



## Deprivation of Esophageal Boluses and Dry Forage Intake in Large-type Goats

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**ABSTRACT :** In goats fed on dry forage twice a day, an esophageal fistula was used to investigate the physiological factors present in the marked suppression of dry forage intake that occurs after 40 min of feeding. The animals used in this study were five large-type male esophageal- and ruminal-fistulated goats. Roughly crushed alfalfa hay cubes with any large remaining chunks removed were used as feed for this research. The study was conducted under both normal feeding conditions (NFC) and sham feeding conditions (SFC). In the NFC control, the esophageal fistulae were closed by plugs and the animals ate dry forage in the normal manner. In the SFC treatment, before starting the experiment the plugs for closing the esophageal fistula were removed and the cannulae for collecting boluses were fitted into the fistulae. Therefore, the esophageal boluses were removed via an esophageal fistula before they entered the rumen. In the NFC control, eating rates sharply decreased in the first 40 min of feeding and were subsequently maintained at low levels. However, eating rates in the SFC treatment remained high after 40 min of the feeding period had elapsed and the goats ate continuously during the 2 h feeding period. In comparison with the NFC control ( $1,794 \pm 203.80$  g/2 h), cumulative dry forage intake in the SFC treatment ( $3,182 \pm 381.69$  g/2 h) was 77.4% greater ( $p < 0.05$ ) upon conclusion of the 2 h feeding period. In the SFC treatment, cumulative bolus output ( $6,804 \pm 469.92$  g/2 h) was about twofold the cumulative dry forage intake due to cumulative salivary secretion volume ( $3,622 \pm 104.13$  g/2 h) upon conclusion of the 2 h feeding period. The result indicates that large amounts of secreted saliva during dry forage feeding act in conjunction with consumed feed to form the ruminal load responsible for ruminal distension. The increased plasma total protein concentrations were higher in the SFC treatment than in the NFC control. However, plasma and ruminal fluid osmolalities increased in the NFC control during and after feeding but were mostly unchanged in the SFC treatment. In comparison with the NFC control ( $3,440 \pm 548.04$  g/30 min), thirst level in the SFC treatment ( $1,360 \pm 467.02$  g/30 min) was 60.5% significantly less ( $p < 0.05$ ) upon conclusion of the 30 min drinking period. The results of the present study indicate that in the second hour of the 2 h feeding period, dry forage intake is regulated by factors produced when boluses enter the rumen. (**Key Words :** Sham Feeding, Dry Forage Intake, Salivary Secretion, Thirst Level, Ruminal Distension, Large-type Goats)

### INTRODUCTION

In countries such as Japan, ruminants are offered dry forage twice a day to raise productivity. Saliva in sheep and goats is secreted in large volumes during the initial stages of dry forage feeding (Sato, 1975; Sunagawa et al., 2003). Saliva acts as a lubricant in the mouth and esophagus and assists in the mastication, swallowing, remastication and reswallowing of dry forage. Saliva also acts as an alkali and

serves to buffer the decrease in the pH of ruminal fluid due to the volatile fatty acid production of microbial fermentation in the rumen.

In goats fed on dry forage for 2 h twice a day, eating rates were rapidly decreased in the first 30 or 40 min of the 2 h feeding period and remained at low levels during the remaining time (Sunagawa et al., 2002; 2003; 2007). Sunagawa et al. (2003) reported that a suppression of dry forage intake during the early stages of feeding in goats was caused by feeding-induced hypovolemia (decreased circulating blood volume), which was produced by the accelerated secretion of parotid saliva. However, the mechanism responsible for the suppression of feed intake after 40 min of feeding is unclear.

In the second hour of the 2 h feeding period, both ruminal fluid osmolality and ruminal distension increased

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Received January 21, 2010; Accepted March 28, 2010

but it is unclear whether or not either of these factors suppressed dry forage intake. Campling and Balch (1961) reported that feed intake was decreased when a balloon was inserted into the rumen and inflated with water in cows fed on hay. Grovum (1995) reported that the increases in ruminal fluid osmolality by intraruminal infusion of the same dose of hyperosmotic NaCl, polyethylene glycol-400 (PEG), sodium acetate or sodium propionate resulted in the same-sized decreases in alfalfa pellet intake by sheep. However, Anil et al. (1993) reported that in cows, if a balloon inserted in the rumen was not filled with enough water or if the increase in ruminal fluid osmolality was insufficient, the amount of dry forage or silage intake was not decreased. From these reports, it remains unclear as to whether or not feed intake is depressed by ruminal distension and increases in ruminal fluid osmolality that occur with dry forage intake under normal feeding conditions.

The objective of this study was to clarify the physiological factors in the marked suppression of dry forage intake that occur after 40 min of feeding. In order to clarify this, an esophageal cannula was fitted into the esophageal fistula to prevent feed from entering the rumen.

## MATERIALS AND METHODS

### Animals

Five large-type male goats (crossbred Japanese Saanen/Nubian, aged 3 to 5 years, weighing  $72.6 \pm 4.32$  kg) fitted with esophageal and ruminal fistulae were used in this study. The goats were maintained in individual metabolism cages (length 2 m  $\times$  width 1 m  $\times$  height 2 m) that allowed for the separate collection of urine and feces. The laboratory room was maintained under thermoneutral conditions (room temperature  $25.7 \pm 0.54^\circ\text{C}$ ; relative humidity  $87.3 \pm 1.89\%$ ). About 3 months before the start of experiments, the esophageal fistulation and ruminal cannulation was performed using the method described by Kato et al. (1983).

