Effects of Different Dietary Fiber and Protein Levels on Digestion and Metabolism, Plasma Biochemical Parameters, and Body Weight Gain in 2-Year-Old Yanqi Horses: Postprint

Authors: Liu Kai, Zhao Fang, Li Xiaobin, Chen Xueji, Arslan, Yang Kailun

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Abstract

This experiment aimed to investigate the effects of diets with different fiber and protein levels on digestion and metabolism, blood biochemical indices, and body weight gain in 2-year-old Yanqi horses, providing a theoretical basis for the scientific feeding of Yanqi horses. Twelve 2-year-old male Yanqi horses with an average body weight of (254$±$14) kg were selected and randomly divided into 2 groups, with 6 horses per group. A phased grouping experimental design was adopted; the experiment consisted of 2 phases: Phase 1 included Experimental Group I and Experimental Group II, and Phase 2 included Experimental Group III and Experimental Group IV. Experimental Groups I, II, III, and IV were fed diets with fiber and protein levels of 73.52% and 6.99%, 68.82% and 7.55%, 67.32% and 8.04%, and 64.21% and 8.57%, respectively, for a 21-day digestion and metabolism trial, comprising a 15-day preliminary period and a 6-day formal trial period. The results showed that nutrient digestibility increased with increasing dietary protein level, with the digestibility of dry matter, organic matter, calcium, and phosphorus in Experimental Group IV being significantly higher than that in Experimental Group I (P<0.05). In terms of energy metabolism, increasing dietary protein level could improve the metabolizable energy and digestible energy of 2-year-old Yanqi horses, but the differences among groups were not significant (P>0.05). Regarding calcium and phosphorus metabolism, the calcium and phosphorus intake, retention amount, retention rate, as well as fecal phosphorus and urinary phosphorus excretion in Experimental Groups II, III, and IV were all higher than those in Experimental Group I, with the calcium and phosphorus retention rates in Experimental Group IV being 44.34% (P<0.05) and 47.80% (P<0.05) higher than those in Experimental Group I, respectively. However, increasing dietary protein level had no significant effect on plasma total protein, albumin, globulin, urea nitrogen,

glutamine content, or body weight gain in 2-year-old Yanqi horses (P>0.05). Therefore, increasing the protein level in the diet of 2-year-old Yanqi horses can improve nutrient digestibility and retention, with the optimal levels being 64.21% fiber and 8.57% protein, but it has no significant effect on plasma biochemical indices or body weight gain.

Full Text

Introduction

Animals obtain nutrients through their diet, and feed intake directly determines their growth and development. In horses, dietary fiber and protein levels significantly influence feed consumption. Diets with inadequate protein restrict growth and impair performance, while excessively high protein levels may alter the gastrointestinal environment, reduce nutrient utilization efficiency, and cause metabolic disturbances such as acidosis. Therefore, providing diets with appropriate protein levels is crucial for optimizing nutrient utilization and supporting healthy growth in horses [1].

The Yanqi horse is an excellent local breed primarily used for both riding and draft purposes, predominantly distributed in the Bayingolin Mongol Autonomous Prefecture of Xinjiang. Renowned for its speed, draft power, and endurance, this valuable Chinese breed completed an 80 km endurance race in 4 hours, 49 minutes, and 23 seconds [2]. Yanqi horses reach sexual maturity at approximately 2 years of age, a critical period for reproductive development and the onset of athletic potential. Providing appropriate dietary protein levels during this stage is essential for maintaining high reproductive capacity and performance.

Building on previous research, this experiment established four diets with varying fiber and protein levels for 2-year-old Yanqi horses. Through digestion and metabolism trials, we calculated the digestibility and metabolic rates of major nutrients and established mathematical relationships between nutrient intake and retention, providing a scientific reference for feeding practices.

Materials and Methods

1.1 Experimental Period and Location

The experiment was conducted from July to September 2014 at the Baoqi Yanqi Horse Stud Farm in Bayingolin Mongol Autonomous Prefecture, Xinjiang.

1.2 Experimental Animals

Twelve 2-year-old Yanqi stallions with an average body weight of (254$±$14) kg were selected for this study.

