

## Effects of Intraruminal Saliva Flow on Feed Intake in Goats Fed on Alfalfa Hay Cubes

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**ABSTRACT :** Research was carried out to ascertain whether or not the volume of saliva flowing into the rumen regulates dry forage intake in ruminants. Goats with a parotid fistula were fed roughly crushed alfalfa hay cubes, concentrated beef cattle feed and NaHCO<sub>3</sub> twice daily (10:00-12:00, 16:00-18:00). Except for the days on which experiments were conducted, the animals were free access to drinking water. The animals were intraruminally infused every day prior to the morning feeding period with parotid saliva collected from the parotid fistula over a 24 h period. The present experiment consisted of three treatments, non-infusion (NI), intraruminal infusion of parotid saliva (RSI), and intraruminal infusion of warm water (RWI). In the RSI treatment, approximately 4-5 kg of parotid saliva (280-290 mOsm/l) collected over a 24 h period was intraruminally infused 1 h prior to the commencement of morning feeding. In the RWI treatment, parotid saliva was substituted for warm water (36°C). After infusions, the animals were fed on roughly crushed alfalfa hay cubes for 2 h. During feeding, eating and saliva secretion rates were measured. Blood samples were also periodically collected from the jugular vein. After 2 h feeding, water intake was measured for 30 min. These measurements were used to define thirst levels. On the day of the experiment, the animals were not access to drinking water during the morning feeding. It is thought that rumen fill in RSI and RWI treatments was higher than the NI treatment. In comparison with the NI treatment however, cumulative feed intake increased by 39.3% with RSI treatment and by 45.9% with RWI treatment after completion of the 2 h feeding period. After 2 h feeding, thirst level in the RSI treatment showed only a 10% decrease compared to the NI treatment, but thirst level in the RWI treatment decreased 49.8%. Despite the significant differences in thirst levels between RSI and RWI treatments, the cumulative feed intake in both treatments was similar. When comparing accumulated saliva secretion volumes 2 h after feeding, volumes in the RSI treatment were significantly 35.9% lower than the NI treatment while volumes in the RWI treatment were unchanged. However, the volumes of saliva and fluid flowing into the rumen were greater in both RSI and RWI treatments when compared to the NI treatment. The results indicate that the amount of saliva flowing into the rumen is a factor regulating feed intake in ruminants fed on dry forage. (*Asian-Aust. J. Anim. Sci.* 2002. Vol 15, No. 12 : 1738-1746)

**Key Words :** Intraruminal Saliva Flow, Dry Forage Intake, Goats

### INTRODUCTION

In countries such as Japan, ruminants are frequently offered dry feed to raise productivity. Saliva in sheep is secreted in large volumes during the first hour after the commencement of dry forage feeding (Sato, 1975). Saliva acts as a lubricant in the mouth and esophagus, and assists in the mastication, remastication, swallowing and reswallowing of dry forage. Saliva also acts as an alkaline 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 this way, saliva plays an important role in eating and homeostatic regulation of the acid-base balance in ruminal fluid.

In sheep fed on dry forage, large volumes of saliva are secreted in the early stages of feeding. However, secretion volumes sharply declined following these early stages irrespective of continued feeding. Prasetiyono et al. (2000) and Sunagawa et al. (2001) reported that feed intake rates in goats fed alfalfa hay cubes once and twice a day

respectively decreased sharply 30 min following the start of feeding. Silanikove and Tadmor (1989) reported that in cows deprived of water for long periods of time there was a positive relationship between saliva secretion rates and wheat hay intake. Sunagawa et al. (2002) reported that despite being free access to water and salt via a pedal press system, sheep with a parotid fistula consumed substantially less alfalfa chaff than sheep without a parotid fistula. It is therefore thought that saliva secretion volume is a regulating factor in dry forage intake. Until now, experiments investigating the physiological relationship between saliva secretion volume and dry forage intake have not been conducted.

In the present experiment, the animals were prepared with a parotid fistula. Saliva from the fistula was collected and infused into the rumen once each day prior to morning feeding. This experiment was conducted to clarify whether dry forage intake is regulated by the amount of saliva flowing into the rumen.

### MATERIALS AND METHODS

#### Animals

Five goats (1 Japanese Saanen goat, aged 4 years,

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weighing 72.5 kg; 4 crossbred Japanese Saanen/Nubian goats, aged 3-5 years, weighing 72, 83, 90, 97 kg) were used in this experiment. In order to collect parotid saliva, the aperture of one of the parotid ducts was surgically prepared to exteriorize it via the cheek of the animal more than 6 months prior to experimentation. An Atom Disposable Multiple Purposes Tube (o.d. 2.75 mm, 8 Fr, Atom, Tokyo) approximately 10 cm in length or depending on the animal, a fluid infusion tube (o.d. 4.00 mm, Terumo, Tokyo) was inserted into the parotid duct and fixed to the cheek. Furthermore, to enable the return of saliva collected from the parotid fistula, an extension tube (X3-25, Top, Tokyo) was inserted into the dorsal sac of the rumen. The other end of the tube was fixed to the skin. Parotid saliva flowing from the parotid fistula was collected in a plastic bucket. The sheep were maintained in metabolism cages that allowed for the separate collection of urine, feces and saliva.

