

RESULTS

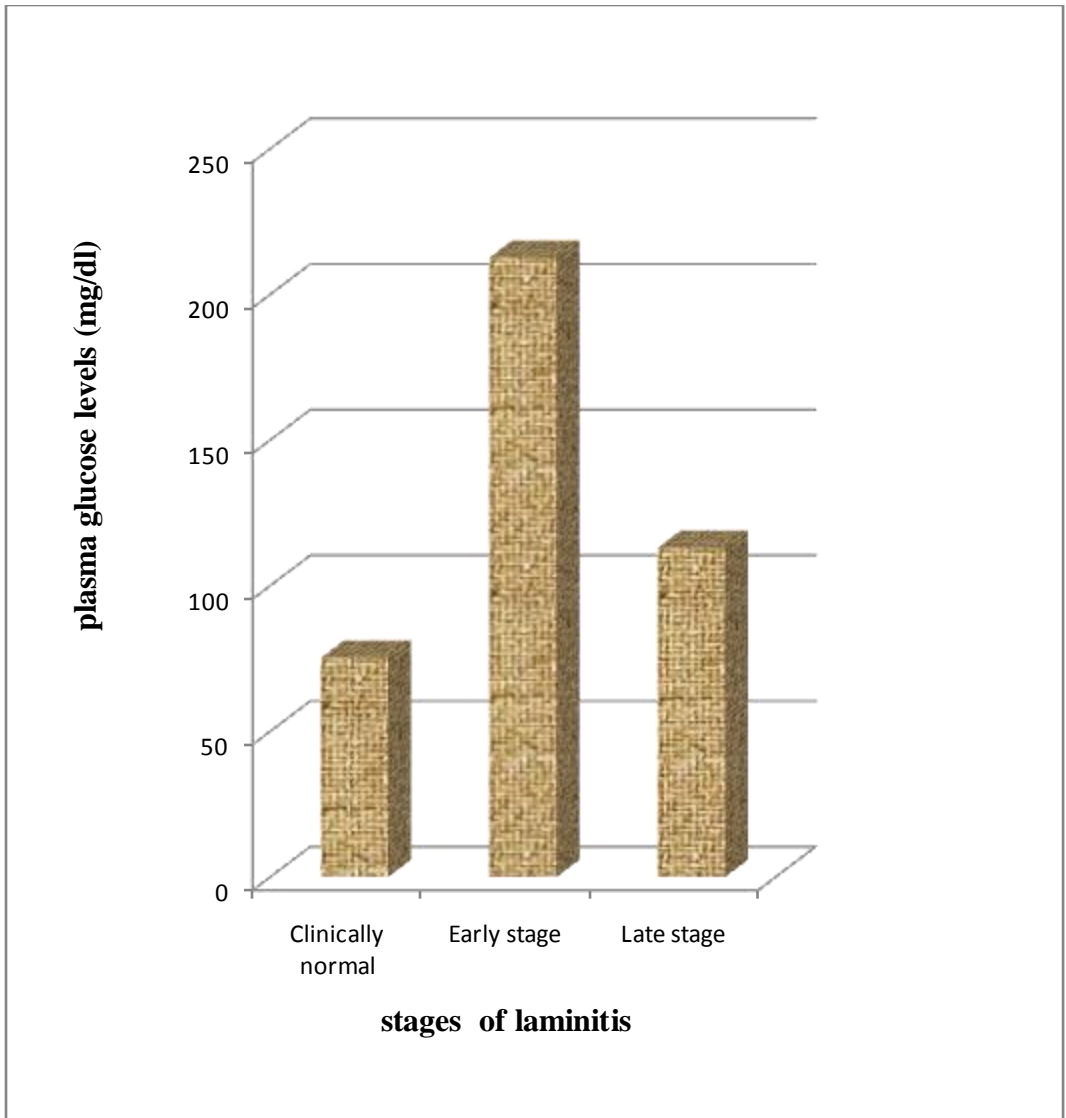
[Table (1)]. plasma glucose concentration and serum insulin level in clinically normal and laminitic horses.

Mean±SE parameters	Clinically normal	Clinically laminitic	
		Late stage	Early stage
Glucose (mg/dl)	75.2 ± 1.5 ^a	212.6 ± 6.6 ^b	113 ± 0.7 ^c
Insulin (μIU/mL)	15.7 ± 0.6 ^a	79.9 ± 1.6 ^b	74.7 ± 2.6 ^b

Data represented as mean value ± standard error (SE).

The values in the same row having the different Small letter are significantly different at $P > 0.05$.

The values in the same row having the sameSmall letter are non significantly different at $P < 0.05$.



[Fig. (1)] . Levels of plasma glucose in clinically normal and laminitic horses



[Fig. (2)] . Levels of serum insulin in clinically normal and laminitic horses.

