Original Research

Physiological, Hematological and Biochemical Indices in Non-Descript Indian Donkeys Working With Different Pack Loads in Brick Kiln Simulated Conditions

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Abstract

Physiological, hematological and biochemical changes were studied in adult non-descript indigenous donkeys (n=4) working with pack loads equivalent to 40% (T1), 50% (T2) and 66% (T3) of their live body weight in a fashion mimicking work at brick kilns. Pre work and post work physiological (RR, RT, PR), hematological (Hb, PCV, RBC, WBC) and biochemical indices (lactate and lactate dehydrogenase enzyme activity in plasma) were estimated. All physiological and hematological indices increased significantly (P<0.01) post work. Post work, plasma lactate levels and lactate dehydrogenase enzyme activities were slightly increased without any significant change in all treatments except for the increased lactate in T2. The donkeys got fatigued at 5, 5 and 4h of work in the three treatments, T1, T2, and T3 completing 9, 9 and 7.5km of distances, respectively working at speed 1.5-2km/h. It is concluded that indigenous donkeys may work comfortably with a pack load up to 50% of their body weight but higher pack load of 66% of their body weight causes early onset of fatigue and should be avoided.

Key words: Biochemical Indices, Fatigue, Indigenous Donkeys, Pack Load, Physiological Indices, Stress, Work Performance

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Introduction

Donkey is the most economical and efficient means of transport. The advantages are its effectiveness as a pack animal in the areas where roads are bad or non-existent, in the hilly and mountainous terrain and in muddy and marshy areas (Hassan and Ibitoye 1993; Pearson *et al.*, 1998). Donkeys are in use for carrying bricks at brick-kilns, transport of clothes by washermen as pack load in India. Donkey owners use them for livelihood without caring much for their health as they are often the poorest of the poor and illiterate (Pal *et al.,* 2002). Lack of knowledge amongst the donkey owners with regard to recognizing stress, pain, and fatigue signs combined with excessive greed for earning profits by overloading at the brick-kilns, often leads to development of multiple health problems as a consequence of their work requirements (Pritchard *et al.,* 2005). To avoid cruelty to donkeys and obtain optimum work output, they require being loaded optimally for an optimum duration. Only a few experimental studies (Pal *et al.,* 2002; Pal *et al.,* 2012; Olaifa *et al.,* 2012; Kumar *et al.,* 2016a; Kumar *et al.,* 2016b) are available with regard to work under pack load in donkeys and comprehensive information on changes in all the three indices (physiological, hematological and biochemical) is not available.

The experiment was conceptualized to study the performance characteristics in terms of the physiological, hematological and biochemical stress indices at three pack loads in the donkeys in a manner as used at brick kilns.

Materials and Methods

Four non-descript indigenous donkeys of 7-10 years of age weighing 110-142 kg were used for carrying pack load equivalent to 40% (T1), 50% (T2) and 66% (T3) of their live body weight for a maximum duration of 6h or until onset of fatigue, whichever was earlier. The experimental design was based on switch over of the three treatments in same donkeys with at least a month's gap in between the trials to remove the residual/adaptive effect. The donkeys walked @1.5-2 km/h on tar road in the morning hours with ambient temperature ranging between 8-26°C in T1; 26-41°C in T2; and 21-37°C in the T3.

Intermittent rest was given at every 500m distance during which the unloading and reloading of pack load was done as at brick-kilns. In T1 and T3, the animals worked for four consecutive days (total number of observations=16 in each) while in case of T2, the experiment of four consecutive days was repeated twice at a gap of one month within the season (total observations=32). Fatigue was assessed on the basis of fatigue symptoms such as coordination of legs, forward movement, frothing in mouth, excitement, tongue protrusion and unwillingness to continue operation after which they were given rest. They were fed as per the nutrient requirements for working donkeys (National Research Council, 2007) with concentrate feed containing oats 40%, gram 30%, wheat bran 27% and mineral mixture (2%) and table salt (1%) in addition to *ad lib* roughages of green fodder (oats/barley) and dry roughage [sewan grass (*Lasiurus scindicus*)/wheat straw] as available during the season. They had free access to drinking water except during the working period.

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Recording of Physiological Responses

Physiological reactions were recorded at 'pre work' condition in the morning and immediately after completion of work ('post work'). The rectal temperature (°F) was recorded by inserting a digital clinical thermometer. Respiration rate was recorded from the abdominal flank movements per minute. The pulse rate was recorded per min by feeling the pulse on palpation of the external maxillary artery.

