



## Plasma Osmolality Controls Dry Forage Intake in Large-type Goats

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**ABSTRACT :** In large-type goats that were fed on dry forage twice daily, dry forage intake was markedly suppressed after 40 min of feeding had elapsed. The objective of this study was to clarify whether or not increases in plasma osmolality and subsequent thirst sensations produced by dry forage feeding suppress dry forage intake. Eight large-type male esophageal- and ruminal-fistulated goats (crossbred Japanese Saanen/Nubian, aged 3 to 6 years, weighing  $72.3 \pm 2.74$  kg) were used in two experiments conducted under sham feeding conditions. The animals were fed *ad libitum* a diet of roughly crushed alfalfa hay cubes for 2 h from 10:00 to 12:00 h during two experiments. Water was withheld during feeding in both experiments but was available for a period of 30 min after completion of the 2 h feeding period. In experiment 1, an intraruminal infusion of artificial parotid saliva (RIAPS) in the control replenished saliva lost via the esophageal fistula and an intraruminal infusion of hypertonic solution (RIHS) in the treatment was carried out in order to reproduce the effects of changing salt content due to feed entering the rumen. In experiment 2, the RIHS control was conducted in the same manner as the RIHS treatment of experiment 1. The treatment group consisted of RIHS-with an intravenous infusion of artificial mixed saliva (VIAMS) treatment that was carried out for 3 h to prevent increases in plasma osmolality during feeding. The results of the RIHS treatment in experiment 1 showed that ruminal fluid osmolality increased and then an increase in plasma osmolality was observed. This resulted in the production of thirst sensations and the reduction of cumulative dry forage intake to 43.3% ( $p < 0.05$ ) of the RIAPS control. The results of the RIHS-VIAMS treatment in experiment 2 indicated that ruminal fluid osmolality was the same as the RIHS control but plasma osmolality significantly decreased, and thirst level was markedly reduced. This caused a significant increase of 31.4% ( $p < 0.05$ ) in cumulative dry forage intake in the RIHS-VIAMS treatment compared to the RIHS control. These results indicate that increases in ruminal fluid osmolality during dry forage feeding indirectly suppresses dry forage intake by causing an increase in plasma osmolality and subsequently inducing thirst sensations. The results of the present study suggest that marked decreases in dry forage intake after 40 min of feeding are caused by increases in plasma osmolality and subsequent thirst sensations produced by dry forage feeding. (**Key Words :** Ruminal Fluid Osmolality, Plasma Osmolality, Thirst Level, Dry Forage Intake, Large-type Goats)

### INTRODUCTION

The advantages of drying fresh grasses are the ability to store it for long periods of time and transport it to far off markets. Furthermore, dry forage can be stored in large amounts making large-scale livestock management possible in the rearing of ruminants. In normal feeding conditions when goats were fed on dry forage for 2 h twice a day, irrespective of whether drinking water was supplied (Nagamine et al., 2003; Sunagawa et al., 2003; 2008) or withheld (Sunagawa et al., 2002; 2007) during feeding, eating rates decreased rapidly in the first 30 or 40 min of the 2 h feeding period and were remained at very low rates for the remainder of the 2 h feeding period. The low eating rates mean that dry forage intake significantly decreased.

Sunagawa et al. (2003) reported that a suppression of dry forage intake during the early stages of feeding in goats was partly caused by feeding-induced hypovolemia (decrease in plasma volume), which was produced by the accelerated secretion of parotid saliva. However, the mechanism responsible for the suppression of dry forage intake after 40 min of feeding is still unclear.

Grovum (1995) reported that the increase 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, Thang et al. (2010) reported that under normal feeding conditions, ruminal distension, ruminal fluid osmolality, plasma osmolality, and thirst level all increased at the same time during dry forage feeding in large-type goats. Consequently, it is difficult to clarify which factors are mainly involved in the suppression of dry forage intake

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after 40 min of feeding in the experiments conducted under normal feeding conditions (Campling and Balch, 1961; Anil et al., 1993; Grovum, 1995).

Thang et al. (2010) has found that in sham feeding conditions in which swallowed boluses of dry forage intake and secreted saliva were prevented from entering the rumen by removal via an esophageal fistula, eating rates were remained at high levels even after 40 min of feeding period had elapsed in large-type esophageal-fistulated goats fed dry forage twice daily. In addition, the goats continued eating throughout the entire 2 h feeding period and consumed more dry forage than in the normal feeding conditions. This indicates that dry forage intake during the second hour of the 2 h feeding period is controlled by factors produced when feed boluses enter the rumen. On the other hand, in the second hour of the 2 h feeding period, ruminal fluid osmolality and plasma osmolality increased and subsequently the animals became thirsty (Thang et al., 2010). However, it is unclear as to whether or not increases in plasma osmolality and thirst sensations brought about by dry forage feeding are in fact physiologically responsible for suppressing dry forage intake.

The utilization of the esophageal-fistulated goats enables the isolation of factors that are presumed to control dry forage intake. In the present study, experiments were conducted under sham feeding conditions whereby esophageal boluses were removed during feeding to prevent them from entering the rumen. The objective of the study was to determine whether or not increases in plasma osmolality and the subsequent thirst sensations created by dry forage feeding do in fact suppress dry forage intake.

## MATERIALS AND METHODS

### Animals

Eight large-type male esophageal- and ruminal-fistulated goats (crossbred Japanese Saanen/Nubian, aged 3 to 6 years, weighing  $72.3 \pm 2.74$  kg) 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  $22.7 \pm 0.44^\circ\text{C}$ ; relative humidity  $85.7 \pm 1.42\%$ ).

In non-experimental days, 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.

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 extract (NFE), 45.9% neutral detergent fiber (NDF), and 36.6% acid detergent fiber (ADF). The concentrated beef cattle feed (86.9% dry matter) contained, on a dry matter basis, 13.4% crude protein, 3.6% crude fat, 3.7% crude fiber, 71.0% nitrogen-free extract (NFE), 14.6% neutral detergent fiber (NDF), and 5.4% acid detergent fiber (ADF). Alfalfa hay cubes and concentrated beef cattle feed were subjected to draught drying ( $70^\circ\text{C}$ , 24 h). Subsequently, alfalfa hay cubes were ground with a Wiley mill (type 40-525P, Ikemoto, Rika Kougyou, Tokyo). The diameter of the holes of the mill grid was 1 mm. The chemical components of the feeds were quantified using the procedures described by the AOAC (1990).

### Experimental design

*Experiment 1 - The effect of intraruminal infusion of hypertonic solution on dry forage intake in large-type goats during sham feeding* : The eight esophageal- and ruminal-fistulated goats were split into two groups (group A: four animals; group B: four animals). The experiment was carried out in accordance with a cross-over design. In the first experimental stage, group A was the control and received the intraruminal infusion of artificial parotid saliva (RIAPS) while group B as the treatment was subjected to the intraruminal infusion of hypertonic solution (RIHS). This was reversed in the second experimental stage in which group A was subjected to the RIHS while group B was the control and received the RIAPS.

In the RIAPS control,  $3.48 \pm 0.06$  L of artificial parotid saliva, a solution resembling parotid saliva (Sunagawa et al., 2008), was intraruminally infused to replenish saliva removed from the esophageal fistula during sham feeding. The intraruminal infusion of artificial parotid saliva was carried out with a bath tub pump and started concurrently with the commencement of feeding. The artificial parotid saliva had an osmolality of 272.1 mOsmol/L, pH 8.6, and its concentrations of  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Cl}^-$ ,  $\text{HCO}_3^-$ , and  $\text{HPO}_4^-$  were 142.8 mmol/L, 8.8 mmol/L, 7.0 mmol/L, 145 mmol/L, and 40 mmol/L, respectively.

In the RIHS treatment, at the commencement of feeding,  $3.43 \pm 0.04$  L of hypertonic solution was intraruminally infused to reproduce salt content of the feed entering in the rumen under normal feeding conditions using a bath tub pump. The hypertonic solution was made by adding NaCl into the artificial parotid saliva. The hypertonic solution had an osmolality of 869.0 mOsmol/L, pH 8.3, and its concentrations of  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Cl}^-$ ,  $\text{HCO}_3^-$ , and  $\text{HPO}_4^-$  were 435.9 mmol/L, 2.7 mmol/L, 358.8 mmol/L, 145 mmol/L, and 40 mmol/L, respectively.

*Experiment 2 - The effect of intraruminal infusion of hypertonic solution and intravenous infusion of artificial mixed saliva on dry forage intake in large-type goats during*

*sham feeding* : Similar to experiment 1, the animals were divided into two groups (A and B). This experiment was conducted in accordance with a cross-over design. In the first experimental stage, group A was the control and received the intraruminal infusion of hypertonic solution (RIHS) while group B as the treatment was subjected to the RIHS and intravenous infusion of artificial mixed saliva (VIAMS). This was reversed in the second experimental stage in which group A was subjected to the RIHS and VIAMS while group B was the control and received the RIHS.

In both the RIHS control and the RIHS-VIAMS treatment, at the commencement of feeding,  $3.4 \pm 0.03$  L of hypertonic solution was intraruminally infused using a bath tub pump. The hypertonic solution with an osmolality, pH, and concentrations of  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Cl}^-$ ,  $\text{HCO}_3^-$ , and  $\text{HPO}_4^-$  were similar to those in experiment 1.

In the RIHS-VIAMS treatment, the artificial mixed saliva, a solution resembling protein-free mixed saliva (Blair-West and Brook, 1969), was infused intravenously using a motor-driven pump (Cole-Parmer Instrument Co. PA-21, Chicago) over a 3 h period beginning 1 h prior to the commencement of feeding (9:00 h) and continued until the completion of feeding (12:00 h). The intravenous infusion of artificial mixed saliva was to determine either increases in ruminal fluid osmolality or plasma osmolality depressed dry forage intake after 30 or 40 min of the dry forage feeding under normal feeding conditions. The infusion rate was  $16.05 \pm 0.16$  ml/min so the total volume of infusion was  $2,889.7 \pm 29.23$  ml/3 h. The artificial mixed saliva had an osmolality of 220.8 mOsmol/L and its concentrations of  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Cl}^-$ ,  $\text{HCO}_3^-$ , and  $\text{HPO}_4^-$  were 113.9 mmol/L, 7.1 mmol/L, 4.9 mmol/L, 115 mmol/L, and 30 mmol/L, respectively. The pH value of artificial mixed saliva was adjusted to 7.4 by bubbling  $\text{CO}_2$  gas (Blair-West and Brook, 1969).

