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ORIGINAL ARTICLE

Effect of heat stress on endotoxin flux across mesenteric-drained and portal-drained viscera of dairy goat

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Summary

This study was designed to evaluate the effect of heat stress on endotoxin flux across mesenteric-drained and portal-drained viscera of dairy goats. Three Saanen first lactation dairy goats were surgically fitted with indwelling catheters in the portal vein, the mesenteric vein and carotid, and were kept in thermal-neutral and then heat stress environment, for examining the effect of heat stress on endotoxin absorption and redox status. Average net absorption of endotoxin (EU/h) across mesenteric-drained viscera (MDV) and portal-drained viscera (PDV) during the whole period of heat stress increased by 279.05% and 227.92% in relation to thermo-neutral period. Plasma concentration of glutathione peroxidase (GSH-Px) and catalase (CAT) in mesenteric and portal vein, and that of superoxide dismutase (SOD) in mesenteric vein, increased significantly during heat stress. Main conclusions were: (i) net absorption of endotoxin in portal vein is mainly from non-mesenteric tissues both in heat stress and in thermo-neutral condition; (ii) heat stress may lead to the significant decrease in plasma SOD, GSH-Px, CAT flux across PDV and MDV, and the significant increase in endotoxin flux across PDV and MDV; and (iii) the increase in gastrointestinal permeability in dairy goats during heat stress may not be induced by the increase in oxidative stress.

Introduction

Lactating ruminants are more susceptible to heat stress than non-lactating ones, because of the bulk feed intake and large quantity of metabolic heat, coupled with the common weakness of ruminants which is the low capacity of dissipating the heat via skin (Blaxter, 1962). Heat stress results in the significant decrease in dry matter intake (Holter et al., 1997), milk yield (West, 2003), milk quality (Beede and Shearer, 1996) and reproductive performance (Monty and Wolff, 1974). Recent studies showed that the main mechanism of damage caused by heat stress is the epithelial damage of intestinal tract, which leads to large absorption of endotoxin from the intestinal tract. Large amount of endotoxin enters the bloodstream, causing a series of damages such as allergic, organ injury, disseminated intravascular coagulation, or even death (Gathiram et al., 1987, 1988a,b). Endotoxin is the main cause of body damage during heat stress, and therefore the elimination of endotoxin may increase the capacity of heat tolerance of animals (Gathiram et al., 1987, 1988a,b). For lactating ruminants, endotoxin entering systemic circulation during heat stress could be both from intestinal tract and from rumen. However, the percentage of endotoxin from each of these sources remains unknown.

Flanagan et al. (1998) reported that heat increases the flux of cellular free radicals. The relationship

between oxidative status and intestinal injury is quite controversial. Hall et al. (2001) reported that damage to the intestinal tract and the increase in intestinal permeability during heat stress is caused by oxidative stress, while Lambert et al. (2002) reported that the increasing in intestinal permeability during heat stress is directly caused by heat, and consequently leads to the bulk entering of endotoxin which causes ischaemia reperfusion followed by the increase in free radicals. The objective of the present study was to quantify the *in vivo* fluxes of endotoxin across the mesenteric and stomach portions of the portal-drained viscera (PDV) of lactating goats, and to clarify the relationship between oxidative status and intestinal injury.

Materials and methods

Animals and surgery

Three lactating Saanen dairy goats (weighed 30 kg) were surgically fitted with indwelling catheters in the portal vein, the mesenteric vein and carotid. Portal vein catheters were silicone tubes, and the two mesenteric catheters were made of tygon. Two mesenteric venous catheters were inserted into branches between the major venous arch and the small intestine, one for sampling and the other for para-aminohippurate (PAH) infusion. The establishment of catheters (portal vein and mesenteric vein) was described by Huntington et al. (1989). The truncus vagosympathicus which is concomitant to the right carotid was surgically peeled off, and then the carotid was wrapped and sealed in skin by stitching. The carotid catheter was inserted into the dissociative carotid 1 day before sampling.

All experimental procedures involving animals were approved by the Animal Care and Use Committee of Animal Nutrition Institute, Sichuan Agricultural University, and were carried out in accordance with the National Research Council's Guide for the Care and Use of Laboratory Animals.

Feeding and management

The goats were allowed 3 weeks to recover from surgery; during the first 2 weeks they had free access to high quality hay for food intake recovery, and the animals were then fed a basal diet during the whole experimental period. Equal amount basal diet was given at 2 h intervals, the formulation of which is summarized in Table 1. The animals were housed in individual pens in a temperature-controlled house with *ad libitum* access to water and were hand-milked twice daily. Temperature in the house was 24 °C during the recovery period and extended to day 5 of the experimental period. On the sixth day of the experiment (started at 08:00 hours), the temperature was adjusted to 35 °C and kept for 24 h. The relative humidity was kept stable (55%). Feed intake and milk yield were recorded daily.