The animals were fed twice daily at 10:00 h and 16:00 h for 2 h each time. During the morning feeding period (10:00 to 12:00 h), the animals were fed 1.5 to 2.5 kg of roughly crushed alfalfa hay cubes. At 16:00 h each day, the animals were fed 300 g of hay and 200 g of concentrated beef cattle feed and half a spoon of multivitamins. The animals were given 5 kg of water at each meal.

### Experimental design

The 5 animals were divided into two groups (group A: two animals; group B: three animals). The experiment was carried out in accordance with a cross-over design. In the first experiment, group A was the control and maintained under the normal feeding conditions (NFC) while group B

was subjected to the sham feeding conditions (SFC). This was reversed in the second experiment in which group A was subjected to the sham feeding conditions (SFC) while group B received the control and was maintained under the normal feeding conditions (NFC).

The NFC control and SFC treatment were carried out with each group at 1 week intervals to ensure that animals were recovered and to minimize any compounding effects from the previous treatments. The five goats were housed in the same room until the experiment had been completed.

In the NFC control, the esophageal fistulae were always closed by plugs and the animals ate dry forage in the normal manner. In the SFC treatment, the ingested feed left the digestive tract via an esophageal fistula before any gastric, intestinal or metabolic effects occurred. Hence, before starting the experiment, the plug for closing the esophageal fistula was removed and a cannula for collecting boluses was fitted into the fistula. Therefore, all swallowed boluses of dry forage intake and secreted saliva were collected in the cannula through the fistula.

In order to ascertain the physiological state of animals, heart rate, respiration rate, and rectal temperature were measured daily prior to the morning feeding period. Heart rate was measured by counting heart sounds with a stethoscope placed 5 cm behind the left olecranon. Respiration rate was measured by counting respiratory sounds with a stethoscope, and observing and counting thoracic movement that occurs in conjunction with respiration. Rectal temperature was measured using a veterinary thermometer inserted 10 cm into the rectum for about 10 min.

One day before the beginning of each treatment in this study, a polyethylene cannula (o.d. 1.50 mm, No. 5, Imamura Gomu, Tokyo) was inserted into the jugular vein on one side of each goat for collecting blood samples. A three-way tap was attached to the end of each cannula. The cannula was sewn to the skin on the animal's neck and back to secure it and filled with heparin-saline (50 IU/ml) to prevent coagulation of the blood.

During the experiment, feed consumption was measured at intervals of 10 min for the duration of the 2 h feeding period (11:00 to 13:00 h). The animals were deprived of water during feeding in both the control and the treatment. Following the completion of feeding, 5 kg of water was provided for a period of 30 min.

The parameters measured in the present study were rate of eating, cumulative dry forage intake, rate of bolus output, cumulative bolus output, rate of salivary secretion, cumulative salivary secretion, thirst level, hematocrit, plasma osmolality, plasma concentrations of Na, K, Cl, total protein and glucose, ruminal fluid pH, osmolality, and concentrations of Na, K and Cl. The rate of eating (g dry

matter (DM)/10 min) and the cumulative dry forage intake (g DM) were measured during the 2 h of feeding (11:00 to 13:00 h). The roughly crushed alfalfa hay cubes (1.5 to 2.5 kg) was placed in a feed box attached to an 8 kg measuring scale which was used to determine eating rate by measuring the weight of the remaining feed every 10 min for the duration of the 2 h feeding period. The bolus output from the esophageal fistula (g) was measured during the 2 h of feeding (11:00 to 13:00 h) by using a feed box attached to a 12 kg measuring scale which was used to determine the weight of the bolus output from the esophageal fistula every 10 min. The esophageal bolus was a mixture of ingested feed and saliva. Rate of salivary secretion was measured by subtracting the rate of eating from the rate of bolus output at the same time so that cumulative salivary secretion was determined every 10 min. Fluid intake is regulated by thirst mechanisms (Guyton and Hall, 1996; Prasetyono et al., 2000). In the present study, the thirst level (g/30 min) was evaluated quantitatively using water intake for 30 min upon conclusion of the 2 h feeding period.

Blood samples (4 ml) were collected through the polyethylene cannula at 8:55, 10:55, 11:15, 11:30, 12:00, 12:30, 13:00 and 13:30 h. Prior to drawing the samples, a drop of heparin solution (1,000 IU/ml) was placed into a test tube. The blood samples were transferred to these test tubes, which were then placed in ice until plasma separation was carried out by centrifugation (16,260×g, 10 min, 4°C).

Ruminal fluid samples (30 ml) were collected at 8:55, 10:55, 11:15, 11:30, 12:00, 12:30, 13:00 and 13:30 h through the polyvinyl tube fitted in the ruminal fistula and put into test tubes placed in ice until ruminal fluid separation from sediments was carried out by centrifugation (12,320×g, 10 min, 4°C).

All surgical and experimental procedures were approved by the Animal Experimental Ethics Committee of the University of the Ryukyus and were in compliance with the Japanese code of practice for the care and use of animals for scientific purposes.

#### Chemical analysis of feeds

The chemical composition of alfalfa hay cubes and ground concentrated beef cattle feed are shown in Table 1. Alfalfa hay cubes and ground concentrated beef cattle feed were subjected to draught drying (70°C, 24 h) and then were ground with a Wiley mill (type 40-525P, Ikemoto, Rika Kougyou, Tokyo, Japan). The chemical components of the feeds were quantified using the procedures described by the AOAC (1990). The chemical component analysis was conducted in triplicate using a total of three samples for each type of feed. The dry matter (DM) content was quantified by oven drying at 135±2°C for 2 h. The crude protein content was calculated from the nitrogen content of