In both experiments, the controls and the treatments were carried out with each group at 1 week intervals to ensure that animals had recovered and to minimize any compounding effect from the previous treatments. 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 in the experiment 1 and the RIHS control of experiment 2. Two polyethylene cannulae 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 in the RIHS-VIAMS treatment of experiment 2. 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.

Before starting the controls and the treatments in both experiments on experimental days, 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.

On the experimental days, feeding time started at 10:00 h and finished at 12:00 h and during the 2 h feeding period, animals were fed *ad libitum* the roughly crushed alfalfa hay cubes. The animals were deprived of water during feeding in the controls and the treatments of both experiments. Following the completion of feeding, water was provided in a bucket and animals were freely drunk water for a period of 30 min (12:00 to 12:30 h).

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, hematocrit, plasma osmolality, plasma concentrations of total protein, glucose, Na, K, and Cl, 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 (10:00 to 12:00 h). Eating rate was determined by placing the roughly crushed alfalfa hay cubes in a feed box attached to a scale and measuring the weight of the remaining feed every 10 min for the duration of the 2 h feeding period. Rate of bolus output from the esophageal fistula (g/10 min) was measured during the 2 h of feeding (10:00 to 12:00 h) by using a feed box attached to a scale and weighing 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). 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. In the present study, thirst level (g/30 min) was defined as water intake for 30 min upon conclusion of the 2 h feeding period.

Blood samples (4 ml) were collected at 8:55, 9:55, 10:15, 10:30, 11:00, 11:30, 12:00 and 12:30 h through the polyethylene cannula. 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, 9:55, 10:15, 10:30, 11:00, 11:30, 12:00 and 12:30 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.

### Biochemical analysis

Blood samples were placed in capillary tubes and centrifuged using a hematocrit centrifuge (HC-12A, Tomy Seiko, Tokyo; 12,851×g, 5 min) 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), for pH and Cl concentration by a pH/Ion meter F-53 (Horiba Ltd., Kyoto), and for concentrations of Na and K with an atomic absorption flame emission spectrophotometer (AA-6200, Shimadzu Corporation, Kyoto).

### Statistical analysis

A two-way analysis (treatment, animal) of variance was performed and subsequent *t*-tests were used to determine the significance of treatment effects. To analyze the relationships between two parameters, regression analysis was used. For statistical analysis, General Linear Model (GLM) procedures (SAS Inst., Inc., Cary, NC, 1990) were adopted.

All data were analyzed using the following model:

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

Where  $Y_{ijklm}$  = the measured variable on the  $l^{\text{th}}$  replication of the  $j^{\text{th}}$  animal within the  $i^{\text{th}}$  group, the  $k^{\text{th}}$  treatment and the  $m^{\text{th}}$  period;  $\mu$  = the overall mean;  $G_i$  = the effect of the  $i^{\text{th}}$  group;  $A_{ij}$  = the effect of the  $j^{\text{th}}$  animal within

the  $i^{\text{th}}$  group;  $T_k$  = the effect of the  $k^{\text{th}}$  treatment;  $P_m$  = the effect of the  $m^{\text{th}}$  period;  $E_{ijklm}$  = the random error effect.

## RESULTS

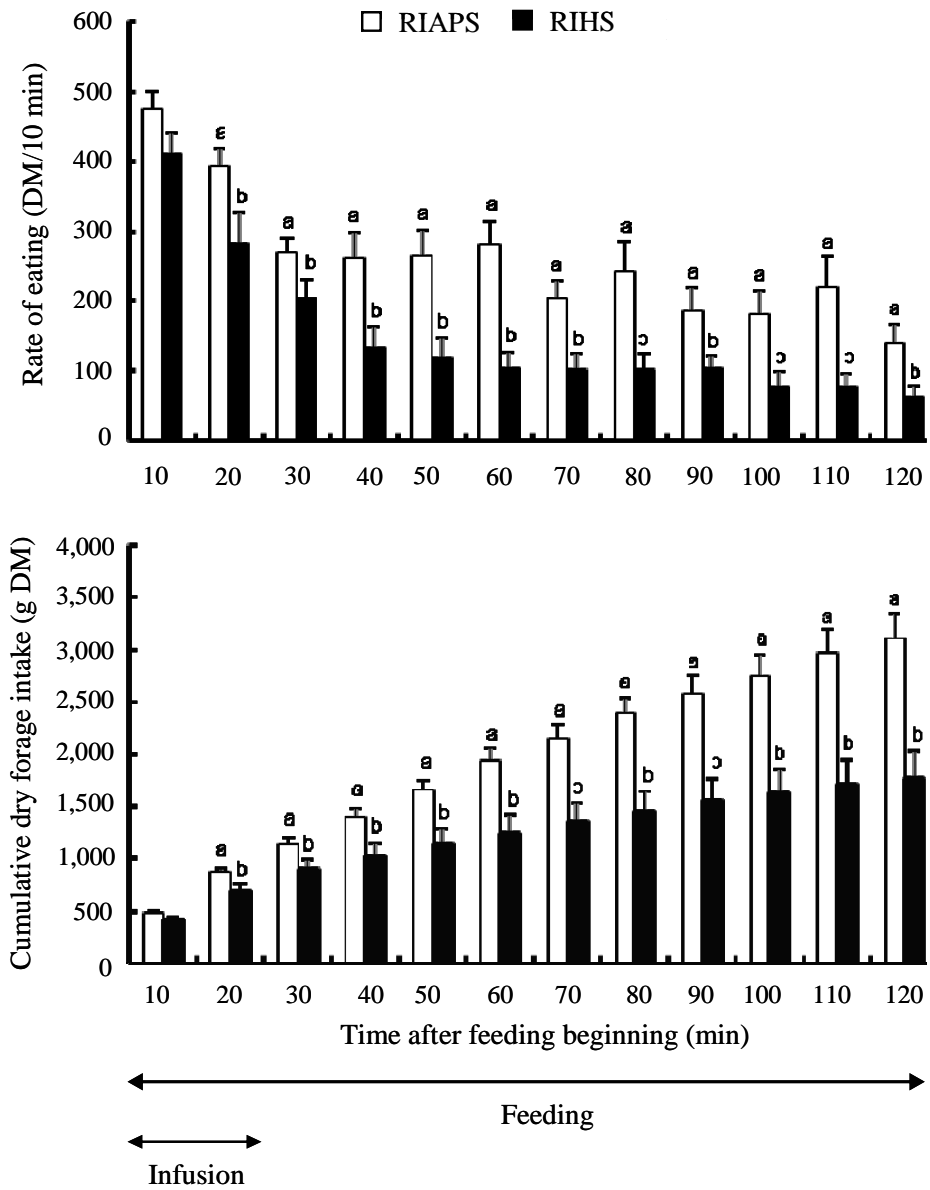
### Experiment 1 - The effect of intraruminal infusion of hypertonic solution on dry forage intake in large-type goats during sham feeding

*Physiological parameters* : The mean values of heart rate, respiration rate, and rectal temperature in the RIAPS control and the RIHS treatment were 72.8±2.88 and 75.0±2.54 beats/min, 17.5±0.91 and 16.5±0.91 breaths/min, and 38.5±0.03 and 38.5±0.10°C, respectively. There were no significant differences between the RIAPS control and the RIHS treatment in the physiological parameters.

*Rate of eating and cumulative dry forage intake* : Figure 1 shows the effect of RIHS on rate of eating and cumulative dry forage intake. Eating rates in the RIHS treatment decreased rapidly in the first 40 min of feeding (0 to 10 min, 409.9±31.66 g; 30 to 40 min, 132.8±30.09 g) and subsequently declined gradually to very low rates (ranged from 61.1±16.84 to 117.0±29.74 g/10 min) for the remainder of the 2 h feeding period. Meanwhile, eating rates in the RIAPS control decreased slowly in the first 40 min of feeding (0 to 10 min, 475.2±25.49 g; 30 to 40 min, 260.3±38.08 g) and then were remained at low rates from 139.1±27.91 to 280.3±33.52 g/10 min during the remaining time of the 2 h feeding period. Compared with the RIAPS control, eating rates in the RIHS treatment were significantly lower ( $p < 0.05$ ) 20 min after the commencement of feeding.

Cumulative dry forage intake in both the RIAPS control and the RIHS treatment progressively increased from after the beginning of feeding to the end of the 2 h feeding period. In comparison with the RIAPS control (3,108.6±237.41 g/2 h), cumulative dry forage intake in the RIHS treatment (1,764.0±261.12 g/2 h) was 43.3% less ( $p < 0.05$ ) upon conclusion of the 2 h feeding period.

*Rate of salivary secretion and cumulative salivary secretion* : The rates of salivary secretion in both the RIAPS control and the RIHS treatment (Table 1) peaked in the first 10 min after feeding was commenced. Thereafter, these rates decreased as the feeding progressed. Salivary secretion rates in the RIAPS control decreased very slowly from 40 min after the commencement of feeding while the rates observed in the RIHS treatment reduced more rapidly. The rates of salivary secretion in the RIHS treatment were markedly lower than those in the RIAPS control throughout the 2 h feeding period. Compared with the RIAPS control, the rates of salivary secretion in the RIHS treatment from 40 to 110 min intervals after the commencement of feeding were significantly lower ( $p < 0.05$ ).



**Figure 1.** The effect of intraruminal infusion of hypertonic solution (RIHS) on rate of eating and cumulative dry forage intake. Values are means±SE of 8 large-type goats. <sup>a, b</sup> Means with different superscript are significantly different (p<0.05) from intraruminal infusion of artificial parotid saliva (RIAPS).