Table 1 Basal diet composition and level of the tested goats

Ingredient	Formulation (%)
Hay	50.0
Corn	20
Wheat bran	15
Soybean meal	8
Rapeseed meal	4.5
Calcium carbonate	0.8
Dicalcium phosphate	0.1
Salt	0.5
Sodium bicarbonate	1
Premix*	0.1
	Nutrient level
DM (%)	87.7
NE_L (MJ/kg)	5.52
CP (%)	12.6
Ca (%)	0.55
TP (%)	0.32
Concentrate/roughage	51:49

DM, dry matter; NE_L, net energy for lactation; CP, crude protein; TP, total P.
*Premix containing $FeSO_4 \cdot 7H_2O$ 170 g/kg; $CuSO_4 \cdot 5H_2O$ 70 g/kg; $MnSO_4 \cdot 5H_2O$ 290 g/kg; $ZnSO_4 \cdot 7H_2O$ 240 g/kg; $5H_2O$ 70 g/kg; $CoCl_2 \cdot 6H_2O$ 510 mg/kg; KI 220 mg/kg; Na_2SeO_3 130 mg/kg; VA 1.620.000 IU/kg; VD3 324.000 IU/kg; VE 540 IU/kg.
Concentrate/roughage was measured value, and all the others were calculated.

Sampling and analysis

The experiment lasted 6 days. On the third day of the experiment, a primed (15 ml) and, then, a continuous mesenteric venous infusion of PAH (15 mg/ml), formulated according to Huntington (1982), was initiated via the distal mesenteric vein catheter to determine portal and mesenteric venous blood flows. Continuous infusion was performed by using a HL-2B calibrated syringe pump (Lanngue Instrument Company, Shijiazhuang, China) with a speed of 12 mg PAH per min (0.8 ml/min). The infusion commenced at 08.00 hours, 2 h after which a spot sampling procedure was administered. Totally six spot samplings were performed at 2-h intervals. At each sampling spot, simultaneous arterial, portal and mesenteric venous blood samples were collected

(5 ml for each sample) slowly into a cuvette containing heparin (200 IU/ml). The average value of the six samples was deemed as the thermo-neutral value. Samples were centrifuged at 1050 *g* for 15 min at 4 °C. Plasma was frozen at −70 °C for further analysis. On the sixth day of the experiment, the procedure carried out on the third day was repeated with minor differences in the times of spot sampling which were 12 instead of 6, at 2-h intervals. Sample collection and treatment was the same.

The PAH concentration of plasma was determined according to Huntington (1982). Plasma concentration of SOD, GSH-Px, CAT, total antioxidative potential (TAP) and malonaldehyde (MDA) was measured correspondingly by using the methods of xanthine oxidation, 5,5′-Dithiobis (2-nitrobenzoic acid), ammonium molybdate, Ferric reducing/antioxidant power and thiobarbituric acid. Reagent kits were bought from Jiancheng Bioengineering Institute, (Nanjing, China). Plasma endotoxin concentration was determined by kinetic turbidimetric assay, using reagent kit bought from Xiamen tachypleus amebocyte lysate, (Xiamen, China). The endotoxin measured with the kit is a lipopolysaccharide.

Rectum temperature and respiration frequency

Rectum temperature and respiration frequency were measured after the first, third and sixth blood sample collection on the third day of the experiment, and the first, sixth and 12th blood sample collection on the sixth day of experiment.

Rectum temperature was measured by inserting a thermometer into the anus upto a depth of 3 cm, and respiration frequency was calculated by counting the undulant times of flank within a certain time slot measured by using a stopwatch.

Calculation and statistics

Portal and mesenteric blood flows (F_{PV} and F_{MV} respectively) were calculated from the following equations:

$$F_{PV} = I/(PAH_{PV} - PAH_A)$$
$$F_{MV} = I/(PAH_{MV} - PAH_A)$$

where I represents PAH infusion rate (mg/h), and PAH_{PV}, PAH_{MV} and PAH_A represent PAH concentrations (mg/l) in the portal, mesenteric and arterial blood. Plasma flows were calculated as the difference between blood flow and packed cell volume. F_{PV} and F_{MV} represent portal and mesenteric plasma flow (l/h).

Net fluxes of endotoxin across PDV and mesentric-drained viscera (MDV) were calculated from the following equations:

$$Absorb_{PV} = (E_P - E_A) * F_{PV} * 1000$$
$$Absorb_{MV} = (E_M - E_A) * F_{MV} * 1000$$

where E_P, E_M and E_A represent the plasma concentrations (EU/ml) of endotoxin in the portal, mesenteric venous and arterial plasma respectively. F_{PV} and F_{MV} represent portal and mesenteric plasma flow (l/h). $Absorb_{PV}$ and $Absorb_{MV}$ represent the net absorption of endotoxin via PDV and MDV respectively. Stomach flux was calculated as the difference between portal and mesenteric fluxes.

Net fluxes of antioxidants (including TAP, SOD, GSH-Px and CAT) across PDV and MDV were calculated from the following equations:

$$Flux_{PV} = (C_P - C_A) * F_{PV}$$
$$Flux_{MV} = (C_M - C_A) * F_{MV}$$

where C_P, C_A and C_M represent the plasma concentrations (EU/ml) of antioxidants in the portal, arterial and mesenteric veins respectively. F_{PV} and F_{MV} represent portal and mesenteric plasma flow (l/h). $Flux_{PV}$ and $Flux_{MV}$ represent the net flux of antioxidants via PDV and MDV respectively.

Mean concentrations of endotoxin and antioxidants within blood vessels of the six sampling spots and the mean net fluxes across portal, mesenteric and gastrosplenic veins were compared using Duncan's multi-comparison (SPSS 13.0; SPSS Inc., Chicago, IL, USA), with a probability of $p < 0.05$ for significance. Feed intake, milk yield, rectum temperature and respiration frequency between heat stress period (HS) and thermo-neutral period (TN) were compared using the *t*-test. Data were presented as mean ± SD.

