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Connection between Circadian Rhythm and Rumen Digestibility of Concentrate and Roughage in Sheep

Chuntao Zhang, Yan Tu, Tao Ma and Qiyu Diao *

Key Laboratory of Feed Biotechnology of Ministry of Agriculture and Rural Affairs, Feed Research Institute of Chinese Academy of Agricultural Sciences, Beijing 100081, China
* Correspondence: diaoqiyu@caas.cn; Tel.: +86-010-8210-6055

Abstract: This study investigated the effects of circadian rhythm on rumen nutrient digestibility using the nylon bag method and the digestibility characteristics of dietary nutrients during the daytime and at night. It also presents modification suggestions for evaluating the nutritional value of raw feed materials. The rumen nutrient digestibility was measured and investigated for correlation with circadian rhythm using the nylon bag method. We established a sheep-feeding program to determine the differences in rumen nutrient digestibility between the daytime and nighttime. The digestibility of 18 standard feed samples was investigated in 6 Hu sheep with ruminal fistulas (body weight: 33.59 ± 0.39 kg, 12 months old). Samples were incubated in the rumen for three periods: daytime (12 h, 6:00 AM to 6:00 PM), night (12 h, 6:00 PM to 6:00 AM), and the entire day (24 h, 6:00 AM to 6:00 AM). The activities of enzymes amylase, lipase and cellulase were higher during the day than at night. The rumen digestibility of organic matter was significantly higher during the daytime than at night compared to the digestibilities of dry matter (DM), crude protein (CP), and ether extract (EE). Among them, the rumen digestibility of DM and CP was higher, but the digestibility of EE was lower during the daytime than at night in cereal feed fed sheep. The rumen digestibility of DM was higher in roughage-fed sheep but lower in rice straw- and corn silage-fed sheep during the day than at night. Circadian rhythms correlate strongly with the activities of major digestive enzymes in the rumen and the rumen digestibility of nutrients. Moreover, the nylon bag method needs to be modified and improved to ascertain the appropriate time for placing the nylon bag in the rumen and thus establish a unified procedure.

Keywords: circadian rhythm; hu sheep; nutrition regulation; nylon bag; rumen; ruminal digestibility

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1. Introduction

The revolution of the planet Earth gives rise to the four seasons of the year, and the rotation of the Earth forms day and night. To survive, all creatures living on Earth must follow natural laws. Nature gives each life a schedule of work followed by rest on Earth, such that their physiological function, nutrient metabolism, and behavior show a 24 h circadian rhythm [1,2]. Currently, the mammalian circadian clock system is believed to be regulated by both the central and peripheral clocks [3–5]. The central circadian clock system is the pacemaker that forms a circadian rhythm network to connect scattered wave sources [6]. The rhythms of each contact point are independent but interact with each other to maintain homeostasis of the internal environment of the body. With advances in research, scientists have discovered that digestion, absorption, and metabolism of various nutrients do not display perfect synchrony during the day and night, with apparent differences in the spatial and temporal distribution in different organs and tissues. This series of activities is affected by the internal circadian clock of the organism as well as the collective influence of the external environment [7,8]. The most prominent and continuous signal is the photoperiod change in the environment caused by the rotation of the Earth. Martelli et al. [9] reported that a prolonged light phase improved the production performance of pigs. The results showed that appropriately prolonging the light duration may enhance the growth performance of fattening pigs. In addition, Wu et al. [10] found that feeding high-protein diets in the morning and low-protein diets at night could markedly improve the growth performance of pigs under similar light conditions with similar feed costs. Andretta et al. [11] adopted a rhythmic feeding pattern, i.e., feeding diets with different nutrient concentrations daily in the morning and evening. It resulted in a balanced nutrient supply and demand in the body, a 12% reduction in fecal and urine N emissions, an increase in N deposition, a 1.0% reduction in feed costs, and a considerable improvement in the carcass quality, demonstrating that the circadian clock system plays an extensive role in regulating metabolism and nutrient absorption. However, the influence of day and night on the digestion of nutrients in the body often is ignored [12,13].

Ruminants rely on complex rumen microflora to digest and absorb feed materials to ensure the availability of nutrients required for the growth and development of the body. When the environmental conditions, e.g., temperature, feeding time, and dietary morphology and composition, are constant, the structure and function of the rumen microflora, as well as the dynamics of the rumen environment, show a specific pattern [1]. Some studies have shown that day and night affect weight gain and nutrient digestibility, but the circadian influence on rumen digestibility remains unknown. Our previous studies have found that although the rumen is not directly exposed to light environment, its physiological and metabolic activities also have certain circadian characteristics, which are mainly manifested by rumen fermentation parameters, enzyme activities and microbial flora activities. When environmental conditions (eating time, diet form and composition, temperature, etc.) are changed, the rumen environment will also change. The rhythm of the corresponding rumen biological clock will also change, breaking the optimal pH of the rumen, resulting in the inhibition of the activity and catalysis of a series of digestive enzymes (amylase, protease, lipase, etc.) and non-digestive enzymes (cellulase, pectinase, etc.) [14]. The current study used the nylon bag method to compare the nutrient digestibility of 18 common feeds in the rumen during the day and night to investigate the effects of circadian rhythm on rumen nutrient digestibility and to establish a scientific sheep-feeding program.