The animals were fed twice a day at 10:00 am and again at 4:00 pm for 2 h each time. Prior to the morning feeding, the collected saliva (3-5 kg) was infused into the rumen via the extension tube using a bath tub pump (Minipondy, KP-30F, Koshin, Tokyo). Following this, 2-3 kg of roughly crushed alfalfa hay cubes, 20 g of NaHCO<sub>3</sub> and 200 g of commercial beef cattle concentrate feed were fed to the animals for 2 h.

Animals were free access to water throughout the day. The alfalfa hay cubes (84.3% dry matter) contained, on a dry matter basis, 18.7% crude protein, 2.4% crude fat, 29.7% crude fiber, 39.7% nitrogen free extracts (NFE), 45.9% NDF and 36.6% ADF. The concentrated beef cattle feed (86.9% dry matter) contained, on a dry matter basis, 13.4 % crude protein, 3.6% crude fat, 71.0% nitrogen free extracts (NFE), 14.6% NDF and 5.4% ADF.

### Experiment

The experiment was carried out in a laboratory with a room temperature of 24-26°C and a relative humidity of 74-84%. This experiment investigated the effect of intraruminal infusion of parotid saliva or warm water (36°C) on cumulative feed intake and eating rates. The control treatment monitored cumulative feed intake and eating rates in the absence of infusion. Animals were deprived of water during feeding on the day of the experiment. Treatments in this experiment were carried out in order, beginning with the no infusion control (NI) followed by the parotid saliva infusion treatment (RSI) and concluding with the warm water infusion treatment (RWI). The treatments were carried out on 2-3 animals at one week intervals to ensure that animals recovered and to minimize the compounding effects of the previous treatments. Respiration frequency, heart rate and rectal temperature were measured everyday prior to the morning feeding

period. The values of these physiological parameters indicated whether an individual was in good health and had no measurable carry-over effects from the previous treatments. In order to take blood samples during the course of the experiment, a polyethylene tube (o.d. 1.5 mm, No. 5, Imamura Gomu, Tokyo) was inserted into the jugular vein of the animals on the day prior to the commencement of experimentation. This tube was fixed in place and filled with heparin-saline solution (50 i.u./ml) to prevent coagulation of the blood. On the day of the experiment, depending on the animal either an extension tube (o.d. 2.0 mm, X-1, Top, Tokyo) or a polyethylene tube (o.d. 2.2 mm, No. 7, Imamura Gomu, Tokyo) was connected to the tube inserted into the parotid fistula. From the open end of the tube, parotid saliva was collected in a graduated measuring cylinder (100 ml). On the days of both the RSI treatment and the RWI treatment, 4.0-5.0 kg of saliva or warm water (36°C) respectively was infused into the rumen using a bath tub pump (Minipondy, KP-30F, Koshinsha) 1 h prior to the commencement of the morning feeding period. The infusion volume for animals weighing 72.5, 83.0, 90.0 and 97.0 kg was 5.0 kg, while the animal weighing 72.0 kg was infused with 4.0 kg. Feeding was begun at 10:30 am and the animals were fed roughly crushed alfalfa hay cubes for 2 h. Eating rates were determined using a measuring scale. The alfalfa hay cubes (2.0-3.0 kg) were placed in a feed box attached to a 6 kg measuring scale. The weight of the remaining feed was measured every 10 min for the duration of the 2 h feeding period. The rate of saliva secretion was measured in a graduated cylinder. Measurements began 10 min prior to feeding commencement, continued every 10 min during the duration of the feeding period and concluded with the last measurement being taken at the end of the 2 h feeding period. To measure the osmolality of saliva, a sample of the collected parotid saliva was taken. This sample was placed in a test tube and refrigerated until measuring. Thirst level (g/30 min) in this experiment was defined as the water intake for 30 min upon completion of 2 h of feeding.

A total of 10 blood samples were taken, 2 each prior to and after the feeding period, and 6 during feeding after 10, 30, 45, 60, 90, 120 min had elapsed. Blood samples (5 ml each) were taken from the jugular vein. Samples were collected in heparinized tubes to prevent coagulation and were subsequently refrigerated. Blood plasma was obtained by centrifugation (16,260×g, 10 min, 5°C).

### Biochemical analysis

Saliva osmolality was measured according to the principle of freezing-point method using an osmometer (Model OM-6010, Kyoto Daiichi Kagaku, Kyoto). Blood samples were placed in hematocrit capillary tubes and centrifuged using a hematocrit centrifuge (HC-12 A, Tomy

Seiko, Tokyo, 16,260×g×5 min) to separate plasma and red blood corpuscle. Hematocrit was determined by a hematocrit reader. Plasma protein and osmolality were measured by a refractometer (Atago, Tokyo) and by an osmometer, respectively.