1.3 Experimental Design

The 12 stallions were randomly divided into 2 groups of 6 horses each using a staged grouping method. Stage 1 comprised trial groups I and II, while Stage 2 comprised trial groups III and IV. Groups I, II, III, and IV received diets with fiber and protein levels of 73.52% and 6.99%, 68.82% and 7.55%, 67.32% and 8.04%, and 64.21% and 8.57%, respectively. Each stage 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 occurred between stages. Horses were fasted and weighed before the adaptation period. During the collection period, all feces and urine were collected every 2 hours. On the final morning, horses were fasted, weighed, and blood samples were collected via venipuncture. All horses were housed under identical environmental conditions. The experimental design and grouping are presented in Table 1 .

1.4 Diet and Animal Management

Throughout the trial, horses were individually housed in stalls (2.5 m × 1.2 m × 2.0 m). The daily ration was divided into five equal portions and offered at 08:00, 12:30, 17:00, 21:00, and 00:00, following a roughage-first-concentrate-second approach with small, frequent meals to ensure complete consumption and adequate water intake. During the adaptation period, horses were released for exercise after feeding, with no access to feed. During the collection period, horses were individually stabled with custom fecal and urinary collection devices, maintained in a standing position, with samples collected every 2 hours. Stalls were cleaned each morning to maintain optimal conditions. Deworming followed the farm' s standard management protocol. The roughage consisted of oat straw chopped to 8 cm lengths, while the pelleted concentrate measured approximately 0.5 cm in diameter and 1.0–1.5 cm in length, with accurate weighing at each feeding. Diet composition and nutrient levels are shown in Table 2 .

1.5 Sample Collection and Preparation

1.5.1 Feed Sample Collection During the trial, samples of pelleted concentrate and oat straw were collected, air-dried, ground through a 40-mesh sieve, and stored for analysis.

1.5.2 Fecal and Urine Sample Collection Fecal samples were collected every 2 hours throughout the collection period using a custom device while horses remained standing. The daily fecal output was thoroughly mixed, and 10% by weight was randomly sampled, placed in labeled bags, air-dried, and weighed. The six-day air-dried samples from each horse were combined, mixed, and 1 kg was retained for analysis. Urine samples were similarly collected every 2 hours using a custom device. The daily urine volume was measured, thoroughly mixed, and 10% was sampled and preserved with 5% concentrated sulfuric acid in plastic bottles. The six-day urine samples were combined, mixed, and 1 L was retained for analysis.

1.5.3 Blood Sample Collection On the final morning of the trial, 10 mL of blood was collected via jugular venipuncture into heparinized tubes. Plasma was prepared by centrifugation at 1,500×g for 20 minutes, aliquoted into 2 mL tubes, and stored at -20°C for analysis.

1.5.4 Body Weight Measurement Horses were fasted and weighed on the morning before the adaptation period and on the morning after the collection period.

1.6 Laboratory Analyses

1.6.1 Nutrient Composition Analysis Dry matter (DM), organic matter (OM), and phosphorus (P) contents in pellets, oat straw, and feces were determined using conventional feed analysis methods [3]. Calcium (Ca) content was measured by the o-cresolphthalein colorimetric method [4]. Urinary P was determined by the phosphorus fixation method [5]. Gross energy (GE) was measured using an HR-15 oxygen bomb calorimeter. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were analyzed using an ANKOM fiber analyzer (USA). Crude protein (CP) was determined using an Elementar Analysen Systeme rapid nitrogen analyzer (Germany).

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

1.7 Data Calculation and Statistical Analysis

Data calculations followed the methods of Yuan Ying [6]. Results are expressed as mean±SD. Data were analyzed using one-way ANOVA in SPSS 16.0, with Duncan' s multiple range test for post-hoc comparisons.

Results

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

As shown in Table 3 , increasing dietary protein level decreased DM, OM, NDF, and ADF intake in 2-year-old Yanqi horses, with group IV significantly lower than group I ($P<0.05$). Conversely, CP, Ca, and P intake increased, with significant differences among groups in Ca and P intake ($P<0.05$), and groups III and IV showing higher CP intake than group I ($P<0.05$). No significant differences were observed among groups in DM, OM, or CP digestion amount ($P>0.05$). Calcium and P digestion amount increased with protein level, with group IV significantly higher than groups I, II, and III (P<0.05). NDF and ADF digestion amount decreased with increasing protein level, though differences were not significant (P>0.05). Digestibility of DM, OM, CP, NDF, ADF, Ca, and P generally increased with dietary protein level, with group IV showing significantly higher DM, OM, Ca, and P digestibility than group I (P<0.05), representing increases of 10.58%, 9.57%, 44.34%, and 47.80%, respectively.