**Table 1.** The effect of intraruminal infusion of hypertonic solution (RIHS) 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)	
	RIAPS	RIHS	RIAPS	RIHS	RIAPS	RIHS	RIAPS	RIHS
10	1,261.3 ±51.94	1,138.8 ±105.48	1,261.3 ±51.94	1,138.8 ±105.48	697.5 ±48.94	652.5 ±79.66	697.5 ±48.94	652.5 ±79.66
20	935.0 ±53.25 <sup>a</sup>	725.0 ±76.74 <sup>b</sup>	2,196.3 ±102.28 <sup>a</sup>	1,863.8 ±179.85 <sup>b</sup>	470.0 ±42.55	391.3 ±30.38	1,167.5 ±75.71 <sup>a</sup>	1,043.8 ±104.49 <sup>b</sup>
30	668.8 ±64.13	525.0 ±72.28	2,865.0 ±143.22 <sup>a</sup>	2,388.8 ±249.02 <sup>b</sup>	350.0 ±47.40	285.0 ±42.38	1,517.5 ±106.36	1,328.8 ±143.03
40	640.0 ±62.42 <sup>a</sup>	358.8 ±71.35 <sup>b</sup>	3,505.0 ±185.86 <sup>a</sup>	2,747.5 ±312.55 <sup>b</sup>	331.3 ±32.37 <sup>a</sup>	201.3 ±43.52 <sup>b</sup>	1,848.8 ±127.20 <sup>a</sup>	1,530.0 ±178.45 <sup>b</sup>
50	685.0 ±52.98 <sup>a</sup>	296.3 ±74.74 <sup>b</sup>	4,190.0 ±209.02 <sup>a</sup>	3,043.8 ±370.86 <sup>b</sup>	372.5 ±32.17 <sup>a</sup>	157.5 ±40.30 <sup>b</sup>	2,221.3 ±155.63 <sup>a</sup>	1,687.5 ±210.34 <sup>b</sup>
60	646.3 ±69.02 <sup>a</sup>	322.5 ±59.00 <sup>b</sup>	4,836.3 ±259.64 <sup>a</sup>	3,366.3 ±429.00 <sup>b</sup>	313.8 ±36.45 <sup>a</sup>	201.3 ±36.47 <sup>b</sup>	2,535.0 ±186.81 <sup>a</sup>	1,888.8 ±243.49 <sup>b</sup>
70	552.5 ±57.22 <sup>a</sup>	278.8 ±61.16 <sup>b</sup>	5,388.8 ±307.00 <sup>a</sup>	3,645.0 ±473.97 <sup>b</sup>	312.5 ±34.73 <sup>a</sup>	158.8 ±35.02 <sup>b</sup>	2,847.5 ±209.29 <sup>a</sup>	2,047.5 ±270.27 <sup>b</sup>
80	538.8 ±85.30 <sup>a</sup>	275.0 ±58.25 <sup>b</sup>	5,927.5 ±358.19 <sup>a</sup>	3,920.0 ±514.41 <sup>b</sup>	252.5 ±47.35 <sup>a</sup>	155.0 ±31.05 <sup>b</sup>	3,100.0 ±240.07 <sup>a</sup>	2,202.5 ±291.17 <sup>b</sup>
90	498.8 ±47.90 <sup>a</sup>	277.5 ±37.31 <sup>b</sup>	6,426.3 ±388.89 <sup>a</sup>	4,197.5 ±545.08 <sup>b</sup>	278.8 ±20.83 <sup>a</sup>	155.0 ±23.53 <sup>b</sup>	3,378.8 ±244.21 <sup>a</sup>	2,357.5 ±303.10 <sup>b</sup>
100	482.5 ±69.56 <sup>a</sup>	230.0 ±54.41 <sup>b</sup>	6,908.8 ±432.95 <sup>a</sup>	4,427.5 ±584.32 <sup>b</sup>	268.8 ±32.70 <sup>a</sup>	140.0 ±28.35 <sup>b</sup>	3,647.5 ±261.25 <sup>a</sup>	2,497.5 ±320.47 <sup>b</sup>
110	565.0 ±81.55 <sup>a</sup>	201.3 ±51.04 <sup>b</sup>	7,473.8 ±466.27 <sup>a</sup>	4,628.8 ±630.55 <sup>b</sup>	303.8 ±46.02 <sup>a</sup>	111.3 ±28.19 <sup>b</sup>	3,951.3 ±277.88 <sup>a</sup>	2,608.8 ±345.95 <sup>b</sup>
120	391.3 ±59.29 <sup>a</sup>	200.0 ±49.89 <sup>b</sup>	7,865.0 ±489.09 <sup>a</sup>	4,828.8 ±670.13 <sup>b</sup>	226.3 ±44.16	127.5 ±30.52	4,177.5 ±283.13 <sup>a</sup>	2,736.3 ±369.34 <sup>b</sup>

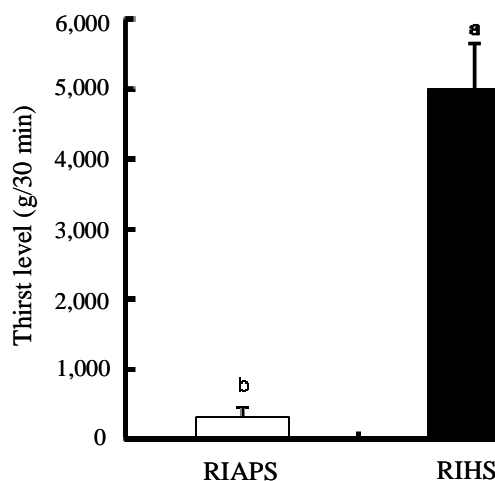
RIAPS = Intraruminal infusion of artificial parotid saliva; RIHS = Intraruminal infusion of hypertonic solution.

Values are means±SE of 8 large-type goats; <sup>a, b</sup> Means in the same row bearing different superscripts differ (p<0.05).

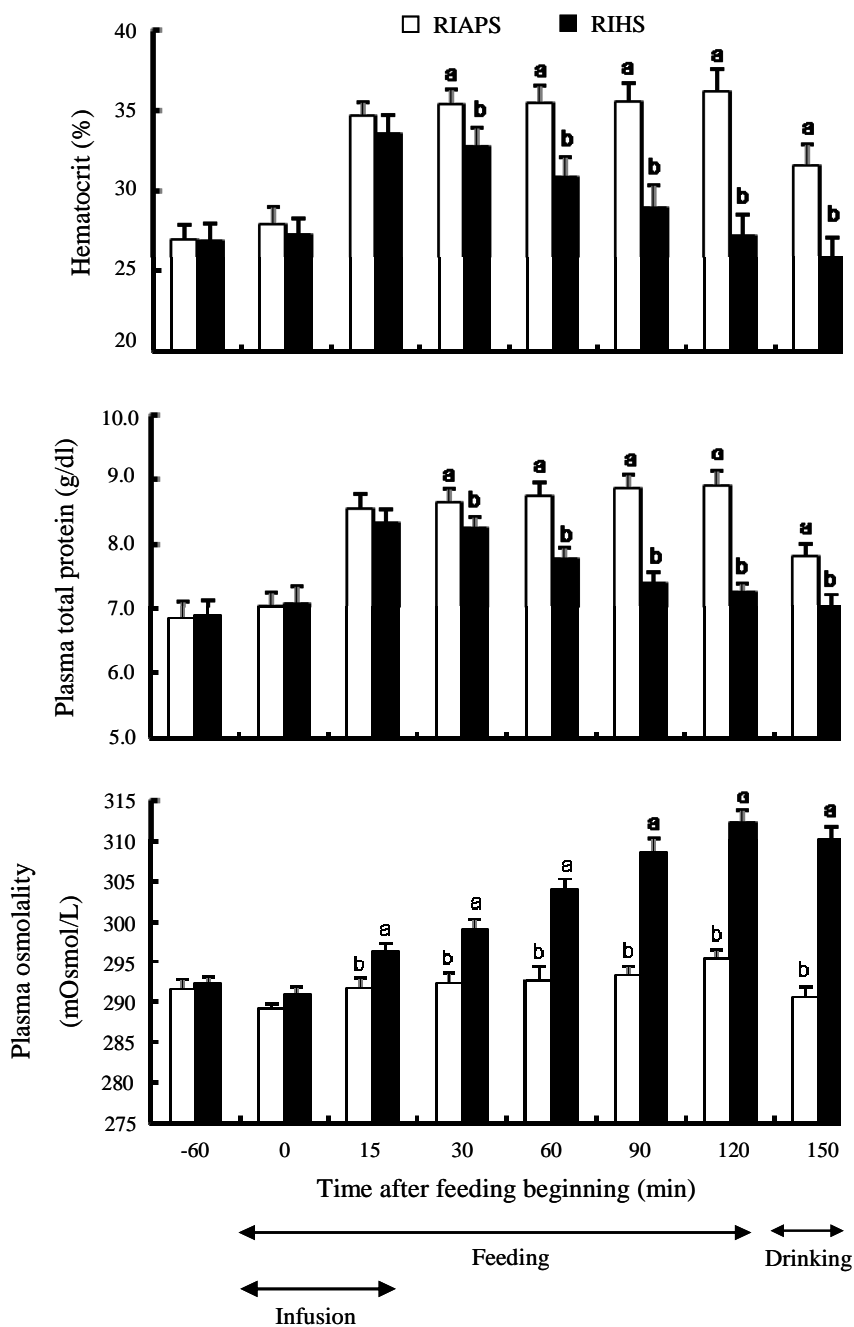
Cumulative salivary secretion volume (Table 1) increased gradually as feeding period elapsed. In comparison with the RIAPS control, cumulative salivary secretion volume in the RIHS treatment was significantly lower (p<0.05) from 40 min after the commencement of feeding.

**Thirst level :** Figure 2 shows the effect of RIHS on thirst level. In comparison with the RIHS treatment (4,993.8±667.85 g/30 min), thirst level in the RIAPS control (293.8±165.41 g/30 min) was 94.1% less (p<0.01) upon conclusion of the 30 min drinking period.

