# EFFECTS OF HEAT EXPOSURE ON WATER METABOLISM AND PASSAGE IN SHEEP

K. Katoh<sup>1</sup>, C. Buranakarl<sup>2</sup>, N. Matsunaga, S.R. Lee, T. Sugawara and Y. Sasaki

Department of Animal Physiology, Faculty of Agriculture, Tohoku University, Tsutsumidori Amamiyamachi, Sendai 980, Japan

## Summary

The present experiment was carried out to investigate the effects of heat exposure on water metabolism and the passage of indigestible particles in sheep. Water intake, respiratory rate, rectal temperature and pH of ruminal fluid and urine were significantly higher ( $P \le 0.05$ ) in the hot environment (32°C) than in the control environment (20°C). Urine osmolality and blood volume were increased, while glomerular filtration rate was decreased, in the hot environment. The liquid flow rate from reticulo-rumen and the excretion of indigestible particles of specific gravity 0.99 (but not 1.27 or 1.38) were increased in the hot environment. From these findings, it is suggested that an increased water intake evoked by heat exposure might affect the flow rate of digesta in sheep.

(Key Words: Water Metabolism, Passage, Sheep, Indigestible Particles, Heat Exposure)

### Introduction

It is well known that changes in the thermal environment induce a number of specific physiological responses in domestic ruminants: changes in food intake, water balance, respiratory and circulatory system and digestive system (Hafez, 1968; Ames, 1979; Christopherson, 1985). The thermal stress would be related to a reduction in animal production, a reduced milk production under hot stress being a typical example.

Hot environment has been known to reduce food intake and urine excretion but increase water intake, respiration rate and rectal temperature. However, the effects of hot environment on the digestive system seem to be more confused. Although reticulum contraction frequency in steers and sheep was markedly reduced after exposure to hot temperature compared with that to cold temperature (Baile and Forbes, 1974; Westra and Christopherson, 1976), it is not established whether the rate of passage or the digestibility of digesta is affected (see Christo-

pherson, 1985).

In the present experiment, we intended to learn the changes in the passage of indigestible particles with different specific gravities in relation to water metabolism in sheep drinking much water when exposed to hot environment.

### Materials and Methods

### Animals and management

Three crossbred castrated male sheep, fitted with a rumen cannula and weighing 42.8 to 46.4 kg, were used. Alfalfa hay cubes (2% of body weight) and water were given once daily from 10:00 h to 12:00 h. They were kept in metabolism cages at an air temperature of  $20 \pm 1$  °C for two weeks before the beginning of the experiment.

## Experimental procedure

Two series of experiments were carried out. Experiment 1 was carried out at an air temperature of  $20 \pm 1$  °C with a relative humidity of 50 %. Experiment 2 was begun 3 days after the environmental temperature was elevated to  $32 \pm 1$  °C with a relative humidity of 50 %. Each period of the experiment was 9 days.

On the second day of each experiment (day 0), Evans blue (0.5 %, 5 ml; Tokyo Kasei, Japan) was injected into the jugular vein at 09:00 h, and blood sampling was followed from another jugular vein at 10, 20 and 45 min after the dye injection. Three kinds of indigestible particles with different

<sup>&</sup>lt;sup>1</sup>Address reprint requests to Dr. K. Katoh, Department of Animal Physiology, Faculty of Agriculture, Tohoku University, Tsutsumidori Amamiyamachi, Sendal 980, Japan.

<sup>&</sup>lt;sup>2</sup>Present address: Department of Physiology, Faculty of Veterisary Science, Chulalongkorn University, Bangkok, Thailand.