### Statistical analysis

The experiments in this research were conducted according to a switchback design. A two-way analysis (animal, treatment) of variance was performed. Following that Duncan's Multiple Range Tests were used to compare treatments. For statistical analysis, GLM procedures (SAS, 1990) were adopted. Data are presented as mean±SD of five sheep.

## RESULT

### Physiological responses

The mean values of respiration frequency, heart rate and rectal temperature before infusion in the three treatments were 26±3.9 breaths/min, 83±9.5 beats/min and 38.8±0.18°C respectively.

### The rate of eating and cumulative feed intake

Figure 1 shows the effect of intraruminal infusion of parotid saliva or warm water on rate of eating and cumulative feed intake. Eating rates in the NI treatment rapidly decreased in the first 40 min of feeding (0 to 10 min 435 g/10 min, 30 to 40 min 79 g/10 min). However, eating rates in the RSI and RWI treatments slowly decreased for the first 60 min. Eating rates in the RSI and RWI treatments during the first 60 min of feeding were larger than those in the NI treatment. Eating rates between the RSI and RWI treatments were not different each other. After 60 min of the feeding period had elapsed, there were no significant differences among three treatments.

In comparison with the NI treatment (1,100±544.1 g/2 h), cumulative feed intakes in RSI and RWI treatments were 39.3% (1,532±648.5 g/2 h) and 45.9 % (1,605±621.7 g/2 h) larger upon conclusion of the 2 h feeding period.

### Thirst level

Figure 2 shows the effect of intraruminal infusion of parotid saliva or warm water on thirst level. Thirst level in the RSI treatment (5,760±1,553 g/30 min) showed only a 10 % decrease and was not significantly different from the level in the NI treatment (6,400±2,618 g/30 min). Thirst level (3,210±1,111 g/30 min) in the RWI treatment was, on the other hand, a significant 49.8% lower than the NI treatment level.

### Plasma osmolality, plasma total protein concentration and hematocrit

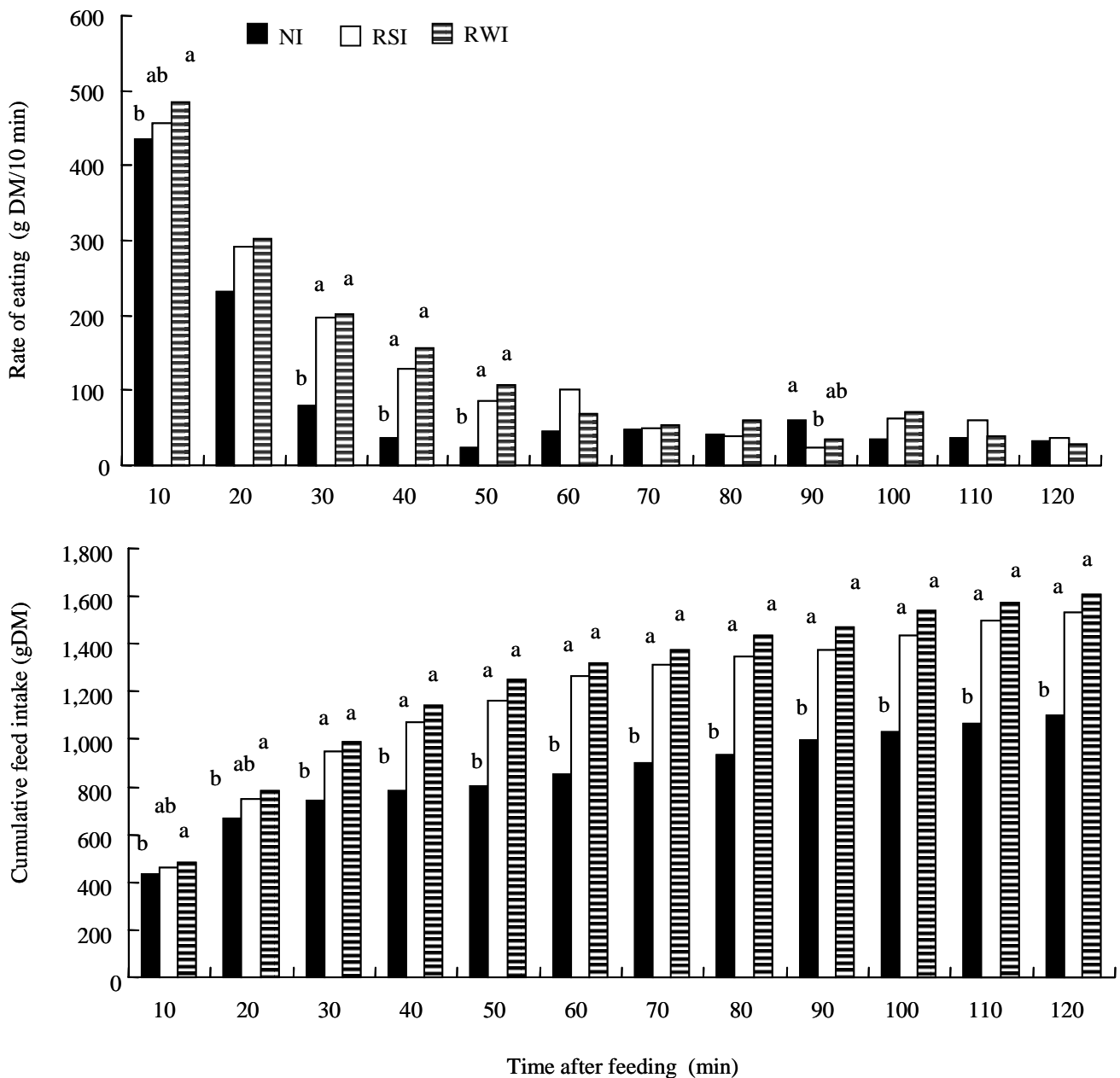
Figure 3 shows the effect of intraruminal infusion of parotid saliva or warm water on plasma osmolality, plasma total protein concentration and hematocrit in the blood sampled at 60 min before feeding and 0, 15, 30, 45, 60, 90, 120, 150, and 165 min after feeding was commenced. Plasma osmolality gradually increased in all three treatments over the course of the 2 h feeding period. In comparison with the NI treatment, plasma osmolality in the RSI treatment was significantly ( $p<0.05$ ) higher throughout the entire period. On the other hand, plasma osmolality in the RWI treatment was not significantly different from the NI treatment during the 2 h feeding period.