Results

Feed intake and milk yield

Feed intake of dairy goats during heat stress significantly decreased ($p < 0.01$). There was no difference ($p > 0.05$) in milk yield between the TN and HS groups (Table 2).

Rectum temperature and respiration frequency

Rectum temperature and respiration frequency of dairy goats during heat stress significantly increased ($p < 0.01$). Respiration frequency

Table 2 Effect of heat stress on DMI and milk yield of dairy goats

Group	DMI (kg/day)	Milk yield (kg/day)
TN group	1.43 ± 0.15^{Aa}	1.13 ± 0.32^{Aa}
HS group	0.84 ± 0.25^{Bb}	0.97 ± 0.29^{Aa}

HS, heat stress period; TN, thermo-neutral period.
Means within a column with an uncommon capitalized superscript are different ($p < 0.01$). Means within a column with an uncommon lowercase superscript are different ($p < 0.05$).

Table 3 Effect of heat stress on rectum temperature and respiration frequency of dairy goats

Group	Rectum temperature (°C)	Respiratory frequency (breaths/min)
TN group	39.8 ± 0.05^{Aa}	28 ± 1.11^{Aa}
HS group	40.8 ± 0.12^{Bb}	138 ± 3.37^{Bb}

HS, heat stress period; TN, thermo-neutral period.
Means within a column with an uncommon capitalized superscript are different ($p < 0.01$). Means within a column with an uncommon lowercase superscript are different ($p < 0.05$).

increased almost four times, from 28/min to 138/min (Table 3).

Rate of plasma flux and endotoxin concentration

With prolonged heat stress, plasma flux rate in the portal and mesenteric veins kept on decreasing. Plasma flux rate in the mesenteric vein in the HS group decreased by 12.91% ($p < 0.05$) and 19.41% ($p < 0.01$) just after 2- and 8-h exposure of heat stress, respectively, in relation to that in the TN group. The maximum decrease (37.86%) happened at 16-h exposure of heat stress. The average rate of plasma flux in the mesenteric vein during the whole period of heat stress in the experiment decreased by 25.13%. The rate of plasma flux in portal vein in HS group began decreasing ($p < 0.01$) after 4-h exposure of heat stress, and reached the lowest point (43.62% lower than in the TN group) at 16-h exposure of heat stress. The average rate of plasma flux in the portal vein during the whole period of heat stress in the experiment decreased by 29.33%. Plasma endotoxin concentration in arterial, portal and mesenteric veins kept on increasing with prolonged heat stress. Plasma endotoxin concentration in the carotid remained stable during the first 10-h exposure of heat stress, after 10-h heat stress, it increased sharply in relation to the TN group (0.26 vs. 0.55 EU/ml, 115.39% increase). Plasma endotoxin concentration began increasing after 6 h in the mesenteric vein ($p < 0.05$) and after 4 h in the portal vein ($p < 0.01$). The average plasma concentration of endotoxin in carotid, portal and mesenteric veins during the whole period of heat stress in the experiment increased by 100%, 308.16% and 277.36% respectively (Table 4).

Endotoxin absorption

Net absorption of endotoxin across MDV and PDV began increasing after 4-h exposure of heat stress.

Table 4 Effect of heat stress on plasma flux rate (l/h) and endotoxin concentration (EU/ml) of dairy goats

	Mesenteric vein		Portal vein		Arterial
	Flux rate	Endotoxin concentrations	Flux rate	Endotoxin concentrations	Endotoxin concentrations
HS (h)					
2	33.65 ± 2.61^{ABb}	0.49 ± 0.11^{Aa}	98.67 ± 6.51^{Aa}	1.02 ± 0.08^{Aa}	0.22 ± 0.05^{Aa}
4	33.42 ± 2.28^{ABb}	0.87 ± 0.08^{Aab}	67.96 ± 7.54^{BCbcd}	1.83 ± 0.11^{Ba}	0.21 ± 0.04^{Aa}
6	33.30 ± 3.24^{ABb}	1.01 ± 0.05^{ABb}	75.81 ± 8.01^{Bb}	3.23 ± 0.31^{Ccd}	0.16 ± 0.01^{Aa}
8	31.14 ± 2.95^{BCcd}	1.61 ± 0.25^{BCc}	67.62 ± 9.06^{BCbcd}	2.98 ± 0.17^{Cc}	0.27 ± 0.03^{Aa}
10	30.60 ± 3.48^{BCcd}	2.41 ± 0.17^{DEFde}	58.03 ± 8.85^{Cde}	3.47 ± 0.20^{Cd}	0.55 ± 0.11^{Bbc}
12	30.36 ± 2.80^{BCcd}	1.93 ± 0.08^{CDcd}	74.14 ± 11.55^{Bbc}	4.07 ± 0.20^{DEe}	0.44 ± 0.06^{Bb}
14	27.16 ± 1.98^{CDde}	1.93 ± 0.09^{CDcd}	63.79 ± 6.01^{BCcde}	3.58 ± 0.23^{CDd}	0.79 ± 0.10^{DEde}
16	24.01 ± 2.63^{De}	2.19 ± 0.10^{CDEd}	55.17 ± 8.55^{Ce}	4.44 ± 0.38^{Ee}	0.54 ± 0.03^{BCb}
18	25.27 ± 2.15^{CDe}	2.31 ± 0.95^{FGfg}	67.96 ± 5.56^{BCbcd}	5.66 ± 0.39^{Ff}	0.68 ± 0.09^{CDcd}
20	25.52 ± 3.34^{CDe}	2.24 ± 0.23^{DEd}	66.67 ± 6.03^{BCbcde}	5.43 ± 0.28^{Ff}	0.86 ± 0.09^{Eef}
22	27.07 ± 2.72^{CDde}	2.80 ± 0.22^{EFGef}	66.33 ± 3.51^{BCbcde}	5.39 ± 0.27^{Ff}	0.95 ± 0.08^{Ef}
24	25.61 ± 3.20^{CDe}	3.32 ± 0.15^{Gg}	67.67 ± 5.51^{BCbcd}	6.52 ± 0.38^{Gg}	0.89 ± 0.12^{Eef}
TN (the average value of the six samples was deemed as the thermo-neutral value)					
	38.64 ± 0.57^{Aa}	0.53 ± 0.07^{Aa}	97.84 ± 1.48^{Aa}	0.98 ± 0.18^{Aa}	0.26 ± 0.09^{Aa}

