



## An Intravenous Replenishment of Salivary Components and Dry Forage Intake in Freely Drinking Large-type Goats

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**ABSTRACT :** Large-type goats eating dry forage secreted large volumes of saliva which resulted in the loss of  $\text{NaHCO}_3$  from the blood and decreased plasma volume (hypovolemia). This research investigated whether or not the loss of  $\text{NaHCO}_3$  from the blood and hypovolemia brought about by dry forage feeding actually depresses feed intake in large-type goats under free drinking conditions. The present experiment consisted of three treatments (NI, ASI, MI). All treatments in this experiment were carried out under free drinking conditions. In the NI control (NI), a solution was not infused. In the ASI treatment, i.v. infusion of artificial saliva was initiated 2 h before feeding and was continued for a total of 3 h concluding 1 h after the commencement of the feeding period. In the MI treatment, mannitol solution was infused to replenish only water lost from the blood in the form of saliva. The hematocrit and plasma total protein concentrations during feeding in the NI control were observed to be higher than pre-feeding levels. This indicated that dry forage feeding-induced hypovolemia was caused by the accelerated secretion of saliva during the initial stages of feeding in freely drinking large-type goats. Increases in hematocrit and plasma total protein concentrations due to dry forage feeding were significantly suppressed by the ASI treatment. While hematocrit during feeding in the MI treatment was significantly lower than the NI control, plasma total protein concentrations were not different. From these results, it is clear that the MI treatment was less effective than the ASI treatment in mitigating the decreases in plasma volume brought about by dry forage feeding. This indicates that plasma volume increased during dry forage feeding in the ASI treatment which inhibited production of angiotensin II in the blood. The ASI treatment lessened the levels of suppression on dry forage feeding, but the MI treatment had no effect on it under free drinking conditions. The results indicate that despite the free drinking conditions, increases in saliva secretion during the initial stages of dry forage feeding in large-type goats caused  $\text{NaHCO}_3$  to be lost from the blood into the rumen which in turn caused a decrease in circulating plasma volume and resulted in activation of the renin-angiotensin system and thus feeding was suppressed. (**Key Words :** Dry Forage Intake, Saliva Secretion, Feeding Induced Hypovolemia, Large-type Goats)

### INTRODUCTION

The advantages of drying fresh grass are the ability to store it for long periods of time and transport it to far off markets. Furthermore, the daily dry matter intake levels in highly productive lactating cows and heifers can be increased by feeding them dry feed rather than fresh grass (Anil et al., 1993). The reason for this is that fresh grass has a water content of more than 80% compared with less than 10% in dry feed. However, the drying process is expensive and leads to the loss of trace nutrients including vitamins. In countries such as Japan where ruminants are raised in barns, most farmers feed their stock a diet of dry roughage twice a day.

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. Prasetyono et al. (2000) and Sunagawa et al. (2003) 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. (2002b) reported that despite being given 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. The mechanisms working to depress dry forage feeding in the initial stages of feeding have never been clarified.

Large-type goats raised on dry forage eat large volumes

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**Table 1.** Chemical composition of alfalfa hay cubes and ground concentrate feed

	Alfalfa hay cubes	Ground concentrate feed
Dry matter (%)	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 extracts (NFE)	39.7	71.0
NDF	45.9	14.6
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

DM = Dry matter, NDF = Neutral detergent fiber.

ADF = Acid detergent fiber.

in order to satisfy their nutritional requirements for maintenance and production levels. During the initial stages of dry forage feeding in large-type goats, copious amounts of saliva are secreted (Sunagawa et al., 2003). Ruminant saliva is mainly composed of water and  $\text{NaHCO}_3$ . Decreases in humoral factors such as circulating plasma volume, urine excretion, plasma  $\text{HCO}_3^-$  concentration, and blood pH were inhibited by an intravenous infusion of an artificial saliva solution composed mainly of  $\text{NaHCO}_3$  (Blair-West and Brook, 1969; Sasaki et al., 1975). These results indicate that large volumes of water and alkaline electrolytes are lost from the circulating blood to the rumen in the form of saliva during the initial stages of feeding in ruminants fed dry forage.

Sunagawa et al. (2007) reported that in large-type meat producing goats whose water intake had been restricted, decreases in circulating plasma volume brought about by increased saliva secretion during feeding caused a decrease in dry forage intake. The present experiment investigated whether or not components lost from the circulating blood to the rumen in the form of saliva in large-type meat producing goats given free access to water, caused a decrease in feed intake during the initial stages of feeding.

