Effects of Intraruminal versus Intravenous Infusions of Acetone on the Ruminating and Masticating Behavior of Goats

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ABSTRACT : Acetone, which is produced from butyric acid when it passes through the rumen wall, was infused into the rumen and jugular veins of three female goats to investigate the role of acetone in ruminating and masticating behavior. The ruminating behavior, as measured by the number of boli and the ruminating time, decreased ($p<0.05$) with intraruminal acetone infusion. However, the ruminating behavior did not change significantly in response to intravenous acetone infusion. Feed intake significantly decreased with intraruminal acetone infusion, but not with intravenous acetone infusion. The concentrations of acetone in the plasma increased significantly ($p<0.05$) with both acetone infusion regions. Ruminal fluid acetone, and isopropyl alcohol (IPA), which is one of the ketone bodies, produced from acetone by bacterial action in rumen, concentrations were significantly increased ($p<0.05$) with both acetone infusion regions. These results suggest that the chemoreceptors sensitive to acetone are more likely to be in the rumen epithelium, portal system, or liver, where they can respond to acetone levels. ***(Asian-Aust. J. Anim. Sci. 2003. Vol 16, No. 2 : 198-203)***

Key Words : Acetone, Ipa, Rumination, Intraruminal and Intravenous Infusion, Goats

INTRODUCTION

The regulation mechanisms via chemoreceptors of ruminating and masticating behavior in ruminants have been investigated by intraruminal (Ulyatt, 1965; Simkins et al., 1967) and intravenous (Oshiro et al., 1998; Oshiro et al., 2000) infusions of volatile fatty acids (VFA). Data from these studies suggest that chemical stimulation of metabolites may regulate the ruminating and masticating behavior via the nervous system in ruminants.

Most metabolite-infusion experiments have focused on the effects of acetic and propionic acid infusion, with the results indicating that chemoreceptors for acetic and propionic acids are in the rumen wall and in the vessel walls of ruminal veins, respectively (Baile and Mayer, 1968; Baile, 1971). In contrast, butyric acid, the third major quantitative VFA in the rumen and blood, has long been considered less effective than acetic and propionic acid in regulating the mechanism of the masticating behavior of ruminants (McDonald et al., 1988). Because most ruminally absorbed butyric acid is metabolized to ketone bodies by ketogenic enzymes of the mitochondria in rumen epithelium (Heitmann et al., 1987), and little appears in blood circulation (Forbes, 1986). However, the feed intake of cattle (Simkins et al., 1967) and sheep (Ulyatt, 1965) decreases with intraruminal butyric acid, with increases in acetone concentrations having been observed at the same time. Thye et al. (1970) have reported that feed intake is negatively correlated with increases in acetone concentrations after feeding in ewes. Oshiro et al. (2000) have reported that the masticating behavior of goats decreases and ruminating behavior increases with intravenous acetone infusion. These results suggest that acetone plays a physiological role in regulating factors for ruminating and masticating behavior such as acetic and propionic acids. However, there have been no investigations to clarify the effects of intraruminal acetone infusion on the ruminating and masticating behavior of ruminants.

Therefore, the present experiment was carried out to clarify the physiological significance of acetone in controlling ruminating and masticating behavior by infusing acetone into the rumen and jugular vein in goats.

MATERIALS AND METHODS

Animals and feeding

Three crossbred mature female goats (Saanen×Native Okinawan, 21.7±1.5 kg BW) were fed alfalfa hay cubes and water at 09:00 every day. Each goat was surgically prepared with a cannula in the jugular vein and a rumen fistula and was housed in a climatically controlled experimental room at 20.3±0.6°C and a relative humidity of 65.4±0.6% in an individual metabolism cage. The test room (10.4 m^2) was continuously provided with light using fluorescent electric bulbs (4 bulbs×40 W, 53.8±7.5 Lux) during the trial. The goats were used that had become accustomed for 4 weeks to the experimental environment and handling. Before the experiment, each goat was attached to chewing sensors to measure jaw movement (1 week before), and a polyethylene

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catheter (o.d. 0.7 mm, 63 mm length, TOP Co., Tokyo, Japan) for blood sampling and infusion was inserted into a jugular vein towards the heart (12 h before).

