Effect of the light and darkness cycle on eating and rumination behaviour in sheep fed on roughage diet alone

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In the present study, effect of the changes in daily light (L) and dark-Abstract ness (D) cycle on rumination behaviour in sheep fed roughage alone at a level of 2.0 or 2.2% dry matter per bodyweight per day in 2 times feeding a day or hourly feeding. The 4 daily lighting treatments were as follows; Treatment I, 12L+12D; Treatment II, 6L+6D+6L+6D; Treatment III, 24L, and Treatment IV, 24D in trial 1, and in trial 2, Treatment I, 12D+12L; Treatment II, 6D+6L+6D+6L; Treatment III, 24D and Treatment IV, 24L. During the sampling period in each lighting treatment, the time spent chewing during eating and ruminating was recorded. The eating behaviour was observed in each hour during lighting period in 2 times feeding a day. Daily eating pattern after hourly feeding was relatively steady in all the 4 lighting treatments. Daily total time spent ruminating tended to increase in Treatments I and II in 2 times feeding a day, and in hourly feeding the tendency was in opposition. There was no rumination at 1 hour after morning and evening feeding in 2 times feeding a day. In hourly feeding, on the contrary, there was no zero time spent ruminating per hour in all the lighting treatments. During continuous lighting, the rumination pattern in the first half (09:00-21:00) and the latter half (21:00-09:00) was very similar after 2 times feeding a day, and that after hourly feeding was relatively steady for 24 hours. From these findings, it was confirmed in ruminant animals that the changes in daily lighting condition, in particular daily darkness pattern, could have clearly influenced on initiation of rumination behaviour.

Keywords: Eating and rumination behaviour, Light and darkness cycle, Roughage diet

Introduction

Most animals have a certain period as about 24 hours in their daily life, i.e. a circadian rhythm, and it is thought to change by the changes of light, temperature, and humidity etc. in the natural environment. The light could be mainly related to the physiological control of whole body through a central nervous system after received through the retina. Then, the sensitivity for natural light is incorporated into some parts of central nervous system in animal body (Sugawara, 1996).

When the external stimuli such as light was controlled artificially, the daily period for activity in the wild rabbits were 24 hours; but the rhythm was affected by the changes in lighting environment (Mech et al., 1996). In the domestic fowl, the lighting situation rather than sound and/or feeding time should control the time for oviposition (Wilson et al., 1964), and so, under the lighting condition during full day, the temperature could be a factor for controlling the oviposition time (Payne et al., 1965). On the other hand, swine, managed under the artificial-lighting situation, would be able to detect 24 hours, so they took more time to sleep in the night time than in the day time (Facto et al., 1959). Similarly, in the mouse being fed under the daily darkness condition, the time spent for exercise did not change during 24 hours (Scott, 1958). Under the natural condition, it is obvious that the lowest body temperature

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will be surely observed at early morning in grazed buffalo, camel, sheep, giraffe and zebu (Bligh and Robinson, 1965). In the human beings, it is also obvious that the body temperature goes down during sleeping as a response to gluco -steroid hormone (Evans, 1949). These phenomena are thought to be wholly incorporated into the 24 hours circadian rhythm. The behaviour, which did not change through external stimuli and also the change in body temperature, could be thought to be induced by a biological clock (Cloudsley-Thompson, 1953, 1961; Hakker, 1964; Aschoff, 1965).

In the sheep, there is a ruminating behaviour as their activity in 24 hours circadian, and it is also well known that they have more intensive rumination time at early morning (before sun rise) than other time under a mild climate (Grovum, 1979). In the present study, the changes in daily pattern of rumination behaviour and the effect of biological clock on daily pattern of rumination were investigated in sheep fed only roughage diet under various conditions in daily light and darkness cycle.

