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MONITORING OF EXPERIMENTALLY INDUCED HYPERLIPIDAEMIA IN DONKEYS

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ABSTRACT

Controlled experiment was conducted to induce hyperlipidaemia in donkeys. For the purpose of the study, 12 male donkeys 4-10 years were housed in the College farm in different pens. Animals were randomly allocated into two groups of equal number. Animals in the two groups were subjected to fasting for 4 and 5 days, respectively. Blood samples were collected several times during three occasions i.e. pre-fasting, fasting and post fasting. Haemoglobin concentration, packed cell volume and RBCs count were evaluated in all animals. Plasma was tested for total proteins, albumin, triglycerides (TG), total cholesterol, urea, creatinine, AST activity level and Bilirubin. Pulse rate, respiratory rate and rectal temperature were also evaluated. General health of animals and abnormal clinical signs were recorded daily. Withholding feed significantly increased mean plasma urea, AST, bilirubin and creatinine level. Total protein and albumin, decreased significantly in animals subjected to fasting for five days, compared with baseline values. Mean plasma TG and total cholesterol concentrations significantly increased with time in feed-deprived donkeys. In conclusion, withholding feed for four and five days significantly increases blood lipid concentrations in donkeys, but individual donkeys respond differently.

Keywords: *Hyperlipidaemia, withholding of feed, donkeys, Sudan*

INTRODUCTION

Disturbances of lipid metabolism that result in accumulation of triglycerides in the blood are common in equine species (Gay *et al*., 1978; Naylor *et al*., 1980; Jeffcott and Field, 1985a; Watson *et al*., 1992a and Watson and love, 1994). Hyperlipaemia is a pathophysiological response to prolonged negative energy balance associated with gross lipaemia (Naylor *et al*., 1980; Watson *et al*., 1992a; Watson and love, 1994). Hyperlipaemia in equine has been thoroughly reviewed elsewhere (Watson, 1994; Hughes *et al*., 2004; Mckenzie, 2011).

Hyperlipaemia is a life-threatening condition in horses, ponies, and donkeys (Moore *et al*., 1994, Mogg and Palmer, 1995; Dunkel and McKenzie, 2003, Hughes *et al*., 2004). If left undetected or untreated, hyperlipaemia may progress to hepatic lipidosis and liver failure with multi-systemic complications (Mogg and Palmer, 1995). The disease is well described in donkeys (Naylor *et al*., 1980; Mair, 1995) with mortality from 86% to 95%, higher than that in ponies (Fowler, 1989).

Suggested predisposing factors include food deprivation or increased metabolic demands, most remarkably in obese ponies, American Miniature Horses, or donkeys (Naylor *et al.*, 1980; Moore *et al*., 1994; Mogg and Palmer, 1995; Frank *et al*., 2002; Dunkel and McKenzie, 2003; Hughes *et al*., 2004).

Various risk factors for the development of hyperlipaemia have been reported; the disease is most common in mares of all breeds, accounting for between 74 and 100% of affected horses (Gay *et al*., 1978 and Watson *et al*., 1992a). Reproductive activity appears to increase the susceptibility of pony mares to hyperlipaemia, with late pregnancy and early lactation being predisposing factors (Gay *et al*., 1978; Jeffcott and Field 1985a &b; Watson *et al.*, 1992a). Reproductive activity does not appear to be a strong predisposing factor in miniature breeds, with 65-71% of affected females reproductively inactive (Moore *et al.*, 1994 and Mogg and Palmer, 1995).

The condition tends to occur in middle-aged, obese donkeys often secondary to an episode of stress (Whitehead *et al*., 1991). Typically identified stressors include debilitating malaise such as laminitis or parasitism, nutritional deprivation and behavioural stress. Higher incidence of hyperlipaemia, were associated with lactation or late

gestation in Ponies (Jeffcott and Field, 1985a and Schotmann and Kroneman, 1969).