2.2 Effects of Different Dietary Fiber and Protein Levels on Energy, Nitrogen, Calcium, and Phosphorus Metabolism

Table 4 shows that GE intake decreased with increasing protein level, with group I significantly higher than groups III and IV (P<0.05). Fecal and urinary energy also decreased, with groups III and IV significantly lower than group I (P<0.05). Digestible energy (DE) and metabolizable energy (ME) increased with protein level, with groups II, III, and IV showing 4.35%, 3.99%, and 5.15% higher DE, and 8.52%, 13.90%, and 15.25% higher ME than group I, respectively, though differences were not significant (P>0.05).

Nitrogen intake increased with protein level, with groups III and IV significantly higher than group I (P<0.05). Fecal nitrogen output showed an initial increase then decrease, with group III significantly higher than groups I and IV (P<0.05). Urinary nitrogen was unaffected by protein level (P>0.05). Nitrogen retention and retention rate showed a similar trend of initial increase then decrease, without significant differences among groups (P>0.05).

Calcium intake increased with protein level, with groups II, III, and IV showing 11.44% (P<0.05), 24.86% (P<0.05), and 37.13% (P<0.05) higher intake than group I. Fecal and urinary Ca outputs did not differ among groups (P>0.05). Calcium retention and retention rate increased overall, with group IV significantly higher than all other groups (P<0.05).

Phosphorus intake increased significantly with protein level (P<0.05). Fecal and urinary P outputs initially increased then decreased, with groups III and IV showing higher fecal P than groups I and II (P<0.05), while group II had the highest urinary P, significantly different from group I (P<0.05). Phosphorus retention and retention rate increased with protein level, with group IV significantly higher than groups II and III (P<0.05), though groups I, II, and III did not differ significantly (P>0.05).

2.7 Effects of Different Dietary Fiber and Protein Levels on Plasma Biochemical Indices

As shown in Table 5 , increasing dietary protein level had no significant effect on plasma TP, ALB, GLB, or UN concentrations (P>0.05), though groups II, III, and IV showed higher values than group I. Plasma UN tended to increase with protein level, but differences were not significant (P>0.05). Plasma Gln concentration decreased with increasing protein level, though again differences were not significant (P>0.05).

2.8 Effects of Different Dietary Fiber and Protein Levels on Body Weight Gain

Table 6 indicates that increasing dietary protein level had no significant effect on final body weight, weight gain, or average daily gain (ADG) (P>0.05). Group II showed the highest weight gain and ADG, while group IV had the lowest values among all groups.

Discussion

3.1 Effects of Different Dietary Fiber and Protein Levels on Nutrient Intake, Digestion Amount, and Digestibility

Nutrient digestibility in animals depends on numerous factors including animal characteristics (age, body weight, condition, performance), environmental conditions, management practices, feed quality, and diet composition (moisture content, concentrate-to-forage ratio, NDF level) [1]. Research indicates that nutrient digestibility is related to diet composition. Slade [7] demonstrated that nitrogen and protein digestibility is associated with DM intake and dietary protein level, with increased DM intake or protein level enhancing CP digestibility. Gibbs [8] reported that horses showed higher apparent nitrogen digestibility from high-protein alfalfa than from low-protein Bermuda grass hay. Karlsson [9] found that increasing dietary oat proportion significantly improved DM, OM, and crude fiber digestibility in horses. Our results align with these findings, showing that nutrient digestion amount and digestibility in 2-year-old Yanqi horses increased with dietary protein level. DM digestibility rose with protein level, peaking in the 8.57% protein group. At this protein level, OM, CP, Ca, and P digestibility reached maximum values of 72.71%, 70.78%, 54.72%, and 44.31%, respectively. Additionally, NDF and ADF digestibility increased with protein level, possibly because dietary protein promoted proliferation of fiber-degrading microorganisms in the cecum, thereby enhancing NDF and ADF digestibility [10]. Therefore, increasing dietary protein level improved nutrient digestibility in 2-year-old Yanqi horses, with optimal utilization achieved at 8.57% protein.

3.3 Effects of Different Dietary Fiber and Protein Levels on Energy, Nitrogen, Calcium, and Phosphorus Metabolism

Energy metabolism reflects the utilization of carbohydrates, proteins, and fats. Research shows that energy requirements vary with age and work status. Coenen [11] confirmed that growing foals aged 3–6 months require 0.88 MJ/kg · W0.75, decreasing to 0.63 MJ/kg · W0.75 at 13–18 months, with 18-month-olds approaching mature maintenance requirements. NRC (2007) [12] recommends that mature horses weighing 400 kg and 500 kg require 0.79 MJ/kg · W0.75 and 0.83 MJ/kg · W0.75, respectively, at 24 months [13]. Our results show that fecal and urinary energy decreased while DE and ME increased with dietary protein level, with maximum values at 8.57% protein, indicating improved energy utilization efficiency.