**Hematocrit, plasma total protein concentration and plasma osmolality :** Figure 3 presents the effect of RIHS on hematocrit, plasma total protein concentration and plasma osmolality. Hematocrit and plasma total protein concentrations in both the RIAPS control and the RIHS treatment increased rapidly during the first 15 min after the commencement of feeding. Subsequently, hematocrit and plasma total protein concentrations gradually decreased in the RIHS treatment while remaining at high level in the RIAPS control for the remainder of the feeding period.



**Figure 2.** The effect of intraruminal infusion of hypertonic solution (RIHS) on thirst level. Values are means±SE of 8 large-type goats. <sup>a, b</sup> Means with different superscript are significantly different (p<0.01) from intraruminal infusion of artificial parotid saliva (RIAPS).



**Figure 3.** The effect of intraruminal infusion of hypertonic solution (RIHS) on hematocrit, plasma total protein concentration and plasma osmolality. Values are means±SE of 8 large-type goats. <sup>a, b</sup> Means with different superscript are significantly different (p<0.05) from intraruminal infusion of artificial parotid saliva (RIAPS).

Compared with the RIAPS control, hematocrit and plasma total protein concentrations in the RIHS treatment were significantly lower (p<0.05 and p<0.01, respectively) from 30 min after the commencement of feeding.

Plasma osmolality in the RIHS treatment increased gradually and reached the highest level of 312.3±1.57 mOsmol/L upon conclusion of the 2 h feeding period. Meanwhile, plasma osmolality in the RIAPS control increased very slowly and peaked (295.5±1.07 mOsmol/L) upon conclusion of the 2 h feeding period. Compared with

the RIAPS control, plasma osmolality in the RIHS treatment was significantly higher (p<0.01) for the duration of the 2 h feeding period.

*Plasma glucose concentration* : Plasma glucose concentrations in the RIHS treatment were slightly lower than those in the RIAPS control for the duration of the 2 h feeding period (Table 2). Compared with the RIAPS control, plasma glucose concentrations in the RIHS treatment were significantly lower (p<0.05) at 15, 30, 90 and 150 min intervals during the 2 h feeding period and upon conclusion

**Table 2.** The effect of intraruminal infusion of hypertonic solution (RIHS) on plasma concentrations of glucose, Na, K, and Cl and ruminal fluid concentrations of Na, K, and Cl

Time after feeding beginning (min)	Plasma glucose (mg/dl)		Plasma Na (mmol/L)		Plasma K (mmol/L)		Plasma Cl (mmol/L)		Ruminal fluid Na (mmol/L)		Ruminal fluid K (mmol/L)		Ruminal fluid Cl (mmol/L)	
	RIAPS	RIHS	RIAPS	RIHS	RIAPS	RIHS	RIAPS	RIHS	RIAPS	RIHS	RIAPS	RIHS	RIAPS	RIHS
-60	63.9 ±1.42	62.9 ±1.26	138.1 ±1.03	138.8 ±0.56	4.8 ±0.09	4.7 ±0.09	99.1 ±0.97	100.4 ±0.53	109.1 ±7.46	122.4 ±7.45	58.0 ±7.82	41.9 ±5.43	36.3 ±4.50	43.2 ±3.81
0	64.6 ±2.06	62.3 ±1.36	137.0 ±0.71	139.3 ±0.67	4.7 ±0.09	4.6 ±0.17	98.9 ±0.85	99.5 ±0.42	104.2 ±6.44	119.7 ±8.27	54.0 ±5.24	39.6 ±5.65	38.4 ±4.41	45.1 ±4.02
15	66.9 ±2.10 <sup>a</sup>	61.6 ±1.46 <sup>b</sup>	140.8 ±1.13	141.9 ±0.74	4.6 ±0.15	4.7 ±0.11	103.4 ±0.96	101.9 ±2.77	108.2 ±6.47 <sup>b</sup>	211.3 ±19.65 <sup>a</sup>	47.4 ±5.65	35.2 ±4.11	35.3 ±4.05 <sup>b</sup>	140.9 ±12.13 <sup>a</sup>
30	65.0 ±2.15 <sup>a</sup>	59.8 ±1.67 <sup>b</sup>	139.3 ±1.19 <sup>b</sup>	142.9 ±0.40 <sup>a</sup>	4.5 ±0.13	4.5 ±0.12	102.8 ±0.88 <sup>b</sup>	105.6 ±0.42 <sup>a</sup>	111.3 ±6.10 <sup>b</sup>	210.6 ±18.49 <sup>a</sup>	46.8 ±5.35	34.7 ±5.00	36.1 ±4.89 <sup>b</sup>	173.8 ±9.77 <sup>a</sup>
60	63.0 ±3.15	60.0 ±1.48	140.4 ±1.32 <sup>b</sup>	145.6 ±0.38 <sup>a</sup>	4.5 ±0.21	4.5 ±0.10	103.6 ±0.84 <sup>b</sup>	107.8 ±0.84 <sup>a</sup>	111.9 ±5.29 <sup>b</sup>	215.1 ±18.31 <sup>a</sup>	45.5 ±5.89	36.0 ±5.37	35.7 ±4.55 <sup>b</sup>	185.5 ±8.56 <sup>a</sup>
90	64.0 ±2.28 <sup>a</sup>	58.8 ±1.32 <sup>b</sup>	139.5 ±1.39 <sup>b</sup>	147.3 ±0.80 <sup>a</sup>	4.7 ±0.21 <sup>a</sup>	4.3 ±0.15 <sup>b</sup>	102.4 ±1.03 <sup>b</sup>	109.4 ±0.75 <sup>a</sup>	109.5 ±5.52 <sup>b</sup>	198.1 ±17.87 <sup>a</sup>	44.6 ±5.56	37.5 ±3.42	36.1 ±4.36 <sup>b</sup>	187.0 ±8.68 <sup>a</sup>
120	64.6 ±2.97	60.3 ±1.35	138.8 ±1.44 <sup>b</sup>	149.3 ±0.45 <sup>a</sup>	4.7 ±0.14 <sup>a</sup>	4.1 ±0.14 <sup>b</sup>	103.6 ±1.36 <sup>b</sup>	109.9 ±0.81 <sup>a</sup>	110.8 ±5.53 <sup>b</sup>	198.2 ±20.60 <sup>a</sup>	45.9 ±5.56	35.4 ±3.90	37.6 ±4.66 <sup>b</sup>	188.2 ±8.32 <sup>a</sup>
150	64.8 ±2.37 <sup>a</sup>	59.5 ±1.57 <sup>b</sup>	137.4 ±1.03 <sup>b</sup>	147.6 ±1.10 <sup>a</sup>	4.5 ±0.11 <sup>a</sup>	3.8 ±0.10 <sup>b</sup>	98.6 ±1.05 <sup>b</sup>	108.4 ±1.19 <sup>a</sup>	117.6 ±6.47	144.7 ±20.52	44.4 ±5.42 <sup>a</sup>	26.6 ±4.81 <sup>b</sup>	39.8 ±5.08 <sup>b</sup>	148.8 ±4.51 <sup>a</sup>

RIAPS = Intraruminal infusion of artificial parotid saliva; RIHS = Intraruminal infusion of hypertonic solution.

Values are means±SE of 8 large-type goats; <sup>a, b</sup> Means in the same row bearing different superscripts differ ( $p < 0.05$ ).

of the 30 min drinking period.

*Plasma concentrations of Na, K and Cl* : Table 2 shows the effect of RIHS on plasma concentrations of Na, K and Cl. Plasma concentrations of Na and Cl in the RIHS treatment increased gradually while they mostly remained unchanged in the RIAPS control during the 2 h feeding period. Compared with the RIAPS control, plasma concentrations of Na and Cl in the RIHS treatment were greater ( $p < 0.01$ ) from 30 min after the commencement of feeding.

Plasma K concentrations in the RIHS treatment tended to be reduced gradually as the feeding period elapsed while those in the RIAPS control mostly remained unchanged during the 2 h feeding period. In comparison with the RIAPS control, plasma K concentrations in the RIHS treatment were significantly lower ( $p < 0.05$ ) from 90 to 150 min after the commencement of feeding.

*Ruminal fluid osmolality and pH* : Figure 4 shows the effect of RIHS on ruminal fluid osmolality and pH. Ruminal fluid osmolality in the RIHS treatment increased rapidly and reached the highest level of  $408.4 \pm 9.79$  mOsmol/L at 30 min after the commencement of feeding and subsequently decreased gradually to the end of the 2 h feeding period. Meanwhile, ruminal fluid osmolality in the RIAPS control mostly remained unchanged during the 2 h feeding period. Compared with the RIAPS control, ruminal fluid osmolality in the RIHS treatment was significantly higher ( $p < 0.01$ ) for the duration of the 2 h feeding period.

Ruminal fluid pH in both the RIAPS control and the

RIHS treatment was slightly higher than compared with pre-feeding levels during the 2 h feeding period. In comparison with the RIAPS control, ruminal fluid pH in the RIHS treatment was significantly lower ( $p < 0.01$ ) for the duration of the 2 h feeding period.

