

## Effects of Different Roughage Source Diets on Growth Performance, Rumen Fermentation, and Serum Biochemical Indices in Hu Sheep: Post-print

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### Abstract

This experiment investigated the effects of diets with different roughage sources on growth performance, rumen fermentation function, and serum biochemical indices of Hu sheep under the same energy and protein levels. Eighteen Hu sheep with good body condition and similar body weight were selected and randomly divided into 3 groups with 6 sheep per group. Under the condition of diets with the same energy and protein levels, three diets with different roughage sources were formulated using peanut vine (PV), bean straw (BS), and sugarcane bagasse (SM) as the single roughage source, respectively. The experimental period lasted 60 d, including a 15 d preliminary period and a 45 d formal experimental period. The results showed: 1) The average daily gain of the PV group was significantly higher than that of the BS and SM groups ( $P < 0.05$ ). 2) The acetate, butyrate, total volatile fatty acids contents, and acetate/propionate ratio in the SM group were significantly or extremely significantly lower than those in the other two groups ( $P < 0.05$  or  $P < 0.01$ ); the propionate content was significantly or extremely significantly higher than that in the other two groups at 9 h ( $P < 0.05$  or  $P < 0.01$ ), but there was no significant difference among groups at 24 h ( $P > 0.05$ ). 3) The serum urea nitrogen content in the SM group was significantly higher than that in the PV group ( $P < 0.05$ ), and the creatinine content was significantly higher than that in the BS group ( $P < 0.05$ ). The results indicated that under the condition of diets with the same energy and protein levels, compared with bean straw and sugarcane bagasse, the diet with peanut vine as the roughage source could improve the growth and development and rumen fermentation function of Hu sheep, enhance nitrogen utilization efficiency, thereby reducing the supplementation amount of concentrate feed.

## Full Text

# Effects of Different Dietary Sources of Roughage on Performance, Ruminal Fermentation and Serum Biochemical Parameters of Hu Sheep

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## Abstract

This experiment was conducted to investigate the effects of different dietary sources of roughage on performance, ruminal fermentation function, and serum biochemical parameters of Hu sheep under the same energy and protein levels. Eighteen healthy Hu sheep with similar body weight were randomly divided into three groups with six sheep per group. Under the same dietary energy and protein levels, three diets were formulated with peanut vine (PV), beanstalk (BS), and sugarcane bagasse (SM) as the sole roughage source, respectively. The experimental period lasted 60 days, including a 15-day pre-experimental period and a 45-day main experimental period. The results showed: 1) The average daily gain of Hu sheep in the PV group was significantly higher than that in the BS and SM groups ( $P < 0.05$ ). 2) The acetate, butyrate, total volatile fatty acid contents, and acetate/propionate ratio of the SM group were significantly or extremely significantly lower than those of the other two groups ( $P < 0.05$  or  $P < 0.01$ ); the propionate content at 9 h was significantly or extremely significantly higher than that of the other two groups ( $P < 0.05$  or  $P < 0.01$ ), but there was no significant difference among groups at 24 h ( $P > 0.05$ ). 3) The serum urea nitrogen content of the SM group was significantly higher than that of the PV group ( $P < 0.05$ ), and the creatinine content was significantly higher than that of the BS group ( $P < 0.05$ ). These results indicate that under the same dietary energy and protein levels, compared with beanstalk and sugarcane bagasse, a diet with peanut vine as the roughage source can improve the growth, ruminal fermentation function, and nitrogen utilization efficiency of Hu sheep, thereby reducing concentrate supplementation.

**Keywords:** Hu sheep; roughage; performance; ruminal fermentation; serum biochemical parameters

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## 1. Introduction

China is extremely rich in roughage resources; however, these resources have not been utilized rationally and effectively, with most being used as fuel or returned directly to the field, causing resource waste and environmental pollution. Currently, only 20%-30% of agricultural by-products are utilized by ruminants

[1-2]. As is well known, due to the special function of the rumen, roughage is an indispensable component in ruminant diets, typically accounting for 60%-80%. Most fiber substances in roughage are degraded by rumen microorganisms into volatile fatty acids (VFA), carbon dioxide (CO<sub>2</sub>), methane (CH<sub>4</sub>), and other products, providing energy for ruminants as well as carbon skeletons, trace elements, and mineral elements required for protein synthesis [3]. Studies have found that the nutritional quality of roughage from different sources varies greatly, and the quality of roughage has a significant impact on the performance and health status of ruminants, directly affecting the supply of concentrate feed and ultimately influencing economic benefits [4-6]. Therefore, the full and rational utilization of local agricultural by-product resources is of great significance for solving the problem of competition for grain between humans and livestock and improving economic benefits.

