Changes in Serum Vitamin E and Trace Mineral Levels and Other Blood Parameters in Growing Thoroughbred Horses During the Period of Pasture Grazing and Stable Feeding

C. E. Lee*, N. K. Park*, S. H. Jin*, Y. J. Kim**, D. H. Kang**
and K. I. Kim***

National Jeju Agri. Exp. Station, RDA*, Korea Racing Association**, Cheju National University***

Thoroughbred 육성마의 방목과 사사기간 중 혈청 비타민 E, 미량광물질 및 기타 화학치의 변화

이종언* · 박남건* · 진신흠* · 김영진** · 강동희** · 김규일***

농진청 제주농업시험장*, 한국마사회**, 제주대학교***

ABSTRACT

Nutritional adequacy of growing Thoroughbred horses raised in an alternate feeding system - grazing during late spring through late fall and stable feeding for the rest of seasons - was assessed by determining vitamin E and trace mineral levels in the serum and blood chemistry related to nutrition and health. During the stable feeding in winter and early spring, 50 growing female horses were fed concentrates (1.4% of their body weight), grass hay (0.62%) and alfalfa hay (0.37%). For the grazing period, the same horses were fed supplementary concentrates (1.1%) during late spring through early summer, and concentrates (1.1%) and alfalfa hay (0.5%) during late summer through late fall. Blood samples were collected before grazing in early spring, and during grazing in early summer through late fall. Serum vitamin E, BUN, GTP, total bilirubin and direct bilirubin levels were increased ($P<0.01$) by grazing compared to those measured before the initiation of grazing. Horses had lower ($P<0.01$) serum Fe contents in early summer than in late fall or in time of stable feeding. Stable feeding increased ($P<0.01$) serum Cu content compared to grazing in both early summer and late fall. In late fall, serum Zn level increased ($P<0.01$) compared to that found in the other seasons. Blood glucose and creatinine levels decreased ($P<0.01$) after grazing. Results indicate that supplementations of some minerals and vitamin E are not always necessary in diets for growing horses and should be done after careful evaluation of diets with regard to concentrations and biological availability of minerals.

(**Key words** : Horses, Vitamin E, Trace minerals, Grazing, Stable feeding)

Ⅰ. INTRODUCTION

Most horses are fed hay, concentrates and supplementary vitamins or minerals in stable during the winter season, but during spring through fall, horses are kept outdoors for grazing or exercise on horse farms in Jeju, Korea. In many cases, horses receive concen-

Corresponding author : Lee, Chong-Eon, 1696, Odeung-Dong, Jeju 690-150, Korea. Phone : 064-741-2558
Fax : 064-742-0154 E-mail : leecc00@rda.go.kr

trates, hay or vitamin-mineral supplements even during pasture grazing. Many horse farmers and care takers tend to supplement diets with minerals and vitamins, without the knowledge of their adequacy.

Vitamin E has an important role in reducing free radicals and in increasing glutathione peroxidase activity, especially following exercise (Ji et al., 1990), and immune function (Baalsrud and Øvernes, 1986) in horses. The supply of dietary vitamin E depends on seasons and feed ingredients used and their quality because it is readily oxidized during the processing and storage. Mäenppää et al. (1988a) reported that serum levels of α-tocopherol markedly decreased in winter in both mares and foals, but increased rapidly after they were transferred to pasture.

Without the knowledge of the contents and biological availability of trace minerals in feeds used, concentrates are usually supplemented with them. When Fe, Mn, Zn and Cu were added to diets containing lower levels of these minerals, bone mineral deposition increased in yearlings (Ott and Asquith, 1989). Trace minerals in soil as well as in feeds may influence horses' mineral nutrition because horses consume soil during grazing, but their biological availabilities are not known.

Blood chemistry, such as blood urea nitrogen (BUN), GOT, GTP, billirubin, glucose, albumin or total protein, reflects general nutrition and health states of animals. BUN, an easily detectable metabolite, is synthesized in the liver, and in a higher rate when horses receive low-quality or excessive protein. Therefore, it can be an indicator of adequacy of protein ingested, as shown by Fonnesbeck and Symons (1969), who reported that BUN concentrations were much higher in horses fed alfalfa hay than in horses fed orchardgrass hay.

Our present study was done to evaluate the adequacy of nutrient intake by determining vitamin E and mineral levels in the serum, and blood chemistry in growing Thoroughbred horses raised in an alternate feeding system - grazing during spring through fall and stable feeding for the other seasons.