Nitrogen metabolism is essential for understanding protein balance in animals. Diet composition, nutrient level, digesta retention time [13], and physiological status affect nitrogen metabolism. Protein digestion begins in the stomach, with absorption primarily in the small intestine [14]. Slade [7] reported that nitrogen retention in non-working horses increased with dietary protein level. Drogoul [15] found that increasing dietary barley proportion improved CP digestibility. Our nitrogen retention results align with these studies. NRC (2007) [12] recommends 7.73 g/kg · W0.75 CP for 400 kg mature horses at 24 months, while Reitnour [16] reported a minimum requirement of 8.09 g/kg · W0.75, and Olsman et al. [17] suggested 9.15 g/kg · W0.75 as optimal. Our 2-year-old Yanqi horses required 14.19 g/kg · W0.75, higher than previous reports, likely due to the low utilization efficiency of oat straw roughage necessitating greater CP intake.

Calcium and phosphorus are major mineral requirements. Calcium ions participate in bone mineralization, muscle contraction, nerve excitability, blood clotting, cell adhesion, and apoptosis [18]. Research in pigs shows that reducing dietary fiber increases P retention [19], while Howe et al. [20] found that high dietary P combined with increased CP intake significantly reduces P retention rate. Equine Ca and P absorption is influenced by diet type, nutrient composition, breed, age, and body weight, with Cymbaluk [21] reporting declining Ca digestibility from 6 to 24 months of age. Our results show that increasing dietary protein and decreasing fiber level enhanced Ca and P retention, consistent with Stanogias et al. [19]. Literature reports Ca retention rates of 51–69% and P retention rates of 30–55% in horses [22]. Our observed retention rates of 17.74–42.58% for Ca and 29.62–44.08% for P indicate that while P retention aligns with Schryver et al. [19], Ca retention was lower, possibly reflecting poor Ca utilization in Yanqi horses requiring further investigation.

3.6 Effects of Different Dietary Fiber and Protein Levels on Plasma Biochemical Indices

Chu Hongzhong [23] reported that serum TP and ALB increased significantly with CP intake in hybrid Yili foals, while Ayishayila [24] found no significant differences in plasma TP, ALB, GLB, UN, or glucose in suckling foals fed supplemental concentrate. Greppi [25] also reported no effect of dietary CP level on equine blood biochemical indices. In our study, plasma TP values were within the normal range (52–79 g/L) [26], but ALB was below the reference range (26–37 g/L), indicating slightly suboptimal nutritional status. Increased dietary protein had no significant effect on plasma TP, ALB, or GLB, consistent with Ayishayila [24], possibly due to the short trial duration. Plasma UN reflects protein metabolism status; while Guilinsheng et al. [27] found decreasing UN with increasing concentrate-to-forage ratio in Holstein bulls, our UN values showed a slight, non-significant increase with protein level, indicating minimal impact. Glutamine maintains intestinal structure and function during gastrointestinal stress and influences protein synthesis and oxidative stress [28]. In our study, plasma Gln showed no significant changes with protein level, likely due to the short feeding period.

3.7 Effects of Different Dietary Fiber and Protein Levels on Body Weight Gain

Animal growth is affected by breed, sex, nutrition, and environment. Chu Hongzhong [23] reported that increasing dietary nutrient level improved ADG and reduced feed-to-gain ratio in 8–11-month-old hybrid Yili foals. Wang Shaosong [29] reported an average mature Yanqi horse weight of 362.79 kg in 1989, substantially higher than our 260 kg average, likely because our 2-year-old horses were not yet mature. The lack of significant effect of protein level on weight gain may be attributed to two factors: first, the 31-day trial period was too brief to detect weight changes; second, prior grazing on low-nutrient pastures may have caused compensatory growth in groups I and II, potentially explaining why group II showed the highest gain and group IV the lowest. In conclusion, increasing dietary protein level improved nutrient digestibility and retention, with optimal performance at 64.21% fiber and 8.57% protein, but did not significantly affect plasma biochemical indices or body weight gain.

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