In all three treatments, plasma total protein concentrations prior to feeding (NI: 6.9±0.52, RSI: 7.0±0.52, RWI: 7.7±1.15 g/dl) had increased 15 mins after the commencement of feeding (NI: 7.8±0.58, RSI: 7.7±0.74, RWI: 8.1±1.12 g/dl). However, plasma total protein concentrations gradually decreased in all treatments for the remainder of the feeding period. Plasma total protein concentration in the NI treatment 1 h prior to the commencement of feeding (6.9±0.52 g/dl), and immediately prior to feeding (7.0±0.47 g/dl), were not significantly different. On the other hand, due to infusion, plasma total protein concentrations in RSI and RWI treatments decreased over the same period from 7.0±0.52, 7.7±1.15 g/dl to 6.8±0.56, 7.3±1.01 g/dl respectively. Plasma total protein concentrations in the RSI treatment were lower than both NI and RWI treatments 30 min after feeding. There were no significant differences between the NI and RWI treatments during feeding.

In all three treatments, hematocrit immediately prior to feeding (NI: 27.1±1.55, RSI: 26.9±1.14, RWI: 29.4±3.91%) had increased 15 min after the commencement of feeding (NI: 32.2±3.44, RSI: 32.0±1.58, RWI: 35.6±4.39%). However, hematocrit gradually decreased in all three treatments for the remainder of the feeding period. Hematocrit in the RSI treatment were lower than both NI and RWI treatments 60 min after feeding. There were no significant differences between the NI and RWI treatments during feeding.

### Rate of saliva secretion and cumulative saliva secretion

Figure 4 shows the effect of intraruminal infusion of parotid saliva or warm water on the rate of saliva secretion and cumulative saliva secretion. The rates of saliva secretion in all three treatments peaked for the first 10 min after feeding was commenced (NI: 135±33.1, RSI: 133±13.6, RWI: 150±20.7 ml/10 min). In comparison with the NI treatment, the rates of parotid saliva secretion in the RSI treatment were significantly ( $p<0.05$ ) lower throughout



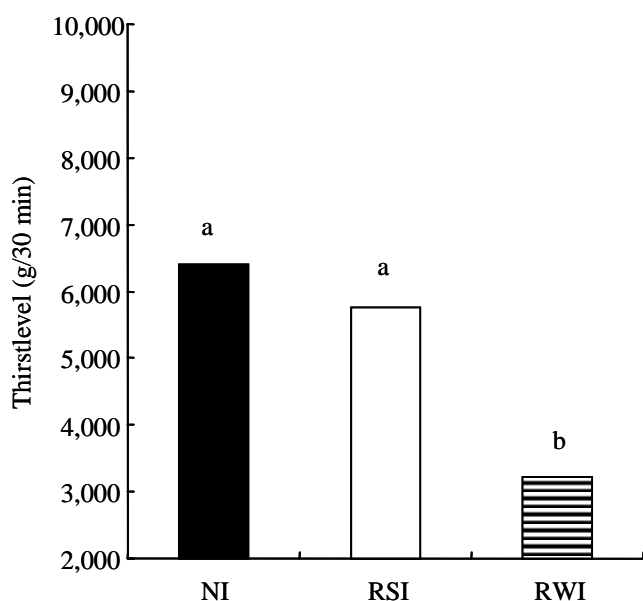
**Figure 1.** The effect of intraruminal infusion of parotid saliva (RSI) or warm water (RWI) on rate of eating and cumulative feed intake. <sup>a,b</sup> Means with different superscripts are from no infusion treatment (NI,  $p < 0.05$ ).

the entire period of feeding. On the other hand, the rates of saliva secretion in the RWI treatment were significantly ( $p < 0.05$ ) lower than those in the NI treatment during the early stage of feeding.

