Effects of Different Dietary Fiber and Protein Levels on Nutrient Digestion, Metabolism, and Plasma Biochemical Parameters in 12-Month-Old Yanqi Horses (Postprint)

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Abstract

This experiment aimed to investigate the effects of diets with different fiber and protein levels on nutrient digestion and metabolism, as well as plasma biochemical indices, in 12-month-old Yanqi horses, to provide a theoretical basis for scientific feeding of Yanqi horses. Twelve 12-month-old Yanqi male horses with an average body weight of (191±20) kg were selected and randomly divided into 2 groups of 6 horses each; using a split-plot design, period 1 consisted of experimental groups I and II, and period 2 consisted of experimental groups III and IV. Each group was fed diets with different fiber and protein levels (the dietary fiber and protein levels for each group were 76.59% and 6.81%, 74.21% and 7.88%, 71.82% and 8.94%, and 69.41% and 10.01%, respectively), and a 21-day digestion and metabolism trial was conducted, including a 15-day preliminary period and a 6-day formal collection period. The results showed that the intake of dry matter, organic matter, crude protein, calcium, and phosphorus in Yanqi horses exhibited an upward trend with increasing dietary protein level, while the intake of neutral detergent fiber and acid detergent fiber showed a downward trend. The digestibility of dry matter, organic matter, crude protein, calcium, and phosphorus (except for group I) as well as digestible energy and metabolizable energy in Yanqi horses increased with increasing dietary protein level, among which the calcium and phosphorus digestibility in groups II, III, and IV increased by 10.78% ($P>0.05$), 19.48% ($P>0.05$), and 7.46% ($P>0.05$), and 10.44% ($P>0.05$), 7.60% ($P>0.05$) compared with group I, respectively. The metabolizable energy of groups III and IV was significantly higher than that of groups I and II ($P<0.05$). Regarding nitrogen, calcium, and phosphorus metabolism, the retention rates of calcium and phosphorus in Yanqi horses also showed an increasing trend, but increasing dietary protein level had no significant effect on nitrogen retention rate ($P>0.05$). There were no signifi-

cant differences in plasma total protein, albumin, globulin, urea nitrogen, and glutamine contents among groups (P>0.05). It was concluded that increasing the protein level in Yanqi horse diets could increase nutrient intake and improve nutrient digestibility and retention, with the optimum being a dietary fiber level of 69.41% and protein level of 10.01%, but had no significant effect on plasma biochemical indices.

Full Text

Effects of Different Dietary Fiber and Protein Levels on Nutrient Digestion and Metabolism, and Plasma Biochemical Indices of 12-Month-Old Yanqi Horses

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Abstract

This study investigated the effects of different dietary fiber and protein levels on nutrient digestion, metabolism, and plasma biochemical indices in 12-month-old Yanqi horses to provide a theoretical basis for their scientific feeding. Twelve healthy Yanqi male horses aged 12 months with an average body weight of (191±20) kg were randomly divided into two groups of six horses each. Using a staged grouping design, trial groups I and II were arranged in period 1, while trial groups III and IV were arranged in period 2. The four groups were fed diets with varying fiber and protein levels (76.59% fiber and 6.81% protein, 74.21% and 7.88%, 71.82% and 8.94%, and 69.41% and 10.01%, respectively). A 21-day digestion and metabolism trial was conducted, comprising a 15-day adaptation period and a 6-day collection period. The results showed that the intakes of dry matter (DM), organic matter (OM), crude protein (CP), calcium (Ca), and phosphorus (P) increased with rising dietary protein levels, while neutral detergent fiber (NDF) and acid detergent fiber (ADF) intakes decreased. The digestibility of DM, OM, CP, Ca, and P (except for trial group I), as well as digestible energy (DE) and metabolizable energy (ME), increased with dietary protein level. Specifically, Ca digestibility in groups II, III, and IV increased by 10.78% (P>0.05), 19.48% (P>0.05), and 7.46% (P>0.05) compared to group I, respectively, while P digestibility in groups II and IV increased by 10.44% (P>0.05) and 7.60% (P>0.05), respectively. The ME in groups III and IV was significantly higher than in groups I and II (P<0.05). Regarding nitrogen, calcium, and phosphorus metabolism, the retention rates of Ca and P showed increasing trends, though increasing dietary protein level had no significant effect on nitrogen retention rate (P>0.05). No significant differences were observed among

groups in plasma total protein, albumin, globulin, urea nitrogen, or glutamine content (P>0.05). In conclusion, increasing dietary protein level enhanced nutrient intake, digestibility, and retention, with optimal levels at 69.41% fiber and 10.01% protein, though it did not significantly affect plasma biochemical indices.