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specific gravities (0.99, 1.27 and 1.38; 300 of each particle) and polyethylene glycol (PEG, 5g; MW = 4,000, Wako Pure Chem., Japan) were injected into the rumen through a rumen cannula as a suspension in tap water (100 ml) at 11:00 h. The indigestible particles were all of the same rod-shape and size (2 mm diameter and 4 mm length) but differed in color. The specific gravity (SG) of the particles was varied by adjusting the ratio of polyethylene, polycarbonate and titanium. The first sampling of rumen liquid was done at 12:00 h and was followed by sampling every 2 hours to 22:00 h and at 09:00 h on the next day.

Rectal temperature, heart rate and respiratory rate were measured twice at 09:00 h and 14:00 h and the venous blood sampling was carried out by a puncture of the jugular vein, on days -1, 0, 1, 4 and 7. Twenty four-hour samples of faeces and urine excreted were collected from 11:00 h to 11:00 h on the next day during both experimental periods.

### Analytical methods and calculations

The collection of particles and the determination of PEG concentration in faeces were as follows. Each 24-hour faeces sample was mixed with I litter of distilled water and placed in a nylon mesh bag (mesh size: 1,6 mm x 1,6 mm). After the filtrate was centrifuged at 12,000 rpm for 10 min, the supernatant was used for the determination of PEG concentration, while the precipitate was used for organic matter content. The residue of the filtrate in a bag was soaked in tap water for several hours. Then the particles were picked out one by one from the bag using tweezers, their SG being indicated by their color. The indigestible particles were kindly donated by Showa Denko Co., Japan. The concentration of PEG in the rumen juice and faeces was determined by the method of Smith (1959).

The VFA concentration in the rumen juice was determined by gas chromatography (Model G80, Yanako, Japan). The concentration of creatinine in the urinc and plasma was determined by a commercial kit (Creatinine kit-Wako, Wako Pure Chem., Japan). The osmolality of urine, rumen juice and plasma was determined by an osmometer (Model 3L, Advanced Inst., U.S.A.). The organic matter content of faeces was determined as follows: a precipitate of faecal filtrate collected on days -1, 1, 4 and 7 was dried at 90 °C to

constant weight and ashed at 600 °C for 6 hrs. The organic matter content (%) was calculated from the weight difference between DM and ash.

The rumen liquid volume and plasma volume were calculated from the extrapolation method of the PEG and Evans blue concentrations. LFR = 0.693 x RLV/LRT, where LFR is the liquid flow rate, RLV is the rumen liquid volume and LRT is the time for the equivalent of half the liquid in the rumen to be transferred to the omasum, while MRT (mean retention time) was calculated from 1/K (Chaiyabutr et al., 1987), respectively. GRF = Cu x Vu/Cp, where the GFR is the glomerular filtration rate, Cu is the urine creatinine concentration. Vu is the urine volume and Cp is the plasma creatinine concentration.  $BV = 100 \times Vp/$ (100-PCV) where BV is the blood volume, Vp is the plasma volume and PCV is the packed cell volume (%).

### Statistical analysis

The results are represented as the mean  $\pm$  S.E. (n = 3). The mean value for each animal was calculated from all the observations through each experiment. The difference between the control and the hot environment was analyzed with paired t-test (Zar, 1984). The difference was considered to be significant when P value was less than 0.05.

### Results

The mean body weight of the sheep (44.5 kg) was not changed when measured before and after the experiment. The animals are all the forage given in both environments.

## Water intake and faeces excretion (table 1)

The mean daily water intake in the hot (32 °C) environment was significantly (P < 0.05) larger than that in the control (20 °C) environment. The total water intake for 9 days in the hot environment was  $32.3 \pm 2.6$  1, which was significantly (P < 0.01) larger than that in the control environment (22.6  $\pm$  3.6 1). There was no significant (0.05 < P < 0.10) difference in the mean daily faeces excretion.