Materials and Methods

Animals and diets: In trial 1, two crossbred (Japanese Corriedale×Suffolk) female lambs, weighing 26.8-32.8 kg, were used. They were kept in metabolism cages throughout the experimental period, and fed timothy hay at a level of 2.0% dry matter (DM) per bodyweight (BW) per day. In trial 2, three crossbred (Japanese Corriedale×Suffolk, 2 castrated male and a female) sheep (40.1±0.6 kg BW) were used. They were kept in metabolism cages, and fed mixed hay (predominantly Italian ryegrass) at a level of 2.2% DM per BW per day. The hays were chopped *ca*. 3-5 cm length before feeding. Fresh

Table 1. Chemical composition of roughage feed

Item	Timothy hay	Mixed hay*
Dry matter	89.1	87.4
Organic matter	92.1**	88.6
Crude protein	12.2	8.8
Crude fat [#]	1.9	2.2
Crude fibre	35.7	35.8
NFE##	42.3	41.8

*Harvested from predominantly Italian ryegrass pasture.

** % of dry matter.

#Ether extracts. ##Nitrogen free extracts.

water was freely available, and each animal had access to mineralized salt lick at all time.

Experimental procedure: The half or 1/24 of daily amount of hay was given to the animals at 2 (07 : 00 and 19 : 00) or 24 times (hourly) a day in trial 1, respectively. In trial 2, the daily 2 times feeding was done at 09 : 00 and 21 : 00, and 24 times feeding was also conducted. Two times daily feeding of hay was done by manually, and hourly feeding was conducted using a hand-made auto-feeder. The four lighting treatments introduced in the two trials were as follows: Treatment I, 12L+12D; Treatment II, 6L+6D+6L +6D; Treatment III, 24L and Treatment IV, 24D in trial 1; and Treatment I, 12D+12L; Treatment II, 6D+6L+6D +6L; Treatment III, 24D and Treatment IV, 24L in trial 2 (Figure 1).

Treatment	т	т	т	т	
	07:00	13:00	19:00	01:00	07:00
	(09:00)*	(15:00)	(21:00)	(03:00)	(09:00)
I (trial 1)	L			D	
(trial 2)	D)		L	
II.	L	D	·		D
	D	L -		D	L
III			L		
			- D		
IV			D		
			L		

* In trial 2. L: lighting, D: darkness

Figure 1 Time schedule for lighting control in a day.

In each lighting treatment, the change-over design was used for each feeding treatment. During the 7-day (trial 1) or 5-day (trial 2) sampling period in each lighting treatment, the time spent chewing during eating (trial 2) and ruminating was measured by the method of Fujihara (1980) using a wire stain gauge attached on the lower jaw (Harumoto and Kato, 1979). The term used in this paper for indicating rumination behaviour is based on the work of Gordon (1955). During the entire experiment, a radio was switched on to avoid some effect of sound and voice from outside of the experimental room. The experimental room was originally dark, and lighting control was done artificially using a fluorescent lamp.

Analytical methods: Chemical composition (% of DM) of the roughage diets were determined by the method of AOAC (Hoitz, 1960).

In trial 2, data were analyzed by analysis of variance using StatView (Version 5. 0. 1, SAS Institute Inc. 1999). The statistical significance of differences between the treatment means were examined by Tukey's test.

Results and Discussion

Diets: As shown in Table 1, the timothy hay used in trial 1 contained relatively high crude protein (CP), and then it was thought to be quite good quality hay. On the other hand, the mixed (predominantly Italian ryegrass) hay used in trial 2 was not so good because CP content was quite low as compared to the timothy hay.

Trial 1

Rumination behaviour: As shown in Table 2, when sheep were receiving the diet at two times a day, the time spent ruminating tended to be lengthened in Treatment I (12L +12d) as compared to the other treatments. In hourly feeding, the time spent ruminating in the two sheep was not always same, and also tended to be long in hourly feeding in all lighting treatments. Then, it can be considered that in hourly feeding, the physical stimuli to the rumen wall were continuously given sufficiently for initiating of rumination. In 2 times feeding a day, daily ruminating time under the light and darkness cycle tended to be longer as compared to that under the continuous lighting or darkness, and there was a reverse tendency in hourly feeding.