Cumulative saliva secretion volumes upon conclusion of the 2 h feeding period were  $828 \pm 131.7$ ,  $531 \pm 73.9$ ,  $811 \pm 86.4$  ml/2 h in NI, RSI and RWI treatments, respectively. Cumulative saliva secretion volume in the RSI treatment showed a significant 35.9% decrease when compared to the NI treatment. However, there was no significant difference between the RWI and NI treatments.

### Saliva osmolality

Figure 5 shows the effect of intraruminal infusion of parotid saliva or warm water on saliva osmolality. Parotid saliva osmolality increased after feeding in all three treatments. Saliva osmolality in the RSI treatment was significantly higher than that in the NI treatment at 20, 100, 110 and 120 min after feeding. On the other hand, saliva osmolality in the RWI treatment prior to, during and after feeding was not significantly different from that in the NI treatment.



**Figure 2.** The effect of intraruminal infusion of parotid saliva (RSI) or warm water (RWI) on thirst level. <sup>a,b</sup> Means with different superscripts are from no infusion treatment (NI,  $p < 0.05$ ).

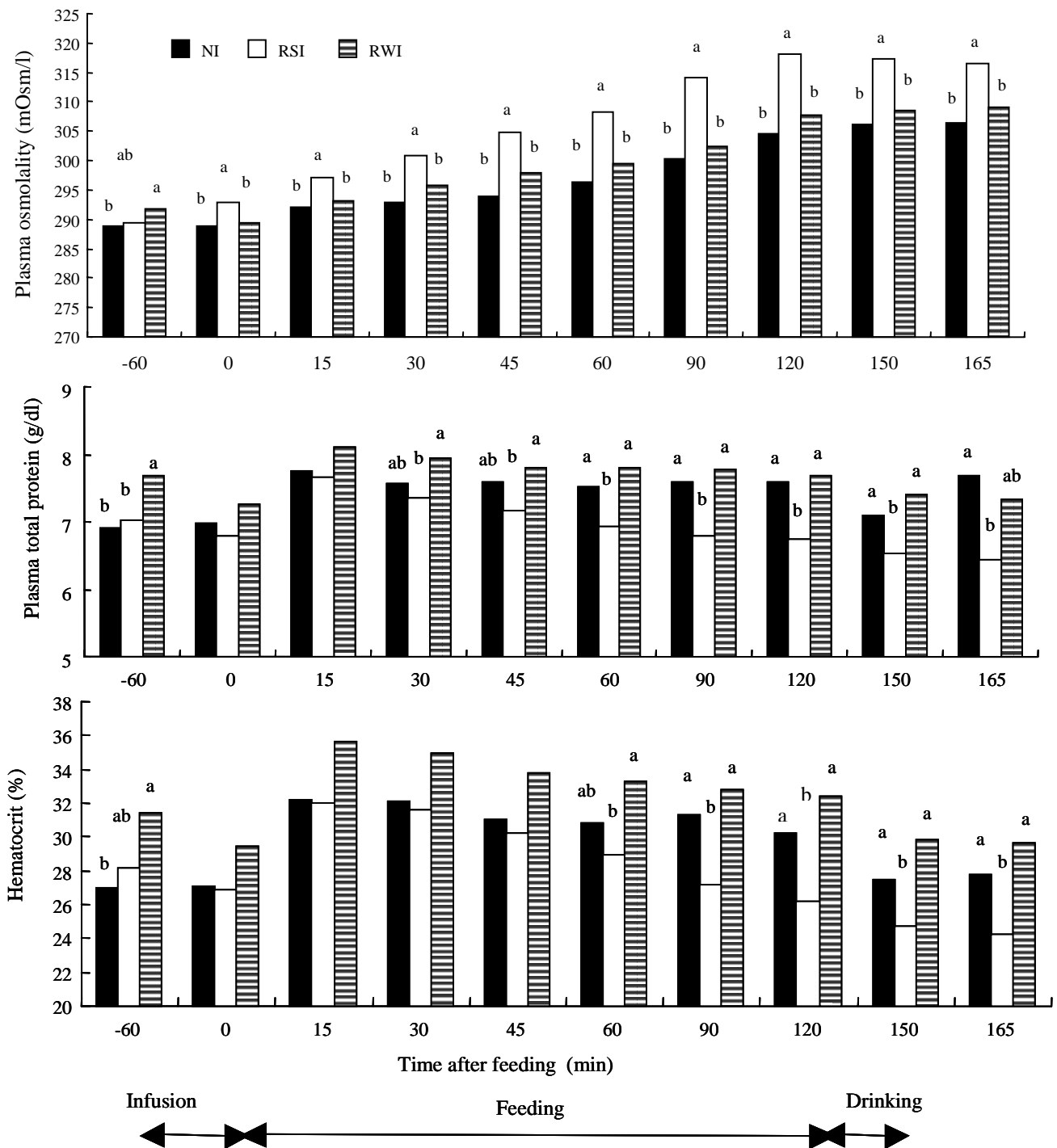
## DISCUSSION

Preliminary experiments were conducted 2 months prior to the present experiment. In the preliminary experiments, animals were raised using two methods. The first method saw the infusion of parotid saliva collected from the parotid fistula into the rumen. In the second method, collected saliva was not infused. Under these conditions, a number of different parameters were measured. The results were that saliva secretion from the unilateral parotid fistula and feed intake during the morning 2 h feeding period in the infusion treatment ( $828 \pm 131.7$  ml/2 h;  $1,100 \pm 544.1$  g/2 h) were significantly greater compared to the non infusion treatment whereby saliva secretion was  $399 \pm 50.7$  ml/2 h and feed intake was  $489 \pm 185.0$  g/2 h. The feed intake in goats with a parotid fistula that were intraruminally infused with saliva, was similar to pre-fistulation values ( $929 \pm 81.8$  g/2 h) (Sunagawa et al., 2002a). Sunagawa et al. (2002b) reported that in an experiment using a pedal press system for the delivery of water and salt, feed intake of alfalfa chaff in non-fistulated sheep was significantly greater than non-intraruminally infused fistulated sheep. Therefore, it is thought that the internal environment of animals maintained with a daily infusion of parotid saliva in the present experiment was similar to non-fistulated animals. In the period 30-60 min after feeding, saliva secretion rates in the NI treatment declined markedly. During this same period of time, eating rates also showed a significant decrease (Figure 1, 4). During actual eating and rumination, saliva secretion rates increased significantly. Saliva secretion rates