Hematology and Biochemical Observations

Blood from jugular vein was collected aseptically with negligible pain to the animal without any disturbance just before the start of work ('pre work') and at 'post work' immediately after completion of work in sterile EDTA tubes. About 1.0ml of the whole blood was used for evaluating hematological parameters (hemoglobin, packed cell volume, red blood cell and white blood cell count) in automated blood cell counter (Ms4, Melet Schloesing Laboratories, France). Plasma was separated from the rest of the blood by centrifugation at 3000 rpm for 15 minutes. Lactate and lactate dehydrogenase enzyme were estimated spectrophotometrically at 340 nm as per the procedure detailed by the kit manufacturer (Spinreact, Spain).

Statistical Analysis

Mean and S.E.M. of pre and post-work parameters were compared by t-test in each treatment as per the methods detailed in Snedecor and Cochran (1967).

Results and Discussion

Physiological Responses

All physiological responses increased significantly ($P<0.01$) in the donkeys in all the three treatments (Table 1).

Table 1: Physiological responses during working with different pack loads in donkeys

Pack Load	**40% Body weight**		**50% Body Weight**		**66 % Body Weight**	
	Pre work	Post work	Pre work	Post work	Pre work	Post work
Rectal Temp (°F)	98.37 ±0.17	101.3±0.16**	98.32±0.10	100.17±0.08**	98.31±0.05	101.49±0.18**
Pulse Rate (min^{-1})	34.63±0.98	53.5±1.58**	37.72±1.18	59.16±1.25**	33±0.46	53.38±0.78**
Respiration Rate (min^{-1})	22.5±0.56	51.06±0.98**	28.19±1.08	51.81±1.32**	24.63±0.45	50±2.03**
Distance covered and Time of work	9km Fatigued in 5h (Fatigue initiated at 4th h)		9km Fatigued in 5h (Fatigue initiated at 4th h)		7.5km Fatigued in 4h (Fatigue initiated at 3rd h)	

***Values between pre and post work differ significantly (p<0.01)*

The donkeys experienced a moderate physiological stress while working with pack loads of 40% and 50% of body weight. Fatigue symptoms such as unwillingness to continue operation, un-coordination between

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hind and forelegs were moderate until having worked for 4h in T1 and T2; and for 3h in T3, but increased intensely at 5h in the donkeys in T1 and T2 and by 4h in T3 after which they were stopped from working. The donkeys walked at normal speed of 1.5-2km/h and a total of 9 km was completed in 5 hours by each animal in T1 and T2. However, the donkeys covered only 7.5 km in 4h and got fatigued, exhibiting unwillingness to work further in group T3. Similar changes in working donkeys have been reported earlier with increased pack load (Pal *et al.,* 2002; Pal *et al.,* 2012; Kumar *et al.,* 2016a, Kumar *et al.,* 2016b). While Kumar *et al.* (2016a) and Kumar *et al.* (2016b) reported higher speed in continuous work, the studies by Pal *et al*. (2002) and Pal *et al.* (2012) represented similar speed under continuous and work-rest cycle. Pal *et al.* (2012) reported continuous work for 4h and work rest cycle of 2.5h work -1h rest-2.5h work under two different pack loads of bricks (40% and 50% of body weight). Both studies established higher stress in the continuous mode and in the heavier load. Goe (1983) observed that donkeys can carry a load of 27-50% of their body weight which is similar to the optimum load observed in the present study.

The increased physiological responses after work indicated physiological adaptive mechanism not only to carry the specific loads but also to mitigate stress caused by work. The physical work increases muscular metabolism and causes increased heat production which increases the core body temperature. The increase in the muscular metabolism also demands higher oxygen requirement which is fulfilled by the increase in respiration and cardiac output. The initial increase in rectal temperature is also a requirement for activating the metabolic processes in the working muscles and other tissues in order to change the internal frictional resistance in muscles and enhance transport of oxygen to working muscles (Astrand and Rodhal, 1970, Pal *et al.,* 2002, Pal *et al.,* 2012). Stimulation of sympathetic nervous system resulting in an increase in catecholamine (adrenaline) level leads to increased heart and respiratory rate, ultimately increasing aerobic and anaerobic glycolysis (Geor and McCutcheon, 1980; Allaam *et al.,* 2014). Intermittent rest helps in gaining essential vitality so as to derive more output from the working animal (Pal *et al.,* 2002; Pal *et al.,* 2012). The increased blood flow as a result of physiological homeostatic mechanism along with the increased respiratory rate dissipates the heat produced in the animal system by increasing the sensible and evaporative heat loss through breath and sweat. The sweating (data not presented), increased after 5 km of work which also contributed to cooling. Pack load, speed and duration of work determine the overall intensity of work (Ram *et al.,* 2012). Physiological responses increased significantly at 10% and 20% draft load in working donkeys in loading car (Dagar *et al.,* 2015).

Hematological Responses

White blood cell counts, red blood cell counts, hemoglobin and hematocrit increased significantly 'post work' in all the three treatment groups (Table 2). Catecholamine release from adrenals increases the flux

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of erythrocytes from spleen into blood during exercise in equines (Hodgson and Rose, 1994; Piccione *et al.*, 2008; Allaam *et al.*, 2014).