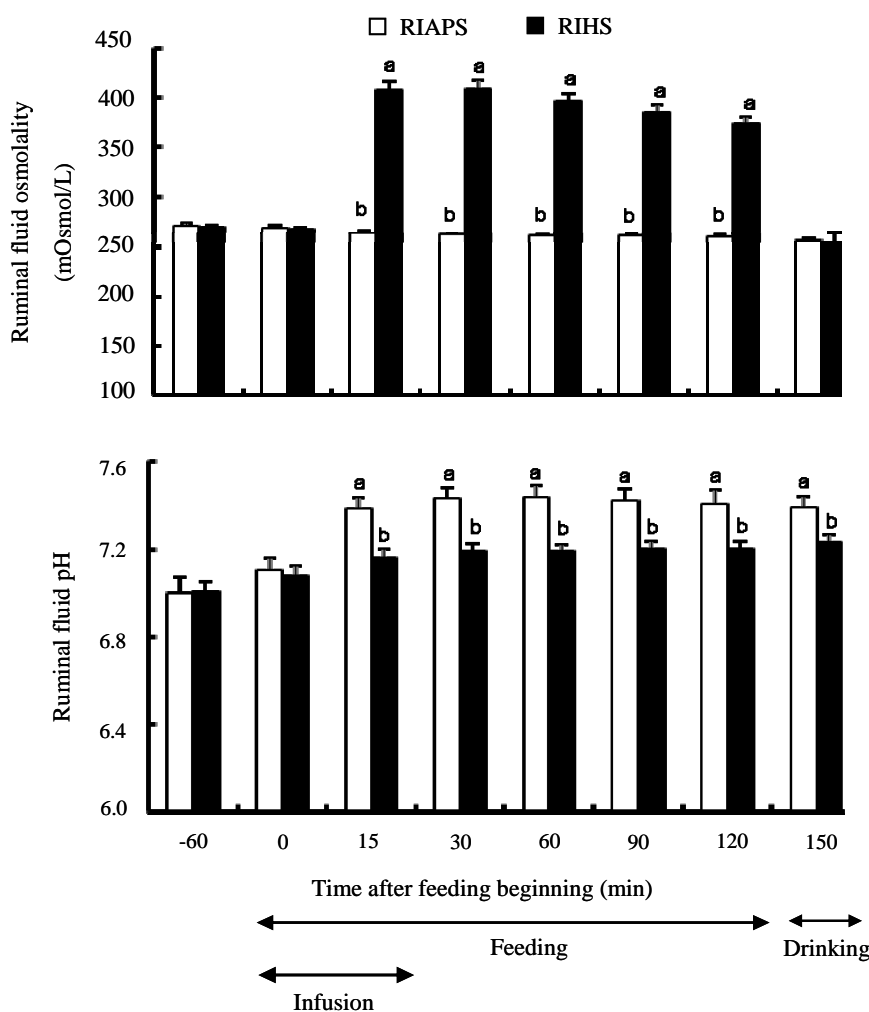
*Ruminal fluid concentrations of Na, K and Cl* : The effect of RIHS on ruminal fluid concentrations of Na, K and Cl is shown in Table 2. Ruminal fluid concentrations of Na and Cl in the RIHS treatment increased rapidly after the commencement of feeding while those in the RIAPS control mostly remained unchanged during the 2 h feeding period. Compared with the RIAPS control, ruminal fluid concentrations of Na and Cl in the RIHS treatment were significantly higher ( $p < 0.01$ ) over the 2 h feeding period.

Ruminal fluid concentrations of K in both the RIAPS control and the RIHS treatment tended to be reduced gradually as feeding period elapsed. Although ruminal fluid concentrations of K in the RIHS treatment were lower than in the RIAPS control, there were no significant differences between the control and the treatment in terms of ruminal fluid concentrations of K during the 2 h feeding period.

## Experiment 2 - The effect of intraruminal infusion of hypertonic solution and intravenous infusion of artificial mixed saliva on dry forage intake in large-type goats during sham feeding

*Physiological parameters* : The mean values of heart rate, respiration rate, and rectal temperature in the RIHS control and the RIHS-VIAMS treatment were  $75.6 \pm 3.06$





**Figure 4.** The effect of intraruminal infusion of hypertonic solution (RIHS) on ruminal fluid osmolality and pH. Values are means $\pm$ SE of 8 large-type goats. <sup>a,b</sup>Means with different superscript are significantly different ( $p < 0.01$ ) from intraruminal infusion of artificial parotid saliva (RIAPS).

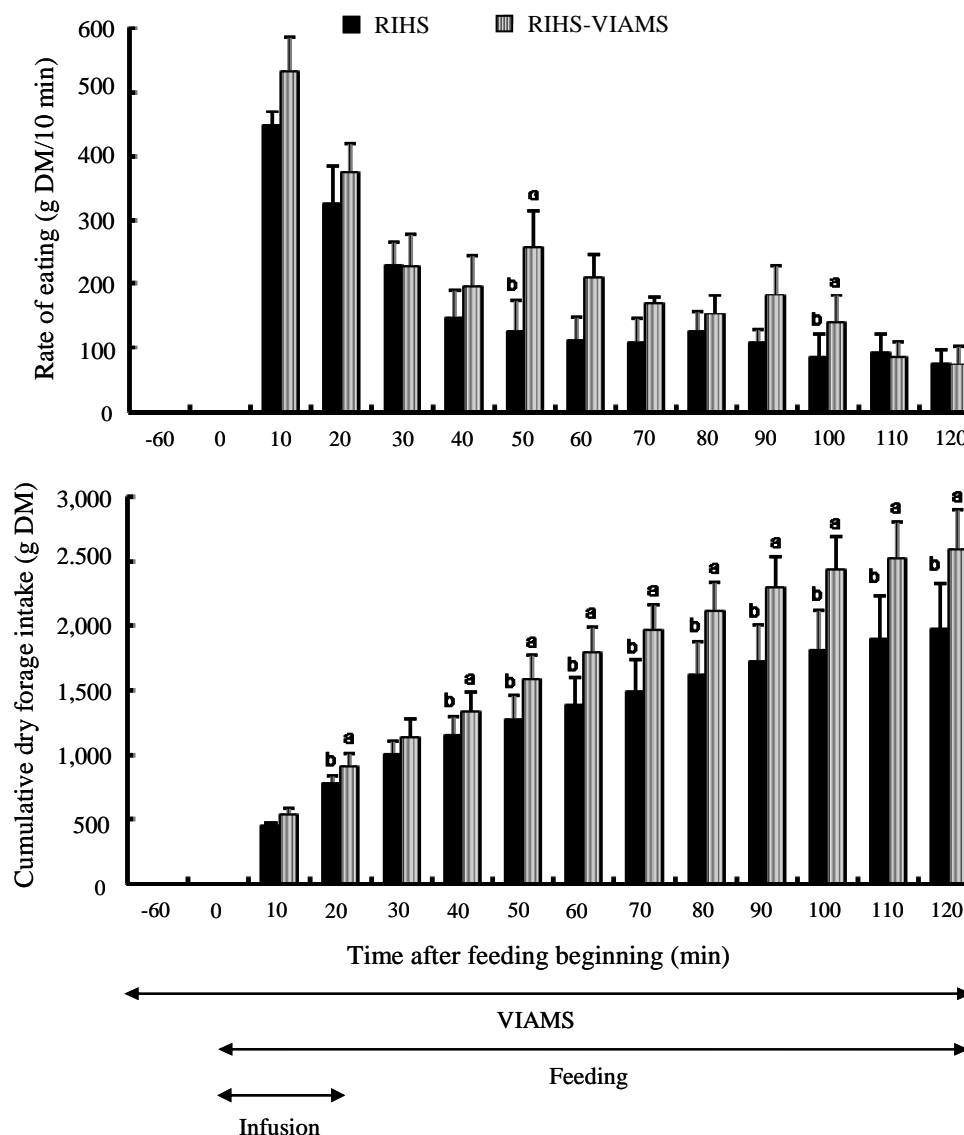
and  $74.4 \pm 3.06$  beats/min,  $16.4 \pm 0.40$  and  $17.6 \pm 0.75$  breaths/min, and  $38.5 \pm 0.14$  and  $38.4 \pm 0.15^\circ\text{C}$ , respectively. There were no significant differences between the RIHS control and the RIHS-VIAMS treatment in the physiological parameters.

**Rate of eating and cumulative dry forage intake:** Figure 5 shows the effect of RIHS-VIAMS on rate of eating and cumulative dry forage intake. Eating rates in both the RIHS control and the RIHS-VIAMS treatment decreased rapidly in the first 30 min of feeding (0 to 10 min,  $448.5 \pm 21.72$  g and  $532.8 \pm 54.00$  g; 20 to 30 min,  $227.6 \pm 37.51$  g and  $225.9 \pm 51.03$  g, respectively). Subsequently, these rates declined gradually to very low rates (ranged from  $74.2 \pm 22.37$  to  $124.8 \pm 48.16$  g/10 min) in the RIHS control while remaining at low rates from  $72.5 \pm 29.64$  to  $256.3 \pm 58.74$  g/10 min in the RIHS-VIAMS treatment for the rest of the 2 h feeding period. Compared with the RIHS control, eating rates in the RIHS-VIAMS treatment were

significantly higher ( $p < 0.05$ ) at 50 and 100 min intervals after the commencement of feeding.

Cumulative dry forage intake in both the RIHS control and the RIHS-VIAMS treatment increased progressively after the commencement of feeding to the end of the 2 h feeding period. In comparison with the RIHS control ( $1,972.6 \pm 359.72$  g/2 h), cumulative dry forage intake in the RIHS-VIAMS treatment ( $2,591.4 \pm 312.18$  g/2 h) was 31.4% greater ( $p < 0.05$ ) upon conclusion of the 2 h feeding period.

**Rate of salivary secretion and cumulative salivary secretion:** The rates of salivary secretion in both the RIHS control and the RIHS-VIAMS treatment (Table 3) peaked in the first 10 min after feeding was commenced. Subsequently, these rates decreased as the feeding progressed. Salivary secretion rates in the RIHS-VIAMS treatment decreased slowly from 50 min after the commencement of feeding while the rates observed in the RIHS control reduced more rapidly. The rates of salivary secretion in the RIHS control were markedly lower than



**Figure 5.** The effect of intraruminal infusion of hypertonic solution and intravenous infusion of artificial mixed saliva (RIHS-VIAMS) on rate of eating and cumulative dry forage intake. Values are means $\pm$ SE of 8 large-type goats. <sup>a, b</sup> Means with different superscript are significantly different ( $p < 0.05$ ) from intraruminal infusion of hypertonic solution (RIHS).

those in the RIHS-VIAMS treatment throughout the 2 h feeding period. Compared with the RIHS control, the rates of salivary secretion in the RIHS-VIAMS treatment were significantly higher ( $p < 0.05$ ) at 50, 70, 90 and 110 min intervals after the commencement of feeding.