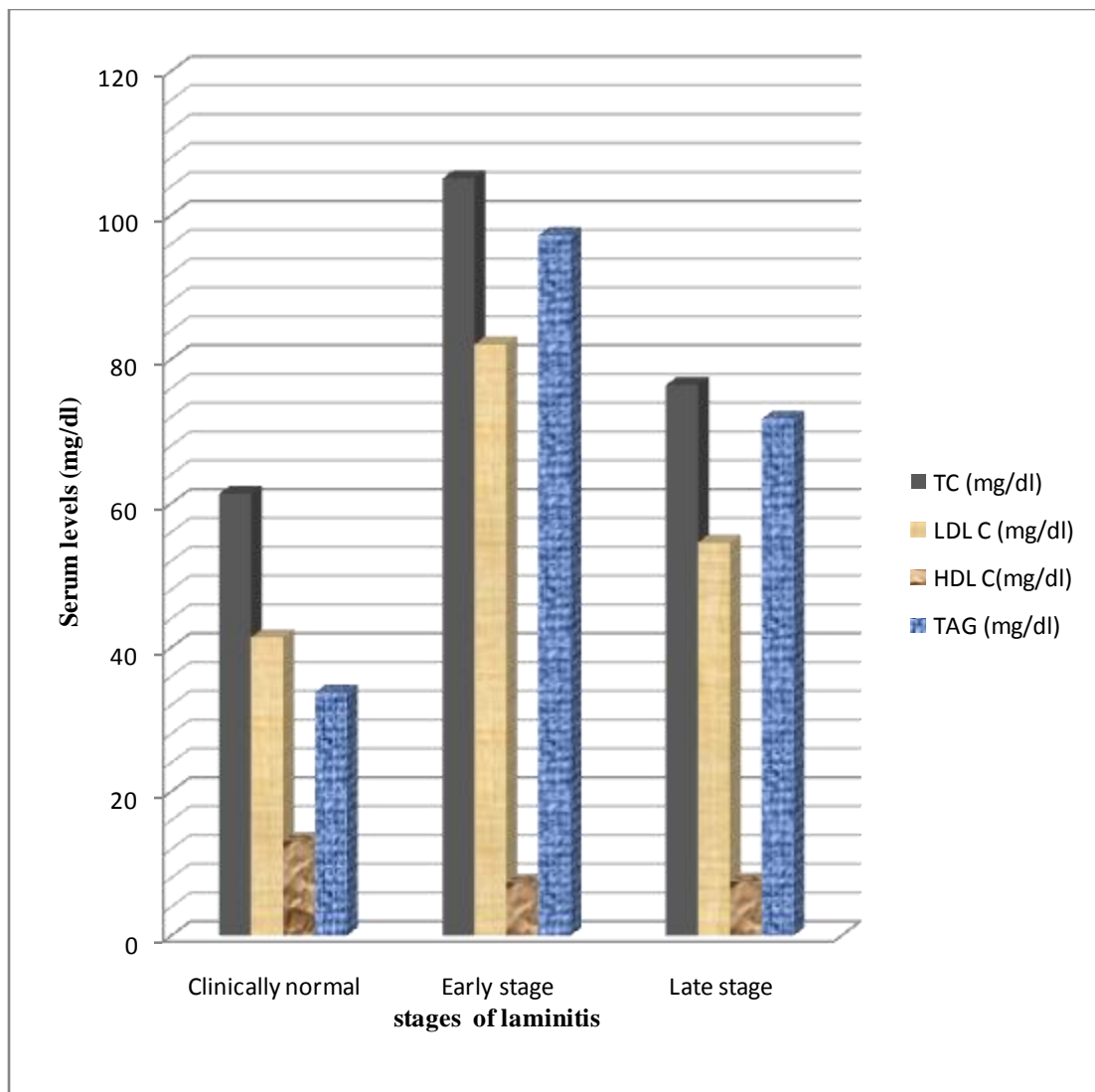
[Table (2)] : Represent total serum cholesterol (TC) , high density lipoprotein cholesterol (HDL - C), low density lipoprotein cholesterol (LDL- C) and triacylglycerol (TAG) concentrations in clinically normal and laminitic horses.

Mean±SE parameters	Clinically normal	Clinically laminitic	
		Late stage	Early stage
TC (mg/dl)	61.3 ± 2.1 ^a	105.1 ± 1.5 ^b	76.4 ± 5.4 ^c
LDL C (mg/dl)	41.36 ± 3.6 ^a	79.02 ± 4.4 ^b	54.52 ± 3.75 ^c
HDL C(mg/dl)	13.2 ± 0.6 ^a	7.42 ± 0.44 ^b	7.54± 0.56 ^b
TAG (mg/dl)	33.7 ± 2.7 ^a	97.2 ± 5.5 ^b	71.7± 3.7 ^c

Data represented as mean value ± standard error (SE).

The values in the same row having the different small letter are significantly different at $P > 0.05$.

The values in the same row having the same small letter are non significantly different at $P < 0.05$.



[Fig. (3)]. Represent total serum cholesterol(TC) , high density lipoprotein cholesterol (HDL - C), low density lipoprotein cholesterol (LDL- C) and triacylglycerol (TAG) concentrations in clinically normal and laminitic horses.