Keywords: diet; Yanqi horse; fiber; protein; digestion and metabolism; plasma biochemical indices

Introduction

Balanced dietary nutrition forms the foundation of animal production. Inappropriate dietary fiber and protein levels or improper roughage feeding can compromise animal health, reduce nutrient utilization efficiency, and even cause disease or mortality. Therefore, appropriate dietary protein and fiber levels are essential for scientific feeding, and the rational use of roughage is crucial for improving metabolic processes and production performance.

The Yanqi horse is an excellent breed of endurance horse in Xinjiang, primarily distributed in Hejing and Heshuo counties of the Bayinguoleng Mongolian Autonomous Prefecture. Characterized by a robust constitution, symmetrical structure, and strong bones, it is a typical riding-draft dual-purpose horse with excellent work endurance, riding stamina, speed, and adaptability to coarse feeding. Yanqi horses reach maturity at approximately 3 years of age, with 12 months being a critical period for physical growth that significantly influences future athletic and reproductive performance. Consequently, scientific feeding management is vital for their growth and production performance. However, research on the nutritional requirements of Yanqi horses is scarce, and no nutritional standards have been established for this breed.

This study utilized 12-month-old Yanqi male horses as experimental subjects. Based on the NRC (2007) nutrient requirements for horses, four diets with different fiber and protein levels were formulated. Using digestion and metabolism trials, we measured the digestibility and metabolic rates of major dietary nutrients in horses and established mathematical relationships between nutrient intake and retention, providing a basis for the scientific feeding of 12-month-old Yanqi horses.

1.1 Experimental Time and Location

The experiment was conducted from July to September 2013 at the Baoqi Yanqi Horse Stud Farm in Bayinguoleng Mongolian Autonomous Prefecture, Xinjiang.

1.2 Experimental Animals

Twelve 12-month-old Yanqi male horses with an average body weight of (191±20) kg were selected and randomly divided into two groups of six horses each.

1.3 Experimental Design

A staged grouping method was employed. The experiment consisted of two periods: period 1 included trial groups I and II, while period 2 included trial groups III and IV. Each group received diets with different fiber and protein levels (76.59% fiber and 6.81% protein, 74.21% and 7.88%, 71.82% and 8.94%, and 69.41% and 10.01%, respectively), with pelleted concentrate as the concentrate feed and oat straw as the roughage . Each period consisted of a 21-day digestion and metabolism trial, including a 15-day adaptation period and a 6-day collection period. A 9-day dietary transition period was implemented between periods 1 and 2. Horses were weighed after overnight fasting before the adaptation period. During the collection period, all feces and urine were collected every 2 hours from each horse. At the end of the trial, horses were weighed after overnight fasting, and blood samples were collected via venipuncture.

1.4 Diet and Management

All horses were housed under identical environmental conditions throughout the trial, with individual feeding stalls and stables. Based on feeding behavior, the daily ration was divided into five meals (at 08:00, 12:30, 17:00, 21:00, and 00:00), fed concentrate first followed by roughage in small, frequent portions to ensure complete consumption. Water was provided ad libitum. Diet composition and nutrient levels are presented in Table 2 .

The premix provided per kilogram of diet: VA 480 IU, VB 816.32 mg, VB 333.2 mg, VB 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.

1.5 Sample Collection and Preservation

1.5.1 Diet Sample Collection

During the collection period, pelleted concentrate and oat straw samples were collected, air-dried, ground through a 40-mesh grinder, and stored for analysis.

1.5.2 Fecal and Urine Sample Collection

Feces: Horses were kept standing throughout the collection period. Using a custom fecal collection device, feces were collected every 2 hours, weighed, thoroughly mixed, and a 10% subsample was placed in labeled bags, air-dried,

and weighed. Air-dried fecal samples from six consecutive days were combined, mixed, and 1 kg was retained for analysis.

Urine: Using a custom urine collection device, urine was collected every 2 hours, thoroughly mixed, and total volume was measured. A 10% subsample was acidified with 5% sulfuric acid, stored in plastic bottles, and sealed. Urine samples from four consecutive days were combined, mixed, and 1 L was retained for analysis.

