Effects of Nutrition and Draught on Reproductive and Adrenal Functions of Female Donkeys

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Abstract

Cyclic ovarian activity and plasma progesterone (P4) concentrations were assessed for 179 days in 5 (free grazing) and 6 (free grazing + high energy and protein-supplemented) normocyclic donkeys. In addition, plasma P4 and cortisol were measured in blood samples collected at 15 min intervals in the same donkeys on days 82-84 and 113-114 when made to carry a cartload weighing about three times their bodyweight for a period of 3h. Cyclic P4 concentrations were measured in samples collected three times weekly. Oestrous cycle lengths and duration of estrous were not influenced by plane of nutrition in both groups of donkeys. Plasma cortisol levels increased significantly ($P<0.05$) in non-supplemented donkeys during the draught period. Changes in plasma P4 levels during draught were influenced by the stage of oestrous cycle; a significant increase ($P<0.01$) being observed in donkeys in luteal phase and a decrease ($P<0.01$) in non-luteal phase. Plasma P4 levels associated with subsequent post-draught oestrous cycles were depressed ($P<0.05$) in both groups, decline being more prominent in donkeys stressed during non-luteal phase than those stressed during the luteal phase. These results demonstrate that draught does interfere with ovarian cyclic and endocrine functions of working female donkeys and this situation might be exacerbated by poor nutrition.

Key words: *Nutrition, draught, progesterone, cortisol, donkey.*

Introduction

Donkeys in many developing countries play an important role in the economies of rural and urban low-income groups. They mainly serve as pack animals but are also widely used in several related activities such as carting, ploughing, weeding and threshing (Ramachandran and Srinivas, 1991; Kumwenda and Mateyo, 1991; Fall et al., 1996). Donkeys are usually put to work before they are three years of age and sustain a productive working life for up to 15 years (Mohamed, 1991). Despite their important role in improving the economy of rural societies, their population worldwide has remained stagnant or has changed little (FAO, 1985, 1988). Although the causes of this stagnation are likely to be multifactorial, (Barrowman, 1991; Ramachandran, 1991) low reproductive performance resulting from poor nutrition probably plays a major role (Roberts, 1971). In Ethiopia for example Mohamed (1991) reported the foaling percentage ranging between 20-30% only, a rate that is considerable small compared to other domestic species.

Donkeys are exposed to work when they are about 2 years of age, the age at which they are expected to reach sexual maturity. When not properly nourished such an early exposure to work could reduce the duration of their working life and impair their long-term reproductive performance (Svendsen, 1991; Barrowman, 1991). Donkeys

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raised in the tropics depend mostly on natural pastures and legumes as the sole source of energy, protein, vitamins and minerals (Canacco, 1991). These feeds are usually of poor quality and their availability is often limited during the dry season. Usually, draught animals perform more efficiently if nutrition inputs are adequate to support their requirements (Mattewman et al., 1991) and poor quality feeding leads to failure to perform various body functions including reproductive and adrenal functions (Giri et al., 1990; Twerda, 1995. There are very few studies in Sub-Saharan countries which have reported on the nutritional requirements of the donkeys in general and on the interaction between feeding and draught stress on one hand and on reproductive performance on the other. Inferences are often made on the basis of studies done on horses in temperate climates. Since donkeys are used mainly as draught animals, it is possible that draught stress when compounded with poor plane of nutrition may impose serious interference with their natural reproductive and adrenal rhythms leading to reduced reproductive efficiency frequently observed in these animals in the tropics.

The present study was therefore conducted to assess the combined effects of nutrition and draught stress on the ovarian and adrenal functions of donkeys raised either under conditions similar to the traditional management system or when slight nutritional intervention is applied.

Materials and Methods

Study location

The study was carried out at Sokoine University of Agriculture (SUA) in Morogoro Region, Tanzania using two paddocks kindly provided by the University farm. Morogoro lies about 550m above sea level and experiences hot climate throughout the year with exception of few months (June - August). Rainfall pattern is essentially bimodal (Feb-May, Nov-Dec) and the average humidity is about 78%. Ambient temperature ranges between 20°C and 35°C. The paddocks used were composed of natural grasses and the commonest grasses were *Hyparhenia, Sporobolus* and *Cynodon* species. Scattered bushes of *Acacia* spp were also present.