Generally, it is well known that rumination behaviour will occur during the time in early morning, darkness in a day in ruminant animals nourished by ordinary feeding regimen, however there is a few investigation of rumination behaviour related to the daily lighting control. In the pre-

Table 2. Daily time spent ruminating of sheep in trial 1. (min.)	Table 2.	Daily time spen	t ruminating of	sheep in	trial 1.	(min.)
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Treatment	I^*	II	III	IV
2 times feeding a day				
Sheep 1	$314 \pm 11**$	$287\!\pm\!33$	298 ± 21	284 ± 37
Sheep 2	$414\!\pm\!17$	$376\!\pm\!46$	263 ± 37	312 ± 37
Ave.	$356\pm\!18$	327 ± 32	$281\!\pm\!22$	293 ± 24
Hourly feeding				
Sheep 1	462 ± 23	$347\!\pm\!45$	$425\!\pm\!63$	348 ± 38
Sheep 2	390 ± 35	$501\!\pm\!26$	532 ± 25	514 ± 25
Ave.	420 ± 24	$440\!\pm\!35$	$479\!\pm\!40$	447 ± 36
*Treatment I, $12L+12$ **Mean \pm S. E.	D; II, 6L+6D	+6L+6D;	Ⅲ, 24L; I	V, 24D.

ing condition was reversed at day and night (Gordon and McAllister, 1970).
Daily pattern of rumination behaviour: As shown in the

vious work, it has been shown with sheep that there is a

little decrease in the time spent ruminating at early morning, i.e., a little change in circadian rhythm, when daily light-

Figures 2 and 3, there was no rumination at 1 hour after morning and evening feeding in two times feeding a day. In hourly feeding, contrary, there was no zero time spent ruminating per hour in all the lighting treatments. In both feeding methods, there was similar tendency in which ruminating time clearly reduced at 07:00-08:00 and 19: 00-20:00 with regardless of the changes in light control program and also there was a peak of ruminating at the first half and the latter half of day. In the lighting control of 6L +6D+6L+6D, there was no peak of ruminating in hourly feeding regimen, however in 2 times feeding there was a peak of rumination time under the lighting treatment (darkness) at the first half of day. During the period of continuous lighting, there was no peak after hourly feeding, but after 2 times feeding there was a peak at the first half and the latter half of day, respectively. In the continuous darkness, there were 2 peaks at the first half and the latter half after hourly feeding, however after 2 times feeding a day there was a peak only at the latter half.

In the present study, the experiment was continuously performed in all the treatment for lighting condition, and so there were no preliminary period in each treatment, and then, it seems that the sheep has been well adapted quickly to the changes in lighting condition. The most intensive rumination behaviour in each lighting treatment for a day was observed at from 06:00 to 07:00,05:00 to 06:00, 04:00 to 05:00 and 03:00 to 04:00 in 2 times feeding, and that in hourly feeding was from 06:00 to 07:00, 06:00 to 07:00,05:00 to 06:00 and 12:00 to 13:00, respectively. After dividing 4 periods of 6 hours a day, the time spent ruminating in each period was shown in Figure 4. In each lighting treatment, the time spent ruminating was longer during darkness period than that during lighting in both feeding treatments. In the continuous lighting, the difference in the ratio of ruminating time to total ruminating time between the periods was small in both feedings, however in the continuous darkness, the difference tended to be small in hourly feeding and to be large in 2

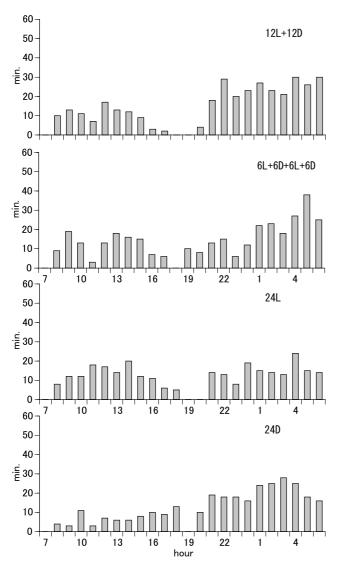


Figure 2 Diurnal pattern of rumination behavior in sheep after 2 times feeding a day in 4 treatments

times feeding a day.