HS, heat stress period; TN, thermo-neutral period. Means within a column with an uncommon capitalized superscript are different ($p < 0.01$). Means within a column with an uncommon lowercase superscript are different ($p < 0.05$).

Table 5 Effect of heat stress on net absorption of endotoxin of dairy goats (10^4 EU/h)

	Mesenteric vein	Portal vein	MDV/PDV
TN group (the average value of the six samples was deemed as the thermo-neutral value)			
	1.05 ± 0.14^{Aa}	7.02 ± 0.84^{Aa}	15.31 ± 4.12
HS group (h)			
2	0.86 ± 0.45^{Aa}	7.84 ± 0.76^{Aa}	11.24 ± 6.21
4	2.20 ± 0.41^{ABb}	11.03 ± 1.63^{ABb}	20.38 ± 5.28
6	2.80 ± 0.278^{BCbc}	23.44 ± 4.74^{CDEde}	12.11 ± 1.19
8	4.20 ± 0.96^{CDEbcdef}	18.49 ± 3.41^{BCcd}	22.90 ± 4.40
10	5.66 ± 0.57^{FGgh}	16.97 ± 2.85^{BCbc}	33.56 ± 2.09
12	4.51 ± 0.32^{DEFGefg}	26.99 ± 5.18^{DEFefg}	17.01 ± 2.24
14	3.09 ± 0.24^{BCDbcd}	17.90 ± 2.50^{BCcd}	17.56 ± 3.26
16	3.95 ± 0.34^{CDEbcdef}	21.43 ± 5.09^{CDcde}	18.98 ± 3.60
18	5.70 ± 2.11^{FGgh}	33.97 ± 4.67^{FGhi}	17.58 ± 8.97
20	3.69 ± 0.73^{BCDbcde}	30.52 ± 3.97^{EFGgh}	12.04 ± 1.06
22	4.96 ± 0.52^{EFGfgh}	29.45 ± 2.82^{DEFfgh}	16.98 ± 2.59
24	6.17 ± 0.35^{Gh}	38.19 ± 4.82^{Gi}	16.27 ± 1.64

HS, heat stress period; MDV, mesenteric-drained viscera; PDV, portal-drained viscera; TN, thermo-neutral period. Means within a column with an uncommon capitalized superscript are different ($p < 0.01$). Means within a column with an uncommon lower-case superscript are different ($p < 0.05$).

Average net absorption of endotoxin (EU/h) across MDV and PDV in the HS group during the whole period of heat stress increased by 279.05% and 227.92% in relation to the TN group. The ratio of endotoxin flux across MDV and PDV at different times of heat stress exposure in the HS group did not differ from the average value of the TN group, with the exclusion of 10-h exposure, which is greater than that in the TN group (Table 5).

Effect of heat stress on plasma antioxidants of dairy goats

The plasma concentrations of SOD (Table 6), GSH-Px (Table 7) and CAT (Table 8) increased significantly in the mesenteric vein during the observation period. Flux of SOD and GSH-Px across PDV, and the flux of GSH-Px across MDV began decreasing after 4-h exposure of heat stress and reached the lowest point at 16-h exposure of heat stress. The flux of CAT across MDV began increasing after 6-h exposure of heat stress and remains at a high level till 18-h exposure of heat stress, then decreased till 22-h exposure of heat stress and remained at the same level as that in the TN group afterwards.

Plasma concentration of TAP (Table 9) in the mesenteric vein at different times of exposure of heat stress in the HS group did not differ from the mean value in the TN group, while that in the portal vein at different times of heat exposure in the HS group was always lower than the mean value in the TN group. The flux of TAP across MDV and PDV at different times of heat exposure in the HS group was always lower than the mean value in the TN group.

The flux of MDA (Table 10) across MDV kept on decreasing from 2- to 18-h heat exposure, and that across PDV kept on decreasing from 4- to 16-h heat exposure, and then increased.