Experimental design

We carried out four different experiments (infusion of intraruminal Ringer's solution, intraruminal acetone, intravenous Ringer's solution, and intravenous acetone). Each experiments was conducted from 09:00 to 21:00. In the intravenous acetone-infusion experiment, acetone containing Ringer's solution was infused at a concentration of 0.0018 mmol0.01 $ml^{-1} kg^{-1} min^{-1}$ for 12 h. In the intraruminal acetone-infusion experiment, the same volume of acetone as that used for the intravenous acetone infusion was single-infused at 09:00. The pH of intraruminal and intravenous infusion solutions were adjusted to 6.8 and 7.0 with 0.1 N NaOH, respectively.

Sampling and analysis

Ruminal fluid (7 mL) was sampled at 0, 1, 3, 6, 9 and 12 h after the start of the metabolite infusion to measure ruminal fluid pH and concentrations of VFA, acetone, and IPA. Blood samples (8 mL) were placed in centrifuge tubes at 0, 1, 3, 6, 9 and 12 h after the start of the metabolite infusion to measure the concentrations of glucose, non-esterealized fatty acids (NEFA), VFA, acetone and IPA. Next, ruminal fluids and blood samples are subjected to a similar procedure and analyzed as described previously (Asato et al., 2002).

The jaw movements of the goats were measured continuously for 12 h from 09:00 to 21:00 using the chewing sensors (TR-756T, NIHON KOHDEN, Tokyo, Japan) with a power supply (SR-601S, NIHON KOHDEN, Tokyo, Japan), and were recorded by polygraph (RJG-4124, NIHON KOHDEN, Tokyo, Japan).

The results are reported as ruminations (number of boli; boli/12 h, ruminating time; min/12 h and bolus time; sec/bolus), mastications (masticating time; min/12 h), and resting time (min/12 h).

Statistical analysis was carried out by the one-way ANOVA procedure of SAS (1985), followed by the Fisher's least squares means method (Snedecor and Cochran, 1989).

RESULTS

The effects of intraruminal and intravenous acetone infusions on the cumulative feed intake at 1, 3, 6, 9 and 12 h after the start of metabolite infusion is given in Figure 1. The cumulative feed intake at 1, 3 and 6 h in the intraruminal acetone infusion was significantly lowered than with the control infusion. However, the total feed intake (g DM/12 h) did not differ significantly between the control and acetone infusions. In contrast, the cumulative

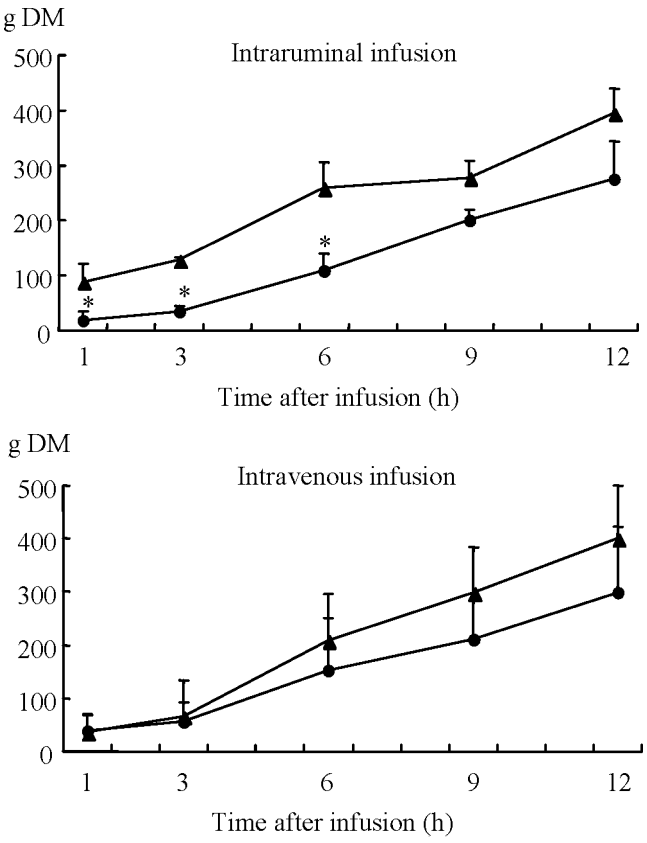

Figure 1. Effects of intraruminal and intravenous acetone infusion on the cumulative feed intake. ●: Acetone infusion, ▲: Control infusion [Ringer's solution infusion, Asterisks indicate the significant difference between acetone infusion (●) and control infusion (▲) at the corresponding sampling time ($p<0.05$)].

feed intake at all time periods in the intravenous acetone infusion did not differ significantly from that with the control infusion.