Ⅱ. MATERIALS AND METHODS

1. Animals and feeding systems

Fifty growing female Thoroughbred horses were fed in an alternate feeding system: stable feeding in winter through early spring and then pasture grazing in spring through late fall (Table 1). During winter and early spring, horses were

Table 1. The feeding systems of growing Thoroughbred horses[1)]

Item	Before grazing[2)] - Early April -	During grazing[2)] - Early June -	During grazing[2)] - Late October -
Average BW (kg)	321	355	401
Age (months)	11 - 14	13 - 16	17 - 21
Feeds supply (%BW)			
Concentrates	1.4	1.10	1.10
Grass hay	0.62	-	-
Alfalfa hay	0.37	-	0.50

[1)] Horses were kept individually during the stable feeding and grouped 6 or 7 horses each per paddock during the grazing period, and horses were individually fed supplemental feeds throughout the entire grazing period.

[2)] Blood samples were taken before, and 45 d and 6 mo after the start of grazing.

kept individually in stables, and fed concentrates (1.4% of their body weight) (Table 2), orchard-grass hay (0.62%) and alfalfa hay (0.37%) (Table 3). Horses were kept on pasture (mixture of orchard grass, perennial ryegrass and white clover) from the middle of April to the end of October. During the grazing period, horses were divided into groups of 6 or 7 horses each, were assigned to one of the paddocks (2,300~2,600 m^2) and individually fed supplementary concentrates (1.1%) during spring through early summer with enough green forages available. They were fed concentrates (1.1%) and alfalfa hay (0.5%) during late summer through late fall. Horses had free access to water.

Table 2. Composition of experimental concentrates (% as-fed basis)

Ingredient	%
Corn	32.1
Soybean meal	18.9
Wheat bran	9.4
Oat	37.7
Salt	0.95
Premix [1]	0.95
Total	100
Analyzed content	
CP (%)	16.9
Ca (%)	0.62
P (%)	0.59
Fe (mg/kg)	121.4
Cu (mg/kg)	9.7
Zn (mg/kg)	54.7

[1] Provided the following per kg of diet: Ca, 2.91 g; P, 0.8 g; Cu, 3.1 mg; Zn, 11.8 mg; Co, 0.076 mg; F, 0.038 mg; retinol, 2,346 IU; cholecalciferol, 950 IU; folic acid, 1.6 mg; inositol 0.75 mg; lactose, 19 mg.

2. Collection of blood, feed and soil samples

Blood samples were collected before horses entered the grazing period in early spring (April), during the grazing in early summer (June, 45 d after the start of grazing) and late fall (October, 6 mo after the start of grazing). Blood samples were taken into tubes without EDTA from the *vena cava* at 07:00 after a 14-h fasting period and centrifuged, and serum samples were collected and stored at −70℃ until analysis. Pasture grass samples were taken from two designated areas (1 m^2) per paddock before and during grazing in early summer and late fall, respectively, dried at 70℃ for 48 hr and ground. Pasture soil samples (top 5~15 cm) were taken from 10 spots per paddock before grazing, pooled and dried at room temperature.

3. Determination of trace mineral, α-tocopherol and blood chemistry

For total trace mineral assays in soil, diet and serum, samples were prepared according to the AOAC procedure (1996). For the extraction of soluble trace minerals in soil, 10 g soil sample was mixed with 50 ml 0.1N HCl, shaken at 30 ℃ for 1 hr and then filtered with no. 6 filter paper. Trace mineral concentrations in the filtrates were determined by an inductively coupled plasma atomic emission spectrometer (ICP-AES; SPECTRO Analytical Instruments, GmbH, Kleve, Germany).

Alpha-tocopherol contents in the serum were determined at 280 nm by the method developed by Liu et al. (1996) using HPLC (model no 616/626, Waters, Miliford, MA) equipped with a μPoracil column (3.9 × 300mm, 10 μm) and UV detector (model no 486, Waters). Blood chemistry was determined using an automatic chemistry analyzer (MARS, InfoPia Co., Ltd, Seoul, Korea) according to the manufacturer's instruction.