In ponies and donkeys hyperlipaemia is usually a primary disease process, and stress and obesity appear to be particularly important predisposing factors (Jeffcott and Field, 1985b; Watson *et al*., 1992a and Mair, 1995). Food deprivation, either accidental, intentional, or relative to the increased metabolic demands of pregnancy or lactation; is a common predisposing factor in primary and secondary hyperlipaemia (Gay *et al*., 1978; Jeffcott and Field, 1985b; Moore *et al*., 1994 and Mogg and Palmer, 1995).

Few detailed case studies in the donkey have been reported, and all have come from the northern hemisphere (Rognerud, 1976; Bossche and Vanden-Bossche, 1988; Mair, 1995). One report from Australia (Tarrant *et al.,* 1998) described hyperlipaemia in a 10 year-old, multiparous, female Jerusalem donkey.

The objective of the current study is to monitor clinical changes in experimentally induced hyperlipidaemia in donkeys due to withholding of feed.

MATERIALS and METHODS

Site of study: The study was carried at the farm of the College of Veterinary Medicine, Sudan University of Science and Technology (SUST), East Nile locality, Hillat Kuku, Sudan.

Ethical approval: The study protocol was approved by the College of Veterinary Medicine Research Board as well as the Deanship of Scientific Research, Sudan University of Science and Technology.

Experimental animals: Twelve male donkeys 4-10 years of age were purchased from local market. Upon their arrival animals were subjected to thorough clinical examination and received antibiotic and anthelmintics. Following adaption period of 10 days, animals were randomly assigned to two groups each of six. Animals in the first group were subjected to fasting for four days, and animals in the second group were subjected to fasting for five days. Water was provided for ad libitum intake.

Blood samples: Seven ml of blood were collected at predetermined time points during three different occasions i.e. pre-fasting (three samples), fasting (3-4 samples) and post-fasting (three samples), via jugular vein puncture into EDTA-coated tubes. Blood was collected between 08:30 and 09:30 AM and kept immediately in ice for transport to the laboratory. Haematological parameters were tested in whole blood while biochemical tests were carried on plasma following centrifugation of whole blood.

Haematological parameters: Haemoglobin, packed cell volume, and total red blood cells count were measured according to Schalm and Jain (1986).

Analysis of Plasma constituents: Concentrations of plasma TG and total cholesterol were measured using the enzymatic colorimetric reagents. Plasma total protein, albumin, urea, creatinine, AST activity level were measured using an in vitro enzymatic colorimetric test kit (Biosystems, S.A. Spain).

Clinical parameters: Pulse rate, respiratory rate and rectal temperature were measured using standard methods (Kelly, 1986).

Statistical analysis: Data were analyzed using GraphPad Prism 5.0 (GraphPad Software). Differences between the three groups were analyzed by using one way ANOVA. The significance is indicated as *$p<0.05$; **$p< 0.01$ and ***$p<0.001$.

RESULTS

Clinical signs: the animals showed dullness, depression, nervousness, coprophagia, weakness, signs of colic (abdominal pain, rolling and grinding of teeth), and lethargy.

Physiological parameters: In the second group there was significant ($P<0. 05$) decrease in respiratory rate in the post-fasting observation period. In the first group there was no significant ($P>0.05$) change in respiratory rate. The other parameters tested expressed no significant ($P>0.05$) fluctuation within the normal range observed in the pre-fasting period as shown in table (1).

Haemtological parameters: There was significant ($P<0.05$) decrease in PCV and haemoglobin concentration in the second group as shown in table (2).

Biochemical parameters: In the first group there was no significant ($P>0.05$) decrease in the total protein concentration during fasting, while the level increased significantly ($P<0.05$) following re-feeding. In the second group there was significant ($P<0.05$) decrease in total protein and albumin concentration during the fasting period (table 3).

As shown in table (3) in the first group, it could be observed that there was no significant ($P>0.05$) increase in total triglycerides and total cholesterol during the fasting period, while there was significant ($P<0.05$) increase during the post fasting period. In the other hand, there was significant ($P<0.05$)

increase in total triglycerides and total cholesterol concentration in the second group during fasting period.

