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Serum Macromineral Levels in Estrual, Fertile, Subfertile and Pregnant Mares Kept Under Two Different Managemental Conditions

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ABSTRACT

This study was conducted on 300 mares kept under one of the two managemental conditions: field (individual management) and farm (organizational management). Mares were categorized as estrual, fertile, subfertile or pregnant. Any possible relationship between fertility and serum levels of sodium, magnesium, phosphorus, potassium and calcium was investigated. The serum sodium level differed significantly ($P<0.05$) among all groups of mares at both conditions, with pregnant mares having the highest and subfertile the lowest levels. Also, independent of the condition, the pregnant mares had significantly higher ($P<0.05$) serum potassium levels compared with subfertile ones. Serum calcium levels were significantly higher in estrual mares when compared with those of pregnant mares under farm management or subfertile mares under field conditions. In each group, mares kept under farm management had significantly higher serum magnesium levels but significantly lower serum calcium levels than those of mares kept under field. In estrual group, mares raised under field condition had significantly higher serum phosphorus levels. These results sufficiently provide the foundation for more rigorous and controlled studies to establish a firm basis for fertility versus serum-mineral-profile relationship. Moreover, due to marginally adequate serum mineral levels in mares kept under both managements, supplementation with mineral mixture was recommended for optimum fertility.

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INTRODUCTION

The equine population in Pakistan is estimated as 0.34 million heads (Anonymous, 2006). Horses are employed in a variety of activities, ranging from recreational ones, like riding, to those more economically driven, like load pulling and mule production. However, fertility problems of mares have been nagging the horse-breeders and stud-owners since long, and it appears that the fertility status of mares in Pakistan is comparatively lower than the world average. Sane *et al*. (1994) reported hormonal dysfunction, genetic disabilities, and managemental factors including the nutrition of brood mare to be the major causes of infertility in mares. Inadequate nutrition has been reported to impair reproduction in farm animals (Rutter and Randel, 1984; Schillo, 1992), delay onset of puberty in heifers (Day *et al*., 1986), induce anestrous in beef cows (Richards *et al*., 1989; Rhodes *et al*., 1996), and prolong postpartum anestrous in dairy cows (Selk *et al*., 1988; Randel, 1990).

It has been reported that the requirements of horses for calcium, phosphorus, and magnesium change in pregnancy owing to increased needs of these elements by the developing fetus (Huntington *et al*., 2005).). Ali *et al*. (2004) revealed higher calcium levels in estrual mares compared with infertile mares. Some studies have also linked the deficiency of various minerals with specific reproductive disorders. Magnesium deficiency delays the uterine involution (Larvor, 1983), and causes fetal loss and irregular estrus cycle in mares. The present study, in an effort to investigate the fundamental biochemical causes, seeks to relate fertility status of a mare with its profile of serum levels of various macrominerals, namely sodium, magnesium, phosphorus, potassium and calcium.

MATERIALS AND METHODS

The present study was conducted on 300 mares in Chenab Breeding Area of Punjab, Pakistan during 2005-2006. The mares were grouped according to their

reproductive status as: estrual, fertile, subfertile and pregnant. The category of each mare was determined according to its record history in conjunction with the findings of rectal examinations. Out of the total, 200 mares were chosen from one intensively managed government farm (farm condition) and 100 mares from studs owned by individual breeders (field condition). Twenty six mares (13 from each group) were excluded from the study as they were either sold by the farmers or moved to other farm. The mares kept at the farm were provided with feed and fodder according to the government approved protocol. On the other hand, the mares kept in the field condition were fed seasonal available fodder as per individual choice of the farmer. Water, however, was available *ad libitum* to the mares at both the managemental conditions.

From each mare, 20 ml of blood was drawn in a clean sterile test tube. The test tube was allowed to stand for approximately 3 hours in slanting position at room temperature for the serum to separate. The serum samples were stored at –20°C in a freezer in sterilized plastic labeled bottles. Later on, for biochemical macromineral analysis, these samples were thawed at room temperature and serum levels of sodium, magnesium, phosphorus, potassium and calcium were determined using commercial kits in chemistry analyzer (Map Lab Plus). For each mineral, duly calibrated procedures were adopted as specified by the respective kit manufacturer.

