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Total darkness activated intestinal clock system and improved intestinal barrier function in growing rabbits

Yao Li[1], Jiali Chen[1], Fuchang Li[1] and Lei Liu[1*]

Abstract

The aim of study was to investigate the effects of dark environment on production performance, intestinal barrier function and clock-related gene expression in rabbits. Forty weaned rabbits with similar body weight (35-day-old) were randomly divided into 2 treatments (20 replicates per treatment, 1 rabbit per replicate: normal light group (12 L and 12 D) or total dark group (24 D). The experimental period lasted for 10 days, with an adaptation period of 3 days and a subsequent formal experimental period of 7 days. The results showed that feed-to-weight ratio of rabbits in total dark group was significantly decreased compared with normal light group. Dark treatment significantly increased gene expression of claudin-1, mucin1 in duodenum, occludin-1, claudin-1, zona occludens 1 (ZO1), junctional adhesion molecule 2 (JAM2) and interleukin 10 (IL10) in jejunum, claudin-1, mucin1, ZO1 and IL10 in ileum and clock, melatonin 1 A, melatonin 1B, and period1 in cecum compared with normal light group. Total dark treatment increased alpha diversity via increasing chao1 index, observed species index and faith_pd index of cecal flora. Total dark treatment significantly reduced percentage of *Deferobacterium* at phylum level in cecum, but significantly increased percentage of *Rumenococci* at genus level. There is an insignificant increasing tendency of acetic acid and propionic acid content of soft feces in total dark group. In conclusion, total dark treatment improves feed conversion efficiency in rabbits and activates cecum clock system, which increased diversity of bacterial flora and production of short-chain fatty acids, then increases intestinal barrier function.

Clinical trial number

Not applicable.

Keywords Growing rabbit, Total darkness, Intestinal barrier, Short-chain fatty acids, Clock system

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Introduction

Light is an important factor of life existence. The lighting system is inseparable from the actual production of animal husbandry. The reproductive activity of hares depends on the length of light duration.

Light regime of 16 L:8D reduces the seasonal effect and improves the annual performance of brood does. The activity of mice increased significantly in the dark, showing a regular rhythm. There was a tendency to increase body weight in the 0 L:24D photoperiod [15]. Domestic rabbits have nocturnal behavior with similar hares, and their retinas are rich in photoreceptor cells (e.g., optic rod cells and optic cones) [9]. Rabbits have peak activity at sunset and sunrise [19], and are better adapted under dark conditions. Long period of darkness increases intake, improves feed conversion, reduces blood glucose concentrations, and increases more social behaviors in young rabbits [7].

Intestinal barrier is mainly composed of mechanical, biological, chemical and immune barriers, which is involved in the nutrition metabolism and immunity [16]. Chronic weekly light/dark shifting alters the circadian phenotype of the colon tissue and results in colon leakiness and loss of colonic barrier function [21].

Photoperiod affects the gene expression of biological clock in testis and colon and the composition of intestinal flora [3]. The intestinal microbial population was regulated by the photoperiod. Specifically, the abundance of Clostridiales in the small intestine significantly increased when mice were exposed to prolonged darkness [26]. Although light is important for reproductive performance of brood does, light may be optional for production of growing rabbits. The present experiment was conducted to investigate the effects of darkness on the production performance, intestinal barriers and clock system of growing rabbits.

Table 1 Compositon and nutrient levels of the basal diet (air-dry basis) %

Ingredient	Content	Nutrient levels[2)]	Content
Corn	18.00	DM	88.43
Soybean meal	21.00	CP	17.65
Soybean oil	1.00	CF	14.33
Wheat bran	16.00	NDF	31.25
Peanut shell	5.00	ADF	18.27
Rice husk powder	9.00	EE	2.31
Peanut seedling	27.00	Ash	10.65
Premix[1)]	3.00	Ca	3.20
Total	100.00	P	0.42

[1)]Premix is provided per kilogram of ration: Vitamin A, 8,000 IU; Vitamin D_3,1,000 IU; Vitamin E, 50 mg; Vitamin K_3, 2.3 mg; Vitamin B_1, 1.75 mg; Vitamin B_2, 6.9 mg; Vitamin B_3, 28.45 mg; Vitamin B_5, 6.7 mg; Vitamin B_7, 2.75 mg; Vitamin B_9, 0.6 mg; vitamin B_{12}, 2.2 mg; choline, 420 mg; lysine, 1,500 mg; methionine, 1,500 mg; copper, 50 mg; iron, 100 mg; manganese, 30 mg; magnesium,150 mg; iodine, 0.1 mg

[2)]Values were calculated

Materials and methods

Animals and experimental designs

Forty weaned IRA female rabbits (35-day-old) with similar body weight were randomly divided into 2 treatments (20 replicates per treatment, 1 rabbit per replicate): normal light group (12 L (100 lx) and 12 D) and total dark group (24 D). The experimental period lasted for 10 days, with an adaptation period of 3 days and a formal experimental period of 7 days. All animals used in this study were privately owned by the farm. All rabbits were reared in single cages and had free access to feed and water during the rearing period. Dark group of rabbits with opaque curtains in the rearing space, twice-daily replenishment of feed boxes and waterers. Under illuminated conditions, tasks such as weighing the feed boxes, distributing it into containers, and replenishing waterers were conducted. A screen mesh with aperture of 5 mm was arranged undermeath the cage for fecal collection.

