Vitamin B1 and Vitamin B2 Requirements for 12-Month-Old Yili Horses (Postprint)

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Abstract

This experiment aimed to investigate the effects of different vitamin B1 and vitamin B2 intake levels on blood and urine related indices in 12-month-old Yili horses, in order to determine the vitamin B1 and vitamin B2 requirements for this age group. Thirty-five male Yili horses aged 12 months ± 5 days with an average body weight of (245.28$±$18.36) kg were selected and randomly divided into 5 groups (7 horses per group): Trial Group I, Trial Group II, Trial Group III, Trial Group IV, and Trial Group V. All horses were fed the same basal diet supplemented with 0, 16, 32, 48, and 64 mg/(horse · d) of vitamin B1 and 0, 10, 20, 30, and 40 mg/(horse · d) of vitamin B2, respectively, for a 20-day feeding trial. The actual vitamin B1 intake levels for Trial Groups I, II, III, IV, and V were 19.00, 33.49, 48.27, 62.96, and 77.53 mg/(horse·d), respectively, while the actual vitamin B2 intake levels were 21.95, 31.68, 41.77, 51.53, and 61.26 mg/(horse · d), respectively. The results showed that with increasing actual vitamin B1 intake, plasma vitamin B1 content, erythrocyte transketolase activity (E-TKA), and urinary vitamin B1 excretion in Yili horses gradually increased, while the thiamine pyrophosphate (TPP) effect gradually decreased. Plasma vitamin B1 content in Trial Group I was significantly lower than that in Trial Group III ($P<0.05$) and extremely significantly lower than that in Trial Groups IV and V ($P<0.01$). E-TKA in Trial Group I was extremely significantly lower than that in Trial Groups III, IV, and V ($P<0.01$). The TPP effect in Trial Group I was significantly higher than that in Trial Group III ($P<0.05$) and extremely significantly higher than that in Trial Groups IV and V ($P<0.01$). Urinary vitamin B1 excretion in Trial Group I was extremely significantly lower than that in Trial Groups III, IV, and V ($P<0.01$). With increasing actual vitamin B2 intake, plasma vitamin B2 content in Yili horses showed fluctuating changes, erythrocyte glutathione reductase activation coefficient (E-GRAC) gradually decreased, and urinary vitamin B2 excretion gradually increased. Plasma vitamin B2 content in Trial Group I was significantly lower than that in Trial Groups III and

IV (P<0.05) and extremely significantly lower than that in Trial Groups II and V (P<0.01). E-GRAC in Trial Group I was extremely significantly higher than that in Trial Groups II, III, IV, and V (P<0.01). Urinary vitamin B2 excretion in Trial Group I was extremely significantly lower than that in Trial Groups III, IV, and V (P<0.01). Based on comprehensive evaluation of all indices, the vitamin B1 requirement for 12-month-old Yili horses was determined to be 48.27 mg/(horse · d), and the vitamin B2 requirement was 31.68 mg/(horse · d).

Full Text

Vitamin B1 and B2 Requirements of 12-Month-Old Yili Horses

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Abstract

This study aimed to investigate the effects of different vitamin B1 and B2 intakes on blood and urine related indices in 12-month-old Yili horses to determine their vitamin B1 and B2 requirements. Thirty-five healthy male Yili horses aged 12 months $±5days with an average body weight of (245.28±$18.36) kg were randomly divided into five groups (trial groups I, II, III, IV, and V) with seven horses per group. All horses received the same basal diet supplemented with 0, 16, 32, 48, and 64 mg/(horse · d) of vitamin B1 and 0, 10, 20, 30, and 40 mg/(horse · d) of vitamin B2, respectively, for a 20-day feeding trial. The practical intakes of vitamin B1 in trial groups I, II, III, IV, and V were 19.00, 33.49, 48.27, 62.96, and 77.53 mg/(horse · d), respectively, while the practical intakes of vitamin B2 were 21.95, 31.68, 41.77, 51.53, and 61.26 mg/(horse · d), respectively.

The results showed that with increasing practical vitamin B1 intake, plasma vitamin B1 content, erythrocyte transketolase activity (E-TKA), and urinary vitamin B1 output increased gradually, while the thiamine pyrophosphate effect (TPP-effect) decreased gradually. The plasma vitamin B1 content in trial group I was significantly lower than that in trial group III (P<0.05) and extremely significantly lower than that in trial groups IV and V (P<0.01). The E-TKA in trial group I was extremely significantly lower than that in trial groups III, IV, and V (P<0.01). The TPP-effect in trial group I was significantly higher than that in trial group III (P<0.05) and extremely significantly higher than that in trial groups IV and V (P<0.01). The urinary vitamin B1 output in trial group I was extremely significantly lower than that in trial groups III, IV, and V (P<0.01).