Animals and their management

A total of 11 mature normocyclic donkeys were used in this study. Before animals were allotted to experimental groups, they were acclimatized to the experimental procedures for a period of 38 days. During this period they were ear-tagged and grazed freely in the paddocks during the day and at night, they were housed together in a well-ventilated animal house. The average grazing time per day was 8 hours (from 0800 - 1600 h) and at about 1300h in the afternoon, the animals were given drinking water. Donkeys were also examined for bacterial infections and sick animals treated with appropriate antibiotics. They were dewormed using Febendazole (Panacur^{R}, Coopers). Deworming was subsequently done at 6 weeks interval.

Activities performed on the animals during the acclimatization phase included handling, weighing, jugular blood sampling and rectal examinations. These activities helped to acclimatize the donkeys to subsequent experimental procedures. Animals were weighed once a week, in the morning before being taken to graze. In order to assess their ovarian function, rectal examination was done twice within the first 14 days of acclimatization period. Subsequently, they were all synchronized using Prostaglandin (Estrumat$^{(R)}$), 2 ml injected intramuscularly containing 263μg cloprostenol per ml and signs of oestrus were observed 24h later. The animals, which did not respond to the first synchronization, were given a second prostaglandin treatment after 48 hours of first treatment. After synchronization, all cycling animals were selected and bled every other day (5 ml in heparin) for a period of 21 days to provide plasma for baseline hormonal data before they were allotted to experimental treatments. Blood sample collection was always done between 0730 - 0800h before the animals were taken to graze with care taken to minimize excitement. Plasma obtained from the blood was stored at -20°C to wait for analyses of progesterone (P_4).

At the end of acclimatization period, animals were allotted to two groups on bases of their haematological and body weight values and grazed in separate paddocks adjacent to each other. The first group of 5 donkeys was subjected to traditional system of grazing (i.e. grazing without supplementation). The second group (6 donkeys) were grazed and additionally supplemented with concentrate (1.8 kg each) constituting of 17%

cotton seed cake and 83% wheat feed which contained 7.7% CP and 12MJ/kg DM ME. Supplementation continued throughout the study period, which lasted for 179 days. During this period, jugular blood samples were taken 3 times a week for the measurement of plasma P4. Donkeys were also weighed at weekly intervals and checked daily for a period of 3 h in the morning and afternoon for signs of heat (oestrus). The number of animals on heat and duration of oestrus were recorded and later confirmed by progesterone values.

At two occasions, on days 82-84 (week 12) and 113 -114 (week 16) of the experimental phase, animals in both groups were each made to pull a cart-load of sand bags weighing about 3 times of its body weight. Donkeys were made to pull the load in an open ground within the university premises for a period of 3 h covering about 7.8 km (or 2.6 km/h). During this period of draught, frequent blood samples were collected using indwelling cannular (i.d 0.86 mm, o.d. 1.27 mm, Portex Ltd, Hyme, Kent, U.K) fitted in the external jugular vein one hour before commencement of the work as previously described by Mutayoba et al. (1996). Five ml blood was collected in heparinized tubes at 15 minutes intervals for 30 minutes before and 3h during the working period. At each bleeding, 5 ml of sterile saline was injected back into the animal. The collected blood was centrifuged and plasma obtained was frozen at -20°C until needed for hormonal determination.

Pasture samples were collected once a month from both paddocks. This was done at five different areas of each paddock by cutting off forages at root-stem junction. The collected samples were fresh weighed, oven dried and then ground and stored for chemical analysis.

Plasma Cortisol Determination

Plasma cortisol concentration was determined in 50 µl plasma aliquots by ^{3}H-dextran - charcoal competitive Radioimmunoassay supplied by World Health Organization (WHO) Matched Reagent Programme and validated for use in donkey plasma according to Mutayoba and Gombe (1989). All immunoassay reagents were kindly provided by the Reproductive Biology Unit (RBU), Department of Animal Physiology, University of Nairobi, Kenya. The intraassay CV's was 4.1% (n=8) at 8.6 nmoll^{-1} and the interassay CVs were 8.1% (n=5) and 11.5% (n=5) at 25.7 and 14.6 nmoll^{-1}, respectively. The minimum limit of detection calculated from 2 standard deviation of maximum binding (Bo) was 1.30 nmoll^{-1}.

Plasma Progesterone Determination

Plasma progesterone (P_4) concentration in donkeys were measured by a solid phase ^{125}I-radioimmunoassay using Coat A-Count-P4-kit, supplied by the International Atomic Energy Agency (IAEA), Vienna, Austria and kindly provided by the Department of Veterinary Surgery and Therigenology, SUA. The intraassay CV's was 4.5% (n=8) at 6.9 nmoll^{-1} and the interassay CV for 8 assays was 12.3% at 12.3 nmoll^{-1}. The minimum limit of detection calculated from 2 standard deviation of BO was 1.20 nmoll^{-1}.