In the present study, there was no clear change in daily total rumination time among 4 lighting treatments and also 2 feeding regimes, and then the effect of daily lighting condition on daily rumination time could not be shown obviously, however, a circadian pattern of the time spent ruminating was slightly observed under the various lighting conditions, when 24 hours was divided 4 periods. In the 12L+12D or 6L+6D+6L+6D lighting conditions, daily ruminating time was greater during the darkness than during lighting, and this would support the result that rumination mainly has been done during the night time a day. Furthermore, the difference in ruminating pattern in the Treatments I (12L+12D) and II (6L+6D+6L+6D) clearly showed the fact that sheep had quickly responded

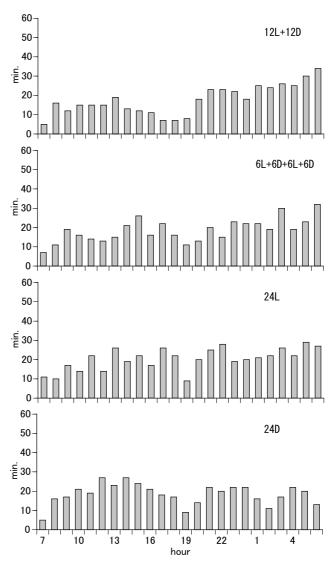
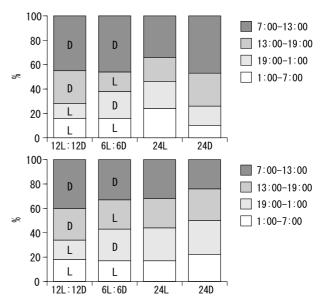
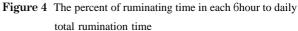


Figure 3 Diurnal pattern of rumination behavior in sheep after hourly feeding in 4 treatments





Treatment	reatment I		I	II		III		IV	
Feeding	2 times/d	Hourly	2 times	Hourly	2 times	Hourly	2 times	Hourly	
Feed intake*	$39.8\pm~2.8^{1,a}$	52.6 ± 4.1^{b}	40.5 ± 2.0^{a}	55.0 ± 1.6^{b}	40.7 ± 9.1^{ab}	50.9 ± 0.8^{b}	43.3 ± 5.5^{ab}	51.7± 1.2 ^b	
Eating time**	$365.2 \pm 16.2^{\circ}$	288.3 ± 23.8^{ab}	$335.0 \pm 12.9^{\text{bc}}$	282.5 ± 8.7^{a}	315.8 ± 10.8^{b}	328.7 ± 19.9^{bc}	$353.5 \pm 10.3^{\circ}$	$341.4 \pm 15.4^{\text{bc}}$	
Eating rate [#]	2.0 ± 0.1^{a}	$3.2\pm~0.4^{cd}$	$1.9\pm~0.1^{a}$	$3.0 {\pm} 0.1^{d}$	$2.5\pm 0.1^{\rm bc}$	2.5 ± 0.2^{bc}	2.1 ± 0.2^{ab}	$2.5\pm 0.1^{\rm bc}$	

Table 3. Eating behavior in sheep fed on roughage diet alone (trial 2).

*g/BW^{0.75}/day. **min./day. #g DM/min. ¹Mean \pm S.E. of 3 sheep. ^{a-d}Means within the same row with different superscripts are significantly different (P<0.05).

Table 4. Rumination behavior in sheep fed roughage diet alone (trial 2)