**Table 1.** Chemical composition of alfalfa hay cubes and ground concentrated beef cattle feed

	Alfalfa hay cubes	Ground concentrated beef cattle feed
Dry matter (DM) (%)	84.3	86.9
Chemical composition (% of DM)		
Crude protein	18.7	13.4
Crude fat	2.4	3.6
Crude fiber	29.7	3.7
Nitrogen-free extract (NFE)	39.7	71.0
Neutral detergent fiber (NDF)	45.9	14.6
Acid detergent fiber (ADF)	36.6	5.4
Na	0.10	0.25
K	2.39	0.71
Cl	0.47	0.31
Ca	1.40	0.78
Mg	0.29	0.25
P	0.23	0.48

the feed determined by a Kjeldhal technique (AOAC, 1990). The crude fat content of feed was determined by continuous extraction with ethyl ether for 16 h using a Soxhlet extraction apparatus. Crude fiber was determined by subjecting the residue from ether extraction to successive treatments with boiling sulfuric acid (1.25%) and sodium hydroxide (1.25%). Nitrogen-free extract (NFE) was calculated by subtraction of the sum of moisture, ash, crude protein, crude fat and crude fiber content from 100. The acid-detergent fiber (ADF) and neutral-detergent fiber (NDF) were determined as described Van Soest et al. (1991). For concentrated beef cattle feed, prior to adding the neutral-detergent solution, the sample was boiled and pre-treated with  $\alpha$ -amylase at 40°C. The samples were charred, ignited and reduced to ash at 550°C in an electric furnace. The mineral content of the ash was then measured using an atomic absorption spectrophotometer (AA-6200, Shimadzu, Tokyo). The Cl content was measured using ion chromatographic methods.

#### Biochemical analysis

Blood samples were placed in capillary tubes and centrifuged using a hematocrit centrifuge (HC-12A, Tomy Seiko, Tokyo; 5 min, 12,851×g) to determine hematocrit by hematocrit reader (Tomy Seiko, Tokyo). Plasma total protein concentration and osmolality were measured using a refractometer (Atago, Tokyo) and an osmometer (OM-6010, Kyoto Daiichi Kagaku, Kyoto), respectively. Plasma glucose concentration was measured using a Spotchem EZ (SP-4430, Arkray, Tokyo). The plasma concentrations of Na, K and Cl were measured using a Spotchem EL (SE-1520, Arkray, Kyoto).

Ruminal fluid was analyzed for osmolality with an osmometer (OM-6010, Kyoto Daiichi Kagaku, Kyoto) and for pH and concentrations of Na, K and Cl by a pH/Ion meter F-53 (Horiba Ltd., Kyoto, Japan).

**Statistical analysis**

This experiment was conducted in accordance with a cross-over design. The values collected were subjected to an analysis of variance (ANOVA). Fisher’s LSD was used to determine the significance of treatment effects. In the ANOVA table for cross-over design, the sources of variance included animals (A), group of animals (G), treatments (T) and error (E). For statistical analysis, General Linear Model (GLM) procedures (SAS Inst., Inc., Cary, NC, 1990) were adopted.

All data except for data collected from rate of bolus output, cumulative bolus output, rate of salivary secretion and cumulative salivary secretion in the SFC treatment were analyzed using the following model:

$$Y_{ijkl} = \mu + G_i + A_{ij} + T_k + E_{ijkl}$$

Where  $Y_{ijkl}$  = the measured variable on the  $l^{th}$  replication of the  $j^{th}$  animal within the  $i^{th}$  group and the  $k^{th}$  treatment;  $\mu$  = the overall mean;  $G_i$  = the effect of the  $i^{th}$  group;  $A_{ij}$  = the effect of the  $j^{th}$  animal within the  $i^{th}$  group;  $T_k$  = the effect of the  $k^{th}$  treatment;  $E_{ijkl}$  = the random error.