in the NI treatment peaked immediately following the start of feeding, but then subsequently declined reaching pre-feeding levels 60 min after feeding had commenced. This return to pre-feeding secretion rates at the 60 min mark of the feeding period coincided with the lowest eating rates. These results suggest the existence of a physiological control mechanism between feed intake of hay and saliva secretion volumes.

When parotid fistulated sheep, fed once a day with approximately 2 kg of alfalfa hay cubes and 50 g of  $\text{NaHCO}_3$ , were intraruminally infused with approximately 3 l of warm water, plasma osmolality did not increase with feeding (Sato, 1975). Additionally, the marked suppression of saliva secretion that occurs with normal feeding conditions was not induced. From this result, it is thought that post-feeding saliva secretion suppression is caused by an increase in plasma osmolality with feeding. When an amount of warm water, equal to that of the volume of saliva collected from the parotid fistula in the course of one day, was infused into the rumen, similar to the NI treatment, little or no change was observed in plasma osmolality up until the 45 min mark. Saliva secretion rates that coincided with feeding slowly decreased in both NI and RWI treatments. In the RWI treatment, despite there being no significant difference in plasma osmolality, saliva secretion rates from 10-40 min after feeding were significantly lower (Figure 3, 4). It is thought that parotid saliva secretion rates were suppressed as the degree of ruminal fill increased. In this experiment, when parotid saliva collected from the unilateral parotid fistula 1 h prior to the morning feeding period (280-290 mOsm/l) was infused into the rumen, as was the case with normal feeding, plasma osmolality increased with feeding (Sunagawa et al., 2001). Except for the 10 min following the start of the feeding period, saliva secretion rates in RSI were suppressed continuously for 2 h of feeding. Sato (1975), Warner and Stacy (1977) reported that there is a negative correlation between saliva secretion rates, ruminal fluid osmolality and plasma osmolality. From these reports, it is thought that the significant decrease in parotid saliva secretion that occurred after 20 min of the feeding period had elapsed in the RSI treatment was, in addition to ruminal fill increases, due to increases in plasma osmolality during feeding that were brought about by intraruminal infusion of saliva.