4. Statistical analysis

Data were subjected to analysis of variance

Table 3. Nutrients contents in forages and soil (dry matter basis)

Item	No.	CP (%)	NDF (%)	ADF (%)	Ca (%)	P (%)	Fe (ppm)	Cu (ppm)	Zn (ppm)
Alfalfa hay	10	18.3	49.4	34.0	1.36	0.21	197.8	9.4	28.2
Orchardgrass hay	6	11.4	73.2	42.4	0.23	0.18	120.2	6.6	32.5
Pasture grass									
Before grazing (Apr.)	16	17.1	49.1	27.1	0.41	0.33	122.1	5.0	27.1
During grazing (Jun.)	16	17.5	47.9	26.9	0.43	0.33	295.2	5.6	35.5
During grazing (Oct.)	16	17.9	50.0	26.9	0.42	0.35	514.0	7.5	41.6
Pasture soil									
Total contents	8	-	-	-	-	-	52233	45.7	144.7
Soluble contents	8	-	-	-	-	-	92.8	0.22	3.22

(ANOVA) on SAS package (SAS, 1988). The main source of variation for all variables was three seasons (or feeding systems) in ANOVA. Duncan's multiple range test was used to compare mean values of each feeding regime, when F-value was significant ($P<0.05$). A Pearson correlation analysis was done to calculate correlation coefficient (r) between blood parameters.

Ⅲ. RESULTS

Serum α-tocopherol content was increased ($P<0.01$) during grazing in early summer and late fall compared to that found before grazing. Horses that are on grazing had higher ($P<0.01$) α-tocopherol contents in early summer than in late fall (Table 4). Horses had lower ($P<0.01$) serum Fe contents in early summer than in late fall or in the time of stable feeding. Interestingly, serum Fe content was remarkably increased ($P<0.01$) in late fall compared to those found in the other seasons. Stable feeding increased ($P<0.01$) serum Cu content compared to grazing in both early summer and late fall. Serum Zn levels were highest ($P<0.01$) in late fall and followed by that in spring and summer in order. Serum urea nitrogen (BUN), GTP, total

Table 4. The effects of an alternate feeding system on serum vitemin E and trace mineral contents in growing Thoroughbred horses[1)]

Item	Before grazing[2)] - Early April -	During grazing[2)] - Early June -	During grazing[2)] - Late October -
Vitamin E (mg/L)	0.96 ± 0.03^{c}	1.35 ± 0.05^{a}	1.16 ± 0.03^{b}
Fe (μmol/L)	122.8 ± 6.8^{b}	83.9 ± 4.0^{c}	229.7 ± 6.8^{a}
Cu (μmol/L)	16.0 ± 0.8^{a}	12.7 ± 0.6^{b}	12.1 ± 0.5^{b}
Zn (μmol/L)	21.7 ± 1.2^{b}	14.3 ± 0.6^{c}	33.9 ± 1.2^{a}

[1)] Values are means ± SE of 50 growing horses.

[2)] Blood samples were taken before, and 45 d and 6 mo after the start of grazing.

[abc] Means in the same row not sharing the same superscripts differ ($P<0.01$).

bilirubin and direct bilirubin concentrations were increased (P<0.01) by grazing compared to those found in stable feeding. Stable feeding increased serum glucose and creatinine, when compared to grazing. Grazing horses in early summer had lower serum Ca contents than those in other seasons (Table 5). A Pearson correlation analysis showed a high correlation between serum total protein contents and albumin (r = 0.94), P (r = 0.82), Ca (r = 0.90), glucose (r = 0.81), urea (r = 0.65) or creatinine (r = 0.74) concentrations (Table 6), and between glucose and albumin (r = 0.78), P (r = 0.70), Ca (r = 0.80) or creatinine (r = 0.70) (Table 6).

Ⅳ. DISCUSSION

Our data demonstrated that serum α-tocopherol concentration was increased during grazing, indicating that green forages in summer season is a good source of vitamin E, as also shown by Mäenppää et al. (1988b). The decreased serum α-tocopherol contents in late fall, compared to those measured in early summer, appeared to be due to the lack of forages available on the pasture. In late fall, horses were fed a supplementary alfalfa hay (0.5% of their body weight) to compensate the decreased forage intake (Table 1).

The normal range of plasma α-tocopherol concentration for yearlings is 1~3 mg/L (Frape, 1998). The serum α-tocopherol concentration (0.96 mg/L) during the stable feeding in the present study appeared to be at the lower nutritional limit, suggesting that its supplementation is required for growing horses during the concentrates-hay feeding period. Similarly, Mäenppää et al. (1988) showed that the winter

Table 5. The effects of an alternate feeding system on blood chemistry in growing Thoroughbred horses[1)]