The results for the ruminating behavior, masticating behavior, and bolus time in the four experiments are given in Table 1. The number of ruminations, ruminating time and masticating time significantly decreased with intraruminal acetone infusion. However, ruminating behavior and masticating time were not affected by the intravenous acetone infusion. The bolus time tended to decrease with intraruminal acetone infusion. In addition, the resting time increased in inverse proportion to the decreases in ruminating behavior with intraruminal acetone infusion.

The ruminal fluid pH, plasma glucose, plasma NEFA and ruminal fluid VFAs (acetic acid+propionic acid+ butyric acid) concentration in the four experiments are given in Figure 2. These variables did not change significantly in the four experiments.

The concentrations of acetone and IPA in the plasma and ruminal fluid in the four experiments are given in Figure 3. Concentrations of plasma acetone immediately

Table 1. Effects of intraruminal and intravenous acetone infusions on the ruminating and masticating behavior

Infusion regions	Intraruminal		Intravenous	
Treatments	Control infusion	Acetone infusion	Control infusion	Acetone infusion
Number of ruminations (boli/12 h)	383±170[a]	205±85[b]	277±108[ab]	227±71[ab]
Ruminating time (min/12 h)	371±104[a]	193±80[b]	287±82[ab]	232±56[ab]
Masticating time (min/12 h)	121±13[a]	60±17[b]	110±13[ab]	104±51[ab]
Resting time (min/12 h)	228±100[a]	467±91[b]	314±82[ab]	385±28[ab]
Bolus time (sec/bolus)	63.3±19.0	53.6±9.3	63.7±13.4	60.8±9.3

[a,b,c] Means within the same row with different superscripts are significantly different ($p<0.05$).
Control infusion : Ringer's solution infusion.

increased ($p<0.05$) after intraruminal and intravenous acetone infusion, and remained at high levels until the end of the experimental period. With the intraruminal acetone-infusion experiment, ruminal fluid acetone concentrations immediately increased ($p<0.05$) after infusion, and then gradually decreased from 1-12 h. However, the acetone concentrations after infusion were significantly greater ($p<0.05$) at all time periods in comparison with the control values. With intravenous acetone infusion, ruminal fluid acetone concentrations were significantly greater ($p<0.05$) from 6-12 h than the control values. The plasma IPA concentrations did not change significantly during the 12 h period in the four experiments. With intraruminal acetone infusions, ruminal fluid IPA concentrations increased significantly ($p<0.05$), reaching a peak concentration at 6 h after infusion. The ruminal fluid IPA concentrations were significantly greater ($p<0.05$) from 1-12 h than the control values. With intravenous acetone infusion, ruminal fluid IPA concentrations were significantly greater ($p<0.05$) from 3-12 h than the control values.

DISCUSSION

It has been considered that butyric acid is less effective than acetic and propionic acids in regulating the masticating behavior of ruminants (McDonald et al., 1988). However, the results of this study clearly show that intraruminal acetone infusion significantly depresses feed intake and ruminating behavior.

It is a well-established that most absorbed butyric acid from the rumen wall is converted to the ketone bodies when it passes the rumen wall. Simkins et al. (1967) and Ulyatt (1965) have reported that plasma ketone concentrations (acetone basis) increase to 0.71-1.02 mM/L and 0.98-1.79 mM/L by butyric acid infusion, respectively. These studies also indicated that decreasing the feed intake during butyric acid infusion is associated with increased plasma acetone concentrations. However, in the present study, the cumulative feed intake was not significantly depressed, even though plasma acetone concentrations increased to 0.95 mM/L (average from 1-12 h after infusion) in response to intravenous acetone infusion. In contrast, in the intraruminal acetone-infusion experiment, the cumulative feed intake was significantly lowered at 1, 3, and 6 h after infusion, and plasma acetone concentrations increased to 1.05 mM/L (average from 1-6 h after infusion) at that time. This different response of the feed intake between intraruminal and intravenous acetone infusions may indicate that increased plasma acetone concentrations are not a primary factor in the feed-intake depression in this experiment.