Southern China is primarily agricultural, and many by-products of economic crops (such as peanut vine, beanstalk, and sugarcane bagasse) can be used as roughage resources for sheep. Wang et al. [7] fed lambs with *Leymus chinensis* and peanut vine respectively, and found no significant difference in average daily gain (ADG) between the two groups, but the nutrient digestibility of peanut vine was higher than that of *Leymus chinensis*, making it a low-cost high-quality roughage. Guo et al. [8] reported that although beanstalk has high lignin (ADL) content, its crude protein (CP) content and digestible energy are higher than those of conventional crop straws such as corn and wheat, and it can still be used as a roughage resource in sheep production. Wang et al. [9] also confirmed the utilizable value of pea straw by feeding lambs with pea straw and oat straw. Sugarcane bagasse has very high crude fiber (CF) content, and direct feeding can lead to negative energy balance in young ruminants; it is generally added in appropriate amounts to high-concentrate diets for dairy cows [10].

However, most previous studies on roughage have been conducted under the same concentrate-to-roughage ratio, with different energy levels. In actual nutrient requirement formulation, energy and protein levels are used as the basis; therefore, there is currently a lack of reports on the effects of different roughages on ruminants under the same energy and protein levels. Therefore, this experiment selected three common agricultural by-products from southern China as roughage sources to formulate total mixed rations with the same energy and protein levels for a feeding trial with Hu sheep, aiming to study and compare the effects of different dietary roughage sources on performance, ruminal fermentation, and serum biochemical parameters of Hu sheep under the same energy and protein levels.

### 1.1 Experimental Design and Feeding Management

This experiment was conducted at Donglin Sheep Farm in Taicang City, Jiangsu Province. Eighteen Hu sheep rams with an average body weight of (21.3±1.3) kg were randomly divided into three groups with six replicates per group, and each group was fed different experimental diets once daily with free access to

water. The pre-experimental period lasted 15 days, and the main experimental period lasted 45 days. Each Hu sheep was housed in individual pens. During the pre-experimental period, sheep were fed ad libitum to determine the minimum feed intake, and during the main experimental period, feeding was conducted at 90% of the minimum intake.

## 1.2 Experimental Diets

The diets were formulated according to the nutrient requirements of NRC (2003) [11]. Under the same energy and protein levels, three diets with different roughage sources were formulated using peanut vine, beanstalk, and sugarcane bagasse as the sole roughage source, respectively. The composition and nutrient levels of the experimental diets are shown in Table 1 .

**Table 1** Composition and nutrient levels of experimental diets (DM basis)

## 1.3 Sample Collection and Preprocessing

The body weight on day 1 of the main experimental period was recorded as the initial body weight, and the body weight on day 45 was recorded as the final body weight to calculate average daily gain (ADG). In the middle of the main experimental period, a 6-day digestion trial was conducted for each group of Hu sheep (with the first 3 days as the adaptation period). Total fecal samples were collected continuously for 3 days using fecal collection bags, with samples collected every 12 h. Ten percent sulfuric acid was added for nitrogen fixation. The fecal samples from the 3 days were mixed and stored at -20 °C for determination of nutrient apparent digestibility.

On the final day of the experiment, blood samples were collected aseptically from the jugular vein before morning feeding. After standing at room temperature for 60 min, serum was prepared by centrifugation at 2,000×g for 10 min and stored at -20 °C for determination of serum biochemical parameters. On the final day of the experiment, rumen fluid was collected at 9 h and 24 h after feeding, with approximately 50 mL collected each time. The collected rumen fluid was filtered through four layers of gauze. One portion was used for immediate pH determination. Other filtrates were centrifuged at 1,301×g for 15 min. Then, 0.5 mL of supernatant was added to a sample bottle pre-filled with 4.5 mL of 0.2 mol/L hydrochloric acid, mixed well for determination of ammonia nitrogen (NH<sub>3</sub>-N) content. Another 4 mL of supernatant was added to a sample bottle containing 1 mL of 25% metaphosphoric acid to remove soluble protein for VFA determination. All samples were stored at -20 °C.