Statistical Analysis

The data were analyzed by the use of SAS computer statistical package using the General Linear Model (GLM). The statistical model included two main effects i.e. plane of nutrition and draught stress.

Results

Changes in body condition of supplemented and non-supplemented donkeys

Supplemented and non-supplemented donkeys showed similar changes in body weights and haemogram picture during the experimental phase. Within the first 2 weeks of the experimental period, body weight of supplemented donkeys increased by 0.4±0.7 kg (±SEM) above their baseline values of 143.5±2.3 kg, whereas, an increase of 0.4±1.3 kg was observed in non-supplemented donkeys above baseline weight of 139.7±3.0 kg. This increase was significant ($P<0.05$) within both groups. Thereafter, both groups recorded a decline in their body weights reaching a minimum -0.2 ±1.1 kg by week 6 before improving slightly during the last 8 weeks of the study period. Changes in haemogram parameters (Packed Cell Volume (PCV) and Haemoglobin (Hb) concentration) paralleled changes in body weights in both groups.

Reproductive patterns of donkeys

Oestrous cycles and duration of oestrus

Length of oestrous cycle and duration of oestrus in supplemented and non-supplemented donkeys over 8 cycles observed during the experimental period were similar in both groups (Table 1). Oestrous cycle length ranged between 16.0 ±1.1 days and 25.0 ±2.2 days (mean 21±2.0 days) and the duration of oestrus ranged between 5.0±0.3 days and 7.0±0.7 days (mean 6±0.7 days).

Table 1. Mean Oestrous Cycle Length (OCL) and Duration of oestrus (OL) of supplemented and non-supplemented donkeys

Oestrous cycles	*Supplemented (n=6) (Days)*		*Non-supplemented (n=5) (Days)*	
	OCL	*OL*	*OCL*	*OL*
1	*24±2.4*	*5±0.3*	*25±2.2*	*6±0.3*
2	*16±1.5*	*5±0.2*	*16±1.1*	*6±0.3*
3	*19±1.9*	*5±0.5*	*21±2.0*	*7±0.7*
4	*18±2.4*	*5±0.4*	*20±1.1*	*6±0.7*
5	*19±0.8*	*5±0.2*	*18±1.1*	*5±0.7*
6	*20±1.2*	*5±0.3*	*21±1.1*	*7±0.7*
7	*23±2.2*	*5±0.3*	*17±0.9*	*7±0.7*
8	*24±1.4*	*5±0.2*	*20±0.9*	*5±0.3*

Plasma progesterone associated with oestrous cycles

Plasma progesterone (P4) profiles in samples collected at two-day intervals during the experimental phase in 3 supplemented and 3 non-supplemented representative donkeys are depicted in Fig. 1. Changes in daily plasma P4 values correlated with the normal stages of oestrous cycle. During the first 3 - 4 cycles, which occurred before donkeys, were subjected to draught, plasma P4 values in all donkeys were low during estrus (up to 0.5 nmoll^{-1}) and high during the luteal phase (up to 37.5 nmoll^{-1}). However, the luteal P4 values were generally higher in supplemented than in non-supplemented donkeys.

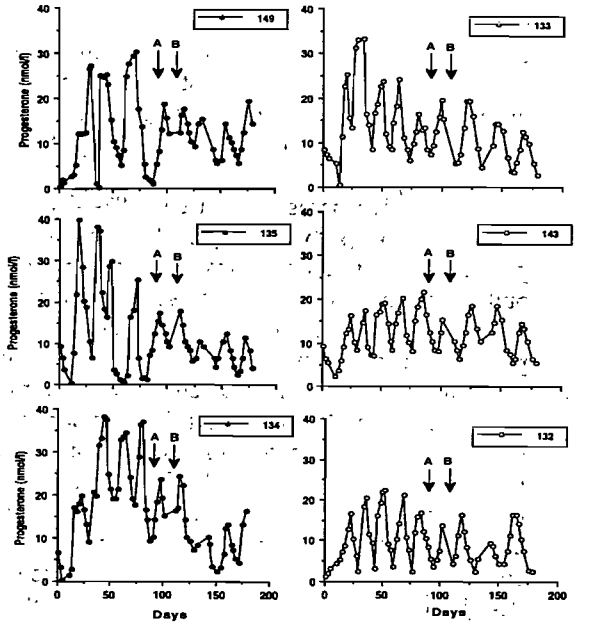

Figure 1: Cyclic changes in daily plasma P4 in supplemented (–•–) and non-supplemented (–o–) donkeys. A and B denote the days when donkeys were subjected to draught

During the period of draught, changes in plasma P4 values in both supplemented and non-supplemented donkeys were comparable and depended on the stage of oestrous cycle of individual animals. Hence, the plasma P4 values of both groups were analysed together and are presented in Figure 2.