2. Materials and Methods

The Chinese Academy of Agricultural Sciences Animal Ethics Committee (permission number: IFR-CAAS20200817) in Beijing, China, supported all experimental plans and procedures. The academy's standards for using animals in research were strictly followed.

2.1. Concentrate

The two types of concentrate were used in this test: (1) oil meals, including flaxseed meal, rapeseed meal, sunflower meal, cottonseed meal, soybean meal, and distillers dried grains with solubles; and (2) grain feeds, including wheat, barley, oat, and corn husk. We purchased the concentrate from the Graminifeng Company, and the nutrients in the concentrate are listed in Table 1.

Table 1. Test the concentrate organic nutrients levels (%, dry matter).

Items	Wheat	Barley	Oat	Soybean Meal	Cottonseed Meal	Rapeseed Meal	Flaxseed Meal	Sunflower Meal	DDGS	Corn Husk
DM	90.18	89.40	94.60	88.74	91.40	90.30	94.49	94.64	88.02	92.01
CP	12.59	10.94	19.72	47.50	56.97	39.04	36.46	29.88	28.53	9.47
EE	1.45	4.60	9.14	3.12	1.30	2.37	5.48	8.29	7.67	2.50
NDF	9.89	13.14	17.40	12.88	15.14	37.09	48.58	44.40	24.32	64.89
ADF	3.34	5.62	8.75	9.11	9.30	24.78	30.62	29.65	15.40	19.30

The full name of the abbreviation in the table is DM: Dry matter; CP: Crude protein; EE: ether extract; NDF: Neutral detergent fiber; ADF: Acid Detergent Fiber. The follow as the same. The concentrate in the table was purchased from Inner Mongolia Fuchuan Feed Technology Co., Ltd., Inner Mongolia., China. for the air-dried basis.

2.2. Roughage

Roughage includes crop straw (corn stalk, rice straw and soybean straw) and forage (alfalfa, oat grass, corn silage, peanut vine and *Leymus chinensis*). We purchased the roughage from the Beijing Sanshi Dairy Farm, Beijing., China. and the nutrients in the roughage are listed in Table 2.

Table 2. Test the roughage nutrient levels (%, dry matter).

Items	Corn Silage	Oat Grass	Peanut Vine	Alfalfa Hay	*Leymus chinensis*	Corn Stalk	Soybean Straw	Rice Straw
DM	92.34	94.60	90.60	90.72	90.93	92.57	90.82	91.28
CP	11.07	7.76	9.67	13.29	6.11	4.21	3.98	3.25
EE	1.79	2.99	3.21	3.19	2.93	1.62	1.79	2.47
NDF	45.16	49.74	42.81	53.50	65.93	64.89	64.55	69.39
ADF	22.89	26.73	32.83	42.18	40.62	41.42	39.02	42.48

The concentrate in the table was purchased from Inner Mongolia Fuchuan Feed Technology Co., Ltd., Inner Mongolia., China. for the air-dried basis.

2.3. Animals and Diets

Six Hu sheep with permanent rumen fistula (33.59 ± 0.39 kg, 12 months of age) were purchased in Huzhou, China. This study was carried out at the Nankou Pilot Base of the Chinese Academy of Agricultural Sciences in Beijing, China (40°21′19.7″ N, 116°45′53.3″ E; 49–74 m height) from November to December 2020 and lasted for 30 days. The area has a flat topography and four distinct seasons. To accurately perceive the influence of day and night, they were kept outdoors to more effectively study the impact of the natural diurnal cycle of day and night. The experimental sheep were fed outdoors in a single pen on a concrete floor, which prevented Hu sheep from eating anything other than diet, and it is given in equal amounts of concentrate and roughage, thus making the experiment more accurate.

The diet was fed twice a day in a concentrate-to-roughage ratio of 56:44 (Table 3), the first at sunrise and the second at sunset, each with 50% of concentrate and roughage fed throughout the day, and clean water was freely available. During the experiment, the daily feed intake and gain of Hu sheep were the same, and the difference was not significant.

Table 3. The composition and nutrient levels of diets (%, dry matter).

Items	Ingredients		
	Concentrate	*Leymus chinensis*	Diets
DM	94.77	90.93	93.08
OM	88.93	93.26	90.84
ME	11.52	5.93	9.06
CP	19.27	7.60	14.14
EE	3.43	2.45	3.00
NDF	18.46	65.90	39.33
ADF	14.34	40.62	25.90
Ash	11.07	6.74	9.16
Ca	0.81	0.50	0.71
P	0.49	0.08	0.31

Concentrate produced by the Graminifeng Company, Beijing., China. concentrate mainly consists of: corn, soybean meal, expanded soybean, cotton meal, corn DDGS, calcium hydrogen phosphate, stone powder, sodium chloride, lysine, vitamin A, vitamin D3, ferrous sulfate, copper sulfate and so on. *Leymus chinensis* purchased from Beijing Sanshi Dairy Farm, Beijing, China for the air-dried basis. The ratio of concentrate to *Leymus chinensis* was 56:44. The calculated metabolizable energy, and the rest are measured values.