Table 2: Hematological responses during working with different pack loads in donkeys

Pack Load Treatment	Hematological parameters	Hb (g/dl)	PCV (%)	RBC (millions/mm3)	WBC (x1000/mm3)
T1	Pre work	11.06±0.10	30.31±0.27	7.39±0.11	13.22±0.25
T1	Post work	12.65±0.19**	33.49±.043**	8.22±0.09**	15.21±0.19**
T2	Pre work	10.95±0.13	29.88±0.35	7.31±0.11	12.94±0.30
T2	Post work	12.53±0.20**	32.83±.041**	8.11±0.10**	14.96±0.27**
T3	Pre work	10.84±0.13	38.89±0.89	6.53±0.14	12.09±0.58
T3	Post work	12.56±0.59**	45.71±1.78**	7.48±0.22**	15±1.04**

***Values between pre and post work differ significantly (p<0.01); T1: pack load equal to 40% of live body weight, T2: pack load equal to 50% of live body weight; T3: pack load equal to 66% of live body weight*

A pack load of 50% of their body weight at a speed of 1.7m/s in the donkeys also caused similar changes in the donkeys (Olaifa *et al.*, 2012). Adaptation to the work regime could have also caused the change, in addition to the climatic variations, feed and breed differences. The pre work hematological responses studied during the morning hours were in agreement with the normal range observed in donkeys (Garba *et al.*, 2015; Laus *et al.*, 2015; Burden *et al.*, 2016). Additionally, these increases are also a result of hemo-concentration in the circulating blood. Post-exercise hematocrit and total circulating hemoglobin increase progressively as hemo-concentration occurs due to dehydration in terms of water loss in sweat in working and exercising equines (Allaam *et al.*, 2014). The cause of leukocytosis in the present study could be the influence of corticosteroids which are released from adrenals in response to stress in working and exercising equines (Allaam *et al.*, 2014).

Plasma Lactate and Lactate Dehydrgenase Activity

Plasma lactate was higher after work in all the three treatments but increased significantly 'post work' only in treatment T2 (Pack load 50% of body weight, Table 3). Lactate did not increase significantly in T1 and T3 because the speed of work was low when compared to speed of race horses (Lekeux *et al.*, 1991, Zobba *et al.*, 2011; Allaam *et al.*, 2014). Significant increase was observed only in T2 during work at high ambient temperature with a pack load of 50% of their body weight which is due to reversible catalytic conversion of pyruvate to lactate by LDH enzyme within a threshold of 4.0 mM/l (Lekeux *et al.*, 1991). Similar to our observations, Moolchandani and Sareen (2015) reported increased lactate after work in donkeys. Post work lactate dehydrogenase activity was non-significantly higher than the pre work activity (Table 3). The mean activity of LDH enzyme was well within the normal range as illustrated by Laus *et al.* (2015); Ljubodrag *et al.* (2015) in donkeys and Allaam *et al.* (2014); Assenza *et al.* (2016) and Aros *et al.* (2017) in horses.

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Table 3: LDH and lactate changes during working with different pack loads in donkeys

Parameters	Pre work	Post work
T1		
LDH (IU/l)	908±34.82	994±20.37
Lactate (mMl/l)	0.99±0.04	1.03±0.04
T2		
LDH (IU/l)	898±20.05	946±22.78
Lactate (mM/l)	0.92±0.03	1.20±0.06*
T3		
LDH (IU/l)	903±43.28	1036±50.87
Lactate (mM/l)	0.97±0.06	1.14±0.07

** Values between pre and post work differ significantly (p<0.05) LDH: Lactate dehydrogenase enzyme activity; T1: pack load equal to 40% of live body weight, T2: pack load equal to 50% of live body weight; T3: pack load equal to 66% of live body weight*

The slight increase in LDH activity in the working donkeys could be attributed to overt damage or transiently increased permeability of muscle fiber membranes in horses (Lekeux *et al.*, 1991; Zobba *et al.*, 2011; Allaam *et al.*, 2014, Assenza *et al.*, 2016). It could also be a general systemic response to work induced temperature and muscular activity (Zobba *et al.*, 2011).

Conclusion

Various pack loads induced significant increase in the physiological responses in donkeys within the physiological capacity of the donkeys at slow working speed of 1.5-2km/h. The hematological indices also increase significantly, but lactate and lactate dehydrogenase activity is not affected although there is a significant increase in lactate during working with a pack load of 50% of body weight. The higher load of 66% of body weight augments an early onset of fatigue than the lower loads, and hence should not be used in pack donkeys.

Conflict of Interest

The authors declare that they have no conflict of interest.

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