The composition and nutrient levels of diet are shown in Table 1. The growth performance and feed intake of rabbits were measured throughout the experimental period. All experimental procedures were approved by the Animal Care and Use Committee of Shandong Agricultural University (No. 2024092), and the "Guidelines for Experimental Animals" of the Ministry of Science and Technology (Beijing, China).

Analysis of production performance

At the beginning of the experiment, the initial body weight and daily feed intake were recorded. At the end of the experiment, the terminal weight was recorded, and the average daily feed intake (ADFI) and average daily gain (ADG) were calculated. ADFI = total feed intake/test days. ADG = (terminal weight - initial weight)/test days. Feed-to-weight ratio (F/G) = ADFI/ADG.

Analysis of slaughter performance

At the end of the trial, 6 rabbits from each treatment were randomly selected, slaughtered by cervical dislocation, and exsanguinated. Carcass, liver and spleen were weighed. The relative weight = tissue or organ weight/pre-slaughter live weight × 100%.

Analysis of intestinal morphology

After the rabbits were slaughtered, the tissue samples of duodenum, jejunum and ileum were collected for further analysis. These samples were processed, embedded, and stained according to the procedures of Liu et al. [12]. In brief, 2 pieces were fixed with 4% paraformaldehyde, which was replaced with fresh 4% paraformaldehyde every 7 days. The sections were stained with haematoxylin-eosin. The tissue sections were prepared and stained by Wuhan Saiwei Biotechnology Co., Ltd.

Analysis of gut microbiology

16 S rRNA gene amplicon sequencing analysis and macrogenomic sequencing are currently the main high-throughput sequencing-dependent methods for the study of gut microorganisms, with 16 S rDNA sequencing allowing for precise quantification of all strains of gut microorganisms and macrogenomic sequencing allowing for the enrichment of important coding genes or pathways that can be identified [20]. Diversity analysis is an important part of amplicon sequencing analysis, α diversity mainly measures the abundance and diversity of microbial communities in a single sample. In addition, functional prediction of microbial communities can be realized based on amplicon sequencing results, and commonly used software includes PICRUSt2 [4] and Tax4Fun [1]. Macrogenome-wide analysis is a high-throughput sequencing analysis of the total DNA of the microbiota in a sample, including bacteria, archaea, protozoa, viruses and fungi.

Quantification of short-chain fatty acids (SCFAs)

The content of short-chain fatty acids in soft feces was determined by gas chromatography-mass spectrometry (GC-2010 Plus, Shimadzu Corporation, Japan). 0.1 g of the sample was taken in a 10 mL centrifuge tube, 2 mL of water (10% aqueous phosphate solution) was added, stirred to make the sample homogeneous, 2 mL of extract was added to extract for 5 min, and then the sample was centrifuged at 2400×g for 15 min. After centrifugation, the ether phase was taken, and then 1 mL of ether to extract the sample for two times with the same steps was added, then the three times of extraction were combined, and then the volume was set and put into the GC-MS instrument for determination and analysis [30].

The chromatographic conditions were: injection temperature 250 ℃, injection volume 1 μL; carrier gas (N2), purge flow rate of 3.0 mL/L, shunt ratio of 8:1, pressure 54.2 kPa. The heating program: from 80 ℃ to 190 ℃ at 10 ℃/min and maintained for 0.50 min; and then 40 ℃/min to 230 ℃ and maintained for 4 min, and the whole program took 16.5 min. The whole program took 16.5 min, and the test was stopped at 17 min.

Analysis of gene expression

Total RNA extraction and quantitative reverse transcription polymerase chain reaction (qRT-PCR) were conducted as described previously [13]. The concentration and purity of RNA were quantified by a biophotometer (Roche, Basel, Switzerland). Glyceraldehyde 3-phosphate dehydrogenase (**GAPDH**) was used as the internal reference for the target genes. The primer sequences are shown in Table 2. PCR data were analyzed using the $2^{-\Delta\Delta CT}$ method [18].

Data analysis

All the data were analyzed with a T test by using the Statistical Analysis Software (SAS version 8e, Cary, NC) general linear model (**GLM**). All the values were expressed as mean ± SEM. Differences were considered significant at $P < 0.05$.

Results

Effect of total dark treatment on growth performance and slaughter performance of rabbits

F/G of rabbits in total dark group was significantly reduced compared to normal light group ($P < 0.05$, Table 3). ADFI and ADG were not significant different between two groups ($P > 0.05$). The indices of small intestine, cecum, liver and spleen as well as the rate of total and semi-clean carcasses were not significant difference between two groups ($P > 0.05$).