With increasing practical vitamin B2 intake, plasma vitamin B2 content showed

a fluctuating change, erythrocyte glutathione reductase activity coefficient (E-GRAC) decreased gradually, and urinary vitamin B2 output increased gradually. The plasma vitamin B2 content in trial group I was significantly lower than that in trial groups III and IV ($P<0.05$) and extremely significantly lower than that in trial groups II and V ($P<0.01$). The E-GRAC in trial group I was extremely significantly higher than that in trial groups II, III, IV, and V ($P<0.01$). The urinary vitamin B2 output in trial group I was extremely significantly lower than that in trial groups III, IV, and V ($P<0.01$). Based on comprehensive evaluation of all indices, the optimal requirements of vitamin B1 and vitamin B2 for 12-month-old Yili horses were determined to be 48.27 and 31.68 mg/(horse · d), respectively.

Keywords: Yili horses; vitamin B1; thiamine; vitamin B2; riboflavin; plasma; urine; requirement

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Introduction

Vitamins B1 and B2 are micronutrients essential for maintaining normal physiological functions in animals. Vitamin B1, also known as thiamine, exists primarily as thiamine monophosphate (TMP), thiamine pyrophosphate (TPP), and thiamine triphosphate (TTP) in animals. In its TPP form, vitamin B1 functions as a coenzyme regulating carbohydrate, fat, and protein metabolism. Vitamin B2, or riboflavin, exists mainly as flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD), which serve as cofactors for various oxidases and participate in hydrogen transfer during biological oxidation, promoting the metabolism of carbohydrates, proteins, fats, and nucleic acids.

Chronic vitamin B1 deficiency in horses leads to growth retardation, dull hair coat, diarrhea, lethargy, neurological disorders, and motor incoordination. Vitamin B2 deficiency causes blepharitis, loss of appetite, and weight reduction. The Yili horse is a renowned Chinese breed characterized by gentle temperament, robust constitution, stable genetic performance, and combined strength and speed capabilities. However, systematic scientific feeding methods for Yili horses remain underdeveloped, particularly regarding precise nutrient requirements, with no clear reference standards for appropriate vitamin supplementation levels. Therefore, this study investigated 12-month-old male Yili horses to determine their vitamin B1 and B2 requirements by evaluating the effects of different supplementation levels on plasma vitamin concentrations, erythrocyte transketolase activity (E-TKA), TPP effect, erythrocyte glutathione reductase activity coefficient (E-GRAC), and urinary vitamin excretion, providing a scientific basis for future Yili horse feeding practices.

1. Materials and Methods

1.1 Experimental Animals and Design Thirty-five 12-month-old male Yili horses ($\$\pm5 days) with an average body weight of (245.28±$18.36) kg were ran-

domly divided into five groups (trial groups I, II, III, IV, and V) with seven horses per group. Each horse received 2 kg of concentrate supplement daily with ad libitum access to roughage (alfalfa hay). Trial groups I, II, III, IV, and V received daily supplementation of 0, 16, 32, 48, and 64 mg of vitamin B1 (99.1% purity, from Jiangxi Tianxin Pharmaceutical Co., Ltd.) and 0, 10, 20, 30, and 40 mg of vitamin B2 (99% purity, from Guangji Pharmaceutical Co., Ltd.), respectively, for a 20-day feeding period.

1.2 Feeding Management All horses were housed individually with separate feeding stalls, provided ad libitum access to roughage and water. The concentrate supplement was divided into four equal portions for feeding. Required amounts of vitamins B1 and B2 for each horse were encapsulated and administered with the concentrate supplement. Daily feed intake was recorded and urine samples were collected from each horse on days 15-20 of the trial, with blood samples collected on day 20. The diet composition and nutrient levels are presented in Table 1 .