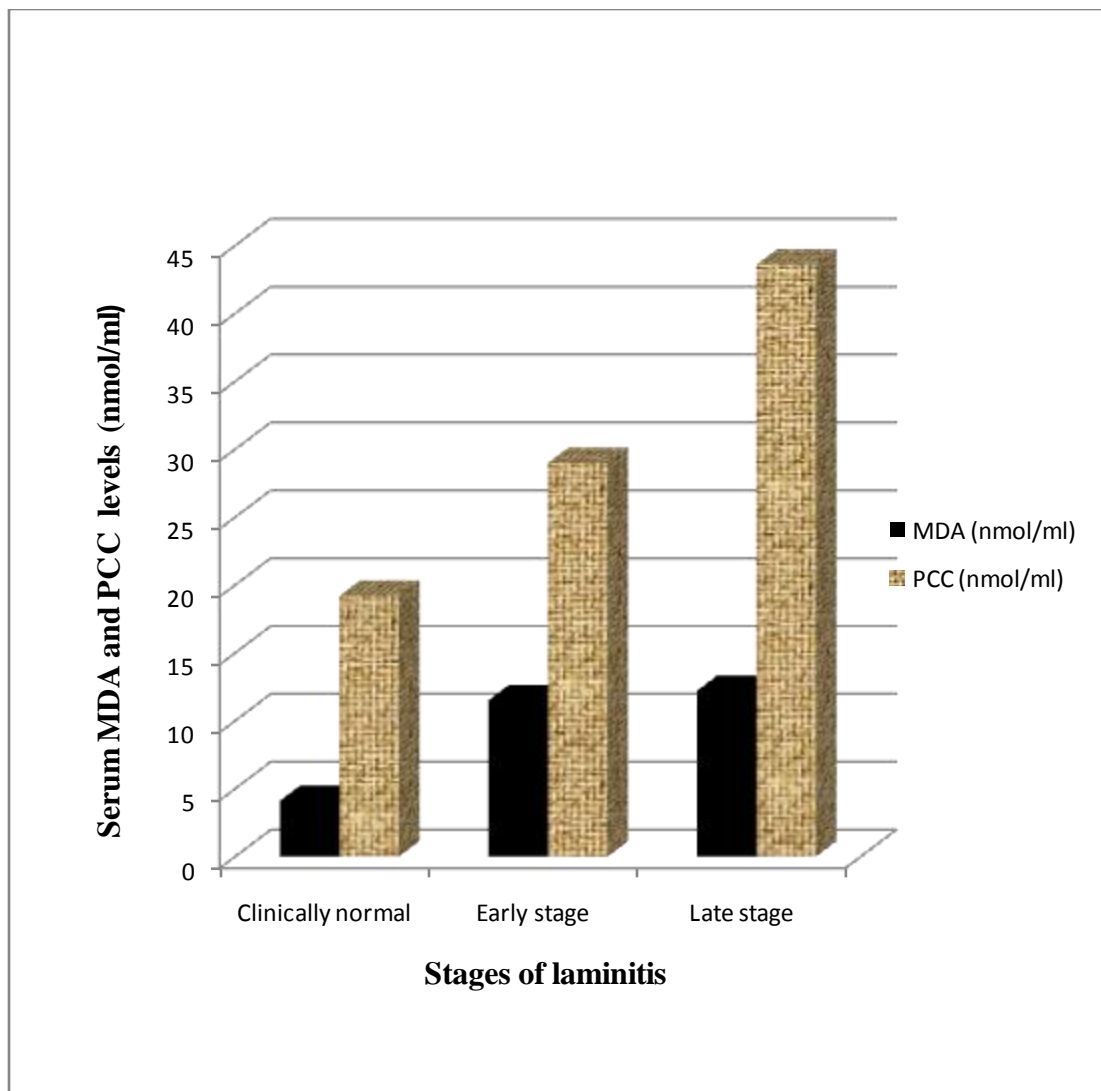
{Table. (3)}: Serum malondialdehyde concentration (MDA) and protein carbonyl content (PCC) in clinically normal and laminitic horses.

Mean±SE parameters	Clinically normal	Clinically laminitic	
		Late stage	Early stage
MDA (nmol/ml)	4.1± 0.3 ^a	11.5± 1.9 ^b	12.2± 1.4 ^b
PCC (nmol/ml)	19.2 ± 1.0 ^a	29± 0.3 ^a	43.6± 3.0 ^b

Data represented as mean value ± standard error (SE).

The values in the same row having the different small letter are significantly different at $P > 0.05$.

The values in the same row having the same small letter are non significantly different at $P < 0.05$.



[Fig. (4)]. Serum malondialdehyde concentration (MDA) and protein carbonyl content (PCC) in clinically normal and laminitic horses.

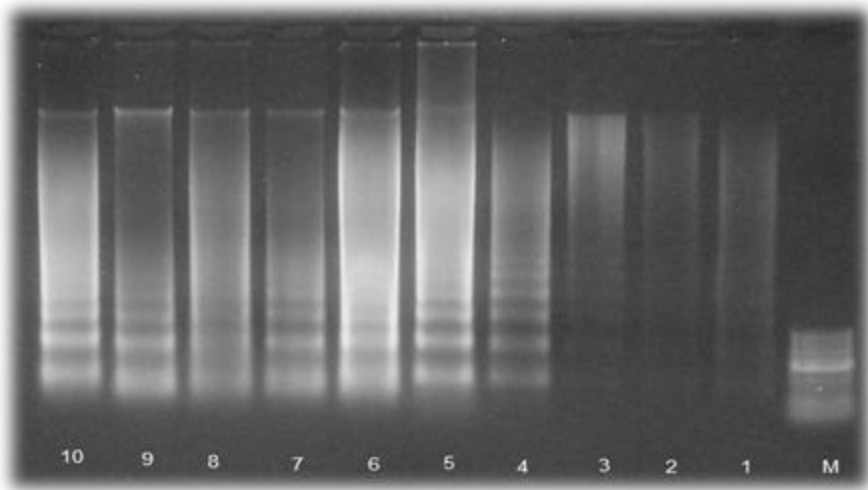


Photo (1) :- Electrophoretic pattern of fragmented DNA from whole blood in clinically normal and laminitic horses.

Fragmented DNA was electrophoresed on 1% TAE agarose gel stained with ethidium bromide. Lane M is 50 bp DNA ladder. Lane (1-4) clinically normal animals. Lane (5-10) clinically laminitic animals in the late stage.