Table 2 Primer sequences of related genes

Genes	GenBank accession No.	Primer sequences(5′–3′)	Product size/bp
GAPDH	NM_001082253	F: TGCCACCCACTCCTCTACCTTCG R: CCGGTGGTTTGAGGGCTCTTACT	163
Occludin–1	XM_008262318	F: CTTGCCTGGGACAGAACCTA R: AGCCATAACCGTAGCCGTAA	121
Claudin–1	NM_001089316	F: GGAGCAAAAGATGCGGATGG R: AATTGACAGGGGTCAAAGGGT	93
ZO1	XM_051822263.1	F: CGGATGGTGCTACAAGTGATGA R: CGCCTTCTGTATCTGTGTCTTCA	138
Mucin1	L41544.1	F: TATACCGCAAGCAGCCAGGT R: GCAAGCAGGACACAGACCAG	135
JAM2	XM_017346699.2	F: ATATCGCAGGTGTCCTGGAA R: GAGCATAGCACACGCCAAG	122
IL10	NM_214041.1	F: CACTGCTCTATTGCCTGATCTTCC R: AAACTCTTCACTGGGCCGAAG	136

Table 3 Effect of total dark treatment on growth performance ($n=20$) and slaughter performance ($n=6$) of rabbits

sample	Treatment group		*P*-value
	Normal Light	Total Dark	
ADG(g)	60.20 ± 2.48	63.06 ± 2.88	0.4671
ADFI (g)	206.17 ± 6.32	197.22 ± 5.32	0.3005
F/G	3.44 ± 0.08[a]	3.15 ± 0.10[b]	0.0396
Liver index (%)	2.75 ± 0.12	2.99 ± 0.13	0.1928
Spleen index (%)	0.07 ± 0.00	0.06 ± 0.00	0.6195
Small intestine index (%)	3.45 ± 0.10	3.59 ± 0.11	0.4048
Cecum index (%)	1.20 ± 0.04	1.26 ± 0.06	0.3631
Total clear-bore rate (%)	48.82 ± 0.72	47.21 ± 0.52	0.0994
Semi-clearance rate (%)	52.58 ± 0.69	51.19 ± 0.53	0.1428

In the same row, values with different small letter superscripts mean significant differences ($P>0.05$). The same as below

Effects of total dark treatment on intestinal morphology and expression of intestinal barrier-related genes of rabbits

Total dark treatment had no significant effect on the intestinal morphology of duodenum, jejunum and ileum in rabbits ($P>0.05$, Table 4). Total dark treatment significantly increased the gene expression of claudin-1 and mucin1 in duodenum, occludin-1, claudin-1, Zona Occludens 1 (**ZO1**), Junctional adhesion molecule 2 (**JAM2**) and interleukin-10 (**IL10**) in jejunum and claudin-1, mucin1, ZO1 and IL10 in ileum compared with control group ($P<0.05$, Fig. 1). The gene expression of occludin1, ZO1, JAM2 and IL10 in duodenum, mucin1 in jejunum and occludin1 and JAM2 in ileum were not significant different between two groups ($P>0.05$).

Effect of total dark treatment on the expression of clock related genes in rabbit cecum

As shown in Fig. 2, total dark treatment significantly increased the gene expression of CLOCK, melatonin 1 A (**MT1**), melatonin 1B (**MT2**) and period1 (**per1**) in cecum compared with normal light group ($P<0.05$). Total dark treatment had no significant effect on bmal1, and cryptochrome (**CRY1**) gene expression in cecum ($P>0.05$).

Effects of total dark treatment on the alpha diversity of intestinal flora of rabbits

As shown in Table 5, total dark treatment increased the alpha diversity via increasing the chao1 index, observed species index and faith_pd index of cecal flora of rabbits compared with normal light group. Total dark treatment had no effect on the shannon index, simpson index, pielou's evenness index and good's coverage index of intestinal flora of rabbits ($P>0.05$).

Changes in the composition and structure of intestinal flora

At phylum level, total dark treatment significantly reduced the percentage of *Deferobacterium* phylum in cecum compared with normal light group ($P<0.05$, Table 6). There were no significant different in the proportion of *Firmicutes*, *Bacteroidota*, *Verrucomicrobiota*, *Desulfobacterota*, *Proteobacteria*, *Cyanobacteria*, *Actinobacteriota*, *Synergistota*, *Campylobacterota*, *Patescibacteria*, *Tenericutes*, *Spirochaetota*, *Fusobacteriota*, and *Acidobacteriota* between two groups ($P>0.05$). At genus level, the percentage of *Rumenococci* in cecum was significantly increased after total dark treatment ($P<0.05$, Table 7). There were no significant different in *Akkermansia*, *Lachnospiraceae*, *Eubacterium*, *Clostridium*, *Phascolarctobacterium*, *Bacteroides*, and *Parasutterella* between two groups ($P>0.05$).