## MATERIALS AND METHODS

### Animals

Six large-type male goats (6 Crossbred Japanese Saanen/Nubian male goats, 4 to 6 yr. weighing  $87.5 \pm 2.4$  kg) were used in this experiment. The goats were maintained in individual metabolic cages (length 2 m  $\times$  width 1 m  $\times$  height 2 m) that allowed for the separate collection of urine, feces and saliva. The laboratory room was maintained under thermoneutral conditions ( $24.9 \pm 0.3^\circ\text{C}$ ,  $77.8 \pm 1.3\%$  relative

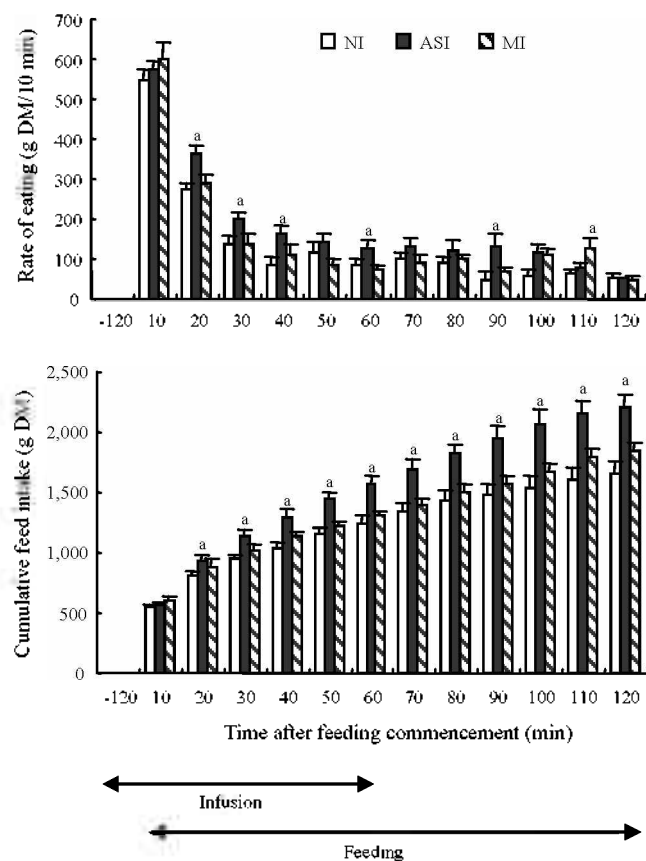
humidity).

The animals were fed twice a day at 1030 and again at 1600 for 2 h each time. During the morning 2 h feeding period (1030 to 1230), the animals were fed 2 to 3 kg of roughly crushed ( $3 \times 3 \times 1$  cm) alfalfa hay cubes. At 1600 each day, the animals were fed again with 0.8 kg of alfalfa hay cubes, and 200 g of commercial ground concentrate. The animals were given free access to water.

The chemical composition of alfalfa hay cubes and ground concentrate feed is indicated in Table 1. Alfalfa hay cubes were ground with a Wiley mill (type 40-525P, Ikemoto Rika Kougyou, Tokyo, Japan). The chemical components of feeds were quantified using the procedures described by the Official Methods of Analysis (AOAC, 1990). The crude protein content was calculated from the nitrogen content of the feed and was determined by a technique originally devised by Kjeldahl (AOAC, 1990). The crude fat was determined by subjecting the feed to a continuous extraction with ethyl ether for 16 h using a Soxhlet. The crude fiber was determined by subjecting the residual feed from ether extraction to successive treatments with boiling sulfuric acid and sodium hydroxide of 1.25%. When the sum of the contents of moisture, ash, crude protein, crude fat and crude fibre was subtracted from 100, the difference was calculated as the nitrogen-free extracts (NFE). The acid-detergent fibre (ADF) and neutral-detergent fibre (NDF) were determined using techniques originally devised by Van Soest (Van Soest, 1991). Both the alfalfa hay cubes and the commercial concentrate were ignited and reduced to ash at  $500^\circ\text{C}$ . The mineral contents of the ash were then measured using an Atomic absorption spectrophotometer (AA-6200, Shimadzu, Tokyo).

### Experiment

Six animals were split into two groups of three animals each. Each group received the non-infusion control (NI), the artificial saliva i.v. infusion treatment (ASI), and the mannitol i.v. infusion treatment (MI) concurrently according to a Latin square design. A minimum recovery period of 1 wk was allowed between each treatment for all animals (Weisinger et al., 1977). The six goats were housed in the same room until the experiment had been completed. Each treatment in this research was conducted over a 3 h period (0830 to 1130). On the day of each treatment, the i.v. infusion of artificial saliva or mannitol solution (17 to 19 ml/min) was conducted with a motor-driven pump (Cole-Parmer Instrument Co. PA-21, Chicago) over a 3 h period beginning 2 h prior to the commencement of morning feeding (0830) and continuing until 1 h of the feeding period had elapsed (1130). The goats used in this experiment were fed on the same type of feed (alfalfa hay cubes, commercial ground concentrate) from 1 month prior to the start of experimentation until its conclusion. In order



**Figure 1.** The effect of intravenous infusion of artificial saliva (ASI) or mannitol solution (MI) on eating rates and cumulative feed intake. Values are means  $\pm$  SE of 6 large-type goats. <sup>a</sup> Means with different superscripts differ ( $p < 0.05$ ) from the non-infusion control (NI).

to ascertain the physiological state of the animals, respiration frequency, heart rate, and rectal temperature were measured each day prior to the morning feeding period. In addition to this, feed intake was also measured daily. The values of these physiological parameters indicated that an individual animal was in good health and had no measurable carry-over effects from the previous treatments. The data gathered from all 6 goats used in the experiment was statistically analyzed as a repeated measurement.