1.5.3 Blood Sample Collection

On the final morning of the trial, blood was collected from each horse after overnight fasting via jugular venipuncture into heparinized tubes (10 mL). Plasma was prepared by centrifugation at 1,500×g for 20 minutes, aliquoted into 2 mL tubes, and stored at -20°C for analysis.

1.6 Laboratory Analyses

1.6.1 Nutrient Analysis

DM, OM, and P contents in concentrate, oat straw, and fecal samples were determined using conventional feed analysis methods. Calcium content was measured by the o-cresolphthalein colorimetric method. Urinary phosphorus was determined by the phosphorus fixation method. Gross energy (GE) was measured using an HR-15 oxygen bomb calorimeter. NDF and ADF contents were analyzed using an ANKOM fiber analyzer. Crude protein content was determined using an Elementar Analysen Systeme rapid nitrogen analyzer.

1.6.2 Plasma Biochemical Indices

Plasma total protein (TP), albumin (ALB), and urea nitrogen (UN) were measured using kits from Zhongsheng Beikong Biotech (catalog numbers 2090-2003, 2074-2003, and 2102-2003, respectively). Globulin (GLB) and glutamine (Gln) were measured using kits from Beijing Huaying Biotech (catalog numbers HY-N0013 and HY-60057, respectively).

1.7 Data Calculation and Statistical Analysis

Data calculations followed the methods of Yuan Ying. All data are expressed as mean ± standard deviation. Statistical analysis was performed using SPSS 16.0 software, with Duncan' s multiple range test used for post-hoc comparisons.

Results

2.1 Effects of Dietary Fiber and Protein Levels on Nutrient Intake, Digestion, and Digestibility

As shown in Table 3 , OM and CP intakes increased with dietary protein level, with CP intake in group IV significantly higher than in groups I and II ($P<0.05$). Conversely, NDF and ADF intakes decreased with increasing protein level, with ADF intake in group IV significantly lower than in groups I and II ($P<0.05$).

Calcium and phosphorus intakes increased with protein level, with group IV showing significantly higher Ca and P intakes than groups I, II, and III ($P<0.05$).

Digestion amounts of DM and OM increased with dietary protein level, though differences among groups were not significant ($P>0.05$). Crude protein digestion increased significantly with protein level, with groups IV and III higher than groups I and II ($P<0.05$). Calcium and phosphorus digestion also increased with protein level, with group IV significantly higher than groups I, II, and III ($P<0.05$).

Digestibility of DM, OM, and CP showed upward trends with increasing protein level, but differences were not significant among groups ($P>0.05$). Digestibility of NDF and ADF increased from groups I to III ($P>0.05$). Calcium and phosphorus digestibility increased with protein level, with group IV significantly higher than groups I, II, and III ($P<0.05$).

2.2 Effects of Dietary Fiber and Protein Levels on Energy Metabolism

Table 4 shows that GE intake increased with dietary protein level, with groups II, III, and IV showing 3.71% ($P>0.05$), 3.98% ($P>0.05$), and 7.42% ($P>0.05$) higher GE intake than group I, respectively. However, increasing protein level had no significant effect on fecal or urinary energy ($P>0.05$). Both DE and ME increased with protein level, with ME in groups III and IV significantly higher than in groups I and II ($P<0.05$).

Plotting GE intake against DE yielded a linear regression equation for 12-month-old Yanqi horses: $y = 0.8106x - 0.4092$ ($R^2 = 0.7049$) [Figure 1: see original paper].

2.3 Effects of Dietary Fiber and Protein Levels on Nitrogen Metabolism

Table 5 indicates that nitrogen intake increased with dietary protein level, with group IV significantly higher than groups I and II ($P<0.05$). Fecal nitrogen output did not differ significantly among groups ($P>0.05$), while urinary nitrogen output in groups III and IV was significantly higher than in groups I and II ($P<0.05$). Nitrogen retention showed an upward trend with protein level, increasing by 19.15% ($P>0.05$), 21.28% ($P>0.05$), and 34.04% ($P>0.05$) in groups II, III, and IV compared to group I, respectively. Nitrogen retention rate remained around 53.00% across all groups, with no significant differences ($P>0.05$).

The linear regression equation between nitrogen intake and retention was $y = 0.4813x + 0.1184$ ($R^2 = 0.5577$) [Figure 2: see original paper].