Effect of total dark treatment on soft feces production and short-chain fatty acid content in soft feces in rabbits

As shown in Table 8, total dark treatment significantly increased the propionic acid content in soft feces compared with normal light group ($P<0.05$). Total dark

Table 4 Effect of total dark treatment on intestinal morphology of rabbits ($n=6$)

	Item	Treatment group		*P*-value
		Normal Light	Total Dark	
Duodenum	Chorionic height (μm)	1052.00 ± 28.63	1129.57 ± 41.06	0.2041
	Depth of crypts (μm)	184.70 ± 14.39	178.30 ± 19.94	0.8212
	Intestinal wall thickness (μm)	136.68 ± 2.42	161.72 ± 12.88	0.161
	V/C[1)]	5.84 ± 0.63	6.69 ± 0.68	0.4112
Jejunum	Chorionic height (μm)	678.32 ± 91.28	711.26 ± 67.25	0.7902
	Depth of crypts (μm)	192.90 ± 8.72	177.71 ± 18.23	0.4461
	Intestinal wall thickness (μm)	213.38 ± 14.58	195.33 ± 22.93	0.5107
	V/C	3.58 ± 0.61	4.09 ± 0.43	0.5415
Ileum	Chorionic height (μm	694.86 ± 44.23	726.37 ± 27.64	0.5464
	Depth of crypts (μm)	142.30 ± 6.99	159.10 ± 11.36	0.2624
	Intestinal wall thickness (μm)	259.18 ± 12.79	262.80 ± 8.08	0.8097
	V/C	4.98 ± 0.51	4.69 ± 0.41	0.6731

V/C = villus height/crypt depth

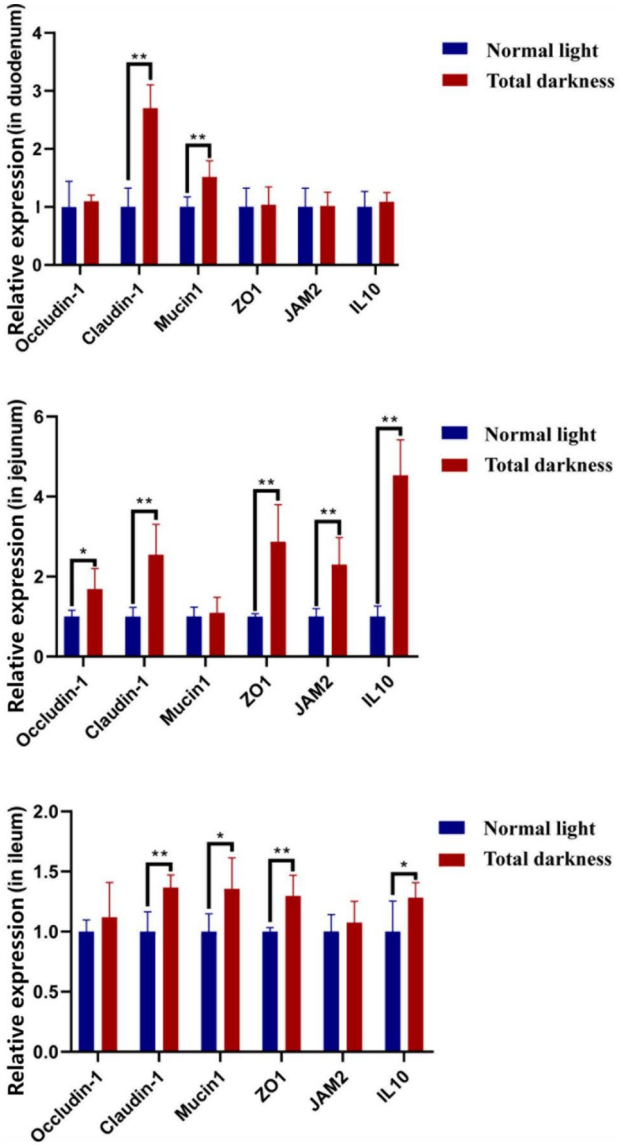

Fig. 1 Effect of total dark treatment on the gene expression related to the intestinal barrier function in rabbits ($n=6$). * Indicates significant difference ($P<0.05$), ** indicates extremely significant difference ($P<0.01$)

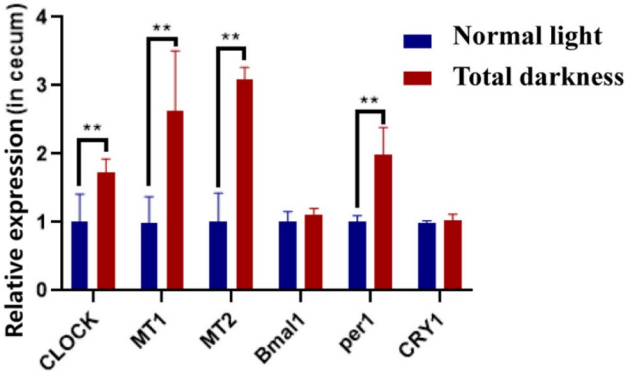

Fig. 2 Effect of total dark treatment on clock-related gene expression in rabbit cecal ($n=6$). ** indicates extremely significant difference ($P<0.01$)