One day before the commencement of each treatment in the experiment, polyethylene cannulae (o.d. 1.50 mm, No.5, Imamura Gomu, Tokyo) were inserted into the jugular veins on both sides of each goat. One was used for infusion, and the other was used for collecting blood samples. A three-way tap was attached to the end of each cannula. The cannulae were sewn to the skin on animal's back to fix them in place. They were filled with heparin-saline (50 IU/ml) to prevent coagulation of the blood. On the day of the experiment, the intravenous infusion of artificial saliva or mannitol solution (17 to 19 ml/min) was conducted with a motor-driven pump (Cole-Parmer Instrument Co. PA-21,

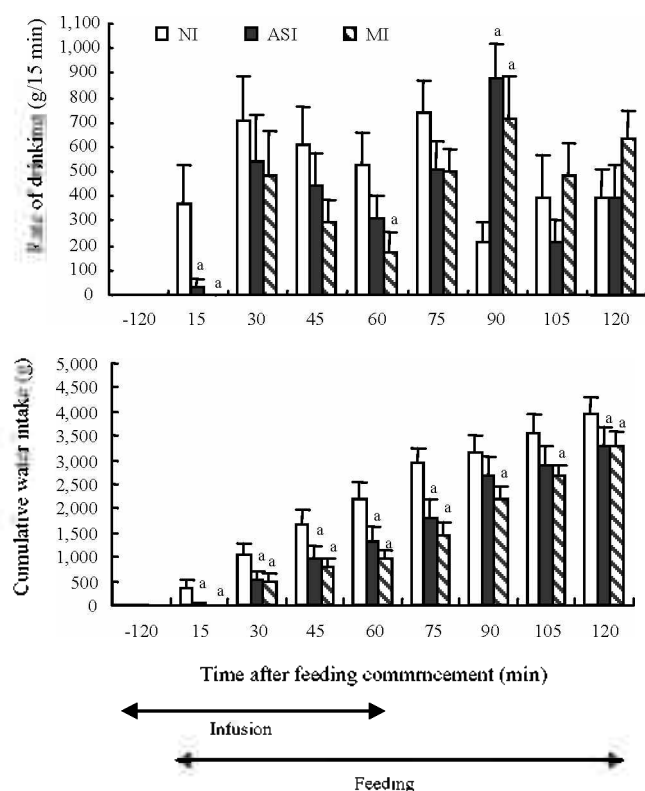
Chicago) over a 3 h period beginning 2 h prior to the commencement of morning feeding (0830) and continuing until 1 h after feeding commencement (1130). The infusion rate for animals weighing 72.0 and 72.5 kg was 17.0 ml/min, while the rate for animals weighing 83 to 97 kg was 19.0 ml/min. The animals were given water freely during morning feeding on the day of the experiments in the control and both treatments. Feeding was initiated at 1030 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 to 3.0 kg) were placed in a feed box attached to a 6 kg measuring scale. As animals eat the feed, the weight of the remaining feed decreases continuously. The weight of the remaining feed was measured every 10 min for the duration of the 2 h feeding period. The decrease in remaining feed as indicated by the scales read every 10 min was used to calculate feed intake. Figure 1 shows eating rates (g/10 min). Drinking rates were measured every 15 min during the 2 h feeding period. Drinking rates were calculated by measuring the weight of water in the animal's drinking bucket every 15 min. Blood samples (5 ml) were collected through the polyethylene cannula. Prior to drawing the samples, a drop of heparin solution (1,000 IU/ml) was placed in a test tube. The blood samples were transferred to these test tubes which were then placed on ice until plasma separation could be carried out. The blood was sampled at 0930, 1030, 1045, 1100, 1115, 1130, 1200, 1230, 1300 and 1315. Blood plasma was obtained by centrifugation (16,260 $\times$ g, 10 min, 4°C).