Treatment	I		Ι	I	Ι	II	I	V
Feeding	2 times/d	Hourly	2 times	Hourly	2 times	Hourly	2 times	Hourly
Rum. Time*	545.5 ± 25.3^{1}	584.2 ± 20.8	553.9 ± 25.2	550.5 ± 17.4	605.5 ± 28.2	566.6 ± 26.0	545.3 ± 26.8	557.7±11.8
No. of boli**	551.0 ± 24.9^{a}	572.4 ± 14.9^{ab}	$582.3 \pm 26.7^{\text{abc}}$	613.2 ± 21.7^{bc}	662.3±28.0°	623.8 ± 20.2^{bc}	593.3 ± 28.6^{abc}	$622.4 \pm 15.9^{\circ}$
Rum. Periods#	$12.3\pm~0.8^{a}$	$23.5 \pm 1.2^{\rm f}$	15.4 ± 0.4^{b}	20.1 ± 0.6^{e}	$15.9\pm 0.8^{\rm bc}$	18.1 ± 0.9^{cde}	$17.6\pm~0.8^{cd}$	19.7 ± 0.9^{de}
Cyclic rate##	$59.5 \pm 1.3^{\rm bc}$	$61.3\pm~1.8^{\circ}$	57.4 ± 1.7^{abc}	54.1 ± 1.2^{a}	55.1 \pm 2.0 ^{ab}	54.7 ± 2.1^{ab}	55.2 \pm 0.8 ^{ab}	53.9 ± 0.8^{a}

*Daily total rumination time (min/day). **Daily number of boli. #Daily number of rumination periods.

^{##}Daily total rumination time (sec.)/no. of boli regurgitated. ¹Mean \pm S.E. of 3 sheep.

^{a-f}Means within the same row with different superscripts are significantly different (P < 0.05).

to the changes in daily lighting condition after both feedings, and this is in good agreement with the result of Gordon and McAllister (1970).

In this experiment, daily time spent ruminating was longer in hourly feeding than in 2 times feeding a day with regardless of the treatment of daily lighting condition and this would be due to continuous tactile stimulus to the rumen wall through the ingested feed particles. There was a tendency with some changes in daily time spent ruminating through the changes of daily light condition in this experiment, but the reason why changes in lighting condition increase or decrease daily ruminating time could not be elucidated yet. However, it will be clear that the time spent ruminating tended to be longer during darkness period as compared to the during light in Treatments I and II (light and darkness cycles), i.e., the animal would quickly be able to change circadian pattern after responded to change in lighting environment.

From these findings, it was confirmed in ruminant animals that the changes in daily lighting condition, in particular daily darkness pattern, could have clearly influenced on initiation of rumination behaviour.

Trial 2

Eating behaviour: As shown in Table 3, intake and the rate of intake were greater in hourly feeding than in 2 times feeding a day, and there was a tendency to increase in eating time at 2 times feeding a day as compared to that at hourly feeding. Feed intake and the rate of eating tended to be greater and daily eating time tended to be shorter in Treatments I and II as compared to that in Treatments III and IV at hourly feeding. Daily time spent eating (282, 5-365, 2 min.) in the present study was in the normal range (200-400 min.) in sheep reported by other workers (Dulphy et al., 1980), however, the values were fairly greater than those reported earlier in sheep fed similar Italian ryegrass or timothy hay (103.7-206.9 min.) at same feeding level (Fujihara, 1980; Fujihara and Nakao, 1982). Consequently, the rate of eating in the present experiment was quite slow as compared to the values in the previous experiments mentioned above.

Rumination behaviour: The values of rumination behaviour is shown in Table 4, and daily total ruminating time tended to be higher in Treatment IV (605.5 min.), and in treatment III the value tended to be lower at 2 times feeding a day as compared to that after hourly feeding. In our previous experiment, it has been observed that daily time