There was an appreciable difference in the changes in ruminal fluid acetone concentrations between the intraruminal and intravenous acetone-infusion experiments. With the intravenous acetone infusion, ruminal fluid acetone concentrations increased linearly after 1 h of infusion, reaching a maximum 12 h later. In contrast, with the intraruminal acetone infusion, ruminal fluid acetone concentrations immediately increased after infusion, and then decreased gradually from 1-12 h, clearly indicating that cumulative feed intake is significantly depressed 1-6 h after intraruminal acetone infusion when ruminal fluid acetone concentrations are high (Figure 3). As such, we concluded that increased ruminal acetone concentrations were responsible for the depression in feed intake in this experiment.

This study further shows that the response of the ruminating behavior to acetone infusion is the same as that of feed-intake behavior. In intraruminal acetone infusion, the number of ruminations and the ruminating time significantly decreased compared with the control infusion. Oshiro et al. (2000) have reported that ruminating behavior significantly decreases after infusions of intravenous acetic and butyric acids, respectively, and observed that plasma acetone concentrations increased to 0.76 mM/L and 1.16 mM/L, respectively. These data seems to indicate that increased plasma acetone concentrations are responsible for the ruminating behavior depression. However, this previous experiment did not consider the ruminal fluid acetone levels, making it difficult to determine whether increased plasma acetone concentrations are the only factor contributing to decreases in the ruminating behavior.

It appears that in this previous experiment the ruminal acetone concentrations were increased, as in our intravenous acetone-infusion experiment it was confirmed that ruminal acetone concentrations increase as the plasma