The artificial saliva, a solution resembling mixed saliva, consisted of 115 mM  $\text{NaHCO}_3$ , 5 mM KCl and 30 mM  $\text{Na}_2\text{HPO}_4$ . The mannitol (41.9 g, 230 mmole) was dissolved in sterilized water (1 L). The pH values of both solutions were adjusted to 7.4 by bubbling  $\text{CO}_2$  gas (Blair-West and Brook, 1969; Sasaki et al., 1975). In preliminary experiments, the relationships between cumulative feed intakes over a 2 h period and infusion rates of 12 to 20 ml/min of artificial mixed saliva were examined. It was found that the optimum infusion rates of artificial parotid saliva to increase feed intake were 17 to 19 ml/min. Thus, these rates were adopted for this experiment.

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.

#### Biochemical analysis

Blood samples were placed in a hematocrit centrifuge (HC-12A, Tomy Seiko, Tokyo, 5 min, 16,260 $\times$ g) to separate plasma and red blood cells. A hematocrit reader (Tomy Seiko, Tokyo) was used to determine hematocrit. Plasma total protein concentration and osmolality were measured



**Figure 2.** The effect of intravenous infusion of artificial saliva (ASI) or mannitol solution (MI) on drinking rates and cumulative water intake. Values are means $\pm$ SE of 6 large-type goats. <sup>a</sup> Means with different superscripts differ ( $p < 0.05$ ) from the non-infusion control (NI).

using a refractometer (Atago, Tokyo) and an osmometer (OM-6010, Kyoto Daiichi Kagaku, Kyoto), respectively. The plasma concentrations of Na, K and Cl were measured using Spotchem EL (SE-1520, Arklay, Kyoto).

### Statistical analysis

The parameters for each animal were measured prior to and again after feeding in all three treatments of the experiment (NI, ASI, MI). The measurements were taken, at the same points in time for each animal. The values from these measurements were used to compare the control and the treatments. A two-way analysis (animal, treatment) of variance was performed. After this, Linear comparison was used to compare treatments. For statistical analysis, GLM procedures (SAS Inst., Inc., Cary, NC, 1990) were adopted. Data are presented as means $\pm$ SE of the 6 goats.

All data were analyzed using the following model:

$$Y_{ijkl} = \mu + \alpha_i + \beta_j + \gamma_k + (\beta\gamma)_{jk} + \varepsilon_{ijkl}$$

Where  $Y_{ijkl}$  = a value from measurement on the  $l^{\text{th}}$  replication of the  $i^{\text{th}}$  animal in the  $j^{\text{th}}$  time and the  $k^{\text{th}}$  treatment;  $\mu$  = the overall mean;  $\alpha_i$  = the effect of the  $i^{\text{th}}$  animal;

$\beta_j$  = the effect of the  $j^{\text{th}}$  time;  $\gamma_k$  = the effect of the  $k^{\text{th}}$  treatment;  $(\beta\gamma)_{jk}$  = the interaction effect between time and treatment;  $\varepsilon_{ijkl}$  = the random error effect.

## RESULTS

### Eating rate and cumulative feed intake

Figure 1 shows the effects of ASI and MI treatments on eating rate and cumulative feed intake. Eating rates in the NI control rapidly decreased in the first 40 min of feeding (0 to 10 min, 545 g/10 min; 30 to 40 min, 86 g/10 min). However, eating rates in the ASI treatment decreased more slowly over the first 40 min than those in the NI control. Eating rates in the ASI treatment at 20, 30 and 40 min intervals after feeding were higher ( $p < 0.05$ ) than those in the NI control, respectively. Eating rates in the MI treatment were not significantly different from those in the NI control.

In comparison with the NI control (1,648 $\pm$ 111.5 g/2 h), cumulative feed intake in the ASI treatment was 33.7% (2,203 $\pm$ 114.9 g/2 h) greater ( $p < 0.05$ ), but that in the MI treatment was not significantly different (1,841 $\pm$ 71.3 g/2 h) upon conclusion of the 2 h feeding period.