Table 6 Effect of heat stress on plasma superoxide dismutase (SOD) of dairy goats

	MDV		PDV	
	Concentrations (U/ml)	Flux (10^6U/h)	Concentrations (U/ml)	Flux (10^6U/h)
HS group (h)				
2	109.13 ± 2.91^{ABCabc}	3.67 ± 0.19^{ABb}	105.52 ± 1.57^{Bb}	10.40 ± 0.53^{Aa}
4	105.98 ± 2.93^{Aab}	3.54 ± 0.20^{Bbc}	108.51 ± 1.38^{ABb}	7.37 ± 0.73^{Abc}
6	105.82 ± 3.18^{Aab}	3.52 ± 2.78^{Bbc}	93.75 ± 1.06^{Dcd}	7.10 ± 0.67^{BCbc}
8	106.48 ± 2.98^{Aab}	3.31 ± 0.24^{BCbcd}	96.94 ± 1.42^{CDc}	6.55 ± 0.79^{BCDcd}
10	109.09 ± 3.24^{ABCabc}	3.33 ± 0.29^{BCbcd}	90.73 ± 0.70^{Dd}	5.26 ± 0.79^{De}
12	106.99 ± 8.95^{ABab}	3.23 ± 0.16^{BCDcde}	91.43 ± 1.25^{Dd}	6.77 ± 0.97^{BCbcd}
14	108.68 ± 4.48^{ABCab}	2.95 ± 0.12^{CDEdef}	104.48 ± 8.57^{Bb}	6.67 ± 0.83^{BCDbcd}
16	111.82 ± 2.85^{ABCbcd}	2.68 ± 0.23^{Ef}	107.25 ± 2.46^{ABb}	5.91 ± 0.80^{CDde}
18	114.93 ± 1.69^{BCcd}	2.90 ± 0.21^{CDEef}	113.74 ± 3.00^{Aa}	7.72 ± 0.48^{Bb}
20	116.86 ± 1.55^{Cd}	2.98 ± 0.35^{CDEdef}	107.86 ± 4.01^{ABb}	7.18 ± 0.41^{BCbc}
22	110.47 ± 1.68^{ABCabc}	2.99 ± 0.29^{CDEdef}	106.54 ± 3.27^{ABb}	7.06 ± 0.20^{BCbc}
24	106.33 ± 1.64^{Aab}	2.72 ± 0.30^{DEf}	103.78 ± 3.17^{BCb}	7.01 ± 0.37^{BCbc}
TN group (the average value of the six samples was deemed as the thermo-neutral value)				
	105.78 ± 3.04^{Aa}	4.09 ± 0.09^{Aa}	108.53 ± 1.05^{Ab}	10.62 ± 0.24^{Aa}

HS, heat stress period; MDV, mesenteric-drained viscera; PDV, portal-drained viscera; TN, thermo-neutral period. Means within a column with an uncommon capitalized superscript are different ($p < 0.01$). Means within a column with an uncommon lower-case superscript are different ($p < 0.05$).

Table 7 Effect of heat stress on plasma glutathione peroxidase (GSH-Px) of dairy goats

	MDV		PDV	
	Concentrations (U/ml)	Flux (10^6 U/h)	Concentrations (U/ml)	Flux (10^6 U/h)
HS group (h)				
2	213.26 ± 9.25^{ABbc}	7.16 ± 0.24^{ABab}	$248.24 \pm 9.17^{ABCabcd}$	24.45 ± 0.72^{Aa}
4	200.49 ± 4.71^{Aa}	6.69 ± 0.39^{BCbcd}	286.96 ± 40.08^{Ee}	19.49 ± 3.32^{Bb}
6	207.46 ± 6.19^{ABb}	6.90 ± 0.49^{ABbc}	223.89 ± 5.06^{Aa}	16.95 ± 1.41^{BCbc}
8	209.71 ± 8.3^{ABbc}	6.51 ± 0.36^{BCDbcde}	232.71 ± 7.03^{ABab}	15.69 ± 1.65^{Cc}
10	219.64 ± 20.54^{ABbcd}	6.68 ± 0.42^{BCbcd}	272.88 ± 10.87^{CDde}	15.77 ± 1.75^{Cc}
12	209.30 ± 18.06^{ABbc}	6.32 ± 0.16^{BCDcdef}	239.32 ± 8.04^{ABCabc}	17.68 ± 2.20^{BCbc}
14	213.56 ± 17.94^{ABbc}	5.78 ± 0.13^{CDef}	$254.98 \pm 13.66^{ABCDbcd}$	16.21 ± 0.66^{BCc}
16	229.84 ± 8.30^{BCcde}	5.51 ± 0.50^{Df}	319.10 ± 28.78^{Ef}	17.44 ± 1.16^{BCbc}
18	235.73 ± 6.98^{BCde}	5.95 ± 0.35^{CDdef}	264.49 ± 6.48^{BCDcde}	17.95 ± 1.04^{BCbc}
20	266.11 ± 7.95^{Df}	6.77 ± 0.72^{ABCbc}	267.37 ± 7.33^{BCDde}	17.79 ± 1.13^{BCbc}
22	250.58 ± 10.31^{CDef}	6.77 ± 0.43^{ABCbc}	267.11 ± 6.12^{BCDde}	17.70 ± 0.54^{BCbc}
24	267.75 ± 9.55^{Df}	6.84 ± 0.64^{ABCbc}	$257.49 \pm 0.82^{ABCDbcd}$	17.42 ± 1.44^{BCbc}
TN group (the average value of the six samples was deemed as the thermo-neutral value)				
	201.36 ± 18.95^{Aa}	7.79 ± 081^{Aa}	266.69 ± 17.39^{BCDde}	26.10 ± 1.93^{Aa}

HS, heat stress period; MDV, mesenteric-drained viscera; PDV, portal-drained viscera; TN, thermo-neutral period. Means within a column with an uncommon capitalized superscript are different ($p < 0.01$). Means within a column with an uncommon lower-case superscript are different ($p < 0.05$).