2.4. Experiment Design

The nylon bag method was based on that described by Fu et al. [15]. A nylon cloth with a diameter of 300 mesh or 35~50 μm was cut into a rectangle of 17 × 13 cm, folded in half, and then used nylon or polyester thread to sew twice. A 12 × 8 cm nylon bag was prepared, with the edge of the bag sealed using a soldering iron or on the alcohol lamp to avoid silk. Then, the bag was numbered, rinsed with tap water, soaked for 50 min, dried at 65 °C to constant weight, and set aside.

Three of the six Hu sheep with fistula were used to test concentrate rumen digestibility, whereas the other three Hu sheep were used to test roughage material digestibility in the rumen. At the beginning of the experiment, we placed a nylon bag in the rumen abdominal sac of the sheep. There were three treatments: day rumen incubation of 12 h, the nylon bag was put into the rumen at 6:00 AM and taken out at 6:00 PM; night rumen incubation of 12 h, the nylon bag was put in at 6:00 PM and taken out at 6:00 AM the next day; and entire day and night rumen incubation of 24 h, the nylon bag was put in at 6:00 AM and taken out at 6:00 AM the next day. After each removal, the liquid was washed quickly until colorless, dried at 65 °C for 48 h, and weighed for 24 d after rehydration. The contents of conventional nutrients in the original samples and those at different time points were measured and the escape rate was estimated to correct the weight of feed samples and calculate the effective degradation rate.

After the formal test, rumen juice was collected from the oral cavity using a rumen catheter (ANSCITECH, Wuhan Collebo Animal Husbandry Technology Co., Ltd. Wuhan., China) two hours each before the morning and evening feed. The pH was measured using a portable pH meter (Testo 206-pH2, Testo AG, Lenzkirch, Germany) immediately after filtration through four gauze layers. The remaining samples were transferred to 15 and 20 mL centrifuge tubes and stored in −20 and −80 °C refrigerators at standby to assess rumen fermentation parameters and rumen microbial flora.

2.5. Measurements

2.5.1. Determination of Nutrients

The dry matter (DM) content in each sample was determined using the AOAC assay (method 930.15; AOAC; Feldsine et al. [16]). The N content was determined using the Kjeldahl method. The neutral detergent fiber (NDF) and acid detergent fiber (ADF) contents were quantified using the method described by Van Soest et al. [17]. Based on the reduction in DM weight upon extraction with diethyl ether in a Soxhlet extraction apparatus for 8 h, the ether extract (EE) was measured (method 920.85; AOAC; Feldsine et al. [16]). Calcium content was determined using an atomic absorption spectrophotometer (M9W-700, Perkin-Elmer Corp., Norwalk, CT, USA; method 968.08; AOAN; Feldsine et al. [16]). Phosphorus content was analyzed using the molybdovanadate colorimetric method (method 965.17; AOAC; Feldsine et al. [16]).

2.5.2. Determination of Rumen Enzyme Activity

Rumen juice was collected by mouth 2 h before morning feeding and 2 h before evening feeding to determine rumen enzyme activity. The activities of amylase, lipase and cellulase in rumen juice were determined using an enzyme-linked immunosorbent assay kit (Shanghai Kehua Circadian Engineering Co., Ltd. Shanghai., China) and an enzyme-labelled analyzer (ST-360) according to the manufacturer's instructions.

2.6. Statistical Analyses

The experimental data were sorted preliminarily using Excel, the rumen digestive enzyme activities were analyzed by one-way ANOVA using SPSS25.0 statistical software, and rumen degradation data were analyzed using SAS version 9.4. (SAS Institute Inc., Cary, NC, USA). The least significant difference test was used to assess the differences between the treatments. Duncan's multiple range test was used to compare statistical differences between the three treatments. Differences of $p < 0.05$ were considered significant, and $0.05 \leq p < 0.10$ was considered a tendency.

3. Results

3.1. Day and Night Characteristics of Rumen Enzyme Activity

The activities of the main digestive enzymes in rumen juice during the day and night are shown in Table 4. We measured the activities of the three major digestive enzymes in the rumen and observed that the actions of amylase, cellulase, and lipase were higher during the daytime than at night. The activities of rumen amylase and cellulase were significantly higher during the day than at night ($p < 0.05$) and were 13.71 and 31.07% higher, respectively, with a direct effect on nutrient digestibility.

Table 4. The activity of three main enzymes in rumen juice during the day and night (n = 15).

Items	Day	Night	SEM	*p*-Value
Amylase (U/g)	35.75	31.44	1.48	0.02
Lipase (U/g)	61.42	59.20	1.57	0.19
Cellulase (U/mL)	4.43	3.38	0.41	0.02

Rumen fluid collected 2 h before late feeding represents the daytime rumen environment, Rumen fluid collected 2 h before morning feeding represents the rumen environment at night. A value of $p < 0.05$ was considered statistically significant.