### Drinking rate and cumulative water intake

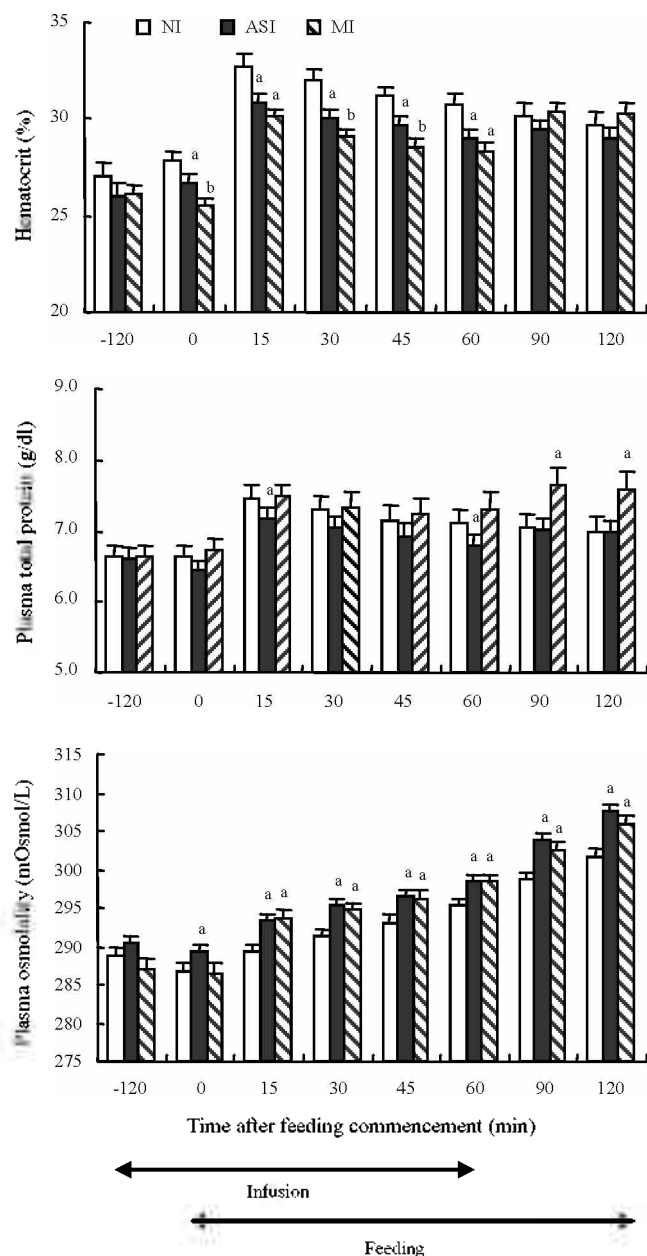
Figure 2 presents the effects of ASI or MI treatment on drinking rate and cumulative water intake after the completion of the 2 h feeding period. In the NI control, drinking behaviors were induced over the first 75 mins of the feeding period. In the ASI and MI treatments however, water intake over the first 75 mins of feeding tended to decrease. Drinking rates in the ASI and MI treatments at 90 min intervals after feeding were higher than those in the NI control, respectively.

In comparison with the NI control (3,953 $\pm$ 364.0 g/2 h), cumulative water intake in the ASI and MI treatments were 16.6% (3,295 $\pm$ 370.9 g/2 h) and 16.8% (3,287 $\pm$ 287.3 g/2 h) less ( $p < 0.05$ ) upon conclusion of the 2 h feeding period.

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

Figure 3 shows the effects of ASI or MI treatment on hematocrit, plasma total protein concentration and plasma osmolality in the blood sampled at 120 min before and 0, 15, 30, 45, 60, 90 and 120 min after feeding has commenced.

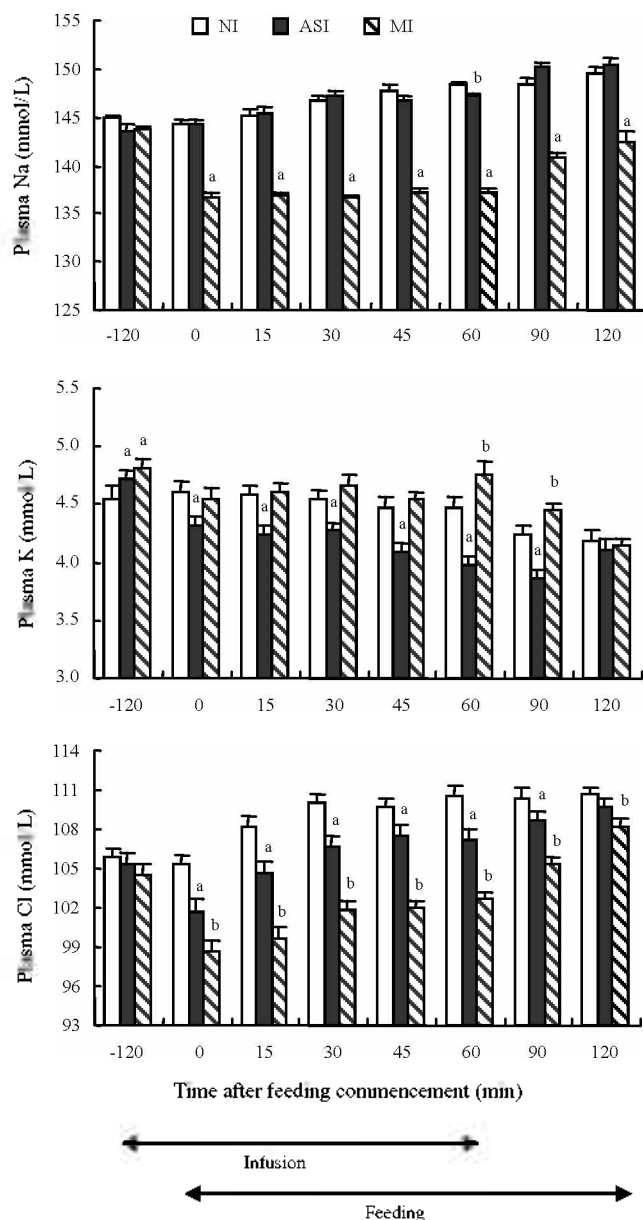
In the NI control and both treatments, rapid increases in hematocrit and plasma total protein concentrations were recorded during the first 15 min after the commencement of feeding. Following this however, hematocrit and plasma total protein concentrations gradually decreased in both treatments for the remainder of the feeding period. Compared with the NI control, the ASI and MI treatments significantly ( $p < 0.05$ ) decreased hematocrit over the first 1



