Changes in microbial nitrogen synthesis in the rumen of lactating Holstein cows by exposure to hot condition

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Changes in microbial nitrogen synthesis in the rumen of lactating Holstein cows by exposure to hot condition

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Abstract

The changes in microbial nitrogen synthesis in the rumen of lactating dairy cows by exposure to hot condition were examined using four Holstein cows. The cows were kept under thermoneutral temperature (18 ℃) for fourteen days and then exposed to hot temperature (28℃) for the following two weeks; the relative humidity in both temperatures was 60%. The results showed that urinary allantoin excretion correlated with dry matter intake ($r= 0.76$, $P< .05$) and nitrogen intake ($r= 0.71$, $P< .05$), while concentration of urinary creatinine correlated with decreased body weight ($r= 0.83$, $P< .05$) as a proof of body tissue mobilization. In the hot environmental temperature, allantoin excretion in urine decreased. However, the utilization rate of nitrogen for microbial nitrogen synthesis during heat exposure increased on average from 63.4 % (at 18℃) to 75.6 % (28℃). Microbial nitrogen synthesis per digestible organic matter intake increased on average from 20.4 (at 18℃) to 23.0 gN (28℃). The utilization rate of nitrogen and digestible organic matter intake for microbial nitrogen synthesis during the heat exposure increased as the heat exposure was prolonged.

Key words: Allantoin, microbial nitrogen, hot temperature, digestibility, dairy cow

Introduction

The newly proposed systems for estimating the nitrogen (N) requirements of ruminants need estimated amount of protein that is digested and absorbed in small intestine. This comprises microbial protein synthesized in the rumen and dietary protein that escapes rumen digestion[2, 21, 28]. Allantoin excreted in urine appears to originate predominantly from nucleic acids synthesized by rumen microorganism[4] and has been used for estimating protein synthesized by the microbes[18]. The excretion of allantoin is affected by dry matter intake (DMI), N intake and their interactions with energy intake[11, 36]. Allantoin affected by environmental temperature was reported in the study under low temperature[13], in which the efficiency of rumen microbial synthesis was increased and the rumen degradation of dietary proteins was decreased. However, no reference was found in hot temperature or in the adapting period from thermoneutral to hot temperature. Hot temperature will reduce DMI, decrease rumen passage rate and increase digestibility coefficients[7]. Moreover, exposing animals to hot temperature will possibly force them into heat stress and low feed intake that will result in reducing digestive tract motility and ruminal contraction rate, and increasing retention time[5, 34, 39]. As a result, rumen fermentation may be suppressed and microbial synthesis in the rumen will be decreased[14].

This study was carried out aiming to make clear the effect of changes in environmental temperature on urinary allantoin and its correlation with nutrients intake. The performance of energy and nitrogen balance of this experiment will be reported in another paper.

Materials and Methods

1. Animal and experimental procedures

Four multiparous Holstein cows with an average bodyweight and daily milk yield of 576 and 36.7 kg, respectively, were used in this study. They were housed in a controlled room with individual tie stalls equipped