Table 8 Effect of heat stress on plasma catalase (CAT) of dairy goats

	MDV		PDV	
	Concentrations (U/ml)	Flux (10^4 U/h)	Concentrations (U/ml)	Flux (10^4 U/h)
HS group (h)				
2	1.07 ± 0.09^{ABb}	3.60 ± 0.06^{ABab}	1.83 ± 0.08^{EFf}	18.06 ± 0.50^{Aa}
4	1.06 ± 0.24^{ABb}	3.57 ± 0.98^{ABab}	2.45 ± 0.16^{Hi}	16.56 ± 0.88^{Aa}
6	1.54 ± 0.15^{CDEdef}	5.12 ± 0.39^{CDEde}	2.25 ± 0.07^{GHh}	17.04 ± 1.27^{Aa}
8	2.24 ± 0.19^{Gi}	6.95 ± 0.44^{Fg}	2.02 ± 0.06^{FGg}	13.65 ± 1.45^{Ba}
10	1.95 ± 0.28^{EFGghi}	5.96 ± 1.11^{DEFef}	1.77 ± 0.09^{Ef}	10.24 ± 1.21^{CDc}
12	2.07 ± 0.06^{FGhi}	6.28 ± 0.49^{EFfg}	1.71 ± 0.09^{Ef}	12.63 ± 1.33^{BCb}
14	1.84 ± 0.35^{DEFGfgh}	4.99 ± 0.98^{CDEde}	1.40 ± 0.02^{De}	8.92 ± 0.71^{DEc}
16	2.17 ± 0.17^{FGhi}	5.18 ± 0.19^{CDEde}	1.15 ± 0.10^{BCcd}	6.29 ± 0.68^{Fd}
18	1.91 ± 0.13^{EFGghi}	4.82 ± 0.29^{BCDcd}	1.31 ± 0.17^{CDde}	8.88 ± 1.08^{DEc}
20	1.72 ± 0.21^{DEFefg}	4.36 ± 0.57^{ABCbcd}	1.02 ± 0.16^{ABbc}	6.79 ± 0.98^{EFd}
22	1.46 ± 0.22^{BCDcde}	3.91 ± 0.21^{ABCabc}	0.82 ± 0.01^{Aa}	5.44 ± 0.26^{Fd}
24	1.21 ± 0.22^{ABCbcd}	3.06 ± 0.19^{Aa}	0.91 ± 0.06^{ABab}	6.19 ± 0.87^{Fd}
TN group (the average value of the six samples was deemed as the thermo-neutral value)				
	0.95 ± 0.14^{Aa}	3.66 ± 0.57^{ABab}	1.75 ± 0.17^{Ef}	17.13 ± 1.86^{Aa}

HS, heat stress period; MDV, mesenteric-drained viscera; PDV, portal-drained viscera; TN, thermo-neutral period. Means within a column with an uncommon capitalized superscript are different ($p < 0.01$). Means within a column with an uncommon lower-case superscript are different ($p < 0.05$).

Discussion

Heat stress and endotoxin

For non-ruminants, endotoxin is mainly absorbed from the intestinal tract. Cronjé (2005) speculated that endotoxin in ruminants is absorbed both from the intestinal tract and from the rumen, and the rumen might be the most important site for endotoxin absorption. Results from the present study confirmed Cronjé's (2005) speculation. In the present study, endotoxin absorption from MDV took only a small percentage (<20%) of the net flux of endotoxin across PDV for all the goats at different thermo-status. Therefore, in goats endotoxin is

Table 9 Effect of heat stress on plasma total antioxidative potential of dairy goats