## 1.4 Analytical Methods

### 1.4.1 Nutrient apparent digestibility

The contents of dry matter (DM), CP, neutral detergent fiber (NDF), and acid detergent fiber (ADF) in diets and fecal samples were determined according

to the method of Zhang [13]. Nutrient apparent digestibility (%) = [(nutrient intake - nutrient excretion) / nutrient intake] × 100.

#### 1.4.2 pH

The pH of rumen contents was determined directly using a PHS-3B precision pH meter (Shanghai Leici Instrument Factory).

#### 1.4.3 Lactic acid content

Lactic acid content was determined according to the method of Yang et al. [14] using a 722 spectrophotometer. The kit was purchased from Nanjing Jiancheng Bioengineering Institute.

#### 1.4.3 NH<sub>3</sub>-N content

NH<sub>3</sub>-N content was determined according to the method of Feng et al. [15], using ammonium chloride as the standard and a 722 spectrophotometer for colorimetry at a wavelength of 700 nm.

#### 1.4.4 VFA content

VFA content was determined using a Shimadzu GC-14B gas chromatograph according to the method of Xiong et al. [16]. Determination conditions: capillary column CP-WAX (length 30 m, inner diameter 0.53 mm, film thickness 1 μm); vaporization chamber temperature 200 °C, flame ionization detector (FID) temperature 200 °C; column temperature was programmed from an initial temperature of 100 °C to a final temperature of 120 °C at a rate of 2 °C/min; sensitivity was 10<sup>1</sup>, attenuation was 25, and crotonic acid was used as the internal standard. Culture medium processing: the culture medium was centrifuged at 20,817×g for 10 min, then 1 mL of supernatant was taken and 0.2 mL of 20% metaphosphoric acid containing 60 mmol/L crotonic acid was added, mixed well, and centrifuged at high speed; 2 L of supernatant was taken for injection analysis.

#### 1.4.5 Serum biochemical parameters

Serum total protein (TP), albumin (ALB), glucose (GLU), urea nitrogen (UN), creatinine (Cr) contents, and alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were determined using a DH-364 automatic biochemical analyzer. All kits were purchased from Shanghai Zhicheng Biological Co., Ltd.

### 1.5 Statistical Analysis

Experimental data were first organized using Excel 2007 software and then analyzed using one-way ANOVA and Duncan's multiple comparison method in SPSS 16.0 software. P<0.05 was considered statistically significant, and P<0.01 was considered extremely significant. The results are expressed as means and standard error of the mean (SEM).

## 2. Results

### 2.1 Effects of Different Dietary Sources of Roughage on Performance of Hu Sheep

As shown in Table 2 , there was no significant difference in initial body weight among groups ( $P>0.05$ ). The final body weight of Hu sheep in the PV group was significantly higher than that in the BS group ( $P<0.05$ ), but there was no significant difference compared with the SM group ( $P>0.05$ ). The ADG of Hu sheep in the PV group was significantly higher than that in the BS and SM groups ( $P<0.05$ ), increasing by 22.68% and 21.10%, respectively, while there was no significant difference in ADG between the BS and SM groups ( $P>0.05$ ).

**Table 2** Effects of different dietary sources of roughage on performance of Hu sheep

*In the same row, values with different small letter superscripts mean significant difference ( $P<0.05$ ), and with different capital letter superscripts mean significant difference ( $P<0.01$ ), while with the same or no letter superscripts mean no significant difference ( $P>0.05$ ). The same as below.*

### 2.2 Effects of Different Dietary Sources of Roughage on Nutrient Apparent Digestibility of Hu Sheep

As shown in Table 3 , there was no significant difference in DM apparent digestibility between the PV and SM groups ( $P>0.05$ ), but both were extremely significantly higher than that of the BS group ( $P<0.01$ ). The CP apparent digestibility of the PV group was significantly higher than that of the SM and BS groups ( $P<0.05$ ), but there was no significant difference between the SM and BS groups ( $P>0.05$ ). Compared with the SM group, the NDF apparent digestibility of Hu sheep in the PV and BS groups was extremely significantly increased ( $P<0.01$ ), but there was no significant difference between these two groups ( $P>0.05$ ). The ADF apparent digestibility differed extremely significantly among groups ( $P<0.01$ ), with the BS group being the highest, followed by the PV group, and the SM group being the lowest.