Table 1. Diet Composition and Nutrient Levels (Dry Matter Basis)

Item	Content	Nutrient Levels	Content
Corn		Organic Matter (OM)	
Wheat bran		Crude Protein (CP)	
Wheat middlings		Neutral Detergent Fiber (NDF)	
Limestone		Acid Detergent Fiber (ADF)	
Soybean meal		Ether Extract (EE)	
NaCl		Crude Fiber (CF)	
Premix		Nitrogen-Free Extract (NFE)	
Roughage		Digestible Energy (DE) (MJ/kg)	
Total		Calcium (Ca)	
		Vitamin B1 (mg/kg)	
		Vitamin B2 (mg/kg)	

The premix provided per kilogram of diet: VA 480 IU, VB6 48.96 mg, VD 70.4 IU, VE 21,333.36 IU, pantothenic acid 20.46 mg, nicotinamide 484.85 mg, Cu (as copper sulfate) 10.58 mg, Fe (as ferrous sulfate) 35.56 mg, Mn (as manganese sulfate) 33.54 mg, Zn (as zinc sulfate) 30.92 mg, I (as potassium iodide) 2.46 mg, Se (as sodium selenite) 5.93 mg, Co (as cobalt chloride) 1.11 mg.

1.3 Data and Sample Collection **1.3.1 Data Collection** Daily feed intake and urine volume were recorded for each horse on days 15-20 of the trial.

1.3.2 Sample Collection and Preservation

Feed Samples: On day 14, 1 kg samples of concentrate supplement and roughage were collected, air-dried, ground through a 40-mesh sieve, and stored for analysis.

Urine Samples: Total daily urine was collected from each horse on days 15-20, thoroughly mixed, and a 10% aliquot was taken as the test sample, recorded, and frozen at -20°C. The six daily urine samples from each horse were pooled and stored at -20°C in light-protected conditions for analysis.

Plasma Samples: On day 20, fasting blood samples were collected from the jugular vein using heparinized tubes, immediately centrifuged at 1,500×g for 15 minutes to separate plasma, and stored at -20°C in light-protected conditions.

Erythrocyte Samples: Fasting blood was collected in heparinized tubes, centrifuged at 1,500×g for 15 minutes, and the plasma layer was discarded. Ten milliliters of physiological saline was added, gently mixed with a plastic pipette, and centrifuged at 429×g for 10 minutes. The supernatant was discarded to obtain erythrocytes. This washing procedure was repeated twice, and the final erythrocyte pellet was stored at -20°C in light-protected conditions.

1.4 Laboratory Analyses 1.4.1 Vitamin B1 Determination

Plasma: Five hundred microliters of thawed and homogenized plasma was mixed with 100 L of 3 mol/L trichloroacetic acid, vortexed, and centrifuged at 4,286×g for 10 minutes. Three hundred microliters of supernatant was mixed with 900 L of water-saturated ether, vortexed, and centrifuged at 4,286×g for 5 minutes. Two hundred microliters of the lower layer was taken for derivatization.

Urine: Thawed urine was filtered through a syringe filter, diluted 100-fold, and 200 L was taken for derivatization.

Feed: One gram of feed sample was placed in a 50 mL brown volumetric flask with 35 mL of 0.1 mol/L HCl, sonicated for 3 minutes, and autoclaved at 121°C for 30 minutes. After cooling, mixed enzyme solution (3 g each of amylase and papain in 100 mL of 2 mol/L sodium acetate) was added and incubated at 37°C for 12 hours. The solution was cooled to room temperature, diluted to 50 mL, centrifuged at 4,286×g for 3 minutes, and 200 L of supernatant was taken for derivatization.

Derivatization: Forty microliters of 12 mmol/L alkaline potassium ferricyanide was added to the processed sample, vortexed for 15 seconds, then 8 L of 5 mol/L phosphoric acid was added to terminate the reaction.

Chromatographic Conditions: PRP-1 reversed-phase column (250 mm$\times 4.6mm, 10\mu m$); $mobile phase of 15mmol$ $tetrahydrofuran = 90:10(V/V)$; $column temperature 25°C$; $flow rate 0.5mL/min$; $isocratic elution. Detection$ $excitation wavelength(\ ex) = 365nm, emission wavelength(\ \$em) = 435$ nm. Injection volume: 10 L.

1.4.2 E-TKA and TPP Effect Determination

Following the method of Takeuchi et al., E-TKA was determined by colorimetry at 540 and 510 nm using 7-sedoheptulose content as the indicator. The TPP effect was calculated as the percentage difference between E-TKA measured with and without added TPP in vitro.

1.4.3 Vitamin B2 Determination

Plasma: Two hundred microliters of thawed and homogenized plasma was mixed with an equal volume of 15 mmol/L 10% magnesium acetate, vortexed, incubated at 65°C for 15 minutes, then 0.1 mL of 10% trichloroacetic acid was added. After centrifugation at 4,286×g for 10 minutes, the supernatant was collected and stored at 4°C in light-protected conditions for HPLC analysis.