Table 5 Effect of total dark treatment on the diversity of cecal microbiota in rabbits ($n=6$)

Sample	Treatment group		*P*-value
	Normal Light	Total Dark	
Chao1	1075.82 ± 30.29	1160.79 ± 21.46	0.0514
Faith_pd	46.20 ± 1.48[b]	53.49 ± 1.89[a]	0.0162
Goods_coverage	1.00 ± 0.00	1.00 ± 0.00	0.7986
Observed_species	1061.34 ± 28.96[b]	1150.86 ± 20.72[a]	0.0362
Pielou_e	0.82 ± 0.01	0.81 ± 0.01	0.8645
Shannon	8.22 ± 0.12	8.28 ± 0.17	0.7635
Simpson	0.99 ± 0.00	0.99 ± 0.00	0.4262

Table 6 Effect of total dark treatment on the proportion of cecal flora at phylum level (%) ($n=6$)

Item	Treatment group		*P*-value
	Normal Light	Total Dark	
Firmicutes	66.69 ± 4.43	67.27 ± 2.51	0.9111
Bacteroidota	15.30 ± 1.83	12.20 ± 2.52	0.3419
Verrucomicrobiota	12.03 ± 4.18	13.59 ± 4.05	0.7938
Desulfobacterota	1.78 ± 0.35	1.83 ± 0.13	0.8923
Proteobacteria	1.18 ± 0.09	0.91 ± 0.14	0.1172
Cyanobacteria	0.84 ± 0.10	1.26 ± 0.36	0.2896
Actinobacteriota	0.83 ± 0.07	0.71 ± 0.08	0.2620
Synergistota	0.08 ± 0.07	0.43 ± 0.18	0.1060
Campylobacterota	0.28 ± 0.09	0.57 ± 0.15	0.1348
Patescibacteria	0.32 ± 0.06	0.26 ± 0.09	0.5829
Deferribacterota	0.032 ± 0.007[a]	0.005 ± 0.002[b]	0.0032
Tenericutes	0.007 ± 0.007	0.002 ± 0.002	0.4835
Spirochaetota	0.005 ± 0.003	0.007 ± 0.003	0.7342
Fusobacteriota	0.003 ± 0.002	0.002 ± 0.002	0.5490
Acidobacteriota	0.002 ± 0.002	0.003 ± 0.002	0.5490

Table 7 Effect of total dark treatment on the proportion of cecall flora at genus level (%) ($n=6$)

Item	Treatment group		*P*-value
	Normal Light	Total Dark	
Akkermansia	12.17 ± 4.26	13.86 ± 4.16	0.7831
Ruminococcus	5.93 ± 0.83[b]	9.03 ± 0.72[a]	0.0180
Lachnospiraceae	4.05 ± 0.53	3.38 ± 0.75	0.4811
Eubacterium	1.55 ± 0.41	0.96 ± 0.30	0.2709
Clostridium	0.87 ± 0.09	0.84 ± 0.13	0.8848
Phascolarctobacterium	0.61 ± 0.27	0.68 ± 0.24	0.8454
Bacteroides	0.69 ± 0.13	0.93 ± 0.39	0.5635
Parasutterella	0.46 ± 0.07	0.46 ± 0.09	0.9888

treatment significantly decreased the hexanoic acid content in soft feces compared with control group ($P<0.05$). Dark treatment had no significant effect on total soft feces production, total hard feces production, acetkc acid, isobutyric acid, butyric acid, isovaleric acid and valeric acid ($P>0.05$).

Table 8 Effect of total dark treatment on feces production and the content of short-chain fatty acids in soft feces ($n=6$)

Item	Treatment group		*P*-value
	Normal Light	Total Dark	
Total soft feces production (g/DM)	25.68 ± 2.42	22.32 ± 1.79	0.2792
Total hard feces production (g/DM)	78.28 ± 4.51	73.76 ± 4.31	0.4782
Acetic acid (mg/g)	9.67 ± 3.12	19.30 ± 1.90	0.0917
Propionic acid (mg/g)	0.94 ± 0.13^{b}	1.40 ± 0.06^{a}	0.0831
Isobutyric acid (μg/g)	76.84 ± 40.17	59.00 ± 1.33	0.7006
Butyric acid (mg/g)	2.67 ± 0.14	2.40 ± 0.18	0.3574
Isovaleric acid (μg/g)	67.67 ± 30.00	62.00 ± 2.33	0.8679
Valeric acid(μg/g)	241.34 ± 76.67	295.50 ± 10.17	0.5562
Hexanoic acid (μg/g)	420.00 ± 36.67^{a}	221.50 ± 8.50^{b}	0.0341
Total short-chain fatty acids (mg/g)	14.08 ± 3.20	23.73 ± 2.16	0.1294

Effect of total dark treatment on feed intake and feces production in rabbits in different times of the day

Ten rabbits were randomly selected from each treatment group, and feed intake, soft feces production and hard feces production were weighed and recorded for each one per hour. Total dark treatment significantly decreased feed intake and hard fecal production at 18:00–0:00 ($P<0.05$). Total dark treatment significantly decreased soft fecal production at 12:00–18:00 ($P<0.05$) (Fig. 3).