Treatment		Ι		П	I	II]	IV
Feeding	2 times/d	Hourly	2 times	Hourly	2 times	Hourly	2 times	Hourly
No. of chews*	$77.7 \pm 6.2^{1, \text{bcd}}$	73.4 ± 2.8^{ab}	80.0±4.1°	67.7 ± 1.5^{cd}	71.4 ± 3.1^{de}	$68.6 \pm 2.6^{\text{abc}}$	61.6 ± 1.5^{a}	$66.6 \pm 1.4^{\text{bcd}}$
Bolus time	52.1 ± 4.8^{b}	52.0 ± 2.2^{b}	49.9 ± 2.4^{ab}	44.5 ± 0.9^{a}	45.8 ± 2.2^{ab}	47.0 ± 1.8^{ab}	45.4 ± 1.2^{a}	45.3 ± 0.9^{a}
Chewing rate	89.8 ± 1.6^{cd}	84.9 ± 1.6^{bcd}	96.0 ± 0.4^{d}	$91.4 \pm 2.1^{\text{bcd}}$	93.6 ± 1.2^{cd}	87.6 ± 0.5^{bcd}	81.6 ± 2.2^{a}	88.2±1.8 ^b
Rum. Index**	78.9 ± 3.5^{cd}	68.6 ± 1.2^{a}	87.2 ± 3.7^{e}	65.0 ± 1.1^{a}	76.1 ± 3.3^{cde}	$71.1 \pm 2.8^{\text{bcd}}$	75.9 ± 2.9^{cd}	68.1 ± 1.9^{ab}

Table 5. Rumination efficiency in sheep fed on roughage diet alone (trial 2).

*Number of chews per bolus. **Time spent ruminating/100g DM eaten. ¹Mean±S.E. of 3 sheep.

^{a-e}Means within the same row with different superscripts are significantly different (P < 0.05).

spent ruminating time in sheep fed Italian ryegrass hay and timothy hay at 2.2% BW as DM basis was 507.8 min. or 565.2 min., respectively (Fujihara, 1980; Fujihara and Nakao, 1982). Gordon and McAllister (1970) has also reported that daily time spent ruminating in sheep fed 600 g hay and 300 g concentrate daily was 570 min. in 2 times (10:00 and 22:00) feeding a day. In the present study, feed intake tended to increase at hourly feeding compared to that at 2 times feeding a day in all the lighting treatments, however there was no tendency in daily time spent ruminating, and this did not support the previous result (Gordon and McAllister, 1970). Daily number of boli at both feedings was higher in Treatment II (continuous darkness) as compared to that in other Treatments. Bolus time was higher, but not significantly, in treatment I at both feedings than that in other Treatments. The number of rumination period tended to increase in hourly feeding, the values in Treatments I and II at hourly feeding were significantly (P < 0.05) greater than those after 2 times feeding a day. The figures of daily rumination periods in the present experiment (12, 3-23, 5/d) slightly tended to be small as compared to those (21.9-23.9) in the previous studies described above (Fujihara, 1980; Fujihara and Nakao, 1982). Cyclic rate (total rumination time (sec.)/number of boli regurgitated) in the present study was in a range of 53.9-61.3, and the figures are in good agreement with the values (58.7-64.0) reported previously (Gordon, 1965; Fujihara, 1980; Fujihara and Nakao, 1982).

Rumination efficiency: As shown in Table 5, the efficiency of rumination for comminuting the food eaten was estimated by measuring the number of chews per bolus, bolus time and the chewing rate, and also rumination index. The number of chews per bolus tended to increase at hourly feeding as compared to 2 times feedings a day

in all the lighting treatments, and the values are slightly higher as compared to the result reported previously (Fujihara, 1980). Bolus time at both feedings in Treatment I was slightly higher than those in other lighting treatments, and chewing rate at both feedings was also higher in Treatment II than those in other lighting treatments. Rumination

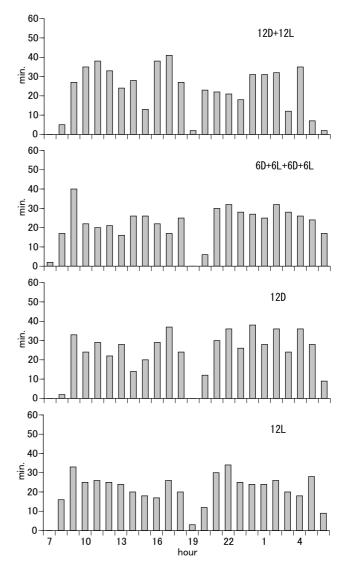


Figure 5 Diurnal pattern of rumination behavior in sheep after 2times feeding a day in 4 treatments

index (Fujihara, 1980) tended to be higher at 2 times feeding a day as compared with that after hourly feeding in all the lighting treatments, the values were comparable to that of previous experiment (Fujihara, 1980).