**Figure 3.** The effect of intravenous infusion of artificial saliva (ASI) or mannitol solution (MI) on hematocrit, plasma total protein concentration and plasma osmolality. Values are means±SE of 6 large-type goats. <sup>a, b</sup> Means with different superscripts differ (p<0.05) from the non-infusion control (NI).

h of the 2 h feeding period. Plasma total protein concentration in the ASI treatment tended to be lower than those in the NI control during the first 1 h of the 2 h feeding period, but the MI treatment concentrations were not different.

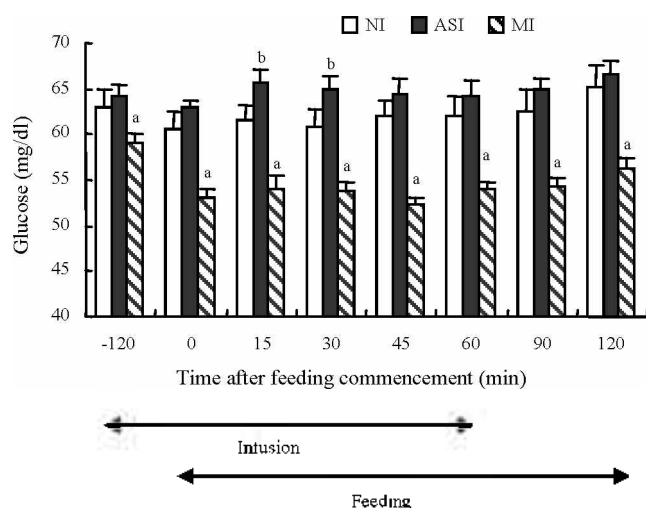
Plasma osmolality very slowly increased in the control and both treatments over the 2 h feeding period. Plasma osmolalities in the ASI and MI treatments were significantly higher (p<0.05) than those in the NI control during the 2 h feeding period.



**Figure 4.** The effect of intravenous infusion of artificial saliva (ASI) or mannitol solution (MI) on plasma Na, K and Cl concentration. Values are means±SE of 6 large-type goats. <sup>a, b</sup> Means with different superscripts differ (p<0.05) from the non-infusion control (NI).

**Plasma concentrations of Na, K and Cl**

Figure 4 shows the effects of ASI or MI treatment on plasma concentrations of Na, K and Cl. Plasma Na concentrations in the ASI treatment were not different from those in the NI control during the 2 h feeding period. However, plasma Na concentrations during feeding in the MI treatment were lower (p<0.05) than those in the NI control. Plasma K concentrations in the ASI treatment were lower (p<0.05) than those in the NI control during feeding, but the MI treatment concentrations were not different. Plasma Cl concentrations during feeding in the ASI and MI



**Figure 5.** The effect of intravenous infusion of artificial saliva (ASI) or mannitol solution (MI) on plasma glucose concentration. Values are means  $\pm$  SE of 6 large-type goats. <sup>a, b</sup> Means with different superscripts differ ( $p < 0.05$ ) from the non-infusion control (NI).

treatments were lower ( $p < 0.05$ ) than those in the NI control. Plasma Cl concentrations in the MI treatment were lower ( $p < 0.05$ ) than those in the ASI treatment.

#### Plasma glucose concentration

Figure 5 presents plasma glucose concentration. Plasma glucose concentrations during feeding in the ASI treatment were not greatly different from those in the NI control, but the MI treatment concentrations were significantly lower ( $p < 0.05$ ).

## DISCUSSION

When sheep and goats commence feeding on dry forage they secrete large volumes of saliva (Denton, 1956; Sunagawa et al., 2003). The saliva secreted by ruminants mainly consists of water and  $\text{NaHCO}_3$  (Argenzio, 1984). Saliva is produced from components in the blood and thus, at the onset of feeding, large amounts of water and  $\text{NaHCO}_3$  move from the circulation into the rumen in the form of saliva. As a result, decreases in circulating plasma volumes, plasma  $\text{HCO}_3^-$  concentrations, and blood pH are observed in ruminants during the initial stages of dry forage feeding (Blair-Weast and Brook, 1969; Sasaki et al., 1975; Denton, 1982; Obara et al., 1994). In the NI control of the present experiment, a decrease in plasma volume estimated by increases in hematocrit and plasma total protein concentrations was apparent within 15 min of the commencement of feeding (Figure 3). The hematocrit and plasma total protein concentrations throughout the entire 2 h feeding period in the NI control were observed to be higher than pre-feeding levels. It is thought that dry forage