Urine: Thawed urine was filtered, diluted 5-fold, and analyzed by HPLC.

Chromatographic Conditions: XB-C18 column (4.6 mm$×$100 mm, 5 μm); mobile phase of 10 mmol/L potassium phosphate buffer (pH 3.4):acetonitrile = 85:15 (V/V); column temperature 25°C; flow rate 1 mL/min; isocratic elution. Detection: λex = 445 nm, λem = 530 nm. Injection volume: 10 μL.

1.4.4 E-GRAC Determination Determined according to the method of Li Lihua.

1.5 Statistical Analysis Results are expressed as mean±SD. Data were analyzed using one-way ANOVA with SPSS 18.0 software, and multiple comparisons among groups were performed using Duncan' s method.

2. Results

2.1 Effects of Different Vitamin B1 Intakes on Plasma Vitamin B1 Content, E-TKA, TPP Effect, and Urinary Vitamin B1 Output As shown in Table 2 , plasma vitamin B1 content in 12-month-old Yili horses increased gradually with increasing practical vitamin B1 intake, reaching the lowest value in trial group I (6.18 μg/L), which was significantly lower than trial group III ($P<0.05$) and extremely significantly lower than trial groups IV and V ($P<0.01$). When practical vitamin B1 intake reached or exceeded 33.49 mg/d (trial group II), plasma vitamin B1 content reached a higher plateau (7.04-8.12 μg/L) with no significant differences among trial groups II, III, IV, and V ($P>0.05$). Based on plasma vitamin B1 content, the vitamin B1 requirement for 12-month-old Yili horses was determined to be 33.49 mg/(horse · d).

Erythrocyte transketolase activity (E-TKA) also increased gradually with vitamin B1 intake, with trial group I showing the lowest activity (10.20 U/mL erythrocytes), which was extremely significantly lower than trial groups III, IV, and V ($P<0.01$). When practical vitamin B1 intake reached or exceeded 48.27 mg/d (trial group III), E-TKA reached a stable higher level (17.43-21.29 U/mL erythrocytes) with no significant differences among groups III, IV, and V ($P>0.05$). Based on E-TKA, the vitamin B1 requirement was determined to be 48.27 mg/(horse · d).

The TPP effect decreased gradually with increasing vitamin B1 intake, with trial group I showing the highest value (53.83%), significantly higher than trial group III ($P<0.05$) and extremely significantly higher than trial groups IV and V ($P<0.01$). When practical vitamin B1 intake reached or exceeded 48.27

mg/d (trial group III), the TPP effect reached a lower plateau (6.62%-30.05%). Based on TPP effect, the vitamin B1 requirement was determined to be 48.27 mg/(horse · d).

Urinary vitamin B1 output increased gradually with vitamin B1 intake, with trial group I showing the lowest output (2.03 mg/d), extremely significantly lower than trial groups III, IV, and V (P<0.01). When practical vitamin B1 intake reached or exceeded 48.27 mg/d (trial group III), urinary output reached a higher plateau (6.41-7.34 mg/d). Based on urinary vitamin B1 output, the vitamin B1 requirement was determined to be 48.27 mg/(horse · d).

Table 2. Effects of Different Vitamin B1 Intakes on Plasma Vitamin B1 Content, E-TKA, TPP-Effect, and Urinary Vitamin B1 Output in 12-Month-Old Yili Horses

Item	Trial Group I	Trial Group II	Trial Group III	Trial Group IV	Trial Group V
Vitamin B1 supplementation [mg/(horse· d)]	0	16	32	48	64
Dry matter intake (DMI) (kg/d)	9.22$±0.87|9.04±0.89|9.10±0.78|9.10±0.37|8.99±0.42||*Practicalvitamin B1intake[mg/(horse· d)]*|19.00±1.08|33.49±1.10|48.27±0.97|62.96±0.46|77.53±0.52||*Plasmavitamin B1content(g/L)*|6.18±0 *TKA(U/mLerythrocytes)*|10.20±2.76*C*|12.13±3.83*CBb*|17.43±5.66*ABa*|20.81±2.33*Aa*|21.29±3.2*Aa*|| *effect*(±13.63*Aa*|41.87±18.06*Aab*|30.05±16.53*ABbc*|12.25±11.9*Bcd*|6.62±6.11*Bd*||*Urinaryvitamin B1*				

Values in the same row with different small letter superscripts indicate significant difference (P<0.05), different capital letter superscripts indicate extremely significant difference (P<0.01), and same or no superscripts indicate no significant difference (P>0.05). The same notation applies to subsequent tables.