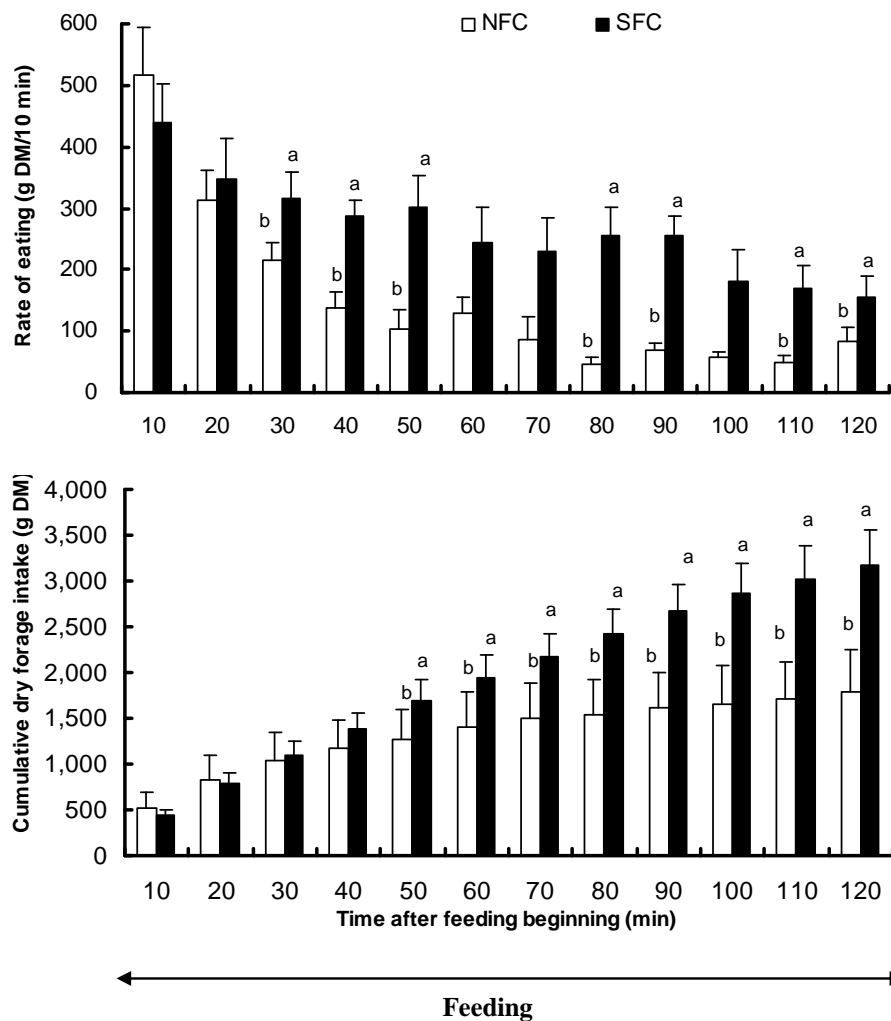
**RESULTS**

**Physiological parameters**

The mean values of heart rate, respiration rate, and rectal temperature in the NFC and SFC treatment were  $76.8 \pm 2.94$  and  $69.6 \pm 3.06$  beats/min,  $18.4 \pm 0.98$  and  $19.2 \pm 0.80$  breaths/min, and  $38.4 \pm 0.07$  and  $38.5 \pm 0.06^\circ\text{C}$ , respectively.

**Rate of eating and cumulative dry forage intake**

Figure 1 shows the effect of the SFC treatment on rate



**Figure 1.** The effect of sham feeding conditions (SFC) on rate of eating and cumulative dry forage intake. Values are means±SE of 5 large-type goats. <sup>a, b</sup> Means with different superscript are significantly different (p<0.05) from normal feeding conditions (NFC).

**Table 2.** The effect of sham feeding conditions (SFC) on rate of bolus output, cumulative bolus output, rate of salivary secretion and cumulative salivary secretion

Time after feeding beginning (min)	Rate of bolus output (g/10 min)	Cumulative bolus output (g)	Rate of salivary secretion (g/10 min)	Cumulative salivary secretion (g)
10	1,096±117.46	1,096±117.46	656±84.95	656±84.95
20	696±137.13	1,792±180.43	350±79.81	1,006±87.61
30	718±86.74	2,510±259.36	402±45.87	1,408±125.68
40	622±48.00	3,132±303.50	336±22.49	1,744±145.83
50	612±56.07	3,744±351.78	310±6.32	2,054±151.15
60	510±32.86	4,254±353.05	266±31.87	2,320±149.16
70	530±80.50	4,784±384.90	300±42.90	2,620±147.34
80	498±43.86	5,282±391.26	242±9.16	2,862±155.38
90	478±49.64	5,760±412.42	222±38.00	3,084±153.05
100	346±63.84	6,106±452.61	166±28.74	3,250±163.52
110	360±37.82	6,466±470.75	190±27.02	3,440±141.53
120	338±66.51	6,804±469.92	182±46.52	3,622±104.13

Values are means±SE of 5 large-type goats.

of eating and cumulative dry forage intake. Eating rates in the NFC control rapidly decreased in the first 40 min of feeding (0 to 10 min, 516±78.78 g; 30 to 40 min, 138±26.53 g) and subsequently declined gradually to very low rates (ranged from 46±12.08 g/10 min to 128±28.35 g/10 min) in the remaining time of the 2 h feeding period. Meanwhile, the eating rates in the SFC treatment slightly decreased in the first 30 min of feeding (0 to 10 min, 440±62.61 g; 20 to 30 min, 316±42.02 g). Eating rates in the SFC treatment from 30 min to the conclusion of the 2 h feeding period except at 60, 70 and 100 min intervals after feeding were significantly higher ( $p<0.05$ ) than those in the NFC control.

From the commencement of feeding until a 40 min interval had elapsed, there were no significant differences between the NFC control and the SFC treatment in terms of cumulative dry forage intake. However, differences in cumulative dry forage intakes between the NFC control and the SFC treatment were significant ( $p<0.05$ ) from 50 min after feeding to the conclusion of the 2 h feeding period. In comparison with the NFC control (1,794±203.80 g/2 h), cumulative dry forage intake in the SFC treatment (3,182±381.69 g/2 h) was 77.4% greater ( $p<0.05$ ) upon conclusion of the 2 h feeding period.

#### Rate of bolus output and cumulative bolus output

The rate of bolus output rapidly reduced in the first 20 min of feeding and then declined gradually to the end of the 2 h feeding period. Cumulative bolus output increased gradually with elapsed feeding period and reached the highest volume (6,804±469.92 g) at the conclusion of the 2 h feeding period (Table 2).

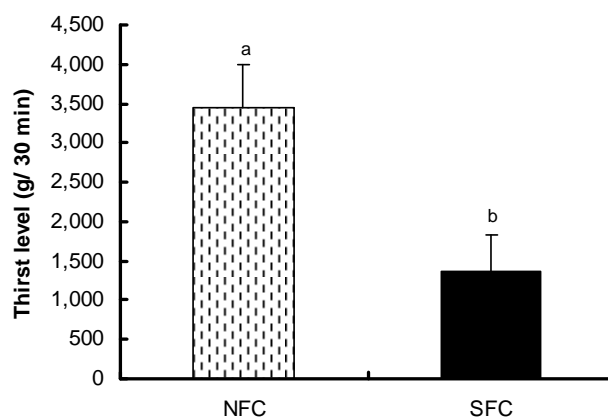
#### Rate of salivary secretion and cumulative salivary

#### secretion

In the SFC treatment, salivary secretion rates decreased sharply 10 min after commencement of feeding and were subsequently maintained at rates from 166±28.74 to 310±6.32 g/10 min for the remainder of the 2 h feeding period. Cumulative salivary secretion volume increased gradually as feeding period elapsed and reached a volume of 3,622±104.13 g upon conclusion of the 2 h feeding period (Table 2).