Discussion

Total darkness improved the feed conversion of growing rabbits

Light has an important role in regulating the physiological functions of animal. Light is closely related to the female rabbit reproduction. Short light, especially continuous darkness, inhibited the development of the reproductive system and delayed sexual maturity in rabbits [31]. However, light condition for a long time seriously harm the production performance of young rabbits [22]. In our study, total dark treatment increased the feed conversion, which is in constant with the previous study indicating that continuous darkness increased growing rabbit feed conversion and social and had better animal welfare [7]. However, these findings are incongruent with a study conducted on broiler chickens, which demonstrated that body weight and feed intake exhibited a linear response to the duration of light exposure, implying that extended light periods lead to increased body weight and feed intake [28].Light duration was negatively correlated with feed conversion ratio in yellow feather broilers, with increased light duration causing an increase in feed intake and decrease in feed conversion ratio [27]. These results imply that light duration have different effect on production performance in rabbits and poultry. In addition, the feeding time or feces (hard fece and soft fece) production time were also altered by dark duration, indicating that rabbit appetite, digestion and absorption process were effected by total darkness.

Total darkness improved the intestinal barrier function of growing rabbits

The intestinal barrier plays a crucial role in facilitating the absorption of nutrients, electrolytes, and water from the intestinal lumen into the bloodstream while maintaining intestinal mucosal homeostasis [17]. It comprises mechanical, chemical, microbial, and immune barriers. The mechanical barrier, which includes intestinal epithelial cells, tight junctions, and the basement membrane, regulates the selective absorption and excretion of substances while preventing pathogen infiltration [18]. Transmembrane proteins such as occludin, claudins, and JAM form selective barriers with neighboring cells through their extracellular domains, regulating adhesion, motility, and permeability, while their intracellular domains interact with cytoplasmic ZO proteins [6]. Claudins are defined as critical determinants of tight junction (**TJ**) permeability, with the tightness of TJs being regulated by the quantity, composition, and mixing ratio of claudins. Occludin also plays a significant role in the maintenance and assembly of TJ proteins. In mice with altered circadian rhythms, the expression of TJ proteins Occludin and claudin-1 in the colonic epithelium exhibits daily oscillations. Furthermore, circadian rhythms modulate intestinal permeability, which is negatively correlated with the expression levels of Occludin and claudin-1 [10].

The results of this study demonstrated that while total darkness had no significant effect on intestinal morphology, it upregulated the gene expression of claudin-1 in the duodenum; claudin-1, ZO1, and JAM2 in the jejunum; and claudin-1 and ZO1 in the ileum, indicating an improvement in the intestinal mechanical barrier function in rabbits under total darkness conditions.

Mucin1, a transmembrane-like glycosylated macromolecules, is abundantly expressed in mammalian epithelial cells, contributes to the formation of the mucus chemical barrier and has a protective function against infections [11].

This study demonstrated that total darkness upregulated the gene expression of mucin1 in the duodenum