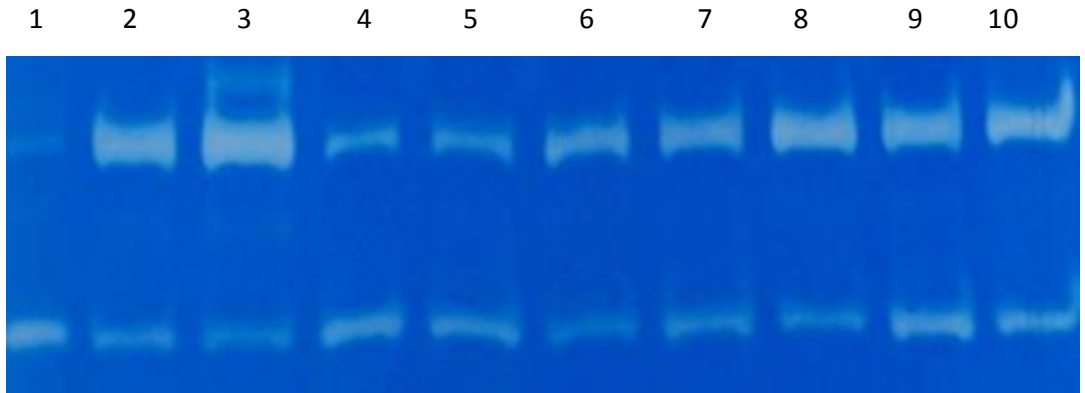
[Table (4):- DNA fragmentation assay.

Mean \pm SE	Clinically normal animals	Clinically laminitic animals
	7.6 \pm 0.3 ^a	15.1 \pm 0.6 ^b

Data represented as mean value \pm standard error (SE).

The values in the same row having the different small letter are significantly different at $P > 0.05$.

The values in the same raw having the same small letter are non significantly different at $P < 0.05$.



[Photo (2)]. Gelatin zymogram (Gel 1) .

Gelatin zymogram of serum samples from clinically normal and laminitic horses: lane 1 is Baby hamster kidney (BHK) standard.lane (2-5) clinically laminitic horses in the late stage. lane (6) clinically laminitic horses in the early stage.lane (7-10): clinically normal horses.

Channel	No	Group	Index	Name	QL	Area(pixel2)	QL-BG	B	BG	Std	Ratio(%)	QL/pixel2	(Q-B)/pixel2	C
1	1	~	1		74025.00	459.00	0.00	✓	74025.00	☺	-----	161.27	0.00	
1	2	~	2		32914.00	244.00	-6436.98	☐	39350.98	☺	100.00	134.89	-26.38	
1	3	~	3		30117.00	199.00	-1976.63	☐	32093.63	☺	91.50	151.34	-9.93	
1	4	~	4		35627.00	242.00	-3401.43	☐	39028.43	☺	108.24	147.22	-14.06	
1	5	~	5		37980.00	267.00	-5080.29	☐	43060.29	☺	115.39	142.25	-19.03	
1	6	~	6		32715.00	230.00	-4378.14	☐	37093.14	☺	99.40	142.24	-19.04	
1	7	~	7		30372.00	214.00	-4140.75	☐	34512.75	☺	92.28	141.93	-19.35	
1	8	~	8		29016.00	233.00	-8560.96	☐	37576.96	☺	88.16	124.53	-36.74	
1	9	~	9		38376.00	266.00	-4523.02	☐	42899.02	☺	116.59	144.27	-17.00	

	1	10	~	10	34653.00	233.00	-2923.96	☐	37576.96	☺	100.00	148.73	-12.55	
	1	11	~	11	46687.00	303.00	-2179.18	☐	48866.18	☺	134.73	154.08	-7.19	
	1	12	~	12	39312.00	254.00	-1651.73	☐	40963.73	☺	113.44	154.77	-6.50	
	1	13	~	13	32092.00	211.00	-1936.92	☐	34028.92	☺	92.61	152.09	-9.18	
	1	14	~	14	34271.00	222.00	-1531.94	☐	35802.94	☺	98.90	154.37	-6.90	
	1	15	~	15	37365.00	239.00	-1179.61	☐	38544.61	☺	107.83	156.34	-4.94	
	1	16	~	16	34286.00	223.00	-1678.22	☐	35964.22	☺	98.94	153.75	-7.53	
	1	17	~	17	39006.00	255.00	-2119.00	☐	41125.00	☺	112.56	152.96	-8.31	

[Fig. (7)]. Denstometric analysis of SDS- PAGE zymography by photocapture microsoft program (gel 1):-

1 2 3 4 5 6 7 8 9 10

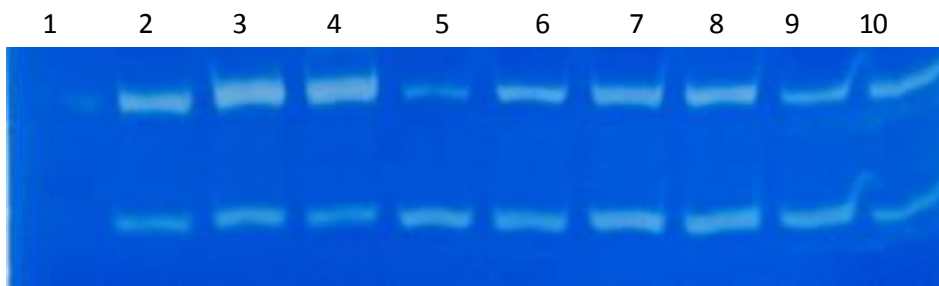


[Photo (3)] . Gelatin zymogram (Gel 2).

Gelatin zymogram of serum samples from clinically normal and laminitic horses: lane 1: BHK standard. lane (2- 4) clinically laminitic animals in the late stage, lane 5: clinically laminitic animals in the early stage .lanes (6- 10) clinically normal animals.