Due to two managemental conditions, four groups of mares, and five parameters under-study; the data was arranged in 2 x 4 x 5 categories. For each category, mean and standard deviation values were determined. For each macromineral, the data of various categories was compared by two-sample t-test.

RESULTS

The mean (± SD) serum macromineral levels are presented as 2 x 4 x 5 category matrix in Table 1. The serum sodium levels differed significantly ($P<0.05$) among all groups of mares at both farm and field conditions. Pregnant mares had the highest serum sodium levels and the subfertile mares had the lowest. In all groups, except pregnant one, serum sodium levels were higher for the mares kept under field management, except the farm pregnant mares, though the difference was significant ($P<0.05$) in estrual group only.

There was no significant difference in serum magnesium levels among the four groups at any managemental condition. The comparison between two managemental conditions, however, revealed that for each group the mares kept under farm management had significantly ($P<0.05$) higher serum magnesium levels than those of their counterparts kept under field management.

For serum phosphorus levels, again there was no significant difference among any pair of groups at farm or field condition. The comparison between farm and field showed that for estrual group the mares at field had significantly ($P<0.05$) higher serum phosphorus levels compared to farm mares.

In terms of serum potassium levels, pregnant mares at farm had significantly ($P<0.05$) higher values as compared to those of fertile and subfertile ones kept under the same managemental condition. At field, however, pregnant mares had significantly ($P<0.05$) higher serum potassium levels than those of subfertile mares only. The managemental condition seemed to have no effect on potassium levels in any group.

For mares kept at farm, the serum calcium levels were significantly ($P<0.05$) higher in the estrual group when compared with pregnant group. However, at field, the estrual mares had significantly ($P<0.05$) higher calcium levels than those of subfertile mares. The comparison between the two managemental conditions suggested that in each group the mares kept under field management had significantly ($P<0.05$) higher serum calcium levels than those of mares managed under farm condition.

DISCUSSION

The minerals of major concern regarding horse feeds are calcium, phosphorus, selenium, and sodium. Deficiencies or toxicities of other minerals may occur rarely if good quality feed with trace mineral salts is provided. Calcium and phosphorus collectively account for 70% of the body's mineral content. Approximately, 90% of calcium and 80% of phosphorus are present in bones and teeth. Therefore, calcium and phosphorus have top priority for proper bone development and maintenance. Also, the ratio of these two minerals is equally important.

In this study, for the mares kept under field management, the subfertile group had the lowest serum calcium levels compared with all other groups, but significantly so when compared with the mares who showed estrus activity. The serum calcium levels in the mares at the farm were even less than those reported earlier (Beaufort Lab, 2008). However, the calcium to phosphorus ratio in the mares, both at farm and field, remained within the normal limits. Subfertile mares in the field group in this study could benefit from calcium supplementation as hypocalcemia in cows has been associated with poor ovarian activity and infertility due to impaired blood flow to ovaries (Kamgaprour *et al*., 1999). The negative effect of calcium and phosphorus deficiency on reproduction in cattle has been reported earlier (Hurley and Doem, 1989).

Sodium makes the principal base of plasma and its function appears to be physiochemical in nature, where in it is responsible to maintain osmotic pressure and acid base balance. A decrease in serum sodium contents in physiological conditions like pregnancy or pathological conditions like pneumonia has been seen (Cornelius and Kaneko, 1963). This study, however, contradicts these reports, as pregnant mares had comparatively higher serum sodium levels. Snow *et al.* (1982) have reported average serum sodium levels of 132.76 m.mol/l, whereas Errington (1937) has reported the serum sodium levels of 161.8 m.mol/l. The value determined for all groups in this study are within the previously reported ranges.