Daily pattern of rumination behaviour: As shown in Figures 5 and 6, at 2 times feeding a day, rumination behaviour tended to increase during darkness period as compared to that in lighting period in Treatment I, and in Treatment II, the change in rumination behaviour tended to be small as compared to that in Treatment I. In Treatment III (continuous darkness), rumination behaviour tended to occur more during the first half of 12 hours than that during the latter half of 12 hours. During the latter half of 12 hours, rumination behaviour tended to increase just after offering, and then it reduced gradually till offering feed at next day with some fluctuations. In Treatment IV (continuous lighting), the hourly pattern was almost the same in the first half of 12 hours and the latter half of 12 hours a day, and the pattern in the latter half of 12 hours was similar to that in The treatment II after 2 times feeding a day. Gordon and McAllister (1970) have shown almost the same daily pattern of rumination in sheep under similar lighting condition (continuous lighting). In Treatment I, rumination behaviour at hourly feeding tended to increase at the late of latter half of 12 hours (at a turning point from darkness period to light period). In Treatment II, rumination behaviour tended to increase during a darkness period than during lighting period after hourly feeding. The changes in daily rumination pattern in Treatment III tended to be larger in the first half than in the latter half after hourly feeding, and in Treatment IV, there was a little change in daily rumination pattern during 24 hours. In the former work (Gordon and McAllister, 1970), it has been reported that the individual variability in daily rumination pattern is very small, and it will not be influenced by the changes in the time for offering feed for animals. According to Pearce (1965), it is obvious that there was also non effect of feeding various feed on the change in daily rumination pattern of sheep.

Here, in comparison with the results of trial 1 in which the order of light and darkness in each treatment was in opposition, daily rumination pattern after 2 times feeding a day changed similarly on the turning points; darknesslighting or lighting-darkness; as in trial 2 (Treatment I).

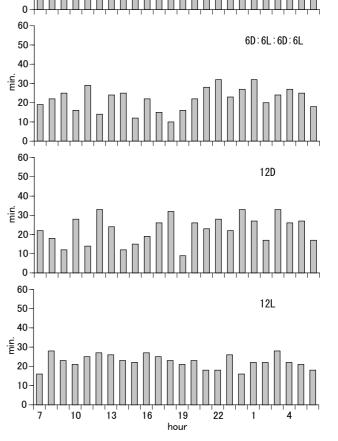


Figure 6 Diurnal pattern of rumination behavior in sheep after hourly feeding in 4 treatments

In Treatment II, when animals were allowed 2 times per day, rumination behaviour at 6 hours after feeding in trial 2 was more prominent as compared with that in trial 1. In Treatment I, rumination behaviour tended to decrease at 6 hours before the point; lighting-darkness; after hourly feeding in both trials. When daily rumination pattern was compared based on the morning time (07 : 00 or 09 : 00) as stating point, the pattern was different between the trials 1 and 2, and also daily ruminating pattern at 3 hours before the morning time as starting point changed, i.e., the rumination tended to increase in trial 1 and to decrease in trial 2. In hourly feeding in Treatment III or IV, daily rumination pattern was relatively steady (continuous lighting), although it was not stable (darkness) in both trials. Gordon and McAllister (1970) reported the peak of ru-