In the first group, urea, AST activity level, bilirubin and creatinine level increased significantly during the fasting period. Bilirubin and creatinine continued to increase significantly at post fasting. In the second group, Urea, AST and bilirubin exhibited non significant change, While, creatinine showed significant increase during the fasting period (table 3).

Table 1: Effect of fasting on physiological parameters

Parameters	Four days fasting				Five days fasting			
	Pre-fasting	**Fasting**	**Post-fasting**	**P-value**	**Pre-fasting**	**Fasting**	**Post-fasting**	**P-value**
RR/min	31.17±4.88	30.33±6.91	30.67±2.93	0.8923	30.33±6.91	33.75±3.53*	30.11±2.81*	0.0207
PR/min	53.28±8.74	49.67±7.32	49.89±4.44	0.2317	49.67±7.32	52.54±3.53	50.78±4.24	0.1955
Temperature °C	38.21±1.09	38.01±0.66	38.11±0.71	0.7663	38.01±0.66	37.87±0.50	38.04±0.61	0.5942

RR= respiratory rate, PR= Pulse rate

Table 2: Effect of fasting on haemtological parameters

Parameters	Four days fasting				Five days fasting			
	Pre-fasting	**Fasting**	**Post-fasting**	**P-value**	**Pre-fasting**	**Fasting**	**Post-fasting**	**P-value**
TRBCs/mm3	7.98±0.81	7.87±0.54	8.12±0.92	0.6113	7.87±0.54	7.80±0.43	7.66±0.61	0.4525
PCV (%)	28.17±3.17	29.50±2.12	28.61±1.65	0.2458	29.50±2.12[a]	29.88±2.27[a]	27.17±2,43[b]	0.0009
Hb g/dl	16.39±2.15	16.28±1.23	15.11±1.23	0.0361	16.28±1.23[a]	15.21±1.02[b]	14.39 ±1.04[c]	0.0001

Table 3: Effect of fasting on biochemical parameters

Parameters	Four days fasting				Five days fasting			
	Pre-fasting	Fasting	Post-fasting	P-value	Pre-fasting	Fasting	Post-fasting	P-value
Total protein g/l	60.50±10.28	57.89±9.06*	68.50±12.63*	0.0128	66.78±5.87[a]	56.88±10.28[b]	53.67±11.44[b]	0.0003
Albumin g/l	21.11±3.92	20.67±7.45	22.28±2.97	0.6283	23.61±3.40[a]	22.67±3.09[a]	20.72±2.95[b]	0.0240
Triglycerides mmol/l	0.64±0.39*	2.13±2.13	3.58±2.77*	0.0003	0.47±0.33a	1.68±1.68b	1.81±1.99b	0.0187
Cholesterol mmol/l	1.53±0.66*	1.76±1.76	2.66±0.84*	0.0154	0.89±0.41a	2.14±1.36b	2.14±1.17b	0.0007
Urea mg/dl	2.96±0.93*	4.81±2.81*	4.01±2.13	0.0369	5.39±1.85	6.23±2.57	5.44±2.46	0.4267
AST UI	18.44±4.55*	28.67±9.82*	24.35±10.44	0.0036	5.84±2.01	6.47±2.81	5.67±4.01	0.6638
Bilirubin mg/dl	0.79±0.68[a]	4.87±4.62[b]	5.05±5.87[b]	0.0064	1.24±0.42	1.69±0.66	2.97±4.06	0.0666
Creatinine mg/dl	1.24±0.42	1.58±0.64*	1.09±0.66*	0.0482	1.09±0.66a	1.67±0.59b	1.24±0.42a	0.0035

DISCUSSION

Given the high mortality associated with hyperlipaemia, it is of considerable importance to veterinarians dealing with susceptible equine populations (Jeffcott and Field, 1985a; Watson and Love, 1994). Hyperlipaemia in the donkey induce mortality from 86% to 95% (Fowler, 1989), higher than that in the pony.

Clinical signs monitored in this study are in accordance with that reported by Watson (1994). Here in the present study, there was significant ($P<0.05$) decrease in respiratory rate in the post-fasting observation period in the second group. The respiratory rate was not below the reference values (26.90±.9.40) for donkeys reported by French, and Patrick, (1995), and Etana and his colleagues (2011).