Channel	No	Group	Index	Name	QL	Area(pixel2)	QL-BG	B	BG	Std	Ratio(%)	QL/pixel2	(Q-B)/pixel2
1	1	~	1		55336.00	368.00	0.00	<input checked="" type="checkbox"/>	55336.00	<input type="radio"/>	-----	150.37	0.00
1	2	~	2		33096.00	312.00	-13819.30	<input type="checkbox"/>	46915.30	<input checked="" type="radio"/>	100.00	106.08	-44.29
1	3	~	3		34996.00	361.00	-19287.41	<input type="checkbox"/>	54283.41	<input type="radio"/>	105.74	96.94	-53.43
1	4	~	4		37852.00	384.00	-19889.91	<input type="checkbox"/>	57741.91	<input type="radio"/>	114.37	98.57	-51.80
1	5	~	5		29488.00	263.00	-10059.20	<input type="checkbox"/>	39547.20	<input type="radio"/>	89.10	112.12	-38.25
1	6	~	6		24677.00	242.00	-11712.43	<input type="checkbox"/>	36389.43	<input type="radio"/>	74.56	101.97	-48.40
1	7	~	7		36915.00	346.00	-15112.87	<input type="checkbox"/>	52027.87	<input type="radio"/>	111.54	106.69	-43.68
1	8	~	8		31304.00	307.00	-14859.46	<input type="checkbox"/>	46163.46	<input type="radio"/>	94.59	101.97	-48.40
1	9	~	9		38520.00	341.00	-12756.02	<input type="checkbox"/>	51276.02	<input type="radio"/>	116.39	112.96	-37.41
1	10	~	10		31029.00	291.00	-12728.54	<input type="checkbox"/>	43757.54	<input checked="" type="radio"/>	100.00	106.63	-43.74
1	11	~	11		38480.00	331.00	-11292.33	<input type="checkbox"/>	49772.33	<input type="radio"/>	124.01	116.25	-34.12
1	12	~	12		30070.00	285.00	-12785.33	<input type="checkbox"/>	42855.33	<input type="radio"/>	96.91	105.51	-44.86
1	13	~	13		30075.00	275.00	-11276.63	<input type="checkbox"/>	41351.63	<input type="radio"/>	96.93	109.36	-41.01
1	14	~	14		28544.00	272.00	-12356.52	<input type="checkbox"/>	40900.52	<input type="radio"/>	91.99	104.94	-45.43
1	15	~	15		29091.00	279.00	-12862.11	<input type="checkbox"/>	41953.11	<input type="radio"/>	93.75	104.27	-46.10
1	16	~	16		30347.00	298.00	-14463.13	<input type="checkbox"/>	44810.13	<input type="radio"/>	97.80	101.84	-48.53
1	17	~	17		34445.00	329.00	-15026.59	<input type="checkbox"/>	49471.59	<input type="radio"/>	111.01	104.70	-45.67
1	18	~	18		46625.00	421.00	-16680.59	<input type="checkbox"/>	63305.59	<input type="radio"/>	150.26	110.75	-39.62
1	19	~	19		40507.00	363.00	-14077.15	<input type="checkbox"/>	54584.15	<input type="radio"/>	130.55	111.59	-38.78

[Fig. (8)]. Denistometric analysis of SDS- PAGE zymography by photocapture microsoft program (gel 2).



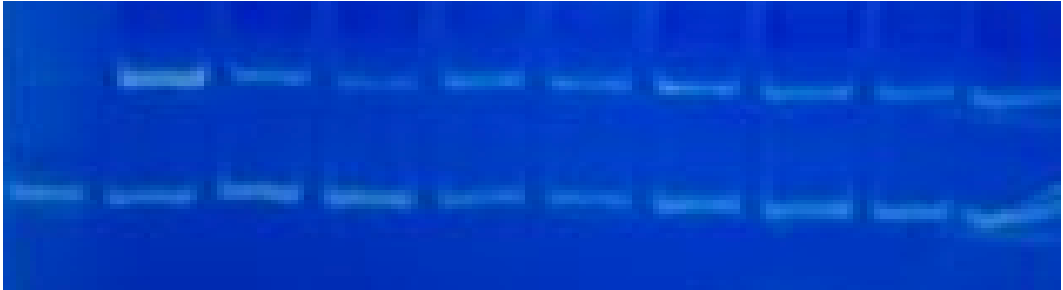
[Photo(4)] . Gelatin zymogram (Gel 3).

Gelatin zymogram of serum samples from clinically normal and laminitic horses. lane 1 : (BHK) standard. lanes (2- 5) clinically laminitic animals in the late stage, lane 6 is clinically laminitic animals in the early stage lane (6-10 are clinically normal animals).