#### Thirst level

Figure 2 shows the effect of the SFC treatment on thirst level. In comparison with the NFC control (3,440±548.04 g/30 min), thirst level in the SFC treatment was 60.5% less (1,360±467.02 g/30 min;  $p<0.05$ ) upon conclusion of the 30 min drinking period.



**Figure 2.** The effect of sham feeding conditions (SFC) on thirst level. Values are means±SE of 5 large-type goats. <sup>a, b</sup>Means with different superscript are significantly different ( $p<0.05$ ) from normal feeding conditions (NFC).

**Table 3.** The effect of sham feeding conditions (SFC) on hematocrit, plasma total protein concentration, plasma osmolality, and plasma concentrations of glucose, Na, K, and Cl

Time after feeding beginning (min)	Hematocrit (%)		Plasma total protein (g/dl)		Plasma osmolality (mOsmol/L)		Plasma glucose (mg/dl)		Plasma Na (mmol/L)		Plasma K (mmol/L)		Plasma Cl (mmol/L)	
	NFC	SFC	NFC	SFC	NFC	SFC	NFC	SFC	NFC	SFC	NFC	SFC	NFC	SFC
-120	26.6 ±1.22	27.2 ±1.12	6.8 ±0.15	7.0 ±0.16	292.2 ±0.86	290.8 ±2.27	64.4 ±2.42	62.0 ±1.58	138.0 ±0.84	138.2 ±0.86	4.5 ±0.23	4.7 ±0.26	101.4 ±0.51	101.6 ±1.03
0	27.0 ±1.17	28.5 ±1.31	6.8 ±0.15	7.1 ±0.18	290.2 ±1.98	289.2 ±2.42	62.2 ±2.52	63.0 ±1.52	137.8 ±1.20	139.6 ±1.03	4.4 ±0.15	4.6 ±0.17	100.2 ±0.80	101.2 ±0.20
15	34.7 ±1.37	34.6 ±0.89	8.1 ±0.10 <sup>b</sup>	8.6 ±0.18 <sup>a</sup>	295.0 ±1.14	292.6 ±2.46	65.6 ±2.68	64.2 ±1.32	139.4 ±0.40	139.0 ±1.22	4.5 ±0.15	4.7 ±0.12	105.2 ±0.37	103.8 ±1.16
30	34.4 ±1.48	36.4 ±0.68	8.0 ±0.17 <sup>b</sup>	8.8 ±0.23 <sup>a</sup>	298.4 ±0.93	294.0 ±1.70	63.4 ±1.60	61.4 ±2.29	142.4 ±0.68 <sup>a</sup>	140.4 ±0.24 <sup>b</sup>	4.4 ±0.13	4.6 ±0.13	107.0 ±0.71	105.8 ±0.66
60	33.9 ±1.68	36.8 ±1.79	7.9 ±0.13 <sup>b</sup>	8.9 ±0.20 <sup>a</sup>	303.0 ±0.63 <sup>a</sup>	297.2 ±1.28 <sup>b</sup>	63.6 ±1.50	61.6 ±1.78	143.4 ±0.68 <sup>a</sup>	141.0 ±0.63 <sup>b</sup>	4.4 ±0.20	4.8 ±0.22	108.0 ±0.71	106.4 ±0.68
90	31.9 ±1.91	36.9 ±1.61	7.6 ±0.13 <sup>b</sup>	9.1 ±0.19 <sup>a</sup>	306.6 ±0.87 <sup>a</sup>	297.2 ±1.66 <sup>b</sup>	62.4 ±2.34	59.2 ±1.71	143.4 ±1.21	141.6 ±0.68	4.1 ±0.21 <sup>b</sup>	4.9 ±0.22 <sup>a</sup>	107.2 ±0.66	106.4 ±1.81
120	32.0 ±1.60	35.9 ±1.73	7.7 ±0.15 <sup>b</sup>	9.1 ±0.19 <sup>a</sup>	311.6 ±1.08 <sup>a</sup>	298.2 ±1.53 <sup>b</sup>	65.8 ±1.53	59.8 ±3.12	144.2 ±0.73 <sup>a</sup>	142.2 ±0.58 <sup>b</sup>	4.3 ±0.44	4.5 ±0.24	107.8 ±0.73	106.0 ±1.10
150	29.5 ±1.61	33.0 ±1.33	7.4 ±0.18 <sup>b</sup>	8.4 ±0.11 <sup>a</sup>	311.8 ±0.92 <sup>a</sup>	294.6 ±1.36 <sup>b</sup>	67.0 ±1.64 <sup>a</sup>	58.0 ±1.97 <sup>b</sup>	144.2 ±0.86 <sup>a</sup>	139.2 ±0.86 <sup>b</sup>	4.0 ±0.19	4.5 ±0.16	106.6 ±0.68 <sup>a</sup>	103.2 ±0.80 <sup>b</sup>

NFC = Normal feeding conditions; SFC = Sham feeding conditions; Values are means±SE of 5 large-type goats.

<sup>a, b</sup> Means in the same row bearing different superscripts differ ( $p < 0.05$ ).

### Hematocrit, plasma total protein concentration and plasma osmolality

In the NFC control and the SFC treatment, hematocrit and plasma total protein concentrations (Table 3) increased rapidly during the first 15 min after commencement of feeding. Subsequently, however, hematocrit and plasma total protein concentrations gradually decreased in the NFC control while being maintained at high level in the SFC treatment for the remainder of the feeding period. Compared with the NFC control, hematocrit in the SFC treatment was higher, but not significantly different, during the 2 h feeding period. Plasma total protein concentrations in the SFC treatment were significantly higher ( $p < 0.05$ ) than in the NFC control during the 2 h feeding period.