There was fluctuation (within the normal range) in the values of pulse rate and rectal temperature observed in the pre-fasting period as shown in table (1). Here the values are also within the recommended reference values recommended by other researchers (French, and Patrick, 1995, and Etana *et al.*, 2011).

There was significant ($P<0.05$) decrease in PCV and haemoglobin concentration in the second group (table 2). It could be observed that in the two groups there was no significant ($P>0.05$) increase in the PCV values during fasting, while in post fasting period there was significant ($P<0.05$) increase in PCV level. This result is in agreement with that of Tarrant *et al.* (1998), who reported significant increase in the first day of a 10-year –old, multiparous, female Jerusalem donkey that was presented with a 2-day history of mild depression, in appetence and lethargy. There was increase in PCV (46%) in the first day of hospitalization and then the level declined to (32%) following 12 days of hospitalization. Physiologically, this result could be justified as the animals decrease the intake of water during fasting which may lead to haemconcentration, and following re-feeding the animals tend to drink water as usual, resulting in haemdilution.

In the first group there was no significant ($P>0.05$) decrease in the total protein concentration during fasting, while the level increased significantly following re-feeding. In the second group there was significant decrease in total protein and albumin concentration during the fasting period (table 3).

The fluctuation in total protein and albumin concentration could be attributed to haemconcentration, while in the second group the decrease could be attributed to the negative energy balance.

In the first group (table 3), it could be observed that there was no significant ($P>0.05$) increase in total triglycerides and total cholesterol during the fasting period, while there was significant increase during the post fasting period. On the other hand, there was significant increase in total triglycerides and total cholesterol concentration in the second group during fasting period. This could be attributed to the non significant ($P>0.05$) increase during fasting period in the first group to the fact that most of the animals in this experiment had body score ranging from fair to good.

The plasma total triglyceride concentration measured in the present study remained significantly ($P<0.05$) lower than values reported previously, with a range 0.47±0.33-3.58±2.77. Individual animals showed TG level up to 11.3 mmol/l in the first group and 8.8 mmol/l in the second group during the fasting period. While the primary causes and clinical presentation leading to hypertriglyceridaemia/ hyperlipaemia appear similar among equids, the degree of plasma triglyceride (TG) increase appears to differ substantially. Fasting of healthy animals results in only a moderate increase of plasma total triglyceride. The values reported for fasted health horses (<2.26 mmol/l; Naylor *et al*., 1980) are lower compared to fasted healthy ponies (mean value 8.27 mmol/l, range 0.23-20.34 mmol/l; Bauer, 1983) and fasted health donkeys (4.24±0.56 mmol/l; Forhead *et al*., 1994).

In naturally occurring hyperlipaemia, the elevation of total triglycerides exceeds the values obtained from fasting normal animals (Dunkel and Mckenzie, 2003). Reported mean±SD of total triglycerides (TG) levels reached 25.4±18.1 mmol/l in ponies (range 4.69-79.1 mmol/l; Watson *et al*., 1992a), 14.92 mmol/l in miniature horses (range 5.57-31.98 mmol/l; Mogg and Palmer, 1995), 23.98 mmol/l in miniature horses and miniature donkeys (range 15.11-33.95 mmol/l; Moore *et al*., 1994) and 16.6 ±1.9 mmol/l in donkeys (Forhead *et al.*, 1994).

Total cholesterol level in the study conducted by Tarrant and his colleagues (1998) was higher at the beginning (7.2 mmol/l) and reduced to 2.1 mmol/l following hospitalization for 19 days (reference values 2.8±0.8). Here the level of cholesterol starts to rise during fasting period and continued at higher levels during re-feeding period.