It was reported that the feed intake of alfalfa pellets was regulated by changes in ruminal fluid osmolality (Baile et al., 1969; Kato et al., 1979; Grovum, 1995). The same sized dose of hyperosmotic NaCl, polyethylene glycol-400 (PEG), sodium acetate or sodium propionate produced the same increases in rumen fluid osmolality when intraruminally infused. These increases in rumen fluid osmolality resulted in the same sized decrease in feed intake (Grovum, 1995). On the other hand, when the rumen fluid osmolality was

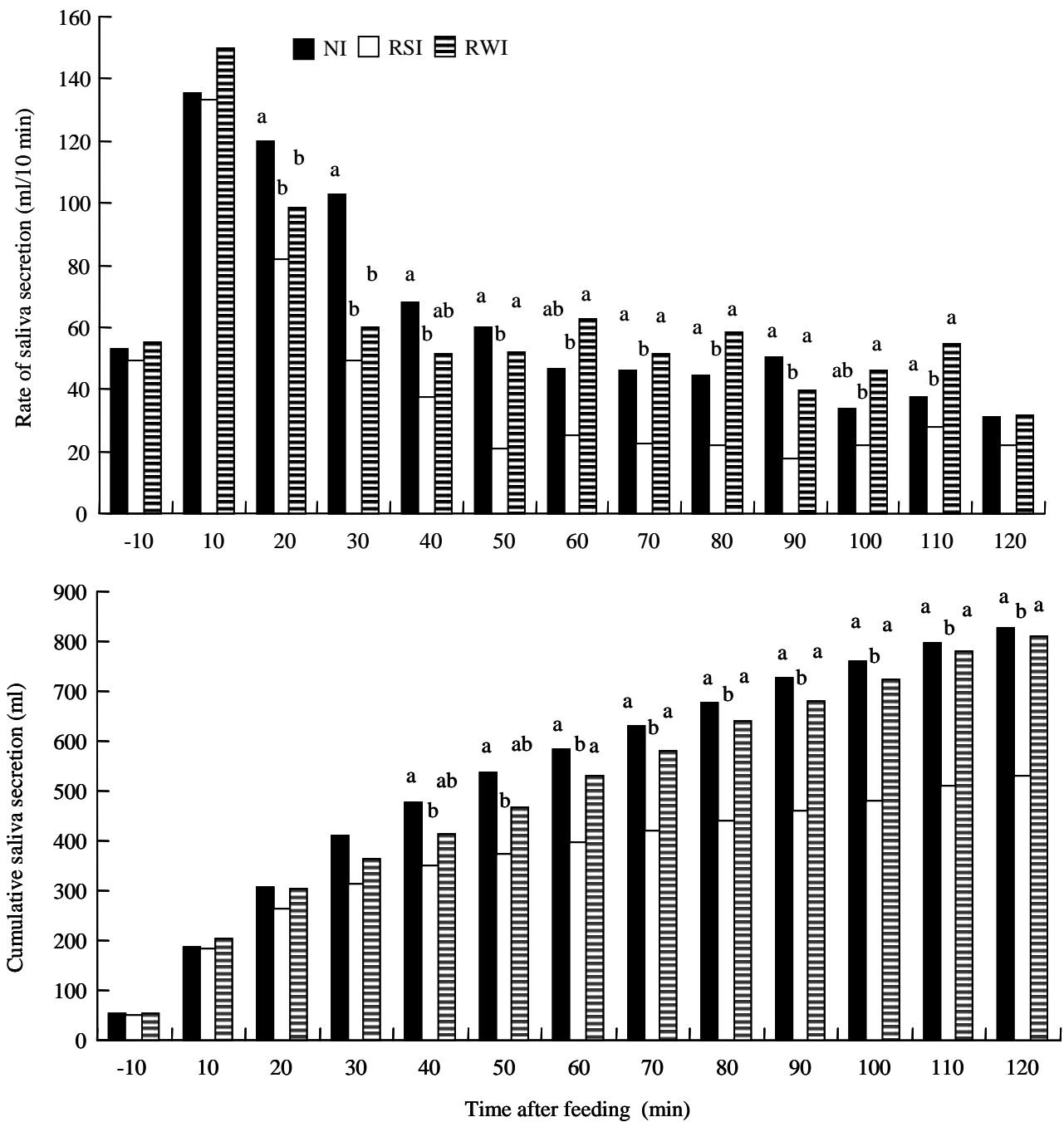


**Figure 3.** The effect of intraruminal infusion of parotid saliva (RSI) or warm water (RWI) on plasma osmolality, plasma total protein concentration and hematocrit. <sup>a,b</sup> Means with different superscripts are from no infusion treatment (NI,  $p < 0.05$ ).

decreased by the intraruminal infusion of an excessive amount of warm water (39.8°C) in sheep fed on concentrated feed, feed intake increased by approximately 30% (Kato et al., 1979). It has been thought that the changes in ruminal fluid osmolality were sensed by the osmoreceptors in the rumen wall and these signals were then transported into the central nervous system (Leek and

Harding, 1975). However, the effect of internal humoral factors on the intake of grass has not been investigated under these experimental conditions.