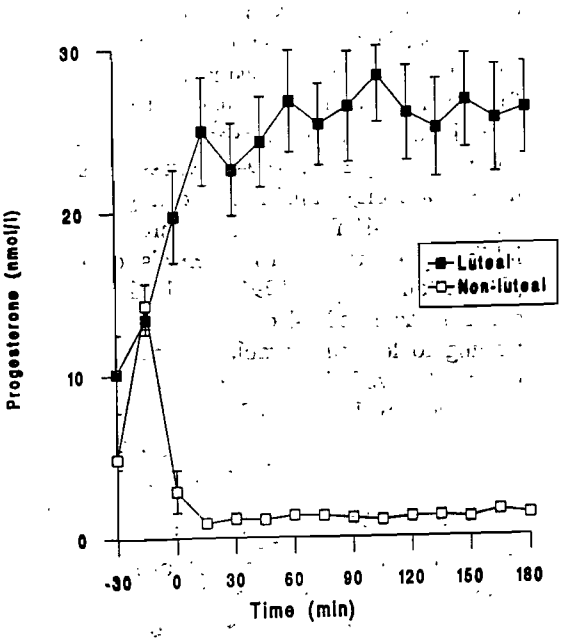

Figure 2: Changes in plasma P4 values in both supplemented and non-supplemented donkeys during draught

Donkeys in luteal phase showed a rapid increase in their mean plasma P4 values within 15 min of draught ($P<0.05$) to reach peak values ($P<0.01$) within 105 min when compared to values before draught. Plasma P4 values in donkeys in non-luteal phase however, showed a marked decline ($P<0.05$) within 15 min of draught and values remained low throughout the entire draught period. Consequently to draught stress (periods depicted as A and B in Figure 1), luteal plasma P4 profiles declined significantly ($P<0.01$) in supplemented donkeys (5 out of 6) when compared to P4 values observed before stress. A similar decline in luteal P4 values was observed only in one out of 4 non-supplemented donkey (No 133, Fig. 1).

Plasma cortisol levels during draught period

The mean baseline plasma cortisol levels in resting supplemented and non-supplemented donkeys in blood samples collected at 15 min interval for a period of 3 hr were 271.6± 33.1 $nmoll^{-1}$ and 272.8± 33.4 $nmoll^{-1}$, respectively. During draught (Fig 3) mean plasma cortisol levels increased rapidly ($P<0.05$) in non-supplemented donkeys within 45 min and remained significantly higher ($P<0.01$) from 135 min - 180 min of draught when compared to similar values of supplemented donkeys. Mean plasma cortisol values in supplemented donkeys did not change significantly from basal values throughout the entire draught period.

Discussion

The main objective of this study was to assess the influence of plane of nutrition and draught stress on the reproductive performance of female donkeys. Observations were made over a period of 5½ months. This period was expected to be adequate for showing any influence of either plane of nutrition or draught stress on reproductive cyclicity of the donkeys. The pastures on which all donkeys were grazed during the experiment were generally of poor quality providing an ideal situation that closely simulates the natural range conditions in periods shortly before the ploughing season in Morogoro. The non-supplemented donkeys were expected to exhibit obvious signs of under nutrition. It was further aimed that the supplement would provide about 22 MJ ME/day; a level that is adequate to meet the theoretical maintenance requirement for resting donkeys weighing 130 kg (Svendsen, 1986; Aboud et al., 1999). This amount is also adequate to provide some additional 6 MJ ME above maintenance.