The reasons for the differences noted regarding hypertriglyceridaemia in different types of equids might be related to fundamental differences in energy metabolism and endocrine responses to dietary influences (Dunkel and McKenzie, 2003). Differences in carbohydrate metabolism between ponies and horses have been reported, including

increased circulating baseline levels of insulin, impaired glucose tolerance (Jeffcott *et al*., 1986) and increased free fatty acid release from simulated pony adipocytes compared to horse adipocytes (Breidenbach *et al*., 1999).

In donkeys, with naturally occurring hyperlipidaemia/ hyperlipaemia, a positive correlation between plasma insulin and STG concentration has been reported (Forhead *et al*., 1994). Activities of lipoprotein lipase and hepatic lipase are higher in hypertriglyceridemic, feed-deprived horses than in fed horses (Frank *et al*., 2003). This suggests that overproduction of triglycerides, possibly complicated by defective catabolism, is the predominant cause of hypertriglyceridemia (Watson *et al*., 1992b).

In the first group, urea, AST activity level, total bilirubin and creatinine level increased significantly ($P<0.05$) during the fasting period. Bilirubin and creatinine continued to increase significantly at post fasting. In the second group, Urea, AST and bilirubin exhibited non significant ($P>0.05$) change, while, creatinine showed significant increase during the fasting period (table 3). Tarrant *et al*., (1998), reported increase in Urea (31.2 mg/dl) and decrease in AST (270 UI).

Azotaemia has been associated with hypertriglyceridaemia in several reports. A statistically significant association has been found between serum creatinine and STG in horses (Naylor *et al.*, 1980) and ponies with hyperlipaemia (Watson *et al.*, 1992a). Eight horses had an elevated serum creatinine (mean 0.46 mmol/l) concurrent with peak STG measured; 12 horses had an elevation of the serum creatinine concentration at least once during their hospitalization (Dunkel and McKenzie, 2003).

CONCLUSION

It is to be concluded that fasting of male donkeys for four and five days (with exclusion of other risk factors), resulted in moderate hyperlipidaemia. Individual variation in the level of total triglycerides was observed within donkeys in relation to body score. Further work was required to test the contribution of other risk factors viz: parasitism, pregnancy and lactation.

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مراقبة ارتفاع الدهون المحدث تجريبيا في الحمير

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أجريت هذه التجربة تحت ظروف سيطرة محددة، لاحداث ارتفاع الدهون تجريبيا في الحمير. من أجل هذا الغرض استخدم 12 من ذكور الحمير تتراوح اعمارهم بين 4-10 سنوات وضعت في حظائر مختلفة بمزرعة الكلية. تم تقسيم الحيوانات عشوائيا الي مجموعتين متساويتين. الحيوانات في المجموعتين تم تصويمها لمدة 4 و 5 أيام، علي التوالي. تم تجميع عينات الدم عدة مرات خلال ثلاث فترات هي: قبل الصيام، أثناء الصيام، وبعد الصيام. تركيز الهيموقلوبين، مكداس الدم، وعدد كريات الدم الحمراء تم تقويمها في كل الحيوانات. تم فحص البلازما لقياس البروتين الكلي، الزلال، الجليسريدات الثلاثية، الكولسترول الكلي، اليوريا، الكرياتنين، البيلروبين، وانزيم AST. وأيضا تم قياس عدد معدل التنفس، درجة الحرارة ومعدل النبض. مع تسجيل علامات الصحة العامة للحيوانات وأية أعراض غير طبيعية بصورة يومية. تصويم الحيوانات ادي الي زيادة معنوية في تركيز اليوريا، الكرياتنين، البيلروبين وانزيم AST في البلازما. في حين أن مستويات البروتين الكلي والزلال حدث فيها انخفاض معنوي في الحيوانات التي تم تصويمها لمدة خمسة ايام عند مقارنتها بالقيم قبل التصويم. أما قيم الجليسريدات الثلاثية والكولسترول الكلي فحدثت لها زيادة معنوية مع الزمن نتيجة للصوم. خلصت الدراسة الي أن تصويم الحيوانات لمدة اربعة وخمسة ايام ادت الي ارتفاع معنوي في تركيز الدهون في البلازما، ولكن استجابة الحيوانات الفردية للتصويم اظهرت اختلافا.