## Respiratory rate, rectal temperature (table 2) and heart rate

The mean respiratory rate in the hot environ-

TABLE 1. EFFECTS OF HEAT EXPOSURE ON WATER INTAKE AND FAECES EXCRE-TION IN SHEFP

	Water intake (ml/day)	Faeces excretion (g/day)
20 °C	2515 ± 402	579 + 21
32 °C	3588 ± 287	653 ± 59
	÷	NS

mean  $\pm$  S.E.; Paired t-test (n = 3); \*: P < 0.05;

NS: Not significant

ment was significantly increased compared to that in the control environment at 09:00 h (P < 0.05) and 14:00 h (P < 0.01). The mean rectal temperature in the 09:00 h (which was measured before feeding) was significantly (P < 0.05) higher in the hot environment than that in the control environment. The mean heart rates in the control and the hot environment were  $65 \pm 2$  and  $66 \pm 3$  beats/min, respectively.

TABLE 2. EFFECTS OF HEAT EXPOSURE ON RESPIRATORY RATE AND RECTAL TEMPERATURE IN SHEEP

	Respiratory rate (breaths/min)		Rectal temperatu.	
	09:00 h	14:00 h	09:00 h	14:00 h
20 °C	41 ± 6	45 ± 7	$38.7 \pm 0.2$	39.0 ± 0.1
32 °C	90 ± 8	111 ± 2	$39.1\pm0.1$	$39.3 \pm 0.1$
	*	**	*	NS

mean  $\pm$  S.E.; Paired  $\ell$ -test (n = 3); \*: P < 0.05; \*\*: P < 0.01; NS: Not significant

## Hematological results (table 3)

The mean blood volume in the hot environment was significantly (P < 0.05) increased compared to that in the control environment. The mean plasma volume was slightly (0.05 < P < 0.10) larger in the hot environment. There was no significant difference in the packed cell volume and plasma osmolality.

TABLE 3. REFECTS OF HEAT EXPOSURE ON PACKED CELL VOLUME, PLASMA OSMOLALITY, PLASMA VOLUME AND BLOOD VOLUME IN SHEEP

	Packed cell vol. (%)	Plasma osmo. (m $\Omega$ sm/kg $H_2\Omega$ )	Piasma vol. (ml)	Blood val. (ml)
20 °C	29.4 ± 2.7	291.0 ± 2.2	1701 ± 22	2433 ± 131
32 °C	29.9 ± 3.2	$298.3 \pm 2.6$	2396 ± 157	$3390 \pm 68$
	NS	NS	NS	*

mean  $\pm$  S.E.; Paired t-test (n = 3); \*: P < 0.05; NS: Not significant

### Function of kidney (table 4)

The mean GFR in the hot environment was significantly (P < 0.05) reduced compared to that in the control environment. On the other hand, the mean urine pH and osmolality in the hot environment were significantly (P < 0.05) increased compared to those in the control environment. Urine volume was not reduced in the hot environment.

### Rumen liquid parameters (table 5)

The mean rumen liquid pH and flow rate from the reticulo-rumen in the hot environment were significantly ( $P \le 0.05$  and 0.001, respectively)

larger than those in the control environment. The mean rumen liquid osmolality was 269.0  $\pm$  4.1 and 275.9  $\pm$  6.7 mOsm/kg  $\rm H_2O$  in the control and the hot environment, respectively. The mean MRT was reduced, but not significantly (0.05  $\leq$  P  $\leq$  0.10), in the hot environment. The mean values of  $\rm T_{1/2}$  (the time at which the PEG concentration was reduced to half) were 42.1  $\pm$  9.1 and 17.4  $\pm$  0.6 hrs in the control and the hot environments, respectively.