Physical and nutritional stresses have been shown to alter the length of oestrous cycle or preventing the preovulatory release of LH with consequence of delayed ovulation. The two factors have also been implicated in shortening of estrus duration and even manifestation of silent heat (Mutayoba et al., 1988). The length of oestrous cycles and duration of estrus observed during the present studies where within ranges reported by others (Allen, 1969; Epstein, 1984; Oviedo, 1986; Henry et al. 1987). It was however, observed that there was no significant differences in the length of oestrous cycles as well as the duration of estrus between the supplemented and non-supplemented donkeys. This was probably associated with the gradual improvement of the quality of pastures, which was associated with the onset of the rainy season that started during the mid-period of the experimental phase. Plasma P4 levels in supplemented donkeys before being subjected to draught stress were generally higher

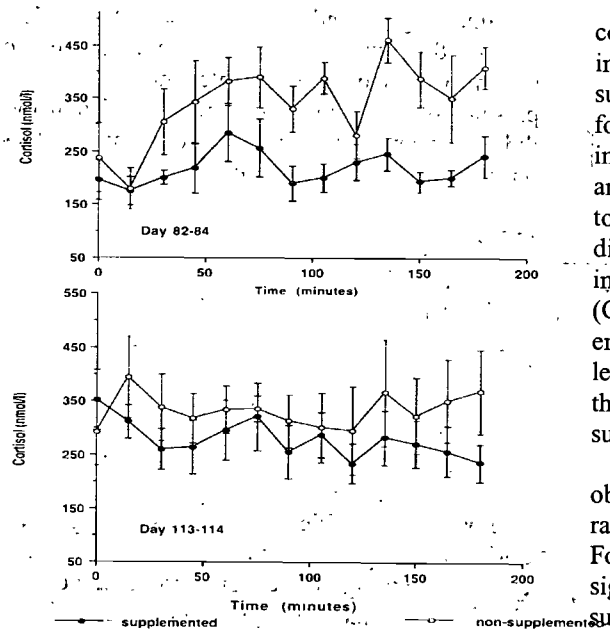

Figure 3: Changes in plasma cortisol levels in supplemented and non-supplemented donkeys during the draught period on days 82-84 and 113-114 of the experimental period

than those of non-supplemented donkeys. These values concur with observations by Hartley et al. (1994) that animals in high plane of nutrition have higher P4 values than animals in low plane of nutrition.

The marked changes in cyclic plasma P4 levels in both groups following draught is an indication that draught-induced stress might affect the synthesis and/or release of P4 from the corpus lutea of cycling donkeys. When donkeys were subjected to draught stress for a period of 3 h, a significant increase in plasma P4 levels was observed on donkeys in luteal phase of the cycle and a significant decline was observed in donkeys in non-luteal phase. These findings suggest that the responsiveness of the ovaries to stress in these animals depend on the stage of the oestrous cycle. Stress-mediated through the release of glucocortcoids is known to suppress pituitary gonadotrophin secretion (Naylor et al., 1990) and may also act directly on the corpus luteal LH receptors altering the secretion of P4 from the corpus luteal (Bambino and Tsueh, 1981). If this occurs during the mid-luteal phase, corticosteroids are known to act as direct inhibitors of luteal P4 synthesis and release which subsequently reduces plasma P4 levels essential for the maintenance of functional and structural integrity of CL during subsequent normal cycle in animals (Duffy et al., 1994). Stress is also known to decrease the available P4 to target organs by displacing P4 from plasma proteins without inhibiting ovarian steroidogenesis or ovulation (Chatterton et al., 1991). Indeed this would enhance increased P4 clearance from the plasma leading to low measurable plasma P4 levels as those observed in donkeys in this study in subsequent cycles which followed draught.

The baseline plasma cortisol levels observed in these donkeys were within normal range for resting donkeys (Mueller et al., 1995). Following a 3hr draught period there was a significant rise in plasma cortisol levels in non-supplemented donkeys but with minimal changes in supplemented animals. Stress of any nature is known to affect the hypothalamic-pituitary-adrenal (HPA) axis leading to an increase or decrease in cortisol secretion (Mutayoba et al., 1994; Hemsworth and Barnett, 1989). Mueller et al. (1995) reported that the levels might reach up to 400 $nmoll^{-1}$ in donkeys after a short period of draught and up to 570 $nmoll^{-1}$ during a prolonged draught stress. Increased in blood cortisol levels during the present study shows that draught imparts considerable stress to working donkeys which might interfere with the ovarian and/or hypothalamo-pituitary functions leading to altered in their reproductive performance. However, it was noted that the responsiveness of the HPA axis to draught stress depended on the nutritional status and body condition of the donkey. Unsuplemented donkeys in poor plane of nutrition seemed to be affected more by stress than those in good condition, probably as a physiological response to increased energy requirement during stress which in undernutritioned animals comes from cortisol-dependent increase in lipolysis and protein catabolism (Ladewig, 1989).

In summary, the present study shows that nutritional and draught stress alter the adrenal function of female donkeys and subsequently affects their reproductive function. These effects are more marked during the period of draught in undernourished donkeys.

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