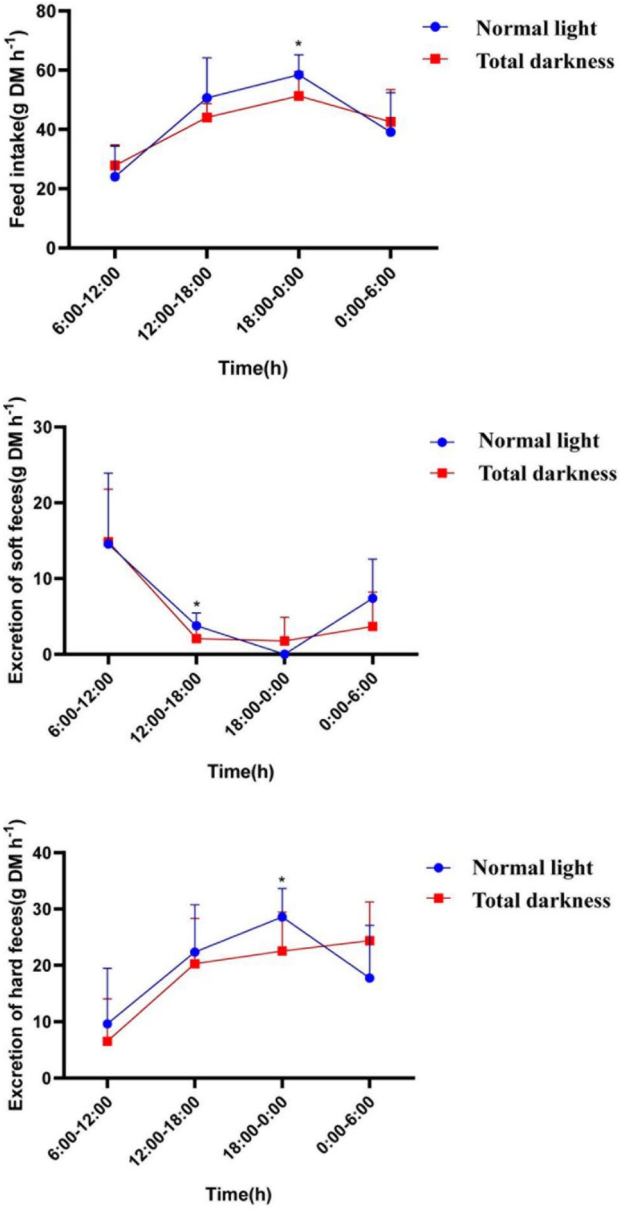

Fig. 3 Effect of total dark treatment on feed intake, excretion of soft feces and excretion of hard feces of rabbits at different times of the day ($n = 10$). * Indicates significant difference ($P < 0.05$)

and ileum compared to the normal light group, indicating a potential improvement in the chemical barrier and protective function against infections in rabbits. Additionally, the impact of total darkness on the intestinal mechanical and chemical barrier functions may be linked to increased cecal short-chain fatty acid (SCFA) concentrations. SCFAs play a critical role in stimulating and protecting intestinal barrier function, supplying metabolic energy, and acting as vital nutrients for mucosal health [5].

Gut microbiota plays critical roles in regulating intestinal homeostasis and integrity. Imbalance of intestinal microecology will cause the colonization and invasion of pathogenic microorganisms in intestine. Intestinal flora can inhibit the colonization and growth of pathogenic bacteria by secreting antimicrobial substances and promoting mucus secretion [14]. In addition, intestinal flora could produce lots of SCFAs by fermentation of partially and non-digestible polysaccharides of carbohydrates and decrease in intestinal ph and redox potential.

In our study, total darkness could increase the proportion of *Ruminococcus*, which is positively associated with acetate and propionate yield [8]. The increased acetic acid and propionic acid levels in soft feces are associated with the increased *Ruminococcus* proportion in total dark treated rabbits. SCFAs are known to have wide-ranging impacts on host physiology, such as maintaining health via regulation of the immune system and maintenance of the epithelial barrier. The soft faeces produced by microbial fermentation in cecum are re-ingested, and SCFAs and other nutrients of soft faeces were absorbed. Therefore, stimulating acetic acid and Propionic acid production by the cecal microbiome in total darkness could be useful for sustaining health and treating diseases [25].

Total darkness activated intestinal clock system of growing rabbits

Intestinal flora is also effected by circadian rhythm. The circadian clock system and the gut microbiota exhibit a sophisticated bidirectional regulatory interplay. Research indicates that light exposure indirectly impacts the intestinal microbiota in mice, leading to changes in the intestinal clock system and functional gene expression. These alterations subsequently influence host-microbiota interactions, ultimately shaping the structure and functionality of the gut microbial community.

Chronic circadian disruption causes alterations in the abundance of gut flora and clock gene expression in mice [26]. The fecal microbial community of clock-mutant mice had lower taxonomic diversity relative to wild-type mice [23]. Photoperiod influences the abundance of specific bacteria in the intestine, leading to differences in the functional characteristics of the gut microbiota in broilers.

This is consistent with our current findings, which demonstrate that rabbits housed in total darkness exhibit higher gut microbiota diversity, as indicated by increased levels of Chao1, Faith_pd, and Observed_species indices [24]. The PCoA results demonstrated that PCoA1 and PCoA2 explained 10.76% and 9.78% of the variation in the cecal microbiota community, respectively, with a cumulative contribution of 20.54% to the total variation. In the plot, distinct colors represent the different treatment groups (D and N), and ellipses denote the range of within-group variability. The significant separation between the treatment groups (D and N) in the PCoA