	MDV		PDV	
	Concentrations (U/ml)	Flux (10^4 U/h)	Concentrations (U/ml)	Flux (10^4 U/h)
HS group (h)				
2	1.85 ± 0.24^{Aa}	6.18 ± 0.34^{ABa}	1.61 ± 0.13^{Bbc}	15.85 ± 0.27^{Bb}
4	1.69 ± 0.19^{ABab}	5.62 ± 0.51^{ABab}	1.27 ± 0.26^{BCde}	8.60 ± 1.68^{DEde}
6	1.52 ± 0.27^{ABCabc}	5.03 ± 0.66^{BCb}	1.64 ± 0.19^{Bb}	12.37 ± 0.25^{Cc}
8	1.23 ± 0.25^{BCcd}	3.79 ± 0.41^{DEcd}	1.35 ± 0.13^{BCcde}	9.05 ± 0.41^{Dd}
10	1.35 ± 0.19^{ABCbcd}	4.08 ± 0.22^{CDcd}	1.08 ± 0.17^{Ce}	6.25 ± 1.06^{EFfg}
12	1.11 ± 0.21^{Cd}	3.32 ± 0.30^{DEde}	1.18 ± 0.17^{Cde}	8.63 ± 0.57^{DEde}
14	1.56 ± 0.31^{ABCabc}	4.26 ± 0.99^{CDbc}	1.45 ± 0.17^{BCbcd}	8.93 ± 1.65^{Dd}
16	1.48 ± 0.19^{ABCbcd}	3.52 ± 0.29^{DEcde}	1.20 ± 0.26^{Cde}	6.69 ± 0.77^{DEFefg}
18	1.13 ± 0.14^{Cd}	2.85 ± 0.48^{Ee}	1.28 ± 0.06^{BCde}	8.15 ± 1.25^{DEdef}
20	1.51 ± 0.32^{ABCabc}	3.81 ± 0.54^{DEcd}	0.68 ± 0.27^{Df}	4.82 ± 2.27^{Fg}
22	1.35 ± 0.13^{ABCbcd}	3.63 ± 0.05^{DEcde}	1.11 ± 0.12^{Ce}	8.15 ± 1.47^{DEdef}
24	1.51 ± 0.13^{ABCabc}	3.83 ± 0.16^{CDEcd}	1.36 ± 0.13^{BCbcde}	8.63 ± 0.21^{DEde}
TN group (the average value of the six samples was deemed as the thermo-neutral value)				
	1.60 ± 0.20^{ABab}	6.20 ± 0.82^{Aa}	2.16 ± 0.07^{Aa}	21.12 ± 0.90^{Aa}

HS, heat stress period; MDV, mesenteric-drained viscera; PDV, portal-drained viscera; TN, thermo-neutral period. Means within a column with an uncommon capitalized superscript are different ($p < 0.01$). Means within a column with an uncommon lower-case superscript are different ($p < 0.05$).

Table 10 Effect of heat stress on plasma malonaldehyde (MDA) of dairy goats

	MDV		PDV	
	Concentrations (nmol/ml)	Flux (10^4 nmol/h)	Concentrations (nmol/ml)	Flux (10^4 nmol/h)
HS group (h)				
2	1.39 ± 0.14^{ABCDbcd}	4.65 ± 0.11^{BCb}	1.45 ± 0.07^{BCDcd}	14.28 ± 0.27^{ABa}
4	1.30 ± 0.16^{ABCabcd}	4.32 ± 0.55^{BCDbc}	1.36 ± 0.10^{ABCbc}	9.22 ± 0.44^{FGf}
6	1.11 ± 0.14^{Aa}	3.67 ± 0.25^{Dde}	1.30 ± 0.16^{ABabc}	9.75 ± 0.63^{Ff}
8	1.16 ± 0.08^{ABab}	3.61 ± 0.45^{Dde}	1.11 ± 0.14^{Aa}	7.43 ± 0.20^{Hhi}
10	1.16 ± 0.21^{ABab}	3.57 ± 0.91^{De}	1.22 ± 0.14^{ABab}	6.97 ± 0.63^{Ii}
12	1.30 ± 0.08^{ABCabcd}	3.92 ± 0.14^{BCDcde}	1.12 ± 0.20^{Aa}	8.25 ± 0.44^{GHgh}
14	1.34 ± 0.08^{ABCDabcd}	3.64 ± 0.11^{Dde}	1.16 ± 0.08^{Aa}	7.36 ± 0.38^{Hhi}
16	1.48 ± 0.21^{BCDEcdf}	3.52 ± 0.17^{De}	1.67 ± 0.14^{DEef}	9.13 ± 0.84^{FGfg}
18	1.53 ± 0.14^{CDEGdfg}	3.84 ± 0.24^{CDcde}	1.71 ± 0.08^{DEFef}	11.61 ± 0.50^{Ee}
20	1.67 ± 0.14^{DEGfgh}	4.22 ± 0.25^{BCDbcd}	1.81 ± 0.14^{EFGfg}	11.20 ± 0.16^{DEde}
22	1.76 ± 0.16^{EGgh}	4.74 ± 0.20^{Bb}	1.95 ± 0.12^{FGg}	12.88 ± 0.26^{CDcd}
24	1.85 ± 0.21^{Gh}	4.70 ± 0.13^{Bb}	1.99 ± 0.08^{Gg}	13.45 ± 0.66^{BCbc}
TN group (The average value of the six samples was deemed as the thermo-neutral value)				
	1.53 ± 0.16^{CDEdfg}	5.63 ± 0.45^{Aa}	1.56 ± 0.07^{CDEde}	15.30 ± 0.83^{Aa}

HS, heat stress period; MDV, mesenteric-drained viscera; PDV, portal-drained viscera; TN, thermo-neutral period. Means within a column with an uncommon capitalized superscript are different ($p < 0.01$). Means within a column with an uncommon lower-case superscript are different ($p < 0.05$).

mainly absorbed from non-MDV, and rumen might be the main site of endotoxin absorption.

Kregel et al. (1988) reported that blood flux of viscera decreased by 40% when colon temperature increased from 37 to 41.5 °C. Therefore, we can exclude the effect of mere circadian rhythm on plasma flux. Hall et al. (2001) reported that heat stress led to the increase in endotoxin in the portal vein, which was also observed in this study. It was noticeable that blood flux of PDV decreased by 29.33% on average during heat stress, with the maximum decrease of 43.62% at 16-h heat exposure. With such significant decrease in visceral blood flow, even if the total absorption of endotoxin is

kept stable, endotoxin concentration in portal vein could increase on a large scale. Therefore, the variation of endotoxin concentration in the portal vein could not be used to elucidate the endotoxin absorption status. We revealed quantitatively the effect of heat stress on endotoxin flux across PDV by studying the concentration balance between arteries and veins as well as the flux rate of blood. Results showed that heat stress lead to the significant increase of endotoxin absorption and endotoxin absorption increased with the time of heat exposure.