Plasma osmolality increased very slowly in both groups over the 2 h feeding period (Table 3). However, plasma osmolality in the NFC control was significantly higher ( $p < 0.05$ ) than in the SFC treatment from 60 min after feeding to the completion of the 2 h feeding period, even after conclusion of the 30 min drinking period.

### Plasma glucose concentration

Compared with the NFC control, plasma glucose concentrations in the SFC treatment were slightly lower over the 2 h feeding period but there were no significant differences between the two groups (Table 3). Nevertheless, plasma glucose concentrations in the NFC control were significantly higher than those in the SFC treatment at the conclusion of the 30 min drinking period.

### Plasma concentrations of Na, K and Cl

Table 3 presents the effect of the SFC treatment on plasma concentrations of Na, K and Cl. Plasma Na concentrations in the SFC treatment were lower than in the NFC control during the 2 h feeding period, but significant differences ( $p < 0.05$ ) between the two groups were found only at 30, 60, 120 and 150 min intervals. Compared with the NFC control, plasma K concentrations in the SFC treatment were slightly higher but not significantly different during the 2 h feeding period, except at 90 min after commencement of feeding. Plasma Cl concentrations in the SFC treatment were slightly lower than in the NFC control during the 2 h feeding period but not significantly different between the two groups.

### Ruminal fluid osmolality and pH

Table 4 shows the effect of the SFC treatment on ruminal fluid osmolality and pH. Ruminal fluid osmolality in the NFC control rapidly increased after commencement of feeding and reached the highest level ( $489.2 \pm 8.68$  mOsmol/L) at the end of the 2 h feeding period, while levels in the SFC treatment were mostly unchanged during the 2 h feeding period. Compared with the NFC control, ruminal fluid osmolalities in the SFC treatment were significantly lower ( $p < 0.01$ ) over the 2 h feeding period.

Ruminal fluid pH in the NFC control rapidly decreased in the first 15 min of feeding and subsequently declined gradually for the remainder of the 2 h feeding period. In contrast, ruminal fluid pH in the SFC treatment was maintained unchanged during the 2 h feeding period.

**Table 4.** The effect of sham feeding conditions (SFC) on ruminal fluid osmolality, pH, and concentrations of Na, K, and Cl

Time after feeding beginning (min)	Ruminal fluid osmolality (mOsmol/L)		Ruminal fluid pH		Ruminal fluid Na (mmol/L)		Ruminal fluid K (mmol/L)		Ruminal fluid Cl (mmol/L)	
	NFC	SFC	NFC	SFC	NFC	SFC	NFC	SFC	NFC	SFC
-120	278.0 ±2.55	264.2 ±5.83	7.02 ±0.06	7.09 ±0.09	87.1 ±13.47	97.7 ±19.51	54.6 ±2.63	53.0 ±3.21	32.6 ±4.00	35.1 ±10.66
0	277.0 ±2.00	269.8 ±5.54	7.11 ±0.05	7.25 ±0.10	91.3 ±14.67	96.2 ±12.46	59.4 ±3.11	56.7 ±5.01	38.0 ±5.21	47.3 ±16.84
15	379.6 ±6.61 <sup>a</sup>	264.4 ±4.86 <sup>b</sup>	6.61 ±0.04 <sup>b</sup>	7.31 ±0.09 <sup>a</sup>	111.6 ±17.93	101.1 ±13.53	66.8 ±5.28	58.0 ±5.06	54.9 ±11.49	47.0 ±15.02
30	421.2 ±18.11 <sup>a</sup>	264.4 ±4.45 <sup>b</sup>	6.56 ±0.06 <sup>b</sup>	7.32 ±0.09 <sup>a</sup>	116.1 ±17.00	99.6 ±11.66	71.8 ±4.96	58.2 ±4.91	71.8 ±11.84	53.7 ±20.30
60	458.0 ±11.22 <sup>a</sup>	261.6 ±5.37 <sup>b</sup>	6.49 ±0.04 <sup>b</sup>	7.35 ±0.09 <sup>a</sup>	122.4 ±20.12	99.9 ±10.14	77.1 ±4.82	58.5 ±4.34	83.7 ±13.41	56.2 ±20.94
90	483.0 ±13.10 <sup>a</sup>	262.6 ±5.76 <sup>b</sup>	6.47 ±0.05 <sup>b</sup>	7.36 ±0.09 <sup>a</sup>	119.9 ±17.49	100.6 ±9.12	80.8 ±4.93 <sup>a</sup>	58.8 ±3.90 <sup>b</sup>	86.0 ±11.64	57.8 ±21.77
120	489.2 ±8.67 <sup>a</sup>	262.8 ±6.13 <sup>b</sup>	6.45 ±0.05 <sup>b</sup>	7.34 ±0.09 <sup>a</sup>	119.0 ±16.84	101.9 ±8.37	83.4 ±5.13 <sup>a</sup>	58.6 ±3.60 <sup>b</sup>	91.3 ±12.97	63.0 ±23.30
150	454.8 ±31.69 <sup>a</sup>	220.0 ±15.55 <sup>b</sup>	6.27 ±0.07 <sup>b</sup>	7.32 ±0.09 <sup>a</sup>	114.7 ±18.42	90.6 ±6.77	85.7 ±5.78 <sup>a</sup>	59.6 ±3.19 <sup>b</sup>	82.0 ±7.25	52.2 ±12.79

NFC = Normal feeding conditions; SFC = Sham feeding conditions; Values are means±SE of 5 large-type goats.

<sup>a, b</sup> Means in the same row bearing different superscripts differ ( $p < 0.05$ ).