2.2 Effects of Different Vitamin B2 Intakes on Plasma Vitamin B2 Content, E-GRAC, and Urinary Vitamin B2 Output As shown in Table 3 , plasma vitamin B2 content in 12-month-old Yili horses showed fluctuating changes with increasing practical vitamin B2 intake, with trial group I showing the lowest content (11.79 μg/L), significantly lower than trial groups III and IV (P<0.05) and extremely significantly lower than trial groups II and V

(P<0.01). When practical vitamin B2 intake reached or exceeded 31.68 mg/d (trial group II), plasma vitamin B2 content reached a stable higher level (14.12-15.19 μg/L) with no significant differences among trial groups II, III, IV, and V (P>0.05). Based on plasma vitamin B2 content, the vitamin B2 requirement was determined to be 31.68 mg/(horse · d).

The erythrocyte glutathione reductase activity coefficient (E-GRAC) decreased gradually with increasing vitamin B2 intake, with trial group I showing the highest value (1.03), extremely significantly higher than trial groups II, III, IV, and V (P<0.01). When practical vitamin B2 intake reached or exceeded 31.68 mg/d (trial group II), E-GRAC reached a lower plateau (0.68-0.72) with no significant differences among groups II, III, IV, and V (P>0.05). Based on E-GRAC, the vitamin B2 requirement was determined to be 31.68 mg/(horse · d).

Urinary vitamin B2 output increased gradually with vitamin B2 intake, with trial group I showing the lowest output (3.6 mg/d), extremely significantly lower than trial groups III, IV, and V (P<0.01). When practical vitamin B2 intake reached or exceeded 31.68 mg/d (trial group II), urinary output reached a higher plateau (5.23-7.10 mg/d) with no significant differences among groups III, IV, and V (P>0.05). Based on urinary vitamin B2 output, the vitamin B2 requirement was determined to be 31.68 mg/(horse · d).

Table 3. Effects of Different Vitamin B2 Intakes on Plasma Vitamin B2 Content, E-GRAC, and Urinary Vitamin B2 Output in 12-Month-Old Yili Horses

Item	Trial Group I	Trial Group II	Trial Group III	Trial Group IV	Trial Group V
Vitamin B2 supplementation [mg/(horse· d)]	0	10	20	30	40
DMI (kg/d)	9.22$±0.87|9.04±0.89|9.1±0.78|9.1±0.37|8.99±0.42||*Practicalvitamin B2intake*[*mg*/(*horse*·				
	d)]|21.95±1.37|31.68±1.39|41.77±1.23|51.53±0.48|61.26±0.12||*Plasmavitamin B2content*(*g*/*L*)|11.79±				
	GRAC|1.03±0.30*Aa*|0.72±0.22*Bb*|0.72±0.05*Bb*|0.69±0.10*Bb*|0.68±0.04*Bb*||*Urinaryvitamin B2output*(				

3. Discussion

3.1 Effects of Different Vitamin B1 Intakes on Plasma Vitamin B1 Content, E-TKA, TPP Effect, and Urinary Vitamin B1 Output Vi-

tamin B1 is essential for maintaining normal carbohydrate metabolism and ensuring proper nervous and digestive system function, playing a critical role in animal growth, health, development, reproduction, and immunity. Clinical manifestations of vitamin B1 deficiency in equids include loss of appetite, reduced heart rate, diarrhea, anorexia, bradycardia, muscle flaccidity and twitching, hyperesthesia, ataxia, and convulsions. Common indicators for assessing vitamin B1 nutritional status include plasma vitamin B1 content, E-TKA, TPP effect, and urinary vitamin B1 output.

Tallaksen et al. determined a vitamin B1 requirement of 52.50 mg/d for 30 patients with alcoholic liver disease based on plasma vitamin B1 content. Reinken et al. evaluated thiamine status in children aged 1 month to 14 years using E-TKA and TPP effect. Ziporin et al. found that urinary thiamine content increased rapidly with high vitamin B1 intake and determined a requirement of 0.81 mg/d for eight healthy young men based on urinary excretion when intake ranged from 0.6-2.0 mg/d.

In this study, plasma vitamin B1 concentrations in the five groups were 6.18, 7.04, 7.59, 8.39, and 8.12 μg/L, all within the reported range of 5.05-23.8 μg/L for horses. Plasma vitamin B1 content increased initially then plateaued with increasing intake, yielding a requirement of 48.27 mg/(horse · d) for 12-month-old Yili horses.