Endotoxin could be eliminated in the liver, and therefore endotoxin concentration in the carotid in heat-stressed goats during the first 8-h of heat stress did not differ from that in thermo-neutral goats. With the time elapse of heat stress, more endotoxin entered the liver which exceeded the detoxifying function and impaired liver function of eliminating endotoxin thereafter. This could explain the significant increase in endotoxin concentration in the carotid after 10-h heat exposure.

Heat stress and antioxidants

The present study revealed that there was a great discrepancy in the redox status of dairy goats evaluated by the concentration and as net flux of antioxidants. Superoxide dismutase activity in the mesenteric vein increased with heat stress if expressed as plasma concentration, while it decreased if expressed as net flux. Nowadays, the redox status in an organism is mainly evaluated by the plasma concentration of different antioxidants (Trout et al., 1998; Calamari et al., 1999; Bernabucci et al., 2002; Bouwstra et al., 2008) without examining the net flux. In fact, blood flow in organisms may redistribute during heat stress, with fewer blood flux in the gastrointestinal tract and more in peripheral circulation for heat exchange. This means that the blood flux across PDV and MDV is quite different before and after heat stress and therefore, it is the net flux, not the concentration, of antioxidants that should be used for evaluating the antioxidant status.

The production and elimination of free radicals should be balanced to maintain a suitable level of free radicals (Rhee, 2006). Antioxidants in organisms, can be classified into enzyme and non-enzyme antioxidants. Antioxidant enzymes include GSH-Px, SOD and CAT, and non-enzyme antioxidants include vitamin E, beta-carotene, vitamin C, cysteine, etc. (Vertuani et al., 2004). Heat stress may trigger the increased amount of free radicals by viscera hypoxia. The increased amount of free radicals depletes large amounts of antioxidants, resulting in the decreased activity of SOD, GSH-Px and TAP, as proved by studies on pigs (Song et al., 2008), broilers (Hall et al., 1999) and dairy cows (Harmon et al., 1997).

In the present study, CAT flux across MDV increased significantly after heat exposure, and then decreased, while that across PDV kept on decreasing during the whole heat stress period. We speculated that the increase in CAT flux across MDV during short period of heat stress could be attributed to the compensatory gene expression induced by H_2O_2. Yu et al. (2007) reported that stress-induced gene expression of CAT1 catalase is mediated by triggering H_2O_2 signal production in Arabidopsis. But no evidence was found in animals.

In the present study, the net flux of plasma SOD, GSH-Px across PDV and GSH-Px across MDV kept on decreasing till 16-h heat exposure, then bounced up to a stable level which was still lower ($p < 0.05$) than that at the thermo-neutral level. The decrease in the fluxes of antioxidants and MDA in plasma during heat stress was mainly because of a decrease in blood flow. Many researchers (Calamari et al., 1999; Bernabucci et al., 2002; Lin et al., 2006; Song et al., 2008) reported that the amount of oxidation products increased with ambient temperature. This was not proved in the present study in which MDA concentration and flux decreased at first and then increased with the time of heat exposure. In the present study, the knee points of the net flux of MDA and GSH-Px across MDV and PDV, and SOD across MDV, happened at 16-h heat stress. Net flux of MDA during the later period of heat stress increased, but was still significantly lower than that at the thermo-neutral level. It could be concluded from this result that no oxidative stress happened during 24-h heat stress.

Heat stress and gastrointestinal permeability

Hall et al. (2001) reported that the increased permeability of the gastrointestinal tract during heat stress was caused by oxidative stress. Result from the present study showed that the significant increase in endotoxin flux across MDV and PDV began at 4-h heat exposure, indicating the significant increase in permeability of the rumen and the gastrointestinal tract at 4-h heat exposure. But MDA concentration at the same time was significantly lower than that in the thermo-neutral level. The present study showed that gastrointestinal permeability of dairy goats increased during heat stress without the occurrence of oxidative stress, suggesting that the increased

permeability of gastrointestinal tract during heat stress was not caused by oxidative injury. Lambert et al. (2002) reported that the increased permeability of everted intestinal sac during heat stress was not caused by oxidative injury. The increase in cortisol during heat stress could increase gastrointestinal permeability (Meddings and Swain, 2000). Hypoxia of viscera caused by heat stress may increase gastrointestinal permeability by ATP depletion (Unno et al., 1996) or by tissue acidosis (Brinnel et al., 1987; Menconi et al., 1997). Therefore, cortisol and hypoxia may be responsible for the increase in gastrointestinal permeability during heat stress, but not oxidative stress.

Conclusion

Net absorption of endotoxin in the portal vein is mainly from non-mesenteric tissues both in heat stress and in thermo-neutral conditions. Heat stress may lead to the significant decrease in plasma, SOD, GSH-Px, CAT flux across PDV and MDV, and to the significant increase in endotoxin flux across PDV and MDV. The increase in gastrointestinal permeability in dairy goats during heat stress was not accompanied by the increase in oxidative stress.

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