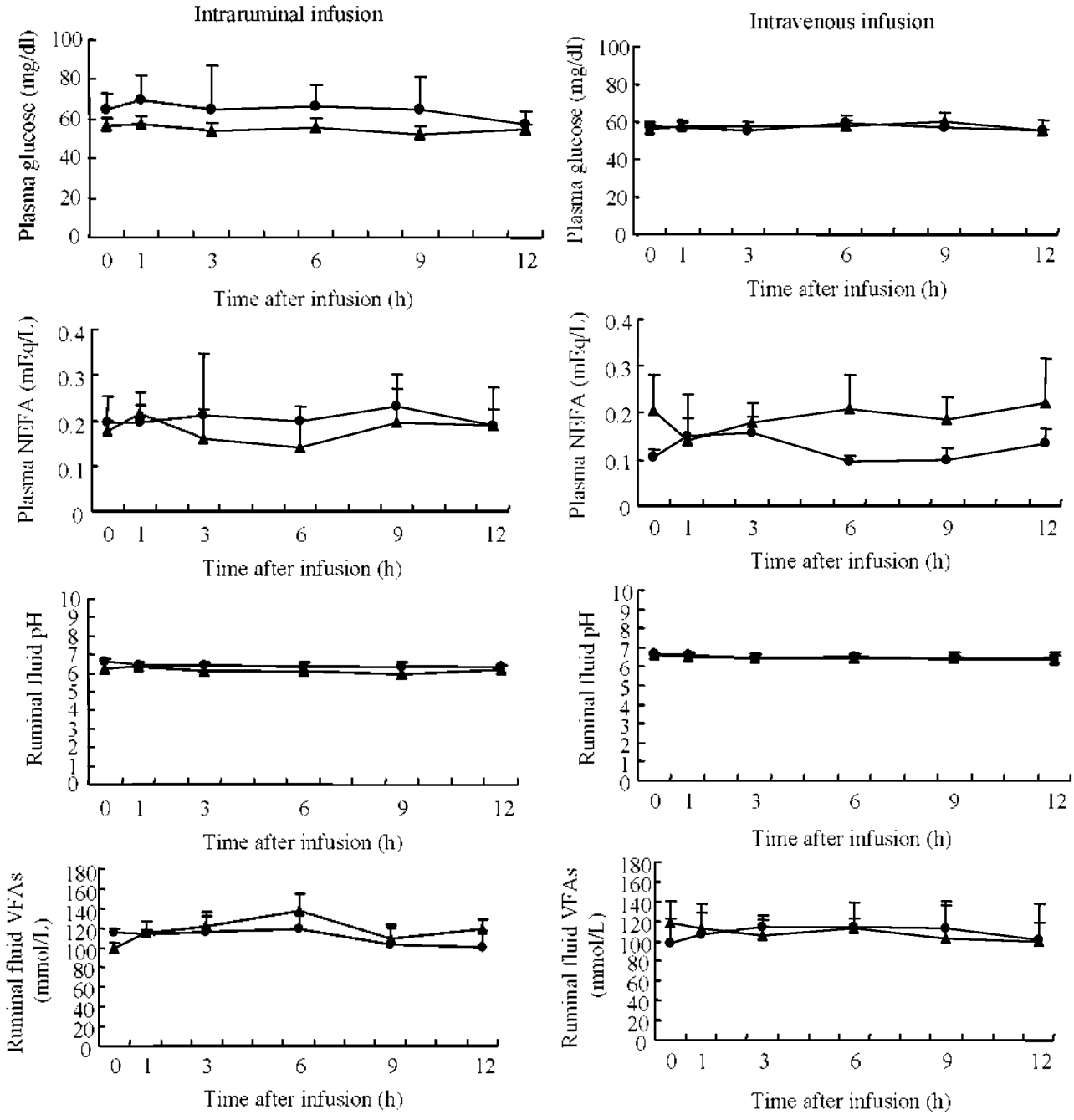

Figure 2. Changes of plasma glucose and NEFA, and ruminal fluid pH and VFAs in intraruminal and intravenous infusion experiment. ●: Acetone infusion, ▲: Control infusion (Ringer' solution infusion)

acetone concentrations increase (Figure 3). In any case, our experiment clearly shows that intraruminal acetone infusion is more effective in controlling ruminating behavior depression than intravenous acetone infusion. Forbes and Barrio (1992) have reported that the afferent signals from chemoreceptors in the rumen epithelium are important in controlling the ruminating and masticating behavior of ruminants.

Simkins et al. (1967) have reported that plasma glucose levels decrease after butyric acid infusion, and concluded that increased ketone by butyric infusion caused increased insulin secretions, which would then be responsible for glucose-level depressions in heifers. However, in the present experiment there was no significant change in plasma glucose concentrations with either intraruminal or intravenous acetone infusion. Probably, differences in feeding ingredients, the infusion volume, the animal species, or some other factor is the cause of these different results.

Plasma NFFA concentrations tend to decrease in intravenous acetone infusion. Some experiments have also

