pack animals. Draft Animal News, 37, 27–33.

Pearson, R. and Quassat, M. (1996): Estimation of the live weight and body condition of working donkeys in Morocco. Veterinary Record, 138 (10),229-233.

Quaresma, M.; Payan-Carreira, R. and Silva, S.R. (2013): Relationship between ultrasound measurements of body fat reserves and body condition score in female donkeys. Veterinary Journal, 197(2), 329- 334.

Rantanen, N.W. (1986): Diseases of the liver. Veterinary Clinical North Animal Equine Practice, 2(1), 105 - 114

Salari, F.; Roncoroni, C.; Altomonte, I.; Boselli, C. and Brajon, G. (2021): Effects of physiological states and diet on blood metabolic parameters in Amata diary donkeys. Animals, 11(11), 1- 11.

Seifi, H.A.; Gray, H.; Mohri, M.; Badiee, A. (2002): Hyperlipidemia in Caspian miniature horses: Effects of undernutrition. Journal of Equine Veterinary Science, 22(5), 205-207.

Sticker, L.S.; Thompson, D.L.; Bunting, L.D.; Fernandez, J.M.; DePew, C.L. and Nadal, M.R. (1995): Feed deprivation of mares: Plasma metabolite and hormonal concentrations and responses to exercise. Journal Animal Science, 73(12), 3696–3705.

Tarrant, J.M.; Campbell, T.M.; Parry, B.W. (1998): Hyperlipemia in a donkey. Australian Veterinary Journal, 76(7),466-469.

Tasker, J.B. (1967): Fluid and electrolyte studies in the horse: III Intake, and output of water, sodium, and potassium in normal horses. Cornell Veterinary, 57(1),649-657.

Toth, B.; Auth, A.; Rompos, L. and Bakos, Z. (2018): Effect of food deprivation on selected parameters of lipid mobilization and hepatic function in healthy Akhal Teke horses. Equine Veterinary Journal, 50(1),98 – 103.

Watson, T.D.; Burns, L. and Love, S. (1992): Plasma lipids, lipoproteins and post-heparin lipases in ponies with hyperlipemia. Equine Veterinary Journal, 24(5),341–6.

Watson, T.D. and Love, S. (1994): Equine hyperlipidemia. Compend Contin Educ Practice Veterinary, 16(3), 89-97.

Williams, S.; Cooper, J.D. and Freeman, S.L. (2014): Evaluation of normal findings using a detailed and focused technique for transcutaneous abdominal ultrasonography in the horse. BMC Veterinary Research, 10 (1), 1-8.

البروتكول العلاجى لمرض فرط الدهون المحدث تجريبيا فى الحمير (ايكواس اسينس)

عبير عبد الوارث ، حنان كمال السيد ، حسين عوض حسين

Emails: abeer199246@yahoo.com and hhussein@aun.eg
Assiut University web-site: www.aun.edu.eg

تعتبر الفصيلة الخيلية الاكثر تأثرا عن غيرها من فصائل الحيوانات باضطرابات التمثيل الغذائى للدهون الجسم (فرط دهون الجسم) وخصوصا الحميرالتى تعد هى الاكثراصابة بذلك المرض. هذا المرض غالبا مايكون اوليا نتيجة الاجهاد وزيادة وزن جسم الحيوان والتى تعتبر من أهم العوامل المحفزة لحدوث ذلك المرض أو يعد مرضا ثانويا نتيجة وجود اى امراض اخرى تؤدى الى انخفاض توازن الطاقة بالجسم (توازن طاقة سلبى). يعد العلاج فى أسرع وقت ممكن أمرا بالغ الأهمية والذى يتكون من استخدام سوائل (محلول سكرى ٥٪ بشكل أساسى) مع حقن الانسولين. تم اعداد الدراسة كتجربة قائمة على الملاحظة و شملت عشرة حمير. تنقسم التجربة (عشرة أيام) الى فترتين ،مرحلة الصيام وهى أربعة أيام ومرحلة ما بعد الصيام وهى تشمل سته أيام أخرى وتم استخدام العلاج لمدة ثلاثة أيام فى مرحلة مابعد الصيام. فى أوقات مختلفة خلال الدراسة ، تم تحديد تأثير مرض فرط دهون الدم وعلاجه على الأتى ذكره: وزن الجسم، معدل دهون الجسم، قياس سمك الدهون تحت الجلد بالموجات فوق الصوتية، تصوير بالموجات فوق الصوتية للكبد، الى جانب قياس بعض مؤشرات الدم. تم ملاحظة الأتى خلال الدراسة: انخفاض قياس سمك الدهون تحت الجلد بشكل ملحوظ فى مرحلة الصيام مع عدم ظهور تغيرات بقياسات الموجات فوق الصوتية للكبد. وجود انخفاض بقياس قطرالوريد البابى للكبد خلال مرحلة الصيام، مع زيادة مستوى الدهون بالجسم خلال الصيام وانخفاض مستوى سكر الدم ثم ارتفاع مستوى سكر الدم بعد الصيام واثناء فترة العلاج. فى الختام: يعد التدخل السريع بالعلاج المناسب والتعامل المتقن فى حالات الاصابة بمرض فرط دهون الدم يساعد فى سهولة اعادة توازن الجسم ومنع أى تغيرات تحدث نتيجة حالة انخفاض الطاقة بجسم الحيوان.