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12D:12L

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Treatment	09:00-15:00	15:00-21:00	21:00-03:00	03:00-9:00
2 times feeding				
I	138.1±8.1 ^{1.b} (25.4) ²	$173.0 \pm 12.9^{\circ} \\ (31.6)$	$109.8 \pm 10.2^{a} \\ (20.0)$	$\begin{array}{c} 125.8 \pm 11.0^{\rm ab} \\ (23.0) \end{array}$
I	122.9±9.1ª (22.2)	139.1±12.9 ^{ab} (25.1)	130.8 ± 8.7^{ab} (23.6)	161.1±11.9 ^b (29.1)
П	110.1±9.1ª (18.8)	154.8±15.9 ^{bc} (26.3)	145.7±6.8 ^b (24.8)	$176.9 \pm 12.0^{\circ}$ (30.1)
IV	125.5 ± 9.4 (23.7)	128.2 ± 01.4 (24.2)	$131.1 \pm 9.0 \\ (24.7)$	145.4 ± 7.8 (27.4)
Hourly feeding				
	141.9±7.3 ^{ab} (24.2)	135.9 ± 9.3^{a} (23.2)	161.1±6.1 ^b (27.4)	148.1±7.0 ^{ab} (25.2)
I	126.3 ± 7.8^{a} (22.9)	111.6 ± 7.5^{a} (20.3)	153.6 ± 7.9^{b} (27.9)	159.1±5.9 ^b (28.9)
Ш	129.7 ± 6.9^{a} (22.6)	131.7±8.3 ^a (22.9)	$149.4 \pm 9.9^{\rm ab} \\ (26.0)$	163.4 ± 8.4^{b} (28.5)
IV	140.6 ± 6.2^{ab} (25.2)	147.8 ± 5.5^{b} (26.5)	128.7 ± 7.4^{a} (23.2)	140.5 ± 6.4^{ab} (25.1)

Table 6. The time spent ruminating for each 6 hours in a day (min.)

¹Mean \pm S.E. of 3 sheep. ²Percentage to daily total ruminating time.

^{a-c}Means within the same row with different superscripts are significantly different ($P \le 0.05$).

mination behaviour in a day will be shifted by the changes in light and darkness cycle, and they have also described sheep had well corresponded quickly to the changes in lighting situation during 4-day accustomed period. They have also reported that rumination behaviour decreased during 10 : 00-16 : 00 hrs in sheep under a natural lighting condition, and in the present study, the rumination behaviour has decreased during similar time (15 : 00-17 : 00 hrs), and there was a relatively small eating activity.

After dividing 24 hours to 4 sections (6 hours) based on the morning time as starting point (09 : 00), rumination time for each 6 hours and the ratio of rumination time in each 6 hours to daily total rumination time are shown in Table 6. The highest rumination time and its ratio (rumination time during 6 hours/daily time spent ruminating) in 2 times feeding a day was observed at 15 : 00-21 : 00, 31.6% and 173 min.; 03 : 00-09 : 00, 29.1% and 161.1 min.; 03 : 00-09 : 00, 30.1% and 176.9 min.; 03 : 00-09 : 00, 27.4% and 145.4 min. in 4 Treatments (light and darkness cycle), respectively. In hourly feeding, the highest rumination time and its ratio to daily rumination time was also observed at 21 : 00-03 : 00, 27.4% and 161.1 min (I).; 03 : 0-09 : 00, 28.9% and 159.1 min (II).; 03 : 00-09 : 00, 28.5% and 163.4 min (III); 15 : 00-21 : 00, 26.5% and 147.8 min. (IV), respectively. The ratio to daily rumination time tended to increase at mid-night in the Treatments II and III. In the Treatment IV, rumination behaviour was similar in each 6 hours period at both feedings, i.e., the rumination occured almost evenly during a day. These findings in trial 2 were different from the results in trial 1, and this would be due to a difference in the starting point (07: 00 and 09: 00).

From the results in the present study, it can be certified that the eating and ruminating behaviour in sheep was changed through a external stimuli which was detected by vision. After hourly feeding, daily eating time was almost the same, and it maybe resulted in similar volume of ration offered 24 times a day. If it is presumed that there was similar amount of digesta in the rumen, the stimuli of ingested feed to the rumen wall could be thought to be equal. However, the ratio of time spent ruminating in each hour was different when the lighting condition (light and darkness) was changed, and then, one of the factors which modulates rumination appearance and length of rumination time in sheep, could be lighting or darkness period in a day. In the daily rumination pattern, the rumination behaviour after 2 times feeding a day was the longest during 15:00-21:00, and the ratio of its time to daily total rumination time was 31.6%. So, it would be concluded that some changes in rumination behaviour through "biological clock" is thought to be not great in ruminant animals.

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