3.2. Rumen Digestibility of the Nutrients in Concentrate and Roughage in 24 h

Table 5 shows the 24 h rumen digestibility of the 10 nutrients in the two types of concentrate. The crude protein (CP) and EE digestibility in the concentrated feed showed the same trend as that of DM. In addition, the ruminal digestibility of DM was different based on the type of feed. The digestibility of grain raw materials was the highest, among which the digestibility of DM for wheat and barley exceeded 80%. The second was the meal protein feed and the last was the by-product feed, i.e., corn husk, related to their low soluble content.

Table 5. Rumen digestibility of concentrate nutrients during whole day (24 h) (%, dry matter).

Items	DM	CP	NDF	ADF	EE
Wheat	88.48 ^{a}	87.30 ^{a}	50.45 ^{a}	60.85 ^{a}	68.83 ^{c}
Barley	84.67 ^{b}	80.99 ^{b}	35.80 ^{c}	40.14 ^{d}	87.19 ^{a}
Oat	68.91 ^{c}	68.31 ^{c}	37.09 ^{c}	31.41 ^{e}	74.09 ^{b}
Soybean meal	55.36 ^{d}	40.67 ^{e}	40.37 ^{b}	41.55 ^{cd}	74.34 ^{b}
Cottonseed meal	49.59 ^{e}	50.95 ^{d}	32.00 ^{d}	43.77 ^{c}	46.96 ^{d}
Rapeseed meal	36.30 ^{g}	26.54 ^{g}	40.48 ^{b}	26.56 ^{f}	31.22 ^{f}
Flaxseed meal	33.50 ^{g}	23.20 ^{h}	49.12 ^{a}	54.26 ^{b}	33.48 ^{ef}
Sunflower meal	29.83 ^{h}	29.61 ^{f}	16.47 ^{f}	19.82 ^{h}	35.24 ^{e}
DDGS	40.46 ^{f}	31.36 ^{f}	17.33 ^{f}	27.35 ^{f}	27.88 ^{g}
Corn husk	24.98 ^{i}	42.95 ^{e}	25.52 ^{e}	22.81 ^{g}	17.19 ^{h}
SEM	1.45	1.39	1.28	1.44	1.38
p-value	0.89	0.79	0.69	0.78	0.70

There were 3 replicates for each feed material and 3 parallel replicates for each replicate. Different letters in the same column indicate significant differences ($p < 0.05$), while no letters indicate insignificant differences ($p > 0.05$).

Rumen digestibility of roughage throughout the day, i.e., for 24 h, is shown in Table 6. Compared to the rumen digestibility of crop straw DM (10.45%), the DM digestibility of alfalfa hay, corn silage, and oat grass was higher (38.75%). The DM digestibility of roughage positively correlated with the digestibility of NDF and ADF.

3.3. Day and Night Degradation Characteristics of Nutrients in Concentrate and Roughage

Table 7 shows the daytime and nighttime ruminal degradation of organic nutrients in concentrates. A distinct feature was that the rumen digestibility of feed nutrients was higher during the day (12 h) than at night (12 h). For example, the difference in mean rumen digestibility of major organic matter components, including DM (35.28 vs. 30.70%), CP (29.44 vs. 24.16%), and EE (32.14 vs. 25.18%), for 12 h during the daytime and nighttime each, indicated that daytime and nighttime directly affected nutrient digestibility in the rumen.

Table 6. Rumen digestibility of roughage nutrients 24 h (whole day) (%, dry matter).

Items	DM	CP	NDF	ADF	EE
Corn silage	57.81 [a]	75.44 [a]	30.86 [b]	19.72 [bc]	88.88 [a]
Oat grass	44.64 [b]	40.71 [b]	19.67 [c]	16.36 [d]	56.84 [c]
Peanut vine	44.60 [b]	42.05 [b]	48.22 [a]	36.51 [a]	73.14 [b]
Alfalfa hay	30.11 [c]	41.24 [b]	19.29 [cd]	21.12 [b]	74.21 [b]
Leymus chinensis	16.76 [d]	17.69 [d]	15.94 [ef]	15.71 [d]	28.46 [d]
Corn stalk	16.58 [d]	25.61 [c]	16.64 d[ef]	15.47 [d]	30.86 [d]
Soybean straw	7.74 [e]	24.67 [c]	14.16 [f]	17.34 [cd]	19.99 [e]
Rice straw	7.02 [e]	14.42 [e]	17.73 [cde]	16.28 [d]	16.36 [f]
SEM	0.42	1.31	0.83	0.94	1.32
p-value	0.40	0.69	0.77	0.32	0.78

There were 3 replicates for each feed material and 3 parallel replicates for each replicate. Different letters in the same column indicate significant differences ($p < 0.05$), while no letters indicate insignificant differences ($p > 0.05$).

Table 7. Rumen digestibility of concentrate nutrients during daytime and nighttime (%, dry matter).