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with individual feeders, waterers and ventilated hoods for gas measurements. The ambient temperature and relative humidity were controlled by computer and monitored continuously. The cows were maintained under thermoneutral temperature (18℃, 60% relative humidity; RH) during 7 -d preliminary experiment to allow them to adapt for a new housing conditions and experimental feeding ration. During this period, no measurements were carried out. The experiment of three weeks was started immediately after the end of the pre-experiment. In the first week of the experiment, the temperature was set on thermoneutral temperature, and in the second and third week, it was lifted to 28℃ (60%RH). The procedure of experiment is presented in figure 1. The cows were fed timothy hay grass (31.4% dry matter; DM), beet pulp (11.9%DM), commercial concentrate (50.6%DM) and soybean meal (6.1%DM) to make crude protein (CP) of 13% and DM of 11.6 MJ ME/kg. Feed was adjusted to meet the metabolizable energy (ME) requirement for maintenance and milk production of cows[1], and given to them four times a day at 0800, 1000, 1600 and 1830h. The daily intakes of DM and nitrogen were measured by collecting orts and drying them with air-draft oven at 60 ℃ for 48 hours. Cows were milked twice daily at 0830 and 1800h, and the production was recorded. Rectal temperature and respiration rates were measured daily at 0800h to detect the physiological responses during exposure to heat. The spot samples of urine and feces, and blood samples were collected twice a day about 30 minutes before milking in the mornings and evenings during the experimental period from week 1 to week 3. Urine samples collected by vulval stimulation were filtered to separate solid contaminants prior to being stored at − 35℃ for chemical analysis. The feces of about 150 g each was grabbed twice a day in the mornings and evenings around the time of the urine collection, and were dried in air-draft oven at 60℃ for 24h. The dried fecal samples were ground to pass 1mm screen for further analysis. Blood samples were collected using catheter planted in vena jugularis at d -9, three days before the sample collection. Soon after collection, part of each blood sample was centrifuged in 10,000 rpm for 5 minutes for hematocrit determination. The rest of each blood sample was centrifuged at 3000 rpm for 10 minutes to obtain the plasma, and the plasma was then stored at − 35℃ until chemically analyzed. Body weight was measured at the d -6, 1, 6, 10 and 14 of temperature elevation. Daily body weight for estimating the total urine excretion was also calculated with daily gain or daily loss at the intervals of the measurements mentioned above. This is based on assumption that daily creatinine excretion is relatively constant due to a function of metabolic weight of animals[11].

2. Chemical analysis

Urine was analyzed to determine allantoin by the procedure of Young and Conway (1942), and creatinine by commercial kit analyzer (Creatinine test, Wako pure chemical, Ltd) based on Jaffe method, both were measured using spectrophotometer (Hitachi U-200, Hitachi, Japan). Total urinary allantoin was converted from total urine excretion calculated based on total collection of urine carried out at the d -6 to -3 (18℃) and the d 8 to 11 (28℃).

Lignin and organic matter (OM) in feeds and feces were analyzed for determining dry matter digestibility (DMD) and OM digestibility by lignin indicator method[3]. Lignin and OM in feeds were determined by 72% sulfuric acid method and ashing at 600℃ for two hours, respectively, while those in feces were determined by near infrared reflectance spectroscopy (NIRS) using the equipment, methods and calibration equation for feces previously reported[30]. The reliability of DMD determined using lignin was evaluated by calculating lignin recovery on total collection that found 104% (sd=1.8%) in the thermoneutral temperature and 97% (sd=3.1%) in the hot temperature, therefore no correction was done on the daily DMD.

3. Prediction of microbial nitrogen supply during heat exposure

Efficiency of N intake to supply microbial nitrogen during heat exposure was calculated by comparing N

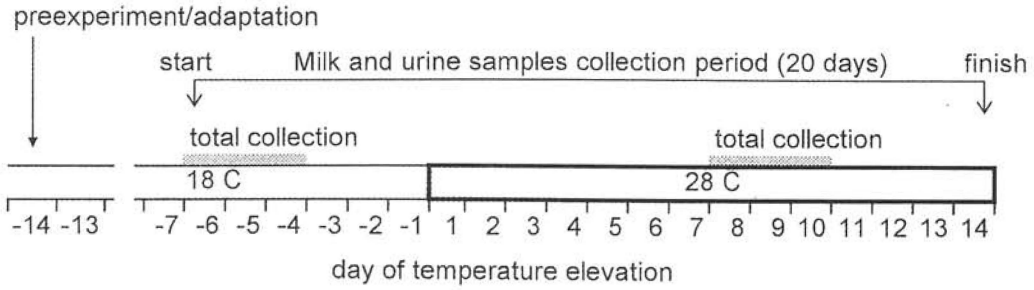

Figure 1. Experimental procedure. At the day 1, the room temperature was elevated to 28℃ from 18℃. Total collection of urine and feces was done twice at day -6 to -1 (18℃) and day 9 to 13 (28℃). Daily spot urine and fecal samples were collected within 30 minutes before milking.