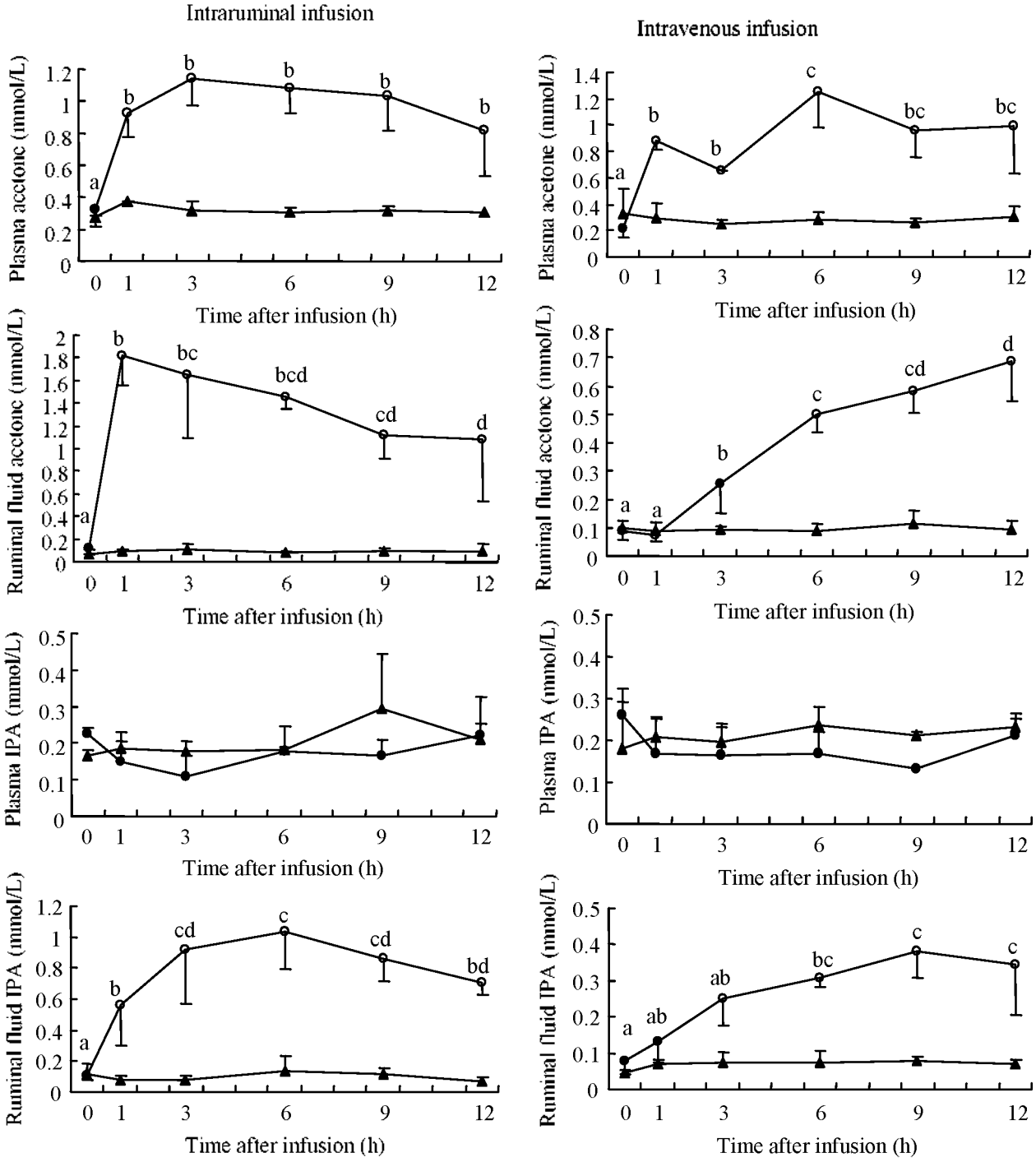

Figure 3. Changes of plasma and ruminal fluid acetone and IPA in intraruminal and intravenous infusion experiments. ●: Acetone infusion, ▲: Control infusion (Ringer's solution infusion), Open circles (○) indicate significantly difference between acetone infusion (●) and control infusion (▲) at the corresponding time ($p<0.05$).

[a,b,c,d] Means with different superscripts indicate significantly different between sampling time in acetone infusion (●,○) ($p<0.05$).

shown that plasma NEFA levels change after infusion of VFAs and ketones infusion. Menahan et al. (1966) have shown that plasma NEFA concentrations significantly decrease after ketone infusion. Thye et al. (1969) have concluded that the decreased plasma NEFA levels are due to the increased plasma ketone levels occurring after feeding. However, Oshiro et al. (2000) have reported that plasma NEFA concentrations increase after infusion of VFAs and acetone. The effects of increases in VFAs and ketone on NEFA concentrations are not yet clear, making further

investigation of the effects of metabolites on NEFA necessary.

The concentrations of ruminal fluid IPA increased after both acetone infusion experiments. In intraruminal acetone infusion, the ruminal fluid IPA levels immediately increased after infusion, indicating that intraruminally infused acetone is quickly converted to IPA. Bruss and Lopez (2000) have observed that IPA can be produced by reducing acetone by rumen bacterial action. Bruss and Lopez (2000) have also hypothesized that acetone in blood circulation can enter the rumen through the rumen wall or in the saliva. In intravenous acetone infusion, the increase in ruminal fluid IPA after infusion clearly supports the above hypothesis. In ruminants, it seems likely that there is a circulation system of acetone and IPA. However, the physiological role of this system is unknown.

CONCLUSION

The different responses to acetone infusion indicate that the chemoreceptor for acetone may be existing in peripheral organs such as the rumen epithelium, portal system, or liver. In addition, it seems likely that afferent signals from the chemoreceptor for acetone in peripheral organs are important in controlling the ruminating and masticating behavior of goats.

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