Item	Before grazing[2)] - Early April -	During grazing[2)] - Early June -	During grazing[2)] - Late October -
BUN (mg/dL)	25.2 ± 0.87^b	31.2 ± 1.4^a	34.2 ± 0.94^a
GOT (IU/L)	378.9 ± 13.4	407.8 ± 12.3	410.7 ± 11.7
GTP (IU/L)	7.0 ± 0.43^b	9.7 ± 0.65^a	10.1 ± 0.50^a
Total bilirubin (mg/dL)	0.35 ± 0.02^c	0.46 ± 0.03^b	0.64 ± 0.03^a
Direct bilirubin (mg/dL)	0.14 ± 0.007^c	0.18 ± 0.009^b	0.25 ± 0.011^a
Total protein (g/dL)	7.0 ± 0.25	6.4 ± 0.25	6.7 ± 0.14
Albumin (g/dL)	4.1 ± 0.10	3.9 ± 0.12	4.0 ± 0.06
Glucose (mg/dL)	120.2 ± 4.3^a	91.2 ± 3.2^c	105.1 ± 2.4^b
Creatinine (mg/dL)	1.4 ± 0.05^a	1.2 ± 0.04^b	1.2 ± 0.03^b
P (mg/dL)	6.7 ± 0.31^a	6.1 ± 0.24^{ab}	5.5 ± 0.14^b
Ca (mg/dL)	14.9 ± 0.55^a	12.9 ± 0.60^b	14.1 ± 0.33^{ab}

[1)] Values are means ± SE of 50 growing horses.

[2)] Blood samples were taken before, and 45 d and 6 mo after the start of grazing.

[abc] Means in the same row not sharing the same superscripts differ (P < 0.01).

Table 6. Correlation coefficient (r) between blood parameters

	GTP	Urea	T-Bil.	Glu.	D-Bil.	GOT	T-Prot	Alb.	P	Ca	Cre.	Fe	Cu	Zn
GTP	1.00													
	0.0													
Urea	0.47	1.00												
	0.001	0.0												
T-Bil	0.40	0.51	1.00											
	0.001	0.001	0.0											
Glu.	0.18	0.51	0.36	1.00										
	0.04	0.001	0.001	0.0										
D-Bil.	0.41	0.51	0.99	0.35	1.00									
	0.001	0.001	0.001	0.001	0.0									
GOT	0.45	0.49	0.26	0.41	0.26	1.00								
	0.001	0.001	0.003	0.001	0.003	0.0								
T-Prot.	0.44	0.65	0.52	0.81	0.52	0.47	1.00							
	0.001	0.001	0.001	0.001	0.001	0.001	0.0							
Alb.	0.43	0.64	0.51	0.78	0.51	0.49	0.94	1.00						
	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.0						
P	0.46	0.59	0.24	0.70	0.24	0.49	0.82	0.77	1.00					
	0.001	0.001	0.007	0.001	0.006	0.001	0.001	0.001	0.0					
Ca	0.39	0.63	0.49	0.80	0.49	0.42	0.90	0.91	0.77	1.00				
	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.0				
Cre.	0.26	0.46	0.38	0.70	0.38	0.25	0.74	0.70	0.66	0.73	1.00			
	0.004	0.001	0.001	0.001	0.001	0.005	0.001	0.001	0.001	0.001	0.0			
Fe	0.16	0.26	0.35	0.14	0.34	0.10	0.09	0.07	-0.03	0.12	-0.01	1.00		
	0.07	0.004	0.001	0.11	0.001	0.27	0.31	0.41	0.71	0.16	0.88	0.0		
Cu	0.14	0.10	-0.07	0.19	-0.06	0.10	0.15	0.08	0.19	0.14	0.11	0.07	1.00	
	0.12	0.26	0.43	0.03	0.50	0.24	0.09	0.37	0.03	0.12	0.22	0.42	0.0	
Zn	0.16	0.27	0.34	0.24	0.33	0.14	0.16	0.13	-0.005	0.20	0.03	0.74	0.25	1.00
	0.07	0.003	0.001	0.007	0.001	0.11	0.08	0.15	0.95	0.02	0.72	0.001	0.006	0.0

serum α-tocopherol level was doubled when horses were transferred to pasture in June. They indicated that about 20% of experimental mares had deficient levels of α-tocopherol (less than 1.2 mg/L) during the winter season.

Serum Fe levels were lower in early summer, but higher in late fall than in the stable feeding, mainly because Fe contents in forages on pasture were higher in late fall than in early spring. Much higher Fe contents in forages during grazing were mainly due to the contamination of soil by grazing (Table 3, also note higher total and lower soluble Fe levels in pasture soils), although the biological availability of Fe in forages or soil is questionable. Serum Fe levels seem to be increased by feeding alfalfa hay (containing 198 mg Fe/kg) because during grazing in early summer, horses did not receive alfalfa hay, and during the stable feeding and grazing in late fall received much higher alfalfa hay (0.5% and 0.37% of their body weight, respectively).