feeding-induced hypovolemia (decrease in plasma volume) was caused by the accelerated secretion of saliva during the initial stages of feeding in freely drinking large-type goats. In the present experiment, the plasma total protein concentrations and hematocrits also increased within 15 min of the commencement of feeding in the ASI and MI treatments (Figure 3). This indicates that i.v. volumes of each solution prior to feeding in the ASI and MI treatments were smaller than the total secretion volumes of mixed saliva. Therefore, it is thought that the ASI and MI treatments replenished the parts of the fluid or  $\text{NaHCO}_3$  lost from the blood in the form of saliva during the initial stages of dry forage feeding.

The sensation of thirst is produced in the brain as a result of the integration of neuronal and humoral information (Fitzsimons, 1979). Neuronal information is transported via the autonomic nerves (especially the vagus) from chemoreceptors in the internal visceral organs. A broad range of internal humoral information is transported via the blood and cerebrospinal fluid. Increased extracellular fluid osmolality, decreases in extracellular fluid volume and arterial pressure, angiotensin II, and dryness of the mouth stimulate the sensation of the thirst (Guyton and Hall, 1996). In the NI control of the present experiment, water intake during the first 1 h feeding period increased due to a dry forage feeding-induced hypovolemia (Figures 2 and 3). Increases in hematocrit and plasma total protein concentrations due to dry forage feeding were significantly ( $p < 0.05$ ) suppressed by the ASI and MI treatments (Figure 3). Plasma total protein concentrations in the MI treatment were not significantly different from those in the NI control. On the other hand, plasma osmolalities in the ASI and MI treatments were significantly ( $p < 0.05$ ) higher than those in the NI control during the 2 h feeding period (Figure 3). Despite this, cumulative water intake during the 2 h feeding period in the ASI and MI treatments was significantly ( $p < 0.05$ ) lower than that in the NI treatment (Figure 2). The result indicates that the level of thirst sensation caused by feeding on dry forage was significantly ( $p < 0.05$ ) reduced through the compensation of circulating plasma loss by the ASI and MI treatments.

Na and Cl are absorbed from the rumen into the blood (Argenzio, 1984). Stacy and Warner (1966), and Warner and Stacy (1972) reported that increases in plasma Na and Cl concentrations in sheep during feeding were dependent upon the absorption of Na and Cl from the rumen. The increases in plasma osmolality associated with feeding in the NI control were very gradual (Figure 3). Argenzio (1984) reported that the majority of plasma osmolality is derived from electrolytic osmolality of Na, K and Cl. Plasma osmolalities in the ASI and MI treatments were significantly higher ( $p < 0.05$ ) than those in the NI control during the 2 h feeding period (Figure 3). In the ASI

treatment of the present experiment,  $\text{NaHCO}_3$ , the main component of artificial saliva, was intravenously infused. In the MI treatment however, only a mannitol solution was infused. Due to this infusion in the ASI treatment, Na lost from the blood into the rumen as a result of increased saliva secretion during the initial stages of dry forage, was replenished. However, K and Cl losses remained unaffected (Figure 3). Na and Cl concentrations in the MI treatment were lower than the NI control and the ASI treatment. The reason for this is thought to be that the blood was diluted by the infusion of the mannitol solution which contains neither Na nor Cl.

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 hyper-osmotic NaCl, polyethylene glycol-400 (PFG), 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 ruminal fluid osmolality was decreased by the intraruminal infusion of an excessive amount of warm water (39.8°C) in sheep fed on alfalfa hay cubes, feed intake increased by 30% (Sunagawa et al., 2002a). In the NI control of the present experiment, water intake in the first half of the 2 h feeding period was greater than both the ASI and MI treatments (Figure 2). From this result, it is thought that ruminal fluid osmolality in the NI control was lower than the ASI and MI treatment as was found by Sunagawa et al. (2002a) when goats were given an intra-ruminal infusion of warm water. The plasma osmolalities in the NI control during feeding were also significantly lower than those in the ASI and MI treatments (Figure 3). However, feed intake in the ASI treatment was significantly higher than the NI control while the MI treatment resulted in feed intake levels virtually the same as the NI control. However, the effect of internal humoral (circulating blood) factors on the intake of dry forage has not been investigated under these experimental conditions.