Channel	No	Group	Index	Name	QL	Area(pixel2)	QL-BG	B	BG	Std	Ratio(%)	QL/pixel2	(Q-B)/pixel2
1	1	~	1		51630.00	378.00	0.00	<input checked="" type="checkbox"/>	51630...	<input type="checkbox"/>	-----	136.59	0.00
1	2	~	2		43184.00	438.00	-1664...	<input type="checkbox"/>	59825...	<input checked="" type="checkbox"/>	100.00	98.59	-37.99
1	3	~	3		47414.00	423.00	-1036...	<input type="checkbox"/>	57776...	<input type="checkbox"/>	109.80	112.09	-24.50
1	4	~	4		34312.00	338.00	-1185...	<input type="checkbox"/>	46166...	<input type="checkbox"/>	79.46	101.51	-35.07
1	5	~	5		28091.00	286.00	-1097...	<input type="checkbox"/>	39063...	<input type="checkbox"/>	65.05	98.22	-38.37
1	6	~	6		26785.00	234.00	-5176...	<input type="checkbox"/>	31961...	<input type="checkbox"/>	62.03	114.47	-22.12
1	7	~	7		45398.00	408.00	-1032...	<input type="checkbox"/>	55727...	<input type="checkbox"/>	105.13	111.27	-25.32
1	8	~	8		37687.00	366.00	-1230...	<input type="checkbox"/>	49990...	<input type="checkbox"/>	87.27	102.97	-33.62
1	9	~	9		33146.00	327.00	-1151...	<input type="checkbox"/>	44664...	<input type="checkbox"/>	76.76	101.36	-35.22
1	10	~	10		29001.00	289.00	-1047...	<input type="checkbox"/>	39473...	<input checked="" type="checkbox"/>	100.00	100.35	-36.24
1	11	~	11		45668.00	360.00	-3503...	<input type="checkbox"/>	49171...	<input type="checkbox"/>	157.47	126.86	-9.73
1	12	~	12		50375.00	406.00	-5079...	<input type="checkbox"/>	55454...	<input type="checkbox"/>	173.70	124.08	-12.51
1	13	~	13		37305.00	302.00	-3944...	<input type="checkbox"/>	41249...	<input type="checkbox"/>	128.63	123.53	-13.06
1	14	~	14		39652.00	315.00	-3373...	<input type="checkbox"/>	43025...	<input type="checkbox"/>	136.73	125.88	-10.71
1	15	~	15		34182.00	265.00	-2013...	<input type="checkbox"/>	36195...	<input type="checkbox"/>	117.86	128.99	-7.60
1	16	~	16		44269.00	342.00	-2443...	<input type="checkbox"/>	46712...	<input type="checkbox"/>	152.65	129.44	-7.15
1	17	~	17		36838.00	287.00	-2362...	<input type="checkbox"/>	39200...	<input type="checkbox"/>	127.02	128.36	-8.23
1	18	~	18		35565.00	268.00	-1040...	<input type="checkbox"/>	36605...	<input type="checkbox"/>	122.63	132.71	-3.88
1	19	~	19		29517.00	253.00	-5039...	<input type="checkbox"/>	34556...	<input type="checkbox"/>	101.78	116.67	-19.92

[Fig. (9)] . Denistometric analysis of SDS- PAGE zymography by photcapture microsoft program (gel 3).

1 2 3 4 5 6 7 8 9 10



[Photo(5)]. Gelatin zymogram (Gel 4).

Gelatin zymogram of serum samples from clinically normal and laminitic horses. lane 1 is BHK standard . lane (2- 4) are clinically laminitic animals in the late stage .lanes (5 - 10) are clinically normal animals.

Channel	No	Group	Index	Name	QL	Area(pixel2)	QL-BG	B	BG	Std	Ratio(%)	QL/pixel2	(Q-B)/pixel2
1	1	~	1		61732.00	400.00	0.00	<input checked="" type="checkbox"/>	61732.00	<input type="radio"/>	-----	154.33	0.00
1	2	~	2		37558.00	349.00	-16303.17	<input type="checkbox"/>	53861.17	<input checked="" type="radio"/>	100.00	107.62	-46.71
1	3	~	3		39297.00	402.00	-22743.66	<input type="checkbox"/>	62040.66	<input type="radio"/>	104.63	97.75	-56.58
1	4	~	4		22021.00	179.00	-5604.07	<input type="checkbox"/>	27625.07	<input type="radio"/>	58.63	123.02	-31.31
1	5	~	5		30037.00	241.00	-7156.53	<input type="checkbox"/>	37193.53	<input type="radio"/>	79.97	124.63	-29.70
1	6	~	6		41708.00	345.00	-11535.85	<input type="checkbox"/>	53243.85	<input type="radio"/>	111.05	120.89	-33.44
1	7	~	7		36232.00	308.00	-11301.64	<input type="checkbox"/>	47533.64	<input type="radio"/>	96.47	117.64	-36.69
1	8	~	8		35398.00	325.00	-14759.25	<input type="checkbox"/>	50157.25	<input type="radio"/>	94.25	108.92	-45.41
1	9	~	9		48274.00	407.00	-14538.31	<input type="checkbox"/>	62812.31	<input type="radio"/>	128.53	118.61	-35.72

1	10	~	10		45382.00	403.00	-16812.99	<input type="checkbox"/>	62194.99	<input checked="" type="radio"/>	100.00	112.61	-41.72
1	11	~	11		29262.00	228.00	-5925.24	<input type="checkbox"/>	35187.24	<input type="radio"/>	64.48	128.34	-25.99
1	12	~	12		30379.00	235.00	-5888.55	<input type="checkbox"/>	36267.55	<input type="radio"/>	66.94	129.27	-25.06
1	13	~	13		36577.00	295.00	-8950.35	<input type="checkbox"/>	45527.35	<input type="radio"/>	80.60	123.99	-30.34
1	14	~	14		31887.00	255.00	-7467.15	<input type="checkbox"/>	39354.15	<input type="radio"/>	70.26	125.05	-29.28
1	15	~	15		28711.00	213.00	-4161.29	<input type="checkbox"/>	32872.29	<input type="radio"/>	63.27	134.79	-19.54
1	16	~	16		45934.00	345.00	-7309.85	<input type="checkbox"/>	53243.85	<input type="radio"/>	101.22	133.14	-21.19
1	17	~	17		47529.00	338.00	-4634.54	<input type="checkbox"/>	52163.54	<input type="radio"/>	104.73	140.62	-13.71
1	18	~	18		48024.00	373.00	-9541.09	<input type="checkbox"/>	57565.09	<input type="radio"/>	105.82	128.75	-25.58
1	19	~	19		44159.00	340.00	-8313.20	<input type="checkbox"/>	52472.20	<input type="radio"/>	97.31	129.88	-24.45

[Fig. (10)]. Denitometric analysis_of SDS- PAGE zymography gel (4) by photocapture microsoft program.