Items		Day	Night	Difference (−,+)	SEM	*p*-Value
Wheat	DM	83.17	76.64	6.53	2.76	0.02
	CP	76.55	75.98	0.57	5.9	0.93
	EE	38.78	52.47	−13.69	8.65	0.15
Barley	DM	64.37	59.92	4.45	2.04	<0.01
	CP	42.74	38.72	4.02	6.36	0.54
	EE	70.84	75.66	−4.82	2.71	0.10
Oat	DM	57.33	54.60	2.73	4.25	0.45
	CP	61.60	55.81	5.79	7.97	0.48
	EE	65.29	65.25	0.04	6.95	1.00
Soybean meal	DM	47.96	40.38	7.58	3.48	0.05
	CP	31.82	21.76	10.06	4.76	0.06
	EE	63.04	56.73	6.31	2.49	0.02
Cottonseed meal	DM	42.53	42.66	−0.13	1.87	0.95
	CP	43.53	43.40	0.13	1.13	0.94
	EE	24.30	12.95	11.35	1.93	<0.01
Rapeseed meal	DM	32.60	24.83	7.77	1.16	<0.01
	CP	25.75	15.47	10.28	1.28	<0.01
	EE	21.95	14.92	7.03	1.89	0.06
Flaxseed meal	DM	26.84	23.78	3.06	4.56	0.51
	CP	20.71	20.48	0.23	0.23	0.97
	EE	22.58	15.24	7.34	4.7	0.16
Sunflower meal	DM	26.46	21.84	4.62	2.06	0.04
	CP	25.38	19.70	5.68	5.69	0.02
	EE	28.82	26.08	2.74	1.59	0.11
DDGS	DM	36.26	34.75	1.51	1.51	0.58
	CP	22.72	21.85	0.87	3.46	0.81
	EE	22.35	16.32	6.03	3.29	0.09
Corn husk	DM	20.20	14.48	5.72	1.85	0.07
	CP	31.12	20.98	10.14	0.73	<0.01
	EE	12.07	5.78	6.29	1.03	<0.01

There were 3 replicates for each feed material at each time point, and 3 parallel replicates for each replicate. A value of $p < 0.05$ was considered statistically significant.

Table 8 shows the effects of daytime and nighttime on roughage-nutrient rumen digestibility. It can be seen that there were differences in the ruminal digestibility of roughage DM, NDF and ADF for day and night reasons. The rumen DM digestibility for

12 h during the day was higher than that for 12 h at night, and the ruminal DM digestibility of alfalfa, *Leymus chinensis*, and peanut vine was increased for 12 h at night than that in the daytime. But the difference was not significant among straw of soybean, rice, oat, and corn stalk as well as corn silage for day and night. In addition, the rumen digestibility of NDF and ADF was related to DM digestibility. The ruminal digestibility of NDF in all forage samples was higher during the day than at night (range: 0.18–8.05); the ADF digestibility trend was similar to that of NDF.

Table 8. Rumen digestibility of roughage nutrients during daytime and nighttime (%, dry matter).

Items		Day	Night	Difference (−,+)	SEM	*p*-Value
Corn silage	DM	48.28	52.55	−4.27	2.05	0.06
	CP	63.02	65.71	−2.69	1.3	0.06
	NDF	19.40	17.64	1.76	2.21	0.44
	ADF	8.82	9.54	−0.72	2.58	0.79
	EE	83.64	83.96	−0.32	0.72	0.67
Oat grass	DM	39.53	40.12	−0.59	1.51	0.71
	CP	36.58	37.96	−1.38	1.2	0.27
	NDF	12.84	12.66	0.18	1.88	0.93
	ADF	9.87	9.51	0.36	2.56	0.89
	EE	53.86	43.91	9.95	1.24	<0.01
Peanut vine	DM	35.16	27.78	7.38	7.39	0.03
	CP	35.92	30.78	5.14	5.15	0.29
	NDF	29.37	27.10	2.27	6.51	0.75
	ADF	14.69	21.72	−7.03	9.59	0.52
	EE	69.02	64.42	4.60	1.74	0.02
Alfalfa hay	DM	29.70	24.65	5.05	2.01	0.03
	CP	40.15	34.96	5.19	1.85	0.01
	NDF	16.27	8.22	8.05	4.32	0.10
	ADF	16.05	8.47	7.58	2.49	0.01
	EE	61.04	57.10	3.94	1.55	0.02
Leymus chinensis	DM	14.28	11.98	2.30	2.3	0.06
	CP	16.56	12.12	4.44	4.44	<0.01
	NDF	4.48	2.81	1.67	1.07	0.15
	ADF	7.23	5.18	2.05	1.04	0.08
Corn stalk	DM	14.31	13.19	1.12	1.11	0.28
	CP	24.78	21.18	3.60	0.8	<0.01
	NDF	12.24	7.12	5.12	1.04	<0.01
	ADF	8.53	6.01	2.52	0.96	0.02
	EE	30.00	27.90	2.10	0.82	0.02
Soybean straw	DM	8.49	8.95	−0.46	0.92	0.62
	CP	19.93	18.07	1.86	0.89	0.06
	NDF	11.86	8.85	3.01	1.31	0.04
	ADF	11.15	7.86	3.29	1.00	0.01
	EE	19.54	8.01	11.53	1.36	<0.01
Rice straw	DM	4.19	6.26	−2.07	0.91	0.04
	CP	7.61	6.12	1.49	0.87	0.11
	NDF	12.66	12.20	0.46	0.46	0.66
	ADF	9.55	10.08	−0.53	0.77	0.50
	EE	9.81	6.85	2.96	1.07	0.02

There were 3 replicates for each feed material at each time point, and 3 parallel replicates for each replicate. A value of $p < 0.05$ was considered statistically significant.