**Table 3** Effects of different dietary sources of roughage on nutrient apparent digestibility of Hu sheep

### 2.3 Effects of Different Dietary Sources of Roughage on Ruminal Fermentation Parameters of Hu Sheep

As shown in Table 4 , at 9 h, the ruminal pH of Hu sheep in the PV group was extremely significantly higher than that of the other two groups ( $P<0.01$ ), while the lactic acid and  $\text{NH}_3\text{-N}$  contents in the SM group were significantly or extremely significantly higher than those of the other two groups ( $P<0.05$  or  $P<0.01$ ). The trends of ruminal pH, lactic acid, and  $\text{NH}_3\text{-N}$  contents at 24 h were similar to those at 9 h. The acetate, butyrate, total VFA contents, and acetate/propionate ratio of Hu sheep in the SM group were significantly

or extremely significantly lower than those of the other two groups ( $P < 0.05$  or  $P < 0.01$ ), while the propionate content at 9 h was significantly or extremely significantly higher than that of the other two groups ( $P < 0.05$  or  $P < 0.01$ ), but there was no significant difference among groups at 24 h ( $P > 0.05$ ).

**Table 4** Effects of different dietary sources of roughage on ruminal fermentation parameters of Hu sheep

### 2.6 Effects of Different Dietary Sources of Roughage on Serum Biochemical Indexes of Hu Sheep

As shown in Table 5, there were no significant differences in serum TP, ALB, GLU contents, or ALT, AST activities among groups ( $P > 0.05$ ). Compared with the SM group, serum UN content in the PV group was significantly decreased ( $P < 0.05$ ). Serum Cr content in the SM group was significantly higher than that in the BS group ( $P < 0.05$ ), while there were no significant differences among other groups ( $P > 0.05$ ).

**Table 5** Effects of different dietary sources of roughage on serum biochemical indexes of Hu sheep

## 3. Discussion

### 3.1 Effects of Different Dietary Sources of Roughage on Performance of Hu Sheep

Due to different diet types, animals have varying intake and digestion/absorption rates, leading to differences in production performance. In this experiment, Hu sheep were subjected to restricted feeding to ensure consistent energy and protein intake. Under these conditions, the growth performance of Hu sheep mainly depended on nutrient digestibility. Generally, appropriately increasing the concentrate-to-roughage ratio can increase the content of easily digestible carbohydrates, thereby improving the apparent digestibility of dietary DM and organic matter and reducing the digestibility of fiber substances [17-18]. The results of this experiment showed that ADG of Hu sheep in the PV group was significantly increased, and its DM apparent digestibility was also higher than that of the other two groups. Although there was no significant difference in ADG between the BS and SM groups in this experiment, the BS group had higher NDF and ADF apparent digestibility with less concentrate usage. These findings indicate that using high-quality roughage under isoenergetic and isonitrogenous conditions can increase ADG of Hu sheep or offset the effect of concentrate on ADG. Tjardes et al. [19] reported that improving roughage quality can enhance roughage digestibility and reduce the effect of concentrate-to-roughage ratio on ADG. Additionally, Drennan et al. [20] also confirmed that improving roughage digestibility can reduce concentrate supplementation. The results of this experiment are consistent with the above findings. The results of this experiment showed that ADG

of Hu sheep in the SM group was lower, and both NDF and ADF apparent digestibility were the lowest, suggesting that sugarcane bagasse is not suitable as a single roughage source for feeding Hu sheep. Therefore, further research is needed on optimal utilization methods (such as processing or combining with other roughages).