2.4 Effects of Dietary Fiber and Protein Levels on Calcium and Phosphorus Metabolism

Table 6 shows that calcium intake increased with dietary protein level, with group IV significantly higher than groups I, II, and III ($P<0.05$). Fecal calcium output also increased with protein level, with group IV significantly higher than groups I and II ($P<0.05$), while urinary calcium did not differ significantly among groups ($P>0.05$). Both calcium retention and retention rate increased with protein level, with group IV significantly higher than the other groups ($P<0.05$).

Phosphorus intake increased significantly with protein level ($P<0.01$). Fecal phosphorus output increased with protein level, with groups III and IV significantly higher than groups I and II ($P<0.05$). Urinary phosphorus was highest in group II, significantly higher than group I ($P<0.05$). Phosphorus retention increased with protein level, with group IV significantly higher than groups I, II, and III ($P<0.05$).

Linear regression equations were established: for calcium, $y = 0.5825x - 74.423$ ($R^2 = 0.6073$) [Figure 3: see original paper], and for phosphorus, $y = 0.1829x + 59.399$ ($R^2 = 0.149$) [Figure 4: see original paper], indicating positive correlations between intake and retention within certain ranges.

2.5 Effects of Dietary Fiber and Protein Levels on Plasma Biochemical Indices

Table 7 shows that increasing dietary protein level had no significant effect on plasma TP, GLB, or ALB content ($P>0.05$), though group IV had higher values than other groups. Plasma UN content did not differ significantly among groups ($P>0.05$), but groups II and IV had lower values than groups I and III. Plasma Gln content showed an upward trend with protein level, though differences were not significant ($P>0.05$).

Discussion

3.1 Effects on Nutrient Intake

Appropriate concentrate-to-roughage ratios are fundamental to scientific feeding and ensure nutrient acquisition. Factors affecting feed intake include animal physiological status, diet type, environment, and feeding techniques. Research indicates that increasing dietary protein level can improve DM intake but reduce roughage intake. For horses, appropriate dietary protein levels not only meet nutritional requirements but also ensure healthy growth. Dulphy et al. reported that decreased diet quality, such as increased crude fiber or reduced CP content, leads to decreased feed intake in horses.

In this study, nutrient intake in Yanqi horses changed with dietary protein level. When fed diets containing 8.94% and 10.01% protein, DM and OM intakes in-

creased. As dietary protein level increased from 6.81% to 10.01% and fiber level decreased accordingly, NDF and ADF intakes decreased. Calcium and phosphorus intakes were also affected, with Ca intake increasing by 12.23%, 26.33%, and 48.44% in groups II, III, and IV compared to group I, respectively. Phosphorus intake increased significantly with protein level. Therefore, increasing dietary protein level (i.e., decreasing fiber level) improved diet quality and enhanced intake of DM, OM, CP, Ca, and P.

3.2 Effects on Nutrient Digestibility

Dietary fiber and protein levels significantly affect nutrient digestion and metabolism in animals. Digestibility of NDF and ADF reflects the ability to utilize structural carbohydrates, while excessive dietary fiber reduces utilization of CP, energy, Ca, and P. Fiber digestibility is influenced by many factors including roughage cell wall structure, feed intake, digesta passage rate, and diet processing. Slade et al. demonstrated that horses' ability to digest dietary nitrogen and CP is related to DM intake and dietary protein level, with CP digestibility increasing as both parameters increase. Hintz et al. reported that increasing the concentrate-to-roughage ratio improved digestibility of DM, OM, CP, NDF, and ADF in ponies.

In this study, nutrient digestibility in 12-month-old Yanqi horses generally increased with dietary protein level. DM digestibility increased gradually, with the most pronounced effect in group IV, followed by groups III and II, which showed 6.97%, 4.89%, and 4.16% improvements compared to group I, respectively. Digestibility of OM, CP, and Ca also increased with protein level. However, P digestibility did not consistently increase with protein level, with group III showing the lowest value, possibly related to physiological status. NDF and ADF digestibility increased with protein level, likely because protein intake promoted proliferation of fiber-degrading microorganisms in the cecum, thereby enhancing fiber digestibility. Additionally, decreasing dietary fiber level (i.e., increasing protein level) improved DM and Ca digestion and increased DE and nitrogen retention. These results indicate that increasing dietary protein level improved nutrient digestibility in 12-month-old Yanqi horses.