Figure 1 shows the rumen nutrient digestibility of the concentrate for 12 h during the day and nighttime each and 24 h the entire day. It is seen that the digestibility of DM, CP, NDF, ADF, and EE was higher during the 12-h daytime than during nighttime. The difference in digestibility between the two phases ranged from 2.8 to 26.91%.

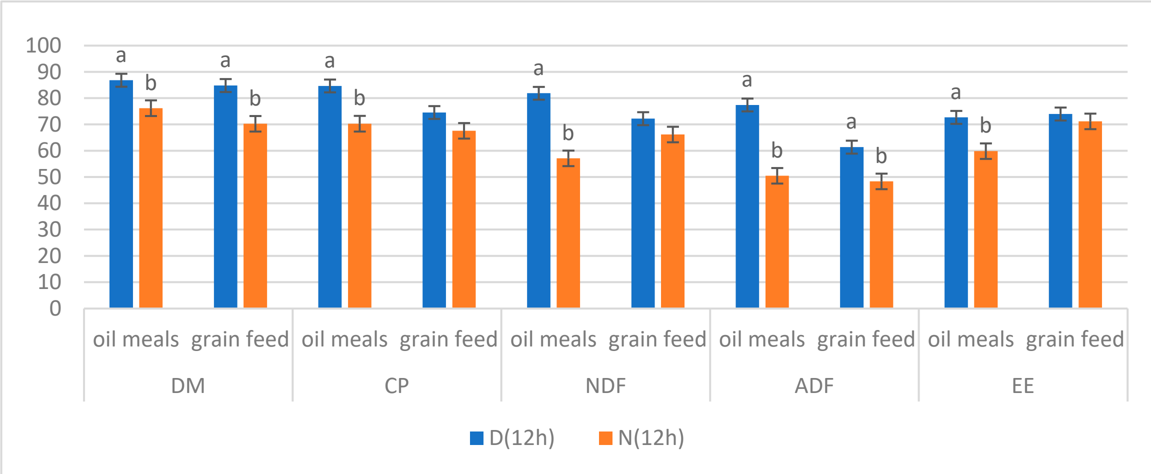

Figure 1. Rumen digestibility of concentrate nutrients during the day and night as a percentage of the whole day, respectively (%, dry matter basis). Different letters in the figure indicate significant differences ($p < 0.05$), while no letters indicate insignificant differences ($p > 0.05$).

Figure 2 shows the rumen nutrient digestibility in the roughage component for 12 h during the daytime or night and 24 h the entire day. As can be seen clearly, the characteristics of DM, NDF, and ADF digestibility in the rumen of crop straw day and night were the same as those of nutrient digestibility in concentrate feed, except for the DM of crop straw. The ruminal degradability of NDF and ADF of roughage varied considerably between the daytime and nighttime. The NDF of crop straw was 17.01% higher during the day than at night, whereas the NDF of roughage grass was 14.68% higher.

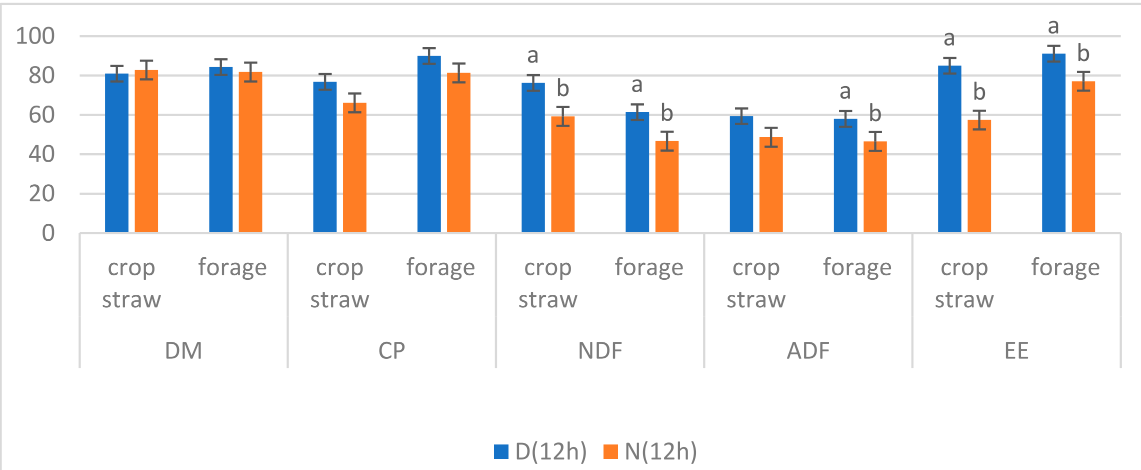

Figure 2. Rumen digestibility of roughage nutrients during the day and night as a percentage of the whole day, respectively (%, dry matter basis). We purchased the roughage from the Beijing Sanshi Dairy Farm., Beijing, China. Different letters in the figure indicate significant differences ($p < 0.05$), while no letters indicate insignificant differences ($p > 0.05$).