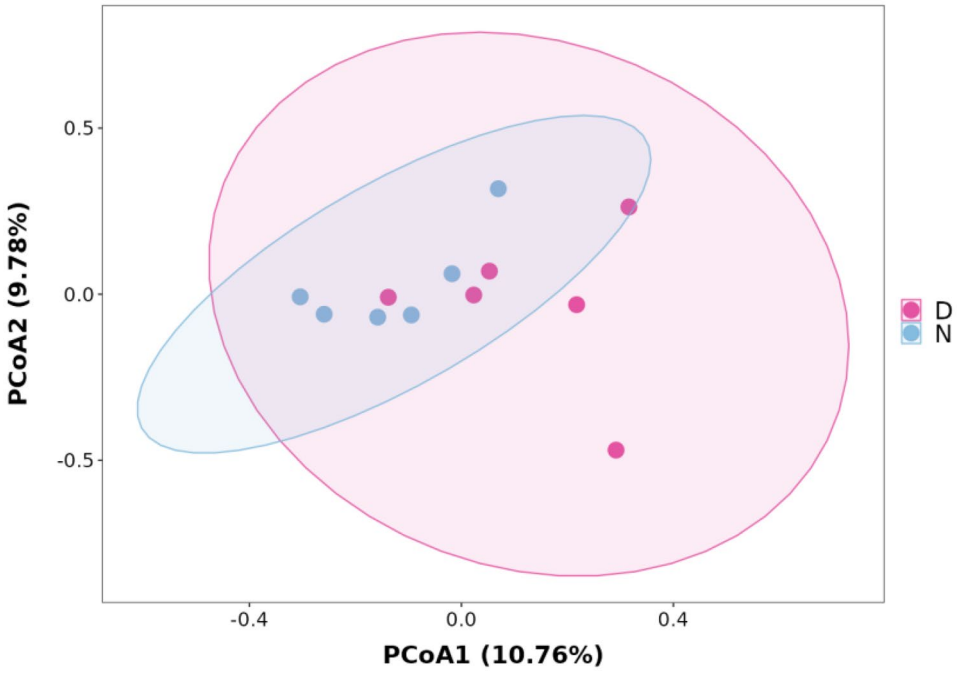

Fig. 4 Principal Co-ordinates Analysis of cecal flora in rabbits

plot indicates that darkness treatment had a notable impact on the microbial community structure in the cecal content of meat rabbits (Fig. 4).

Light resets the circadian phase by rapidly inducing the expression of circadian clock genes. The circadian rhythm encompasses core circadian genes and peripheral clock genes, such as CLOCK, BMAL1, PER, and CRY. The expression of these genes is influenced by light, which is modulated through specific retinal receptors. The article highlights that prolonged exposure to darkness results in the sustained extension of circadian gene expression, whereas light can recalibrate the circadian rhythm [2]. In mice, the PER protein can inhibit the transcription of the CLOCK gene through a negative feedback regulatory mechanism; BMAL1, which is the binding partner of CLOCK in mice, forms a heterodimer with CLOCK (CLOCK-BMAL1) that facilitates positive feedback regulation of gene transcription [29].

Our results showed that total darkness could activate clock system by up-regulating gene expression of CLOCK, MT1, MT2, and per1 in the cecum, which may be one of the contributing factors to the alteration of the gut microbiota diversity.

Conclusion

This study demonstrates that darkness treatment significantly improves feed conversion efficiency and intestinal barrier function in rabbits by activating the cecal circadian clock system and modulating gut microbiota and SCFA production. These findings highlight the novel role of photoperiod manipulation in enhancing gut health and metabolic efficiency, offering potential implications for optimizing animal husbandry practices and improving livestock productivity.

Thesis innovation and follow-up prospects

Currently, research on light primarily focuses on the effects of light intensity, photoperiod, light color, and light sources on the reproductive performance of livestock, while studies on the gut microbiota and cecal fermentation in rabbits are limited. In light of this, the present study investigated the effects of darkness treatment on the production performance, intestinal barrier function, cecal microbial fermentation, and soft feces production patterns in rabbits. Future research should further explore how to optimize host circadian function by modulating the gut microbiota and develop novel therapeutic strategies to address health issues arising from circadian rhythm disruptions.

Abbreviations

ZO1	Zone Occludens 1
JAM2	Junctional Adhesion Molecule 2
IL10	Interleukin 10
MT1	Melatonin 1 A
MT2	Melatonin 1B
Per1	Period1
CRY1	Cryptochrome
TJ	Tight Junction
SCFAs	Short-chain fatty acids
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase

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Author contributions
All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Li Yao, Chen Jiali and Li Fuchang. The first draft of the manuscript was written by Li Yao and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data availability
Materials described in the manuscript, including all relevant raw data, will be freely available to any scientist wishing to use them for non-commercial purposes, without breaching participant confidentiality. The raw sequence data reported in this paper have been deposited in the Genome Sequence Archive (Genomics, Proteomics & Bioinformatics 2021) in National Genomics Data Center (Nucleic Acids Res 2022), China National Center for Bioinformation / Beijing Institute of Genomics, Chinese Academy of Sciences (GSA: CRA020236) that are publicly accessible at https://bigd.big.ac.cn/gsa/browse/CRA020236.

Declarations

Ethics approval and consent to participate
Field studies and other non-experimental studies on animals were conducted in accordance with institutional, national or international guidelines and were approved by the appropriate ethics committees. For all manuscripts containing details, images or videos relating to individuals, written informed consent was obtained from the authors to release these details. Animals are privately owned by the farm and informed consent has been obtained from the animal owner for their use.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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