Serum Cu levels were higher in both early summer (12.7 μmol/L) and late fall (12.1 μmol/L) than before grazing (16.0 μmol/L), indicating that this difference was due to the intake of concentrates (1.1 and 1.1 vs 1.4% of their body weight, respectively), which contain a high Cu content (9.7 mg/kg). Suttle et al. (1996) reported that 16 μmol/L of serum Cu was found when horses were fed a diet containing 20 mg Cu/kg DM and proposed that serum level of 11.5 μmol/L is a marginal value for deficiency that is also shown with a liver concentration of 52.5 μmol/kg wet weight. In the current study, serum Cu levels shown during grazing is unlikely deficient, but supplementary concentrates fed during grazing should contain Cu. Thoroughbred horses under training showed a higher Zn (26 μmol/L) during stable feeding than during grazing (17 μmol/L) (Frape, 1998). Result of our present study also showed that serum Zn levels were markedly influenced by different feeding systems and feeding alfalfa hay, indicating that feeds as well as bioavailability are important factors affecting serum Zn levels. The lower serum Ca level during grazing in early summer is also probably because horses did not receive alfalfa hay, which has a higher Ca content (1.36% of DM).

BUN concentration varies with urea ingestion, liver damage and dietary protein quality and quantity. Fonnesbeck and Symons (1969) showed that BUN concentrations in horses fed alfalfa hay were much higher than in horses fed orchardgrass hay (12.1 vs 23.8 mg/100 mL). In the current study, grazing increased BUN, T-bilirubin and D-bilirubin compared to stable feeding, suggesting that horses consume more protein through young fresh forages during grazing (Table 3). This result suggests that dietary protein in concentrates can be lowered during grazing. In general, plasma glucose levels in horses are relatively stable by hormonal actions (Hintz et al., 1971). However, Baker et al. (1972) reported that when horses were fed diets containing sub-maintenance levels of protein, plasma glucose levels decreased, suggesting that feeding system or feeds may influence the serum glucose level. Our results showed that horses during stable feeding had a higher serum glucose, creatinine and P concentrations, maybe due to higher intake of starch or relatively low consumption of protein during the stable feeding. High correlation coefficients among some blood parameters (e.g., total protein, glucose, P, Ca, BUN and creatinine) indicate that these parameters together reflect nutritional status of horses.

In conclusion, serum vitamin E, BUN, GTP, T-bilirubin and D-bilirubin levels were higher during grazing than before the initiation of grazing. Horses had lower serum Fe contents in early summer than in stable or in late fall. Stable feeding increased serum Cu contents compared to grazing in both early summer and late fall. In late fall serum Zn level increased compared to that found in other seasons. Supplementary feeding of some minerals and vitamin E is not always necessary in growing Thoroughbred horses and should be done after careful evaluation of their contents in diets and their bioavailabilities.

V. 요 약

본 연구는 Thoroughbred 육성마에서 계절별 사양체계에 따른 혈청 비타민 E, 미량광물질 수준 및 혈액 화학치의 변화를 측정하기 위하여 수행되었다. 사사기간(이른 봄) 동안 50두의 육성마(암, 11~14개월령)에게 농후사료(체중의 1.4%), 오챠드그라스 건초(0.62%)와 알팔파 건초(0.37%)를 급여하였다. 방목기간 동안 봄부터 초여름까지는 농후사료를 체중의 1.1% 보충급여 하였고 이 후 가을철 방목종료까지는 농후사료 1.1%와 알팔파 건초 0.5%를 급여하였다. 혈액을 이른 봄(사사기), 이른 여름 및 늦가을(방목기) 3회에 걸쳐 채취하였다. 방목사양은 혈청 비타민 E, 혈중 요소(BUN), GOT, GTP, T-bilirubin 및 D-bilirubin 함량을 증가 시켰다 ($P<0.01$). 혈청 Fe 및 Zn 함량은 가을 방목사양에서가 다른 사양체계에서 보다 높게 ($P<0.01$) 나타났다. Cu는 방목기간에 비해 사사기간 동안에 높았다($P<0.01$). 혈중 glucose 및 creatinine 함량은 방목기간에 비해 사사기간에 높게 ($P<0.01$) 나타났다. 본 연구결과 말 혈청 비타민 E 및 미량광물질 함량과 각종 혈액 화학치는 사양체계 및 급여사료에 큰 차이가 있음을 보여

주었다. 따라서 비타민이나 미량광물질의 추가 공급은 계절별 사양체계 또는 급여되고 있는 사료내 함량 및 이용성 등을 고려하여 이루어져야 할 것으로 사료된다.

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