4. Discussion

4.1. Circadian Rhythm and Rumen Enzyme Activity

Photoperiod changes are the most prominent and long-lasting circadian rhythm signals, affecting the growth and development as well as the metabolism and behavior of livestock. The rumen is not exposed directly to changes in light and dark conditions, but its digestion and physiological metabolism exhibit specific circadian fluctuation characteristics. All the organs and tissues showed regular daily fluctuations. The secretion of major digestive enzymes in the small intestine at night is higher than in the large, but with relatively little enzymatic activity [18], and therefore the basal metabolic rate of the digestive organs is low at night. Only after eating in the morning the activity of digestive enzymes can be mobilized completely, manifested as gastric emptying rate and intestinal absorption rates are faster than at night [19]. Various enzymes secreted by rumen microorganisms are involved in the digestion of food in the rumen, and the number and activity of microorganisms determine the activity of digestive enzymes and the rate of food digestion [20]. Amylase activity directly reflects the number and activity of amylolytic bacteria in the rumen. Similarly, cellulase activity is directly related to the number and activity of cellulolytic bacteria in the rumen [21]. The growth rate of microorganisms is strongly correlated with nutrient uptake, and digestive activity is regulated by enzymes secreted by microorganisms [22].

The circadian rhythm clock has meaningful effects on body metabolism, animal health, and growth [23]. Gordon et al. [24] reported that sheep are more physiologically functioning between 8:00 AM and 12:00 noon than between 12:00 noon and 4:00 PM. In the morning, the oxygen supply in the sheep was sufficient, the energy supply of each organ was good, the enzyme activity was high, and digestion and metabolism were in the best state. The physiological state of sheep during the day and night is incomparable, and the diurnal characteristics of rumen-related digestive and metabolic enzyme activities have rarely been reported [14]. In this experiment, there was no significant difference in the daily feed intake of sheep, with the same standard diet fed during the day and night, excluding interference due to diet. It is necessary to explore their circadian degradation characteristics concerning rumen enzyme activity. The results showed that under consistent light conditions and feeding the exact nutrient amounts in the morning and evening resulted in a similar trend for amylase, lipase, and cellulase activities, with those of rumen amylase and cellulase being significantly higher during the day than at night, at 13.71 and 31.07%, respectively. Thus, the results indicated that the light and dark photoperiods during day and night, respectively, have a direct and practical effect on the activity of rumen digestive enzymes, and the lipase activity was also higher than at night, but the difference was not significant. As the nutrient content fed in one day remains unchanged, adjusting the nutrient proportion such as that of protein, fat, and fiber during the day and night and rationally distributing the nutrients will improve the ability of the animal body to absorb the nutrients and reduce the waste of nutrient feed.

In conclusion, our results show that maintaining the level of total nutrient supply unchanged and adjusting the ratio of nutrients such as protein, fat, and fiber during the day and night can increase the digestion and utilization of nutrients and reduce feed waste.

4.2. 24-h Digestibility of Nutrients in Concentrate and Roughage

Ruminants differ from monogastric animals because of their unique digestive physiological structure, specifically the large rumen. Different types of feed have been observed to have different digestibility in the rumen. Among them, grains had higher digestibility than cakes, and pastures had higher digestibility than straws, which was related to the structural characteristics of the feedstuff. Rumen digestibility of the concentrate is related to the protein content in the raw material. The higher the protein content, the higher the rumen digestibility. The CP digestibility of the grain feed was wheat > barley > oat, and the digestibility of DM showed the same trend. The CP digestibility of oil meals was cotton meal > soybean meal > sunflower meal > rapeseed meal > flax meal. Rapeseed meal contains glucosinolate, erucic acid, and oxazolidinethione, which inhibit the decomposition of rumen microorganisms to a certain extent; therefore, the ruminal digestibility of rapeseed meal is lower than that of other meal-based feeds [25]. The physical structure of the feedstuff also affects the rumen digestibility of nutrients [26]. The outer surface of the nutritional components of cereal feeds is covered with starch granules with a protein matrix, and the degree of binding between the protein matrix and starch granules significantly influences the contact speed between microorganisms and starch [27].

The digestibility of roughage grass was higher at 38.78% compared with that of crop straw at 10.45%. The DM digestibility revealed a positive correlation with the digestibility of other nutrients. In this experiment, roughage grass had higher protein and fat content, whereas crop straw had a higher fiber content.