Cumulative salivary secretion volume (Table 3) increased gradually as feeding period elapsed and reached a volume of  $2,970 \pm 529.46$  g in the RIHS control and  $3,966 \pm 449.07$  g in the RIHS-VIAMS treatment upon conclusion of the 2 h feeding period. In comparison with the RIHS control, cumulative salivary secretion volume in the RIHS-VIAMS treatment was significantly higher ( $p < 0.05$ ) from 50 min after the commencement of feeding.

**Thirst level:** Figure 6 shows the effect of RIHS-VIAMS on thirst level. In comparison with the RIHS control

( $5,000 \pm 603.95$  g/30 min), thirst level in the RIHS-VIAMS treatment ( $2,740 \pm 490.26$  g/30 min) was 45.2% less ( $p < 0.01$ ) upon conclusion of the 30 min drinking period.

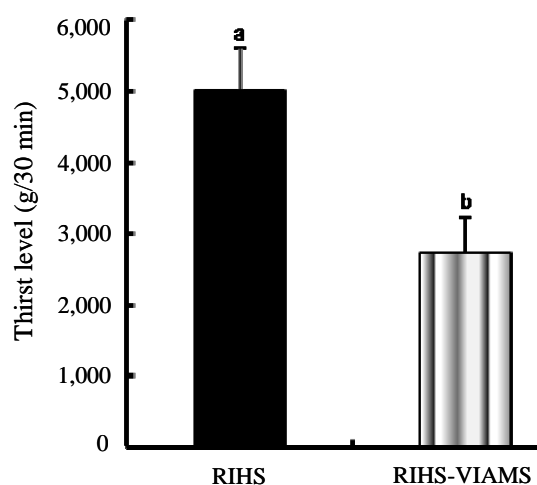
**Hematocrit, plasma total protein concentration and plasma osmolality:** Figure 7 presents the effect of RIHS-VIAMS on hematocrit, plasma total protein concentration and plasma osmolality. Hematocrit and plasma total protein concentrations in both the RIHS control and the RIHS-VIAMS treatment increased rapidly in the first 15 min after the commencement of feeding and subsequently decreased gradually for the remainder of the 2 h feeding period. There were no significant differences between the RIHS control and the RIHS-VIAMS treatment in terms of hematocrit and plasma total protein concentrations over the 2 h feeding period.

**Table 3.** The effect of intraruminal infusion of hypertonic solution and intravenous infusion of artificial mixed saliva (RIHS-VIAMS) 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)	
	RIHS	RIHS-VIAMS	RIHS	RIHS-VIAMS	RIHS	RIHS-VIAMS	RIHS	RIHS-VIAMS
10	1,240 ±116.62	1,380 ±121.04	1,240 ±116.62	1,380 ±121.04	708 ±110.02	748 ±81.45	708 ±110.02	748 ±81.45
20	800 ±97.42	950 ±135.09	2,040 ±209.98 <sup>b</sup>	2,330 ±204.08 <sup>a</sup>	414 ±38.42	506 ±114.04	1,122 ±138.36	1,254 ±95.63
30	600 ±85.09	660 ±81.24	2,640 ±288.70 <sup>b</sup>	2,990 ±274.95 <sup>a</sup>	330 ±45.28	392 ±66.36	1,452 ±176.96 <sup>b</sup>	1,646 ±139.63 <sup>a</sup>
40	380 ±107.94	460 ±69.64	3,020 ±390.70	3,450 ±331.66	208 ±66.59	228 ±35.13	1,660 ±232.96	1,874 ±158.23
50	300 ±122.47 <sup>b</sup>	650 ±136.01 <sup>a</sup>	3,320 ±500.15 <sup>b</sup>	4,100 ±422.79 <sup>a</sup>	152 ±66.06 <sup>b</sup>	346 ±73.18 <sup>a</sup>	1,812 ±293.66 <sup>b</sup>	2,220 ±218.79 <sup>a</sup>
60	370 ±80.00	520 ±64.42	3,690 ±578.88 <sup>b</sup>	4,620 ±406.39 <sup>a</sup>	238 ±46.73	272 ±32.16	2,050 ±335.26 <sup>b</sup>	2,492 ±194.46 <sup>a</sup>
70	290 ±97.98 <sup>b</sup>	510 ±64.03 <sup>a</sup>	3,980 ±651.46 <sup>b</sup>	5,130 ±423.26 <sup>a</sup>	162 ±54.63 <sup>b</sup>	310 ±54.95 <sup>a</sup>	2,212 ±376.60 <sup>b</sup>	2,802 ±222.83 <sup>a</sup>
80	330 ±78.42	420 ±112.47	4,310 ±694.87 <sup>b</sup>	5,550 ±502.49 <sup>a</sup>	182 ±41.16	240 ±79.94	2,394 ±397.75 <sup>b</sup>	3,042 ±288.31 <sup>a</sup>
90	290 ±55.68 <sup>b</sup>	512 ±103.41 <sup>a</sup>	4,600 ±745.49 <sup>b</sup>	6,062 ±565.41 <sup>a</sup>	162 ±32.00 <sup>b</sup>	296 ±57.15 <sup>a</sup>	2,556 ±425.05 <sup>b</sup>	3,338 ±332.98 <sup>a</sup>
100	250 ±83.67	378 ±109.47	4,850 ±801.09 <sup>b</sup>	6,440 ±588.52 <sup>a</sup>	150 ±43.36	214 ±61.77	2,706 ±447.62 <sup>b</sup>	3,552 ±333.16 <sup>a</sup>
110	230 ±81.55	340 ±94.07	5,080 ±880.14 <sup>b</sup>	6,780 ±677.97 <sup>a</sup>	122 ±46.20 <sup>b</sup>	240 ±69.21 <sup>a</sup>	2,828 ±493.11 <sup>b</sup>	3,792 ±399.34 <sup>a</sup>
120	230 ±73.48	260 ±88.60	5,310 ±942.26 <sup>b</sup>	7,040 ±763.45 <sup>a</sup>	142 ±47.16	174 ±56.27	2,970 ±529.46 <sup>b</sup>	3,966 ±449.07 <sup>a</sup>

RIHS = Intraruminal infusion of hypertonic solution; RIHS-VIAMS = RIHS and intravenous infusion of artificial mixed saliva.

Values are means±SE of 8 large-type goats; <sup>a, b</sup> Means in the same row bearing different superscripts differ ( $p < 0.05$ ).

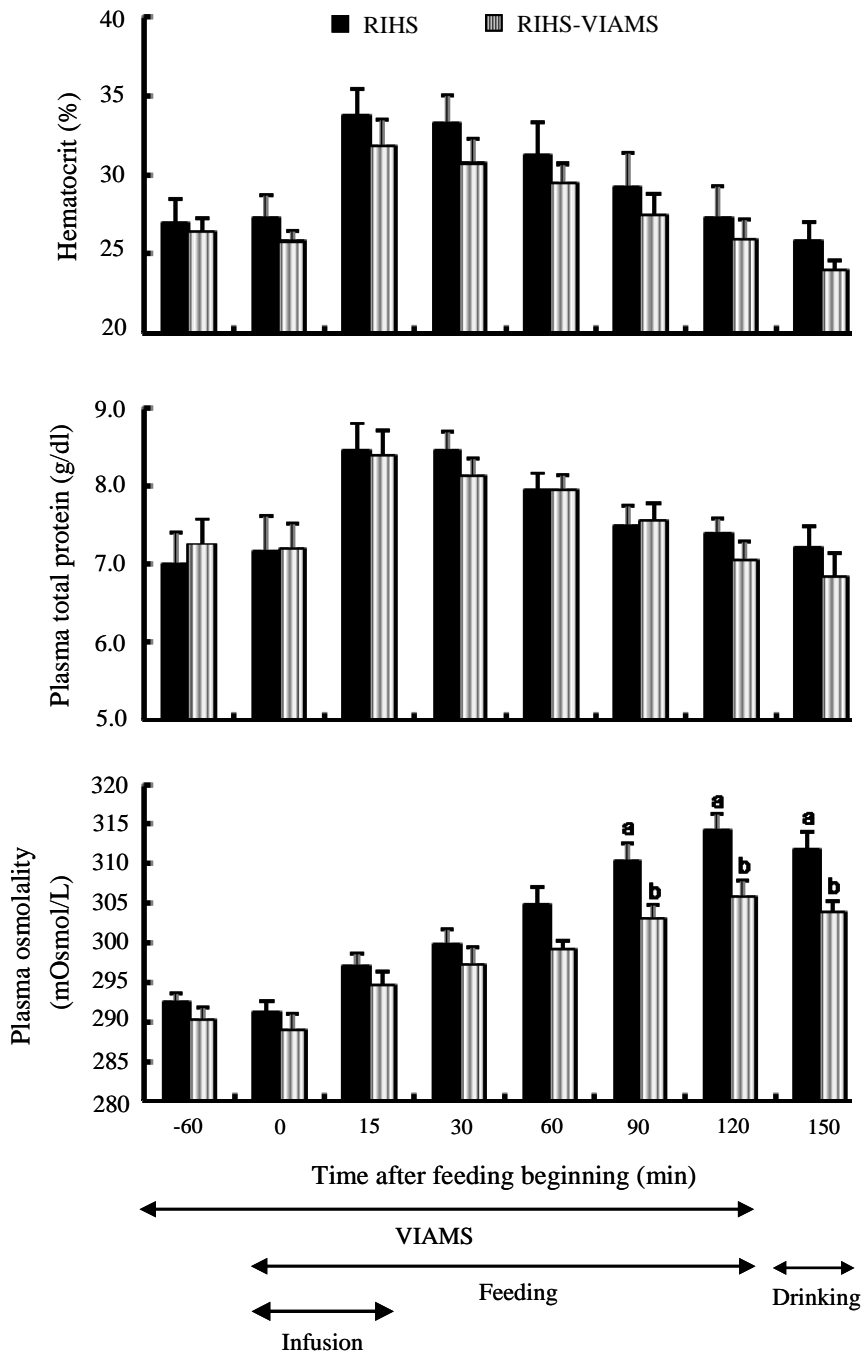


**Figure 6.** The effect of intraruminal infusion of hypertonic solution and intravenous infusion of artificial mixed saliva (RIHS-VIAMS) on thirst level. Values are means±SE of 8 large-type goats. <sup>a, b</sup> Means with different superscript are significantly different ( $p < 0.01$ ) from intraruminal infusion of hypertonic solution (RIHS).