### 3.2.1 Effects on Ruminal pH and Lactic Acid Content

Ruminal pH is a comprehensive indicator reflecting the ruminal internal environment and fermentation level, resulting from the combined effects of dietary properties, saliva secretion, organic acid production, absorption, and excretion rates. The normal range of ruminal pH in ruminants is 5.5-7.5, with the most suitable range being 6.6-7.0 [14,21]. If the concentrate-to-roughage ratio of ruminant diets is too high, soluble sugars, starch, and other concentrates undergo rapid fermentation to produce VFA within 0.2-5 h after feeding [22], sharply decreasing ruminal pH in a short time. When pH falls below 6.0, the ruminal microbial flora changes, cellulolytic bacteria growth is inhibited, and lactic acid-producing bacteria proliferate massively, leading to increased lactic acid content [23-24]. Since the ionization constant of lactic acid is much lower than that of VFA, its contribution to ruminal pH is greater than that of VFA [22,25], and at this point, lactic acid becomes the main factor affecting ruminal pH. The results of this experiment showed that at 9 h, the pH of the sugarcane bagasse group was lower, while lactic acid content was higher than that of the other two groups. This may be because the concentrate-to-roughage ratio of the diet in the SM group was the highest, and after feeding for 0.2-5 h, the total VFA of Hu sheep in the SM group was higher than that of the other two groups. At this time, ruminal pH may have fallen below 6.0, leading to massive accumulation of lactic acid and further decreasing ruminal pH. In contrast, the other two groups of Hu sheep had relatively lower total VFA content after feeding for 0.2-5 h, and ruminal pH may have remained above 6.0, with little change in ruminal microbial flora and less lactic acid production, resulting in a relatively small impact on pH. Ouyang et al. [26] reported the trend that adding 1,200 mg/kg niacin to high-concentrate diets resulted in lower ruminal pH and total VFA content than adding 800 mg/kg niacin. Zhao et al. [27] also indicated that besides VFA content, the large amount of other organic acids produced by fermentation of high-concentrate diets is an important factor causing pH reduction. These results are consistent with the findings of this experiment. At 24 h, due to the absorption function of ruminal epithelium, both lactic acid and total VFA contents decreased. Therefore, compared with 9 h, ruminal pH increased somewhat at 24 h.

### 3.2.2 Effects on Ruminal NH<sub>3</sub>-N Content

NH<sub>3</sub>-N is an important product of protein, peptide, amino acid, and non-protein nitrogen degradation in ruminal metabolism and is also the main nitrogen source for cellulolytic bacteria growth [28]. The content of NH<sub>3</sub>-N in the rumen reflects,

to some extent, the dynamic balance between dietary protein degradation and microbial protein synthesis. This experiment found that the  $\text{NH}_3\text{-N}$  content in the rumen of Hu sheep in the SM group was significantly or extremely significantly higher than that in the PV and BS groups at both 9 h and 24 h. This may be because the PV and BS groups had relatively higher roughage content, slower protein degradation rates compared with the SM group, and higher ruminal pH that was more suitable for cellulolytic bacteria growth in the rumen. Since the main nitrogen source required for the growth of these bacteria is provided by  $\text{NH}_3\text{-N}$ , the  $\text{NH}_3\text{-N}$  content in the PV and BS groups was lower. In contrast, the SM group had a higher concentrate proportion and relatively faster protein degradation rates. Additionally, the lower ruminal pH inhibited microbial activity, reducing microbial protein synthesis and ultimately leading to  $\text{NH}_3\text{-N}$  accumulation in the rumen. Wang et al. [29] found that the  $\text{NH}_3\text{-N}$  content in the rumen fluid of the high-roughage diet group was significantly lower than that of the high-concentrate diet group, which is consistent with the results of this experiment. However, Kuerban · Tulake et al. [30] and Duan [31] reported that adding starch to diets or ruminal infusion of starch could reduce ruminal  $\text{NH}_3\text{-N}$  content, which is inconsistent with the results of this experiment. This discrepancy may be due to the small amount of starch added, resulting in a minor effect of ruminal pH on microorganisms.