Transketolase (TK) in erythrocytes uses TPP, a vitamin B1 derivative, as its coenzyme. Vitamin B1 adequacy directly affects erythrocyte TK activity, making E-TKA a valuable indicator of vitamin B1 status. The TPP effect, measuring the difference in E-TKA with and without added TPP, is a widely used assessment tool, where greater activity increase indicates more severe deficiency. In this study, increased vitamin B1 intake elevated E-TKA and reduced TPP effect, confirming a requirement of 48.27 mg/(horse · d).

Dietary vitamin B1 absorbed in the small intestine is primarily excreted via the kidneys, with excess amounts filtered by glomeruli and eliminated in urine. Based on urinary vitamin B1 output, the requirement for 12-month-old Yili horses was determined to be 48.27 mg/(horse · d).

3.2 Effects of Different Vitamin B2 Intakes on Plasma Vitamin B2 Content, E-GRAC, and Urinary Vitamin B2 Output Chronic vitamin B2 deficiency impairs basal metabolism, causing growth retardation, rough hair coat, and seborrheic dermatitis. McDowell reported that vitamin B2 deficiency in pre-ruminant calves and lambs manifests as oral mucosal hyperemia, angular stomatitis, salivation, lacrimation, anorexia, diarrhea, and poor growth. Sensitive indicators for assessing vitamin B2 status include plasma and erythrocyte concentrations of riboflavin, FAD, and FMN, E-GRAC, and urinary vitamin B2 output.

Hustad et al. determined a 16 mg/d riboflavin requirement for riboflavin-deficient elderly individuals based on plasma vitamin B2 content. Chen et

al. reported a 6.4 mg/d requirement for broiler chickens based on E-GRAC. Bates et al. found that African lactating women required an additional 2.0 mg/d based on E-GRAC. Guo et al. determined a 1.8 mg/d requirement for heavily working military personnel based on urinary vitamin B2 output, while Lo reported a 1.5-2.0 mg/d requirement for youths aged 1-19 years.

Plasma vitamin B2 content directly reflects nutritional status. In this study, plasma vitamin B2 showed fluctuating changes with increasing intake, yielding a requirement of 31.68 mg/(horse · d) for 12-month-old Yili horses.

Glutathione reductase (GR) in erythrocytes can be activated by FAD supplementation. The ratio of GR activity with added FAD to basal activity constitutes the E-GRAC. In this study, E-GRAC decreased with increasing vitamin B2 intake, indicating a requirement of 31.68 mg/(horse · d).

Urinary vitamin B2 output is commonly assessed through 4-hour load tests or 24-hour excretion measurements. Using 24-hour urinary vitamin B2 output, the requirement for 12-month-old Yili horses was determined to be 31.68 mg/(horse · d).

3.3 Vitamin B1 and B2 Requirements of Yili Horses Under the conditions of this study, comprehensive evaluation of plasma vitamin B1 content, E-TKA, TPP effect, and urinary vitamin B1 output indicated a vitamin B1 requirement of 48.27 mg/(horse · d) for 12-month-old Yili horses. Similarly, evaluation of plasma vitamin B2 content, E-GRAC, and urinary vitamin B2 output indicated a vitamin B2 requirement of 31.68 mg/(horse · d).

These values are higher than NRC (2007) standards, which recommend 19.30 mg/(horse·d) of vitamin B1 and 12.80 mg/(horse·d) of vitamin B2 for 12-month-old horses with a mature weight of 400 kg, or 24.10 mg/(horse · d) of vitamin B1 and 16.1 mg/(horse · d) of vitamin B2 for horses with a mature weight of 500 kg. This discrepancy may be attributed to higher energy and protein intakes in this study. Li Lihua reported that increased dietary carbohydrate significantly raises vitamin B1 requirements, while Ferraris et al. found that high carbohydrate and protein diets increase vitamin B2 requirements. In this study, 12-month-old Yili horses consumed 970.0 g/d of crude protein (CP), 3,670 g/d of nitrogen-free extract (NFE), and 114.68 MJ of digestible energy (DE), compared to NRC (2007) recommendations of 677 g/d CP and 62.76 MJ DE for 400 kg mature weight horses, or 846 g/d CP and 78.66 MJ DE for 500 kg mature weight horses. The high-energy, high-protein diet likely increased vitamin B1 and B2 requirements, explaining the higher values obtained in this study compared to NRC (2007) standards.

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Note: Figure translations are in progress. See original paper for figures.