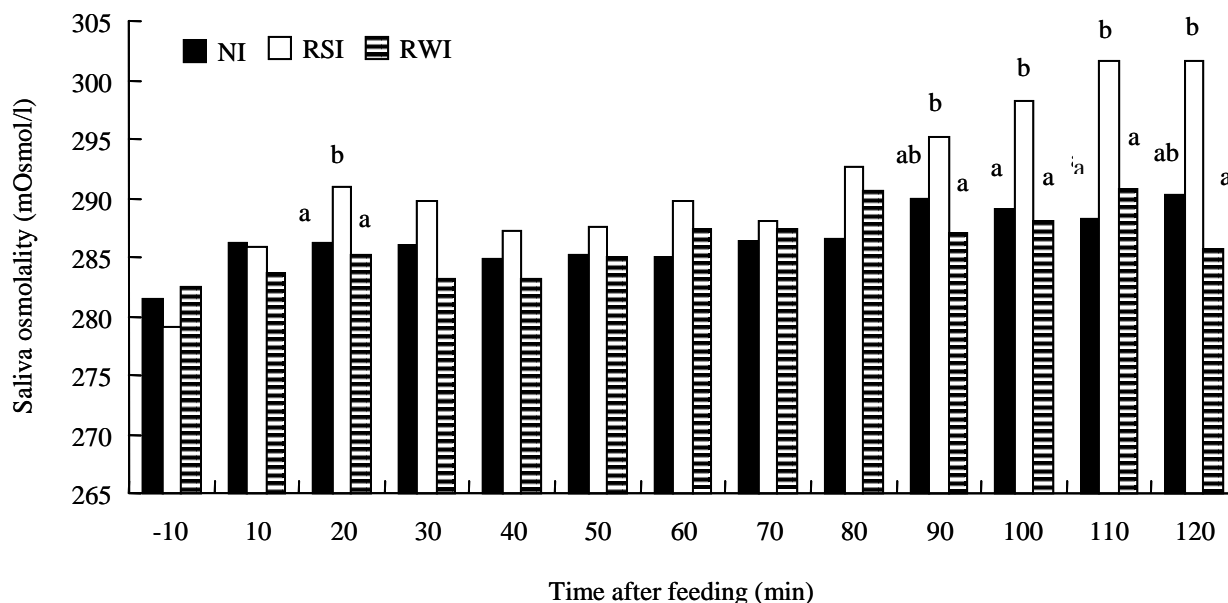
Kato (1977) compared feeding times and feed intake in sheep with an esophagus fistula under normal feeding and sham feeding conditions. The animals were fed fresh grass, hay and concentrated feed. The result of this comparative



**Figure 4.** The effect of intraruminal infusion of parotid saliva (RSI) or warm water (RWI) on rate of saliva secretion and cumulative saliva secretion. <sup>a,b</sup> Means with different superscripts are from no infusion treatment (NI,  $p < 0.05$ ).

research was a tendency for both feeding times and feed intake to increase when fed fresh grass and hay. This difference however, was not significant. This result suggests that despite the consumed feed not entering the rumen, some extraruminal mechanism exists to suppress the feeding behavior. In the present experiment, in comparison with the NI treatment, plasma osmolality in the RWI and RSI treatments was unchanged and higher respectively. In

the first half of the 2 h feeding period, eating rates and cumulative feed intake in both treatments were significantly greater than those observed in the NI treatment. In the first hour of feeding, the RSI and NI values for plasma total protein concentration and hematocrit were approximately equal. However, thirst level in the RSI treatment at the conclusion of feeding was 10.0% lower than that recorded in the NI treatment. Thirst level in the RWI treatment was



**Figure 5.** The effect of intraruminal infusion of parotid saliva (RSI) or warm water (RWI) on saliva osmolality. <sup>a,b</sup> Means with different superscripts are from no infusion treatment (NI,  $p < 0.05$ ).

observed to be 49.8% lower (Figure 2, 3). These results were induced by increases in the volume of fluid flowing into the rumen. It is thought that rumen fill in RSI and RWI treatments was higher than NI treatment. In comparison with NI treatment however, cumulative feeding amounts were 39.3, 45.9% higher in RSI and RWI treatments respectively. Sunagawa et al. (2001) intravenously infused goats fed alfalfa hay cubes twice a day with a mixed artificial saliva, a hypo-osmotic mannitol solution, and an iso-osmotic mannitol solution on different days. The infusions were conducted from 1 h prior to feeding and continued until 1 h of the 2 h feeding period had elapsed. This infusion supplements the fluids in the blood lost through accelerated saliva secretion during the early stages of dry forage feeding. It was reported that thirst levels decreased approximately 13% while accumulated feed intake increased with infusions of mixed saliva (41%), hypo-osmotic mannitol solution (45%) and iso-osmotic mannitol solution (43%). From these results, it is thought that the suppression of feed intake of dry forage in goats is not simply a result of rumen fill but also the thirst that is brought about by the accelerated secretion of saliva in the initial stages of feeding. Moreover, despite the significant differences in thirst levels between RSI and RWI treatments, the feed intake in both treatments was similar. The reason for this is thought to be that saliva consists of not only water but also appetite enhancing substances.

The results of this experiment indicated that dry forage intake is regulated by the amount of saliva flowing into the rumen.

#### ACKNOWLEDGMENT

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