Plasma osmolality in both the RIHS control and the RIHS-VIAMS treatment increased gradually and reached the highest levels of  $314.2 \pm 1.98$  and  $305.8 \pm 2.01$  mOsmol/L, respectively, upon conclusion of the 2 h feeding period. Compared with the RIHS control, plasma osmolality in the RIHS-VIAMS treatment was significantly lower ( $p < 0.01$ ) from 90 min after the commencement of feeding, even upon conclusion of the 30 min drinking period.

*Plasma glucose concentration* : Plasma glucose concentrations in the RIHS control were slightly lower than those in the RIHS-VIAMS treatment for the duration of the 2 h feeding period (Table 4). Compared with the RIHS control, plasma glucose concentrations in the RIHS-VIAMS treatment were significantly higher ( $p < 0.05$ ) at 0, 15 and 90 min intervals.

*Plasma concentrations of Na, K and Cl* : Table 4 shows



**Figure 7.** The effect of intraruminal infusion of hypertonic solution and intravenous infusion of artificial mixed saliva (RIHS-VIAMS) on hematocrit, plasma total protein concentration and plasma osmolality. Values are means±SE of 8 large-type goats. <sup>a, b</sup> Means with different superscript are significantly different ( $p < 0.01$ ) from intraruminal infusion of hypertonic solution (RIHS).

**Table 4.** The effect of intraruminal infusion of hypertonic solution and intravenous infusion of artificial mixed saliva (RIHS-VIAMS) on plasma concentrations of glucose, Na, K, and Cl and ruminal fluid concentrations of Na, K, and Cl

Time after feeding beginning (min)	Plasma glucose (mg/dl)		Plasma Na (mmol/L)		Plasma K (mmol/L)		Plasma Cl (mmol/L)		Ruminal fluid Na (mmol/L)		Ruminal fluid K (mmol/L)		Ruminal fluid Cl (mmol/L)	
	RIHS	RIHS-VIAMS	RIHS	RIHS-VIAMS	RIHS	RIHS-VIAMS	RIHS	RIHS-VIAMS	RIHS	RIHS-VIAMS	RIHS	RIHS-VIAMS	RIHS	RIHS-VIAMS
-60	64.4 ±1.63	65.4 ±1.17	138.2 ±0.49	139.8 ±1.28	4.6 ±0.07 <sup>b</sup>	4.8 ±0.04 <sup>a</sup>	100.0 ±0.63	102.0 ±1.82	128.5 ±6.82	131.9 ±3.06	46.5 ±7.37	62.1 ±2.27	46.9 ±4.38 <sup>a</sup>	35.7 ±3.03 <sup>b</sup>
0	63.4 ±1.63 <sup>b</sup>	66.6 ±1.72 <sup>a</sup>	139.0 ±0.45	137.4 ±1.12	4.5 ±0.24	4.8 ±0.12	99.4 ±0.68	99.2 ±1.46	124.3 ±7.75	138.9 ±4.06	42.9 ±8.25	62.2 ±1.73	48.3 ±3.96 <sup>a</sup>	36.2 ±2.51 <sup>b</sup>
15	62.6 ±2.16 <sup>b</sup>	68.2 ±2.69 <sup>a</sup>	140.8 ±0.58	139.2 ±1.80	4.6 ±0.17	4.7 ±0.15	100.6 ±4.50	103.0 ±1.10	228.2 ±21.64	247.3 ±7.26	32.2 ±5.45	46.9 ±2.22	162.7 ±8.31	157.9 ±8.56
30	60.8 ±2.52	65.2 ±1.24	143.0 ±0.55	142.0 ±1.38	4.5 ±0.13	4.4 ±0.19	106.0 ±0.55 <sup>a</sup>	103.2 ±1.02 <sup>b</sup>	216.5 ±20.93	251.1 ±8.07	27.5 ±5.86	46.2 ±2.44	188.0 ±11.39	177.8 ±11.02
60	61.4 ±1.96	63.4 ±1.89	145.4 ±0.24	142.2 ±1.32	4.5 ±0.12	4.3 ±0.23	109.4 ±0.24 <sup>a</sup>	103.8 ±1.69 <sup>b</sup>	222.2 ±18.66	241.4 ±5.56	30.5 ±7.75	44.5 ±3.53	194.6 ±11.22	172.6 ±9.06
90	59.6 ±1.99 <sup>b</sup>	63.8 ±1.93 <sup>a</sup>	147.8 ±1.07	145.4 ±1.12	4.3 ±0.23	4.2 ±0.20	110.2 ±1.02	103.8 ±2.27	199.5 ±18.45	229.1 ±3.78	33.8 ±4.79	43.1 ±3.05	194.3 ±12.32	165.2 ±8.47
120	61.6 ±1.86	63.4 ±1.91	149.4 ±0.68	145.8 ±1.93	4.0 ±0.19	4.3 ±0.22	111.0 ±0.95 <sup>a</sup>	104.6 ±1.25 <sup>b</sup>	201.4 ±25.20	231.8 ±4.19	31.1 ±5.32	44.3 ±3.23	194.5 ±12.30	160.7 ±8.16
150	59.8 ±2.29	63.2 ±2.99	147.0 ±1.58	146.2 ±0.97	3.8 ±0.15	3.8 ±0.12	109.0 ±1.90	106.6 ±1.72	140.2 ±20.35	180.3 ±5.07	19.7 ±4.67 <sup>b</sup>	35.4 ±2.70 <sup>a</sup>	151.5 ±2.91 <sup>a</sup>	127.3 ±5.95 <sup>b</sup>

RIHS = Intraruminal infusion of hypertonic solution; RIHS-VIAMS = RIHS and intravenous infusion of artificial mixed saliva.

Values are means±SE of 8 large-type goats; <sup>a, b</sup> Means in the same row bearing different superscripts differ (p<0.05).

the effect of RIHS-VIAMS on plasma concentrations of Na, K and Cl. Plasma concentrations of Na and Cl in both the RIHS control and the RIHS-VIAMS treatment increased gradually during the 2 h feeding period. Although plasma Na concentrations in the RIHS-VIAMS treatment were lower than in the RIHS control, there were no significant differences between the control and the treatment in plasma Na concentrations for the duration of the 2 h feeding period. Plasma Cl concentrations in the RIHS control were significantly greater (p<0.05) than in the RIHS-VIAMS treatment at 30, 60 and 120 min intervals.

Plasma K concentrations in both the RIHS control and the RIHS-VIAMS treatment tended to be reduced gradually as feeding period elapsed. There were no significant differences between the RIHS control and the RIHS-VIAMS treatment in terms of plasma K concentrations for the duration of the 2 h feeding period.

**Ruminal fluid osmolality and pH** : Figure 8 shows the effect of RIHS-VIAMS on ruminal fluid osmolality and pH. Ruminal fluid osmolality in both the RIHS control and the RIHS-VIAMS treatment increased rapidly and reached the highest level of 415.6±12.58 mOsmol/L at 30 min in the RIHS control and of 408.0±9.64 mOsmol/L at 15 min in the RIHS-VIAMS treatment after the commencement of feeding and subsequently decreased gradually to the end of the 2 h feeding period. Ruminal fluid osmolality in the RIHS control and the RIHS-VIAMS treatment was not significantly different for the duration of the 2 h feeding period.

There were no significant differences between the RIHS control and the RIHS-VIAMS treatment in terms of ruminal

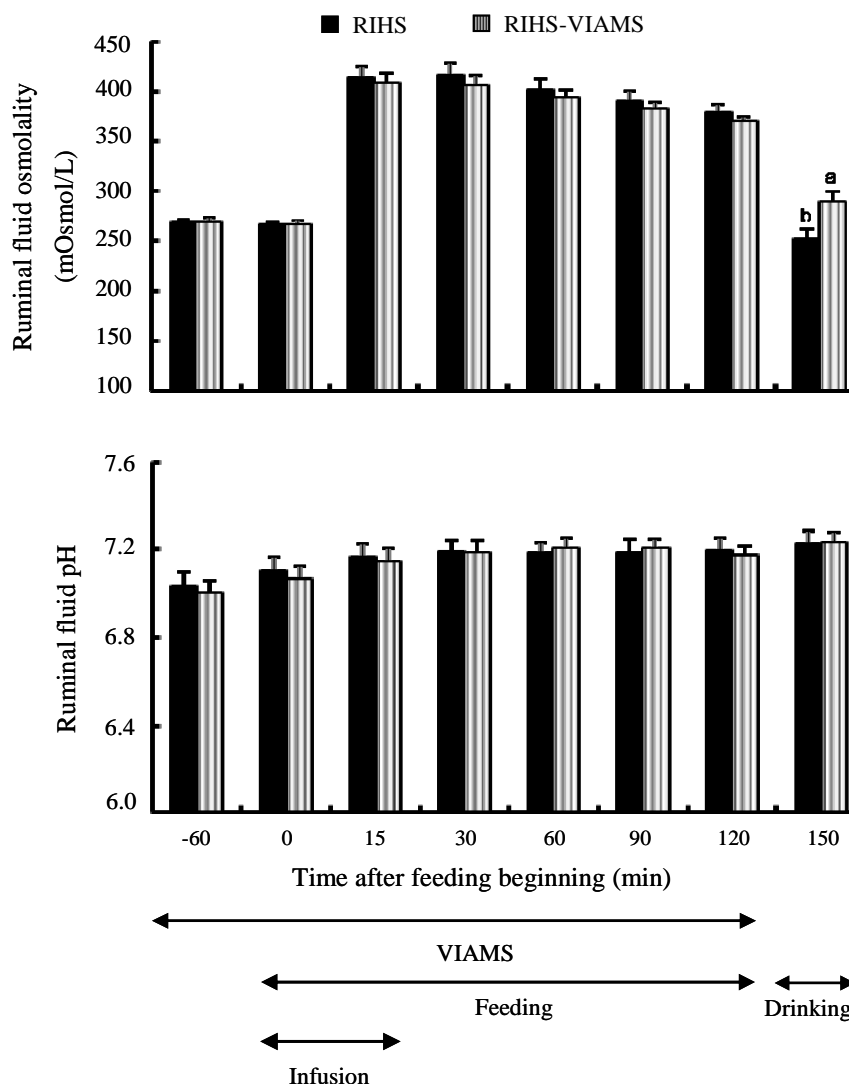
fluid pH during the 2 h feeding period.