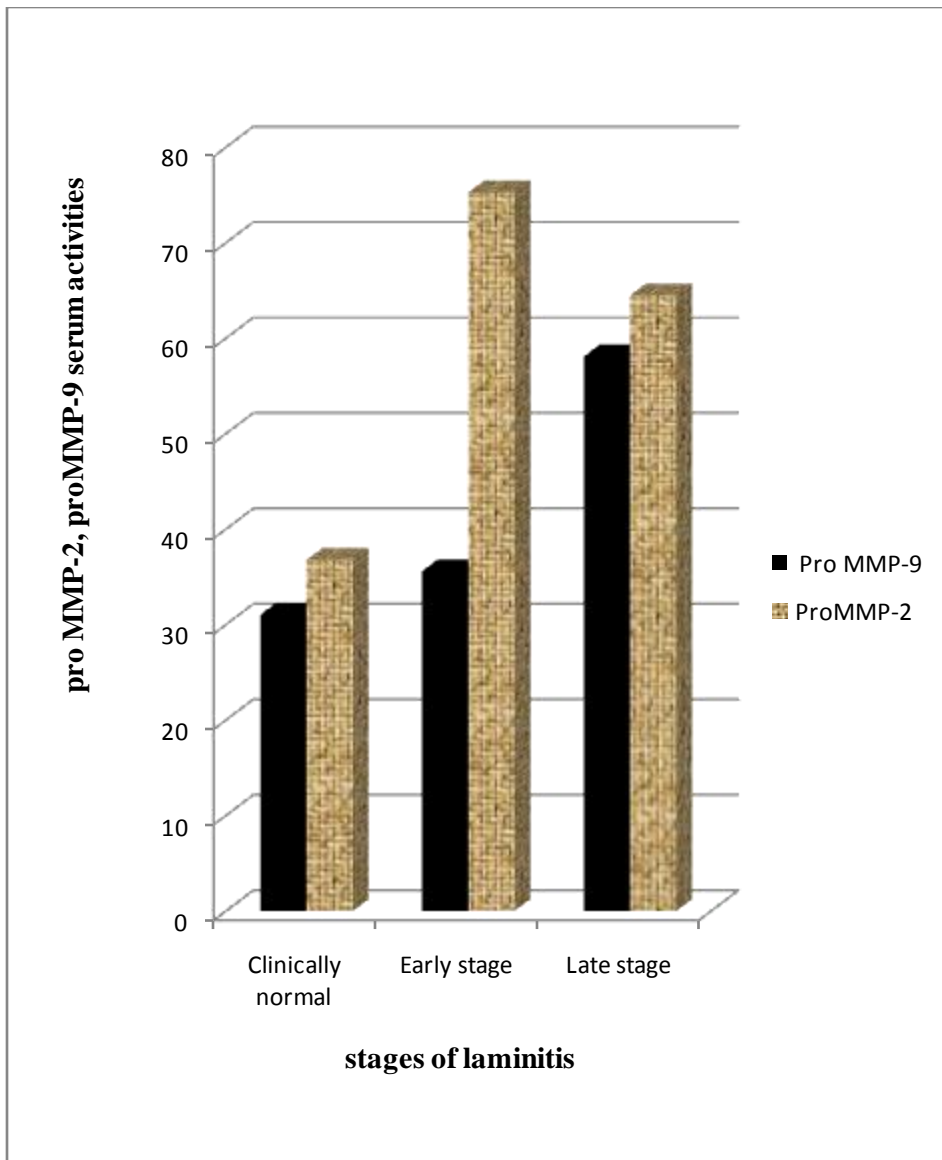
[Table (5)] . Represent serum matrix metalloproteinase-2 (MMP-2) and matrix metalloproteinase-9 (MMP-9) activities in the clinically normal and laminitic horses.

Mean±SE	Clinically normal	Clinically laminitic	
		Late stage	Early stage
parameters			
MMP-2 (72 KD)	36.8 ± 1.3 ^a	75.3 ± 2.0 ^b	64.5 ± 3.8 ^b
MMP-9 (92 KD)	31.05 ± 2.04 ^a	35.6 ± 0.2 ^a	58.09 ± 4.5 ^b