4.3. Circadian Rhythms and Ruminal Degradation

Rumen degradation speed is a crucial factor that affects feed intake in ruminants. It can reflect the difficulty of feed digestion in the rumen. Consumption of large nutrient amounts by sheep at night can easily cause excess nutrients accompanied by insufficient utilization of these nutrients [28,29]. The circadian rhythm characteristics of rumen nutrient digestibility remain elusive, and there are very few research reports on this aspect. Therefore, 10 common concentrates and 8 roughages were selected in this experiment, and the nylon bag method was employed to investigate the effect of circadian rhythm on nutrient ruminal degradation. Using the rumen nutrient degradation rate as the index, we revealed the degradation characteristics of the rumen at 12 h during the day and 12 h at night. The results of this experiment show that the digestibility of various feed nutrients in the daytime was more effective than that at night, which may be because the intensity of animal activities and metabolism is enhanced during the day, while the activity level is weakened at night and the intensity of body consumption is reduced, with all organs and tissues dormant [30]. It is known that feed nutrient digestibility in the rumen is affected by many factors, including biology, chemistry, rumen environment, feedstuff itself, animal species, and the external environment. The feed raw material is usually incubated in the rumen for 4, 8, or 16 h using the nylon bag method, which often ignores the effects of the day, night, and feeding time. The results of 18 feed materials showed that the overall trend of the refined and coarse feeds was that the digestibility of DM, CP, NDF, ADF, and EE was significantly higher during the day (6:00 AM to 6:00 PM) than at night (6:00 PM to 6:00 AM). These results are consistent with the results reported by Diao [31] that the digestibility of nutrients in cow rumen feed raw materials during the day (8:00 AM to 8:00 PM) was significantly higher than that at night (8:00 PM to 8:00 AM). This may be due to the increased activity of metabolism-related enzymes during the day; all organs and tissues are active for better absorption and utilization of nutrients.

The results showed that the digestibility of DM in different feed materials during the day was higher than at night, related to the 'low running' state of the organs and tissues of the body during sleep [32]. The test results reveal that for the same basal diet and light conditions, the digestibility of grain feed and oil meals was significantly higher during the day than at night. However, the difference in digestibility between day and night was not significant for crop straw and forage roughage, which could be related to the structure, nutrient content, and feed antinutritional factors. CP digestibility in protein and cereal feed was significantly higher than that in roughage, possibly because their physical forms largely impacted the effective digestibility of nutrients. The flow rate of feed materials in the rumen is an important factor that affects the rumen degradation rate. Roughage had a low rumen degradation rate and long residence time in the rumen due to its higher lignin content [33].

The rumen is rich in cellulolytic bacteria that can decompose cellulose in the feed, and its degradation products (such as VFA) are the main energy source for animals [5]. The results also showed that rumen degradation rates of NDF and ADF in both crop straw and forage were higher during the day than at night. This result is inconsistent with the assumption that rumen microflora can fully decompose and digest fibrous substances in a quiet night. Based on physiology, animal organs and tissues are in an activated state during the day, and microbial activity is stronger than that at night, which stimulates the secretion and activity of ruminant enzymes; conversely, at night, the rate of feed degradation is lower.

Previously, Shaani et al. [1] conducted a crossover experiment in which rumen fluid was collected at 6:00 AM and 5:00 PM and fermented in vitro for 48 h. Rumen microorganisms were found to be metabolically active during the day. Furthermore, it has been found that the same person empties significantly faster at 8:00 PM than at 8:00 AM after eating the same food [18].

There is a saying in China: 'There is no sheep to cry during the day, and there is no grass at night without fat'. The digestive function of animals is relatively weak at night. As the roughage stays in the rumen for a long time, a sufficient amount should be ensured to make it into the pre-treatment state. When the rumen cellulase activity is strong in the daytime, the digestibility will be increased.

Lipid metabolism has also been observed to have obvious circadian rhythm characteristics that meet the energy demand of the animal body, which indicates that the circadian rhythm system plays a key role in regulating lipid metabolism. Loss of circadian rhythm function or disruption of circadian rhythm leads to an imbalance between feeding cycles and damage to lipid homeostasis [34]. An earlier experimental study showed that nocturnal animals fed a high-fat diet during the 12-h light phase gained significantly more body weight than mice fed the same diet during the 12-h dark phase of the night [23]. The test results further showed that the digestibility of EE in the feed was stronger during the day than that at night.

Hitherto, using the nylon bag method to evaluate the rumen degradation rate of feed nutrients, researchers usually set the rumen incubation time as 6, 8, 12, and 16 h. This approach ignores the effect of circadian rhythm on the degradation rate of nutrients in the rumen. Our experimental results revealed that there is a significant difference in the ruminal degradation rate of nutrients for the circadian rhythm. For example, rumen nutrient digestibility during 12 h in the daytime is significantly higher than that at night. It is necessary to ascertain the appropriate time of the day during the 24 h when placing nylon bags into the rumen will directly affect the accuracy of the rumen degradation rate.

5. Conclusions

1. The activities of amylase, lipase, and cellulase in rumen juice during the day (6:00–18:00) were higher than those at night (18:00–6:00) by 4.17%, 3.75%, and 31.07%, respectively.
2. The average rumen digestibility of the main organic matter components of the concentrate, such as DM (35.28% vs. 30.70%), CP (29.44% vs. 24.16%), and EE (32.14% vs. 25.18%), was higher during the day than at night. This indicates that daytime and nighttime directly affect rumen nutrient digestibility.

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