**Ruminal fluid concentrations of Na, K and Cl** : Table 4 shows the effect of RIHS-VIAMS on ruminal fluid concentrations of Na, K and Cl. Ruminal fluid concentrations of Na and Cl in both the RIHS control and the RIHS-VIAMS treatment increased rapidly after the commencement of feeding and subsequently were maintained at high levels for the remainder of the 2 h feeding period. There were no significant differences between the RIHS control and the RIHS-VIAMS treatment in terms of ruminal fluid concentrations of Na and Cl for the duration of the 2 h feeding period.

Ruminal fluid concentrations of K in both the RIHS control and the RIHS-VIAMS treatment tended to be reduced gradually as feeding period elapsed. Although ruminal fluid concentrations of K in the RIHS control were lower than in the RIHS-VIAMS treatment, there were no significant differences between the control and the treatment in terms of ruminal fluid concentrations of K during the 2 h feeding period.

## DISCUSSION

Large-type goats that were fed on dry forage twice daily and were given free access to drinking water had a decrease in circulating plasma volume, which was estimated by increases in hematocrit and plasma total protein concentrations, brought about by increased salivary secretion during the initial stages of feeding. Plasma osmolality increased after 30 min of feeding due to salt content in the feed entering the rumen (Sunagawa et al.,



**Figure 8.** The effect of intraruminal infusion of hypertonic solution and intravenous infusion of artificial mixed saliva (RIHS-VIAMS) on ruminal fluid osmolality and pH. Values are means $\pm$ SE of 8 large-type goats. <sup>a, b</sup> Means with different superscript are significantly different ( $p < 0.05$ ) from intraruminal infusion of hypertonic solution (RIHS).

2008). Under these feeding conditions, despite the animal's frequently drinking water after 30 min of feeding had elapsed, plasma osmolality continuously increased, and dry forage intake was extremely reduced.

Thang et al. (2010) reported that in the large-type goats under sham feeding conditions in which esophageal boluses were removed via an esophageal fistula before entering the rumen, circulating plasma volume decreased with dry forage feeding but there were no changes in plasma osmolality during feeding. In addition, under sham feeding conditions the animals drank a very small amount of water. After 30 min of feeding had elapsed, eating rates of animals under sham feeding conditions were significantly higher than those under normal feeding conditions. These higher eating rates resulted in a considerable increase in the amount of dry forage consumed by animals under sham feeding conditions. From these results, it was thought that

one of the factors involved in the extreme suppression of dry forage intake after 30 or 40 min of feeding in large-type goats fed dry forage twice daily might be the production of thirst sensations brought about by increases in plasma osmolality due to the ruminal absorption of salt content from the feed entering the rumen.

In experiment 1, the changes in ruminal and blood humoral parameters during feeding in large-type goats fed dry forage twice daily were reproduced under sham feeding conditions. Although ruminal fluid osmolality in the RIHS treatment rapidly increased, changes in humoral parameters including decreases in circulating plasma volume and increases in plasma osmolality in the RIHS treatment were very similar to the changes observed under normal feeding conditions (Sunagawa et al., 2008). It was found that when ruminal fluid osmolality increased in the RIHS treatment, plasma osmolality increased and thirst sensations were

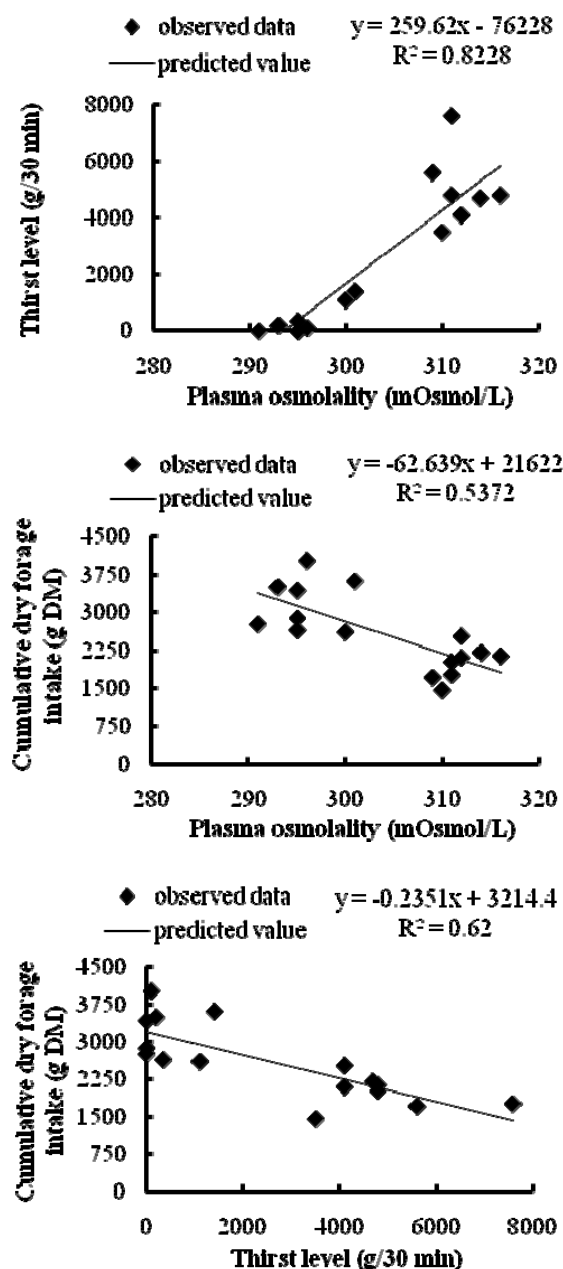
produced. As was observed under normal feeding conditions, eating rates in the RIHS treatment significantly decreased after 40 min of feeding had elapsed. Therefore, cumulative dry forage intake in the RIHS treatment was significantly reduced compared to the RIAPS control due to increases in osmolality of ruminal fluid and plasma, and thirst sensations brought about by an intraruminal infusion of hypertonic solution.

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 (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). The decrease in dry forage intake in the RIHS treatment of the experiment 1 was consistent with the results in the study of 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 goats fed on alfalfa hay cubes, feed intake increased by 30% (Sunagawa et al., 2002). It was thought that the changes in ruminal fluid osmolality were sensed by the osmoreceptors in the rumen wall and these signals were then transported to the central nervous system (Leek and Harding, 1975). However, osmoreceptors have not been found in the rumen wall. In addition, the effect of internal humoral factors on the intake of grass has not been investigated under these experimental conditions.

In experiment 2, while ruminal fluid osmolality in the RIHS-VIAMS treatment was the same as that in the RIHS control, plasma osmolality in the RIHS-VIAMS treatment was significantly decreased due to the intravenous infusion of artificial mixed saliva after 60 min of feeding. Therefore, the thirst level in the RIHS-VIAMS treatment was significantly less than the RIHS control. In ruminants fed dry forage, in contrast to the rapid decrease of circulating plasma volume during the initial stages of feeding, plasma osmolality increased after 40 min of the feeding period had elapsed (Blair-West and Brook, 1969; Sunagawa et al., 2002; 2005). The results of this experiment indicate that the thirst sensations in large-type goats fed dry forage observed after 40 min of the feeding period had elapsed were not in fact caused by decreases in circulating plasma volume but rather by an increase in plasma osmolality (Sunagawa et al., 2008; Thang et al., 2010).

An intraruminal infusion of a hypertonic solution in experiment 2 brought about an increase in plasma osmolality which, when inhibited by an intravenous infusion of artificial mixed saliva, reduced the level of dry forage decreases. In both experiments, the recorded data

was placed into a pool and plasma osmolality correlated negatively with dry forage intake but positively with thirst level (Figure 9). Furthermore, there was also a negative correlation between thirst level and dry forage intake (Figure 9). When plasma volume decreases and plasma osmolality increases, angiotensin II is produced in the blood and vasopressin is secreted; these are the main humoral factors stimulating thirst (Blair-West and Brook, 1969; Fitzsimons, 1979; Mathai et al., 1997; McKinley and Johnson, 2004). In sheep fed on dry forage, it was reported



**Figure 9.** The relationships between plasma osmolality and thirst level, plasma osmolality and cumulative dry forage intake, and thirst level and cumulative dry forage intake upon conclusion of the 2 h feeding period.

that an increase in plasma osmolality in the second hour of feeding suppressed the secretion of parotid saliva (Sato, 1975; Warner and Stacy, 1977). Moreover, McKinley et al. (1979) reported that in sheep fed on dry forage when angiotensin II was infused into the carotid artery, the secretion of parotid saliva was suppressed. Decreases in dry forage intake during intracerebroventricular infusion of angiotensin II are caused by the production of thirst sensations in the brains of ewes leading to excessive water intake and ruminal distension (Sunagawa et al., 2001). It was also reported that intraperitoneal injections of vasopressin decreased dry forage intake in goats (Meyer et al., 1989). These facts indicate that increases in ruminal fluid osmolality during dry forage feeding indirectly suppress dry forage intake by causing an increase in plasma osmolality and subsequently inducing a thirst sensation.

These results suggest that the marked decreases in dry forage intake after 40 min of feeding is partly caused by increases in plasma osmolality and subsequent thirst sensations produced by dry forage feeding.

The present study advances knowledge of the control of dry forage intake in domestic ruminants. Such information is an essential requirement in the development of methods to improve management, welfare and performance.

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