Therefore, ruminal fluid pH in the SFC treatment was significantly higher ( $p < 0.01$ ) than in the NFC control over the 2 h feeding period.

#### Ruminal fluid concentrations of Na, K and Cl

The effect of the SFC treatment on ruminal fluid concentrations of Na, K and Cl is shown in Table 4. Ruminal fluid concentrations of Na and Cl in the NFC control increased gradually after the commencement of feeding while those in the SFC treatment were mostly unchanged during the 2 h feeding period. However, there were no significant differences between the two groups in terms of ruminal fluid concentrations of Na and Cl.

Ruminal fluid concentrations of K in the NFC control also gradually increased after the beginning of feeding while those in the SFC treatment were mainly unchanged. Nevertheless, in the first 60 min after the commencement of feeding, there were no significant differences between the NFC control and the SFC treatment in ruminal fluid K concentrations. In comparison with the NFC control, ruminal fluid K concentrations in the SFC treatment were significantly lower ( $p < 0.05$ ) at 90, 120 min intervals during the 2 h feeding period and at 150 min after the conclusion of the 30 min drinking period.

## DISCUSSION

The new findings in the present study were i) dry forage intake during the second hour of a 2 h feeding period was reduced markedly by factors produced when boluses enter

the rumen; ii) the amount of salivary secretion was larger than dry forage intake and this saliva worked in conjunction with consumed feed to form the ruminal load responsible for ruminal distension; and iii) in comparison with the decrease in plasma volume, the increase in plasma osmolality during the second hour of a 2 h dry forage feeding period was more effective in stimulating the sensation of thirst.

#### Esophageal fistulated animals

Studies using an esophageal fistula in sheep and cattle have been reported previously (Yarns et al., 1964; Alder, 1969; Kato, 1977; Grovum and Chapman, 1988). It was reported that there were many problems in post-operative maintenance of esophageal fistulae and in sample collection from them (Grovum and Chapman, 1988). However, in the present experiment the esophageal fistulae in five goats were successfully maintained and used over long periods of time. Both esophageal and ruminal fistulae were kept in place for an extended period and the animals remained healthy and were not under any stress. Furthermore, the factors that indicated the animal's state of health, namely heart rate, respiratory rate, and rectal temperature, were all within the normal range. Stress level may be indicated by plasma glucose levels which were within the normal range in animals on the SFC treatment. Roughly crushed alfalfa hay cubes with any large remaining chunks removed were used as feed for this research. The final product was similar to lucerne chaff and animals ate this feed without any trouble in swallowing and rumination. In summary, the animals were in a completely normal physiological state.

### Salivary secretion volume and ruminal distension

The ratio by weight of cumulative salivary secretion and cumulative dry forage intake was 1.13 to 1.49 for the duration of the 2 h feeding period in the SFC treatment. Hence, during dry forage feeding the weight of the boluses that was swallowed was 2.14 to 2.45 times the weight of cumulative dry forage intake over the same period. Upon the conclusion of the 2 h feeding period, cumulative salivary secretion volume ( $3,622 \pm 104.13$  g/2 h) was heavier than the cumulative feed intake ( $3,182 \pm 381.69$  g/2 h).

The present experiment showed that this saliva worked in conjunction with consumed feed to form the ruminal load responsible for ruminal distension. Therefore, it is thought that copious amounts of secreted saliva due to dry forage feeding contributed to the marked increase in ruminal distension level in the NFC control during the second hour of the 2 h feeding period.

### Ruminal fluid osmolality

In the NFC control, ruminal fluid osmolality in goats increased significantly with dry forage feeding from  $277 \pm 2.00$  mOsmol/L, prior to feeding, to  $458 \pm 11.22$  mOsmol/L at 60 min after feeding commenced. At the same time, due to the production of volatile fatty acids by microbial fermentation, the ruminal fluid pH significantly decreased from  $7.11 \pm 0.05$  prior to feeding to  $6.49 \pm 0.04$  at 60 min after feeding commenced. It is thought that increases in ruminal fluid osmolality in the NFC control during dry forage feeding was caused by increased ruminal fluid concentrations of Na, K, and Cl due to feed and saliva entering the rumen and by increases in volatile fatty acids produced in the rumen. On the other hand, in the rumen of goats in the SFC treatment, because the feed and saliva were prevented from entering the rumen, ruminal fluid osmolality and pH levels in the second hour of the 2 h feeding period remained the same as pre-feeding levels.

### Thirst level

Thirst is a subjective perception that provides the urge for humans and animals to drink fluids (McKinley and Johnson, 2004). The desire to drink, that is, is completely satisfied only when plasma osmolality or blood volume returns to normal. Increased extracellular fluid osmolality, decreases in extracellular fluid volume and arterial pressure, angiotensin II, and dryness of the mouth stimulate the sensation of thirst in the brain (Guyton and Hall, 1996). Blair-West and Brook (1969) reported that changes in hematocrit and plasma total protein concentration reflected the changes in circulating plasma volume. Sunagawa et al. (2008) reported that in large-type goats fed dry forage, hematocrit and plasma total protein concentration rapidly increased with dry forage feeding while plasma osmolality