3.3 Effects on Energy Metabolism

After feed ingestion, dietary protein, carbohydrates, and fats undergo digestion and metabolism to produce energy, ultimately meeting physiological needs as ATP. Dietary energy content depends on feed composition. Miraglia et al. conducted digestion trials in four ~550 kg horses fed diets with concentrate-to-roughage ratios of 100:0, 75:25, and 50:50, finding that GE intake and DE increased with concentrate proportion.

Our results showed that GE intake in 12-month-old Yanqi horses increased with dietary protein level, consistent with Miraglia et al. Both DE and ME also increased with protein level, with the highest values observed at 10.01%

dietary protein. Barth et al. reported that 190 kg growing horses require 2.87 MJ/(horse · d) on a metabolic body weight basis. NRC (2007) recommends that a 400 kg mature horse at 12 months (257 kg body weight) requires 0.98 MJ/(horse · d). Our digestion trial determined the appropriate energy requirement for 12-month-old Yanqi horses to be 2.78 MJ/(horse · d), similar to Barth et al. but higher than NRC (2007), possibly due to breed and environmental differences.

3.4 Effects on Nitrogen Metabolism

Nitrogen metabolism is a key indicator of dietary nitrogen balance, influenced by diet composition, nutritional level, and animal digestive capacity, with dietary protein level being the direct determinant. Slade et al. found that nitrogen retention in non-working horses increased with dietary protein level. Pfeiffer et al. reported that excessive CP intake and amino acid imbalance increased urinary nitrogen excretion, reducing nitrogen utilization efficiency, with a correlation between CP intake and urinary nitrogen output.

In this study, nitrogen intake and retention in 12-month-old Yanqi horses increased with dietary protein level, consistent with Slade et al., with the highest nitrogen utilization observed at 10.01% dietary protein. Fecal nitrogen output showed little change, while urinary nitrogen increased gradually with protein level, similar to Pfeiffer et al. Reitnour et al. found the minimum CP requirement for horses to be 400 mg DP/(kg BW · d), while Olsman et al. suggested an appropriate requirement of 545 mg DP/(kg BW · d). Our trial determined the appropriate CP requirement for 12-month-old Yanqi horses to be 566 mg DP/(kg BW · d), close to both reported values.

3.5 Effects on Calcium and Phosphorus Metabolism

Calcium and phosphorus are major mineral elements involved in numerous physiological processes. Horses absorb Ca and P primarily through paracellular passive transport and vitamin D-dependent transcellular transport in the intestine. Absorption efficiency is affected by diet type, nutrient composition, Ca:P ratio, breed, and age. Meyer et al. reported that dietary roughage proportion negatively correlated with P digestibility. Stephens et al. found that Ca digestibility increased from 6 months of age but decreased after 24 months. Pagan et al. reported that exercise status did not significantly affect Ca absorption in mature horses.

In this study, Ca and P intakes, digestion amounts, and digestibility in 12-month-old Yanqi horses increased with dietary protein level, consistent with previous research. Schryver et al. reported Ca retention rates of 51-69% and P retention rates of 30-55% in horses. Our study found Ca retention rates of 8.92-29.43% and P retention rates of 38.13-41.09%. The P retention rate was similar to Schryver et al., while Ca retention was substantially lower, possibly related to physiological status and warranting further investigation.

3.6 Effects on Plasma Biochemical Indices

Plasma TP, comprising ALB and GLB, reflects dietary protein level and protein digestion, absorption, and utilization. Plasma UN content is an important metabolic indicator reflecting protein metabolism status and amino acid balance. Chu Hongzhong found that serum UN content in horses negatively correlated with CP intake, decreasing significantly as CP intake increased. In our study, plasma TP in all groups was within the normal range (52-79 g/L), while ALB was lower than the reference value (26-37 g/L), indicating slightly suboptimal nutritional status. Plasma TP, ALB, and GLB increased with dietary protein level, suggesting that increasing concentrate level improved metabolic status. The decreasing trend in plasma UN with increasing protein level indicated improved CP utilization in 12-month-old Yanqi horses.

Glutamine maintains small intestinal structure and function during gastrointestinal damage and is a primary nutrient for small intestinal metabolism, directly or indirectly affecting protein synthesis, degradation, and oxidative stress. In this study, plasma Gln increased with dietary protein level, suggesting that higher protein levels met the needs for protein and amino acid synthesis and metabolism.

In conclusion, increasing dietary protein level in Yanqi horses enhanced nutrient intake, digestibility, and retention, with optimal performance at 69.41% fiber and 10.01% protein, though plasma biochemical indices were not significantly affected.

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