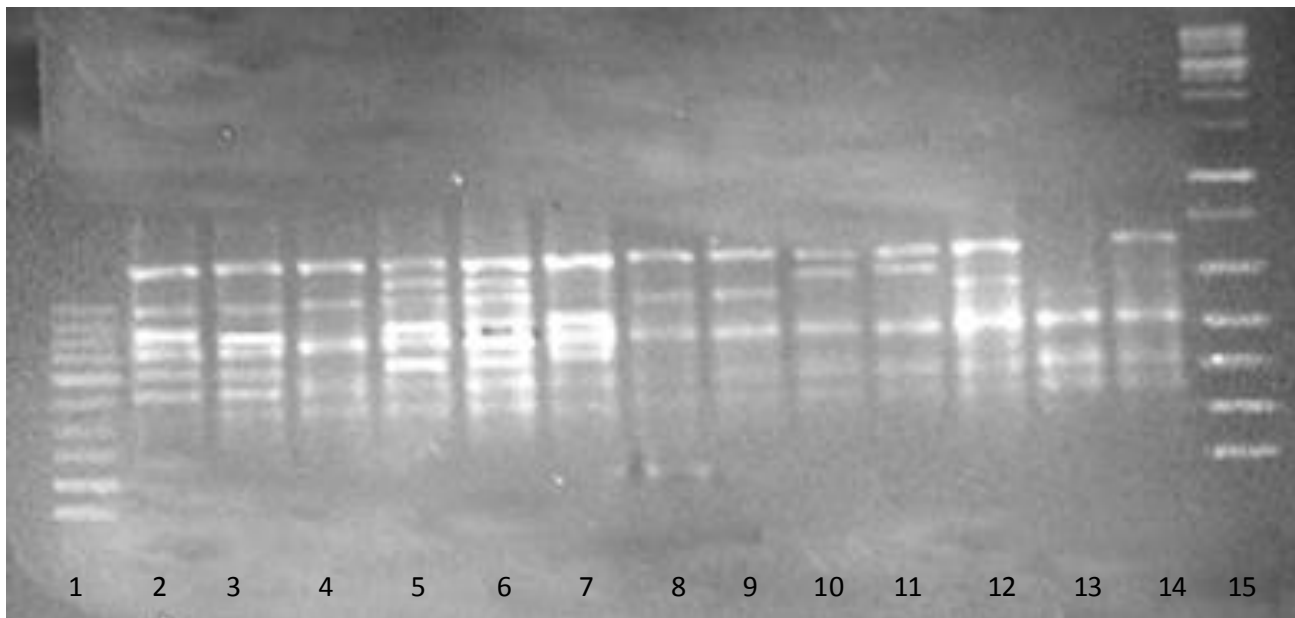
Data represented as mean value ± standard error (SE).

The values in the same raw having the different small letter are significantly different at P > 0.05.

The values in the same raw having the same small letter are non significantly different at P < 0.05.



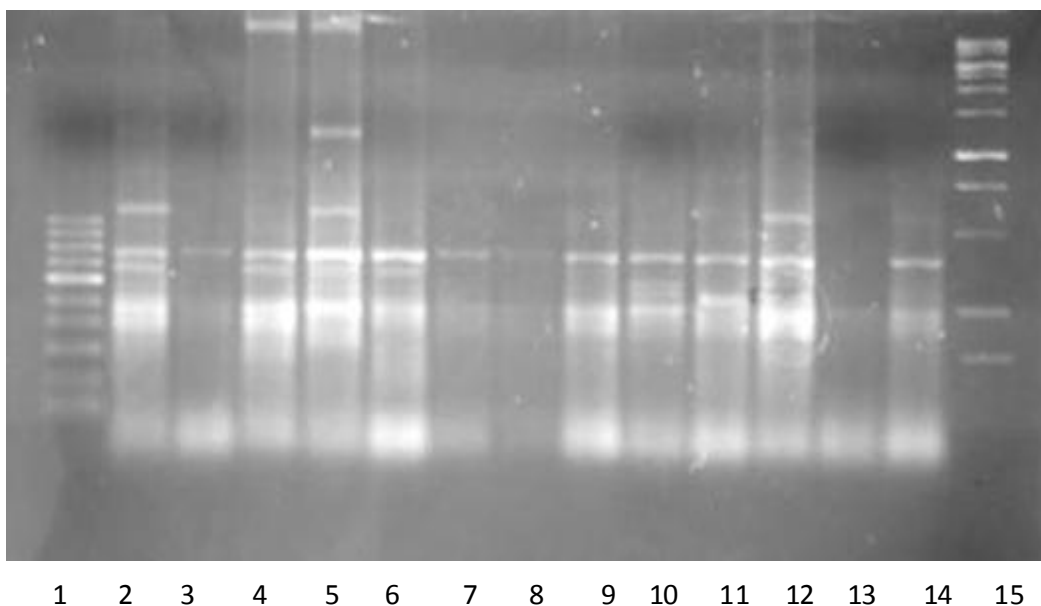
[Fig. (11)]. Represent serum matrix metalloproteinase-2 (MMP-2) and matrix metalloproteinase-9 (MMP-9) activities in the clinically normal and laminitic horses.



[Photo (6)] . Agarose gel electrophoresis for PCR products of ISSR technique by using dinucleotide primers "(GA) 9 C" .

ISSR-PCR profile obtained by using trinucleotid primer . the ISSR reaction products were electrophoresed on 1.5% TBE agarose gel stained with ethidium bromide. The first lane represents 50 bp DNA ladder .Lanes (2 -7) are clinically laminitic animals in the late stage, Lanes (8 – 11) are clinically laminitic animals in the early stage, lanes (12-14) are clinically normal animal . and the last lane represents 15 is 1 Kbp DNA ladder.

Dinucleotide primer "(GA) 9 C" produced 71 bands from which 13 bands are polymorphic (18.3%) and 58 bands are monomrphic, (81.6%)



[Photo (7)] . Agarose gel electrophoresis for PCR products of ISSR technique using trinucleotide primer “(GAG) 6 C”.

ISSR-PCR profile obtained by using trinucleotide primer . the ISSR reaction products were electrophoresed on 1.5% TBE agarose gel stained with ethidium bromide. The first lane represents 50 bp DNA ladder .Lanes (2 -7) are clinically laminitic animals in the late stage ,lanes (8 – 11) are clinically laminitic animals in the early stage, lanes (12-14) are clinically normal animal and the last lane represents 15 is 1 Kbp DNA ladder.

Trinucleotide “(GAG) 6 C” primer produced 52 bands from which 9 bands are polymorphic (15.4%) and 43 bands are monomorphic (84.6%) .