# Ruminal fermentation before and after feeding (table 6)

The mean total VFA concentration and A/P

TABLE 4. EFFECTS OF HEAT EXPOSURE ON URINE VOLUME, URINE PH, URINE OSMOLALITY AND GER IN SHEEP

	Urine volume (ml/day)	Urine pH	Urine Osmo. (mOsm/kg H <sub>2</sub> O)	GFR (µ/kg/min)
20 °C	1202 ± 387	7.68 ± 0.06	1139.9 ± 271.4	962.2 ± 20.9
	841 ± 187	$8.15 \pm 0.02$	$1759.5 \pm 198.7$	445.1 ± 73.3
	NS	*	*	*

mean  $\pm$  S.E.; Paired t test (n = 3); \*: P < 0.05; NS: Not significant

TABLE 5. FFFECTS OF HEAT EXPOSURE ON RUMEN LIQUID PH, RUMEN VOLUME, FLOW RATE AND MRT IN SHEEP

	Rumen liquid pH	Rumen vol.	Flow rate (ml/hr)	MRT (hr)
20 °C	7.68 ± 0.06	7.7 ± 0.3	141 ± 34	60.8 + 13.1
32 °C	8.15 ± 0.02	$8.2 \pm 0.7$	$329\pm38$	25.1 ± 0.9
	*	NS	***	NS

mean  $\pm S.E.$ ; Paired t-test (n = 3); \*: P < 0.05; \*\*\*: P < 0.001; NS: Not significant

TABLE 6. EFFECTS OF HEAT EXPOSURE ON TOTAL VFA CONCENTRATION AND A/P RATIO BEFORE AND AFTER FEEDING IN SHEEP

	Before feeding		After feeding	
	Total VFA conc. (mM)	A/P ratio	Total VFA cone. (mM)	A/P ratio
20 °C	60.0 ± 5.9	$4.3 \pm 0.4$	138.4 ± 15.0	3.9 ± 0.2
32 °C	68.1 ± 6.6	$3.7 \pm 0.2$	76.0 + 10.6	3.5 + 0.1
	NS	NS	NS	半坤

mean  $\pm$  S.E.; Paired t-test (n = 3); \*\*: P < 0.01; NS: Not significant

ratio in the rumen liquid before feeding were not changed in the hot environment. On the other hand, the mean A/P ratio in the rumen liquid after feeding was significantly (P < 0.01) decreased in the hot environment. The mean total VFA concentration after feeding was slightly (0.05 < P < 0.10) decreased in the hot environment.

## Recovery of PEG and indigestible particles (fig. 1)

The PEG recovery in the hot environment was similar to that in the control environment. The recovery of the indigestible particles with SG 0.99 in the hot environment was significantly larger than that in the control environment except on days 1 and 4. There was, however, no significant difference in the recoveries of the other two different kinds of particles (SG 1.27 and 1.38).

### Organic matter content in faeces

The mean values of organic matter content in faeces in the control and the not environment were  $84.8 \pm 2.7$  and  $78.9 \pm 3.4\%$  (based on DM) (0.10 < P < 0.20), respectively.

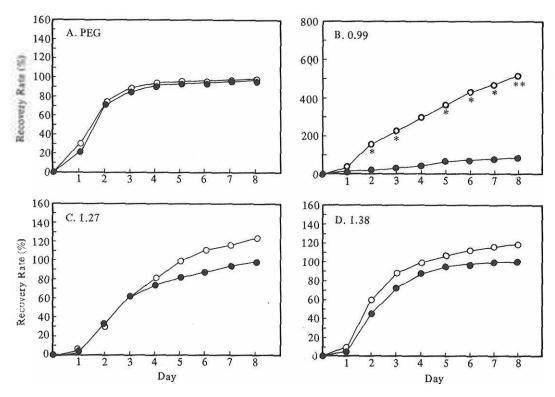


Figure 1. Effects of heat exposure on the cumulative recovery rates of PEG and indigestible particles (SG: 0.99, 1.27 and 1.38). Recovery rates are represented in %, as the total weight of PEG and the total number of indigestible particles recovered in the control environment are 100 %. Paired r-test (n = 3); \*: P < 0.05; \*\*\*: P < 0.01; Control experiment: •; hot environment: •

## Discussion

It is well known that physiological parameters such as water intake, respiratory rate and rectal temperature change when animals are exposed to hot environment (Hofman and Riegle, 1977; Oshiro et al., 1978). In our preliminary experiment, jugular blood pH was increased from 7.45 to 7.55 on day 3 after the environmental temperature was increased from 20 to 30°C, which was accompanied by a decrease in the partial pressure of CO<sub>2</sub> from 37 to 30 mmHg in jugular blood. This respiratory alkalosis was sustained at least for 2 weeks. The reason for an increased pH in the ruminal fluid and urine shown in the present experiment, therefore, would be due to alkalization of blood evoked by respiratory alkalosis in the hot environment.