increased very slowly. Consistent with the results reported by Sunagawa et al. (2008), in both the NFC control and the SFC treatment of the present experiment, after the commencement of dry forage feeding hematocrit and plasma total protein concentration increased to levels higher than pre-feeding levels. Hematocrit and plasma total protein concentrations in the SFC treatment were greater than those in the NFC control. In other words, the level of decrease in circulating plasma volume due to dry forage feeding was larger in the SFC treatment than the NFC control. Saliva is produced from components in the blood and salivary secretion volume has a positive relationship with dry forage intake (Sunagawa et al., 2007). From the proportion of salivary secretion volume and feed intake in the SFC treatment, we estimated that cumulative salivary secretion volume in the NFC control was  $2,033.93 \pm 233.15$  g/2 h. Cumulative salivary secretion volume in the SFC treatment was larger than that in the NFC control. In addition, in the SFC treatment, the saliva secreted during dry forage feeding was removed via an esophageal cannula and did not reach the rumen. Thus, the water content of the saliva was not absorbed into the blood via the rumen. On the other hand, while plasma osmolalities during the initial 30 min of feeding in the SFC treatment were the same as the NFC control, they were observed to be significantly lower than the NFC control in the second hour of the 2 h feeding period. The reason for this was that in the SFC treatment, due to the removal of both consumed feed and saliva via the esophageal cannula, the salts contained in the feed and the saliva were prevented from entering the rumen. Thus, they were not absorbed into the blood via the rumen (Warner and Stacy, 1972; 1977; Argenzio, 1984). Therefore, the result was that water intake at the conclusion of the 2 h feeding period was significantly lower in the SFC treatment compared to the NFC control. The results of the present experiment indicated that in the second hour of dry forage feeding in the NFC control, the decrease in circulating plasma volume in conjunction with the increase in plasma osmolality was responsible for increased thirst level.

### Sham feeding and dry forage intake

In normal feeding when dry forage was fed to ruminants such as in the NFC control of the present experiment, the factors controlling dry forage intake, namely ruminal distension and thirst level, all increased in the second hour of the 2 h feeding period. It is therefore thought that in experiments investigating feed intake suppression conducted under normal feeding conditions, there are a number of factors involved. However, it has never been elucidated which factors are mainly involved in suppression of feed intake.

In the SFC treatment in the present experiment, both



saliva and feed was removed via an esophageal cannula and therefore neither ruminal distension nor ruminal fluid osmolality increased. Microbial fermentation also did not occur. In other words, even after 40 min of feeding, with the exception of a decrease in circulating plasma volume there were no observed changes in the parameters (ruminal distension, plasma osmolality, and ruminal fluid volatile fatty acid concentrations) associated with the suppression of dry forage intake. In the SFC treatment of the present experiment, it was found that the goats continued eating throughout the entire 2 h feeding period and consumed more dry forage than in the NFC control.

The utilization of esophageal fistulated goats enables the isolation of factors that are presumed to control dry forage intake. This isolation allows individual investigation of the effect of each factor on dry forage intake. It is thought that esophageal fistulation of ruminants contributes to the clarification of dry forage intake suppression factors and mechanisms.

#### **Ruminal distension and dry forage intake**

In normal feeding when goats were fed dry forage, such as the NFC control of the present experiment, eating rates rapidly decreased in the first 30 to 40 min of feeding and were subsequently reduced to very low rates for the remainder of the 2 h feeding period (Sunagawa et al., 2003; 2005; 2007; 2008). Sunagawa et al. (2003; 2007) reported that an inhibition of dry forage intake during the early stages of feeding in goats was caused by a feeding-induced decrease in circulating plasma volume. In the present experiment, cumulative dry forage intake in the SFC treatment was the same as the NFC control for the first 40 min after the commencement of feeding. On the other hand, the mechanism at work in the marked suppression of feed intake after 40 min of feeding is unclear.

Campling and Balch (1961) reported that when a balloon was inserted into the rumen and inflated with water, feed intake was decreased in cows fed on hay. The results of the present experiment clarified that copious amounts of saliva were secreted and entered the rumen during dry forage feeding, and this saliva worked in conjunction with consumed feed to form the ruminal load responsible for ruminal distension. From these facts, it is thought that ruminal distension was involved in the decreased dry forage intake observed during the second hour of the 2 h feeding period.

#### **Thirst level and dry forage intake**

Grovum (1995) reported that when the same dose of hyperosmotic NaCl, polyethylene glycol-400 (PEG), sodium acetate or sodium propionate was infused intraruminally to increase ruminal fluid osmolality in sheep,

it resulted in the same-sized decrease in alfalfa pellet intake. In the NFC control of the present experiment, ruminal fluid osmolality increased due to increases in salts contained in the feed and volatile fatty acids produced by microbial fermentation during dry forage feeding. In the second hour of the 2 h feeding period, plasma osmolality increased as a result of ruminal absorption of salts and volatile fatty acids in the NFC control. Because of the decreased plasma volume due to large amounts of saliva secreted during dry forage feeding, plasma total protein concentrations increased in both the NFC control and the SFC treatment. The thirst level in the NFC control was higher than in the SFC treatment because plasma osmolality in the SFC treatment did not change during the dry forage feeding. Prasetiyono et al. (2000) reported that the thirst level was negatively correlated with dry forage intake in goats. From these facts, it is thought that thirst level was also involved in the decrease of dry forage intake during the second hour of the 2 h feeding period.

The results of the present experiment indicate that in the second hour of the 2 h feeding period, dry forage intake is regulated by factors produced when boluses enter the rumen.

#### **ACKNOWLEDGMENTS**

We are grateful to Associate Professor Go Ogura (University of the Ryukyus) and Professor Kunioki Hayashi (Kagoshima University) for their useful suggestions and comments made on the manuscript. We thank Mr. Tomoyuki Namima, Mr. Daisuke Nakama, Mr. Seiya Nagayama, Miss Midori Kidoguchi, Miss Yurie Yamauchi and Miss Nonoko Nakagima for their helpful assistance in experiments and recording the data. We also thank Mr. Glenn McIlvride for his English proof-reading on this manuscript and Professor Takuro Oikawa for his useful advice on statistical analysis. The computation was mainly carried out using the computer facilities in the Research Institute for Information Technology, Kyushu University.

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