Table 1: Mean (± SD) serum levels of macrominerals (mmol/l) in mares during different phases of reproductive cycle, kept under different managemental conditions

Phases of reproductive cycle	Sodium		Magnesium		Phosphorus		Potassium		Calcium	
	Field$^{\lozenge}$	Farm$^{\lozenge}$	Field$^{\square}$	Farm$^{\square}$	Field$^{\bullet}$	Farm$^{\bullet}$	Field$^{\blacksquare}$	Farm$^{\blacksquare}$	Field$^{\circ}$	Farm$^{\circ}$
Estrual	139.6^{Cb} ± 10.7 (10)	128.5^{Ca} ± 5.34 (10)	0.80^{Ab} ± 0.05 (10)	1.0^{Aa} ± 0.13 (10)	1.83^{Ab} ± 0.19 (10)	1.64^{Aa} ± 0.16 (10)	4.4^{ABa} ± 0.88 (10)	4.48^{ABa} ± 0.81 (10)	3.71^{Ab} ± 0.60 (10)	2.48^{Aa} ± 0.25 (10)
Fertile	152.2^{Ba} ± 11.34 (26)	149.1^{Ba} ± 19.62 (55)	0.81^{Ab} ± 0.01 (26)	0.92^{Aa} ± 0.18 (55)	1.69^{Aa} ± 0.14 (26)	1.65^{Aa} ± 0.18 (55)	4.3^{ABa} ± 0.55 (26)	4.38^{Ba} ± 0.64 (55)	3.53^{ABb} ± 0.53 (26)	2.18^{ABa} ± 0.51 (55)
Subfertile	118.2^{Da} ± 7.86 (28)	113.5^{Da} ± 14.81 (16)	0.80^{Ab} ± 0.04 (28)	0.98^{Aa} ± 0.16 (16)	1.69^{Aa} ± 0.15 (28)	1.65^{Aa} ± 0.15 (16)	4.0^{Ba} ± 0.68 (28)	4.39^{Ba} ± 0.81 (16)	3.17^{Bb} ± 0.46 (28)	2.35^{ABa} ± 0.35 (16)
Pregnant	161.3^{Aa} ± 9.09 (23)	163.3^{Aa} ± 12.59 (106)	0.81^{Ab} ± 0.06 (23)	0.99^{Aa} ± 0.14 (106)	1.67^{Aa} ± 0.20 (23)	1.60^{Aa} ± 0.20 (106)	4.7^{Aa} ± 0.72 (23)	5.0^{Aa} ± 0.12 (106)	3.27^{ABb} ± 0.52 (23)	2.14^{Ba} ± 0.41 (106)

Values with different superscripts in the same row (small letters) and in the same column (capital letters) for each mineral differ significantly ($P<0.05$).

Groups sharing similar symbols were compared, while values in parenthesis indicate number of mares in the particular group.

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This study did not reveal any deficiency of magnesium and potassium in the mares at any managemental condition. The serum levels of magnesium were higher in farm mares than the reported average values (Beaufort Lab, 2008). The decreased calcium levels in farm mares can be explained on the grounds that decreasing calcium levels result in increased magnesium levels to combat the phenomenon of loss of tonicity of muscles in general and those of uterus and vagina in particular (Blood and Henderson, 1974). Low serum magnesium levels have been reported (Hurley, 1971; Andrieux-Domont and Hung, 1973) to cause either complete sterility or considerable fetal malformation with resorption and abortion. Serum potassium levels were well within the reported range (Beaufort Lab, 2008), except for the pregnant mares under both managemental conditions which exceeded the higher range. Naheed (2004), however, reported different serum potassium levels in pregnant, fertile and subfertile mares, i.e., 5.19, 2.99 and 4.31 mmol/L, respectively.

Conclusion

It was concluded that overall fertility and pregnancy rate was higher in mares under farm management. Pregnant and subfertile mares, irrespective of the managemental condition, had the highest and the lowest serum sodium levels respectively. Though the mares did not appear to be deficient in minerals, generally the supplementation with mineral mixture supplement containing the minerals studied is likely to help achieve the optimum level of fertility in mares kept under both managemental conditions.

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