In parotid fistulated sheep, parotid saliva is lost from the body causing a reduction in plasma Na concentration and circulating plasma volume. Blair-West et al. (1967) reported that a 48 h intravenous infusion of dextran-saline solution in parotid fistulated sheep prevented decreases in plasma volume. In the ASI treatment of the present experiment, artificial saliva was intravenously infused to replenish  $\text{NaHCO}_3$  and water lost from the blood to the rumen in the form of saliva. The result was that increases in hematocrit and plasma total protein concentrations due to dry forage feeding in the ASI treatment were significantly lower than the NI control (Figure 3). These results indicate that in comparison to the NI control in which water intake increased, the intravenous infusion of artificial saliva, the

main component of which is  $\text{NaHCO}_3$ , significantly mitigated the decreases in circulating plasma volume caused by increased saliva secretion during the initial stages of dry forage feeding. In the MI treatment of the present experiment, a mannitol solution with the same osmolality as artificial saliva was intravenously infused in the same manner as the ASI treatment to replenish only the water lost from the blood in the form of saliva. Plasma Na and Cl concentrations in the MI treatment decreased significantly due to dilution compared to the NI and ASI treatments (Figure 4). While hematocrit during feeding in the MI treatment was significantly lower than the NI control, plasma total protein concentrations were not different. From these results, it is clear that the MI treatment was less effective than the ASI treatment in mitigating the decreases in plasma volume brought about by dry forage feeding. When Blair-West and Brook (1969) intravenously infused artificial saliva equal to saliva secretion volumes for a period of 1 h during feeding in sheep fed once a day on lucerne chaff, the decreases in circulating plasma volume and increases in plasma renin concentration due to dry forage feeding were prevented. McKinley et al. (1979) reported that intravenous infusion of angiotensin II over the dose range 3-20  $\mu\text{g}/\text{h}$  for 15 min caused a dose dependent reduction in parotid saliva secretion in sheep. Conversely, Sunagawa et al. (2005) found that when artificial saliva was intravenously infused from 1 h prior to feeding and continued throughout the feeding period in goats fed alfalfa hay cubes twice a day, a marked increase in saliva secretion occurred despite an increase in plasma osmolality. When Sasaki et al. (1975), using the same infusion method as Blair-West and Brook (1969), intravenously infused female sheep, fed once a day on orchard grass hay, with artificial saliva similar in composition to mixed saliva, decreases of plasma bicarbonate concentration and blood pH due to feeding were completely inhibited. From these reports, it is clear that disturbances among humoral factors such as decreases in circulating plasma volume, increases in angiotensin II production in the blood, and the occurrences of metabolic acidosis are caused by increases in saliva secretion during the initial stages of dry forage feeding in sheep and large-type goats, even those with free access to water.

Sheep and large-type goats eating dry forage secrete large quantities of saliva (Denton, 1956; Stacy and Warner, 1966; Warner and Stacy, 1972; Sunagawa et al., 2003), which decreases plasma volume during the initial stages of dry forage feeding (Blair-West and Brook, 1969). This activates the renin-angiotensin system and decreases urine flow and sodium excretion (Blair-West and Brook, 1969; Sasaki et al., 1974). Angiotensin II is produced in the blood and vasopressin is secreted when circulating plasma volume decreases and plasma osmolality increases in rats and sheep

(Blair-West and Brook, 1969; Fitzsimons, 1979; Mathai and McKinley, 1997). Sunagawa et al. (2001) reported that the significant decreases in feed intake during intracerebroventricular infusion of angiotensin II were caused by production of thirst sensations in the brain of ewes leading to excessive water intake and ruminal distension. Prasetiyono et al. (2000) found that there was a significant positive regression between thirst levels and feed intake in goats fed on alfalfa hay cubes. Meyer et al. (1989) indicated that intraperitoneal injections of vasopressin decreased feed intake in goats. In the present experiment, because of the accelerated secretion of saliva during the initial stages of dry forage feeding, we found that when  $\text{NaHCO}_3$  and water lost from the blood in the form of saliva were replenished, the degree of feeding suppression was less than the NI control in which water intake increased. However, in the MI treatment of this experiment in which only water was replenished, feed intake was not significantly different when compared to the NI control (Figure 1). In this experiment, the plasma glucose concentrations in the MI treatment were significantly lower than the ASI treatment and the NI control (Figure 5). This indicates that the infused mannitol acted as a stimulant causing insulin secretion. Insulin itself is an appetite suppressing hormone. It is thought that this is the reason why feed intake in the MI treatment was almost the same as the NI control. From these reports, in large-type goats fed dry forage under free drinking conditions, the replenishment of artificial saliva to the blood may have inhibited angiotensin II production and vasopressin release usually brought about by dry feed consumption, and thus feed intake increased.

The results of this experiment indicate that despite the free drinking conditions, increases in saliva secretion during the initial stages of dry forage feeding in large-type goats caused  $\text{NaHCO}_3$  to be lost from the blood into the rumen which in turn caused a decrease in circulating plasma volume and resulted in activation of the renin-angiotensin system and thus feeding was suppressed.

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