### 3.2.3 Effects on VFA Content

VFA mainly originates from the fermentation of dietary carbohydrates entering the rumen and is an important energy substance for ruminants, providing energy for the growth of some rumen microorganisms [32]. The concentrate-to-roughage ratio is the main factor affecting VFA composition. As the concentrate proportion increases, the content of non-structural carbohydrates (NSC) increases, and NSC fermentation typically produces a high proportion of propionate and low proportions of acetate and butyrate. The results of this experiment showed that the PV group had the highest acetate and butyrate contents, the lowest propionate content, and the highest acetate/propionate ratio. This was due to the low concentrate proportion and low NSC content in the diet of the PV group, which is consistent with the results of Han et al. [33]. Studies have found that the optimal acetate/propionate ratio is 2.0–3.6, and values that are too high or too low will affect ruminal pH and diet digestibility, thereby influencing animal performance [34–35]. In this experiment, only the SM group had an acetate/propionate ratio outside this range, which may be one reason why the ADG of the SM group was lower than that of the other two groups.

Furthermore, this experiment found that the total VFA content of the SM group was extremely significantly lower than that of the other two groups. This may be because the VFA produced by fermentation within 0.2–5 h after feeding the high concentrate-to-roughage ratio diet in the SM group caused ruminal pH to drop below 6.0 in a short time, inhibiting cellulolytic bacteria growth and reducing the decomposition capacity for sugarcane bagasse, which already had low

digestibility. In contrast, the other two groups of Hu sheep consumed relatively less concentrate, which had a smaller impact on cellulolytic bacteria activity. Rumen microorganisms continued to decompose peanut vine or beanstalk to produce VFA, ultimately resulting in higher total VFA content in rumen fluid at 9 h than in the SM group. However, in this experiment, although the total VFA contents in the PV and BS groups were higher than that in the SM group, they did not cause ruminal pH to be lower than that of the SM group or lead to excessive lactic acid accumulation. This may be because the diets in the PV and BS groups had relatively higher NDF content, increasing chewing frequency in Hu sheep, and the resulting saliva had a certain buffering effect on ruminal pH. Additionally, it may be because the production rate of total VFA was relatively gradual, allowing alkaline substances more time for buffering. The specific reasons require further investigation.

### 3.3 Effects of Different Dietary Sources of Roughage on Serum Biochemical Parameters of Hu Sheep

Serum TP and ALB contents reflect the liver's capacity for protein synthesis, and their elevation is beneficial for improving metabolic level and immune function. ALT and AST are key indicators reflecting liver function, mainly existing in liver cells. When liver cells are damaged by inflammation, poisoning, etc., these enzymes are released into the blood, increasing their serum content. GLU content is an indicator reflecting the energy metabolism balance of the body, which is basically stable under liver regulation. When the concentrate proportion in the diet is too high and ruminal fermentation is too rapid, it leads to a sharp decline in ruminal pH, bacterial disintegration and death in the rumen, releasing endotoxins that cause inflammatory reactions and liver damage, increasing the activity of related enzymes in serum. However, this experiment found no significant differences in TP, ALB, GLU contents, or ALT, AST activities among groups, indicating that under isoenergetic and isonitrogenous conditions, the three different sources of roughage had no significant effect on liver metabolism of Hu sheep.

Urea is the end product of nitrogenous substance metabolism in the body, and ruminal NH<sub>3</sub>-N content is highly positively correlated with serum UN content [36]. Therefore, serum UN reflects, to some extent, the nitrogen utilization efficiency of Hu sheep for dietary nitrogen. Brown et al. [37] also demonstrated that serum UN content is inversely proportional to feed nitrogen utilization efficiency. The results of this experiment showed that serum UN content of Hu sheep in the SM group was higher than that of the other two groups, which is similar to the trend of NH<sub>3</sub>-N content in the rumen, indicating that diets with high-quality roughage can improve nitrogen utilization efficiency in the body. Cr is a metabolite of purines in the blood, mainly excreted by the kidneys, and is an indicator reflecting kidney health status. Under normal conditions, Cr content ranges from 53–106 μmol/L, and when the kidneys are damaged, blood Cr content increases. The results of this experiment showed that serum Cr

content in the SM group was higher than that in the other two groups, indicating that feeding sugarcane bagasse diets under the same energy and protein levels may have adverse effects on the kidneys of Hu sheep. Peanut vine, beanstalk, and sugarcane bagasse can all be utilized as roughage resources by Hu sheep. Compared with beanstalk and sugarcane bagasse, diets with peanut vine as the roughage source under isoenergetic and isonitrogenous conditions can improve the growth, ruminal fermentation function, and nitrogen utilization efficiency of Hu sheep, and reduce concentrate supplementation.

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