Water intake might be enhanced because of an

increased evaporation by panting and salivation in the hot environment. An enhanced water intake and fluid passage rate from reticulo-rumen would then contribute to an increase in water absorption from the lower GI tract into the intravascular compartment resulting in blood volume expansion. It is unlikely that an increase in blood volume might be attributed solely to an increase in plasma volume, since the blood volume was significantly increased while the packed cell volume was not changed. Similar findings were reported in swamp buffaloes and were suggested to be due to stimulation of the sympatho-adrenal system (Chaiyabutr et al., 1987).

An increase in evaporation, furthermore, would force animals to reduce the water loss such as urine, since GFR was decreased and urine osmolality was increased in the hot environment. The decrease in GFR during the summer was reported

in sheep (Nawaz and Shah, 1984). The reduction in GFR might be due to a reduced renal blood flow evoked by an increase in plasma catecholamine concentration. This assumption might be supported by the finding that concentrations of epinephrine and norepinephrine in urine and plasma were increased during the summer in sheep (Oshiro et al., 1976) and during heat exposure in ungulate (Yousef, 1979).

Heat exposure moderately changed the ruminal fermentation in the present study. An increase in water intake and flow of saliva with higher pH induced by respiratory alkalosis might result in a dilution of VFAs and an increase in liquid pH. Earlier reports have demonstrated a decrease in total VFA concentration in hot environment (Weldy et al., 1964; Moody et al., 1967; Kelly et al., 1968). Furthermore, an elevation of ruminal fluid pH during heat exposure agrees with the finding by Mishra et al. (1969). Sano et al. (1979) reported that gluconeogenesis in sheep was decreased after 10 days of heat exposure. It is plausible that a change in ruminal fermentation might affect glucose metabolism.

In our previous paper using sheep and goats (Katoh et al., 1988), the number of indigestible particles recovered from faeces after injection into the reticulo-rumen was increased as the SG of the particles increased, and the recovery rate of particles with a SG smaller than 1.21 was only 7% at an environmental temperature of 22 °C. Most of the SG 0.99 particles were recovered from the reticulo-rumen 10 days after injection into the reticulo-rumen.

In the present study, the recovery of the SG 0.99 particles in the hot environment was 5.4 times larger than that in the control environment. This finding suggests that feed particles with a SG close to that of water could easily pass from the reticulo-rumen of sheep exposed to heat stress. This might be related to an increase in water intake and liquid flow rate from the reticulorumen to the lower GI tract. The reason why the change in liquid passage rate (PEG excretion) through the whole GI tract was smaller than that in the SG 0.99 particles would be as follows. The passage of water from the reticulo-rumen was increased but a large part of the water was trapped in the lower GI tract, while the SG 0.99 indigestible particles flowing out of the reticulo-rumen were not obstructed in the lower GI tract.

Although the separation mechanism in the gut remains unclear, it would be dependent on the motility of the GI tract (Deswysen, 1987; Kaske, 1987).

Previous reports showed that the MRT of solids in the digestive tract increased during heat exposure (Attenbery and Johnson, 1969; Warren et al., 1974). However, it was not confirmed in the present experiment, since any excretion of the indigestible particles was not decreased during heat exposure. It is unlikely that a partial increase in the particle passage as shown in the present study was sufficient to affect organic matter digestibility.

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