intake with estimated microbial N synthesis. Microbial N supply was estimated based on the urinary excretion of purine derivatives (PD)[12] through allantoin excretion, as follows:

$$PDa = (PDe - 0.385 \times W^{0.75}) / 0.85$$

$$PDe = 100/90 \times \text{Allantoin excretion}$$

Estimated microbial nitrogen supply (g per day):

$$= (70PDa) / (0.83 \times 0.116 \times 1000)$$

$$= (PDa \times 0.727)$$

The PDa and PDe are the absorption and excretion of purine derivatives in mmol/d, respectively. The following assumptions were also made:

1. The mean endogenous contribution to urinary PD excretion from degradation of tissue nucleic acids is 0.385 mmol/kg $W^{0.75}$ per day[38].
2. The recovery of absorbed purines as urinary PD is assumed to be 85%, with 15% of the rest that are lost via nonrenal routes, for example saliva[38].
3. Allantoin accounted for proportionately 90% of total urinary PD excretion[35].
4. The digestibility of microbial purines in the intestines is assumed to be 0.83 and the ratio of purine N to total N in mixed rumen microbes is taken as 0.116[9].
5. The N content of purines: 70 mg/mmol[12].

4. Data Analysis

All daily data were averaged from four dairy cows. Daily data of allantoin, creatinine, and DMD were obtained from the averages of the samples collected in the mornings and evenings in order to eliminate the diurnal variation caused by time feeding[25, 35]. The data derived from 7 days in the week under 18 ℃ temperature were adopted as basal values. The daily values of dry matter digestibility (DMD) and urinary allantoin during heat exposure were compared to the basal values. Statistical analysis was done using one-way classification analysis of variance with blocking on animals, and the significance was then analyzed using contrast statement comparing the basal values with the daily values under heat exposure[33].

Results and Discussion

1. Animal performance during heat exposure

After animals were exposed to the hot temperature (28℃) for two weeks, the rectal temperature increased by about 1.4℃ from 38.5℃. Respiration rate doubled approximately (68.5 breaths/min), compared with 30.7 breaths/min at the thermoneutral temperature (18 ℃). However, blood hematocrit dropped from 31.8 to 26.8%. The changes in feed intake, animal performances and allantoin excretion during experimental period are presented in figure 2. The averages of daily DMI, milk yield and body weight of cows decreased by about 44, 41 and 6.6% (= 38 kgBW), respectively. The intake of N, hay and concentrates decreased at the level similar to DMI of about 45% in the thermoneutral temperature. The change in the ratio of roughage to concentrate and N concentration during the experiment was observed to be insignificant. Decreasing daily intake of N linearly correlated with DMI that started to decrease at the d -3 of temperature elevation. Decreasing DMI also highly correlated (r = 0.98) with milk yield.

In the animals exposed to the hot temperature, the increase of respiration rate and rectal temperature, and the decrease of blood hematocrit indicated that they were in heat stress[22, 24]. As predicted, the DMI during heat exposure decreased, which confirmed the results reported in many studies[6, 7, 15]. However, the level of decreasing DMI was higher than 25% reported by Itoh *et al.* (1998) and 20 − 40 % by Sanchez *et al.* (1994). Milk yield was decreased by heat stress and agreed with those previously reported[22, 26, 40], with the higher level of decrease than 19% reported by Itoh *et al.* (1998). Therefore, the present study showed that the level of damage caused by heat stress was higher than those of other similar reports, and it was considered as consequence of high milk yield (36.7 kg/d) and the effect of ventilated hood for respiration gas measurements.

2. Allantoin and dry matter digestibility during heat exposure

Daily allantoin concentration during heat exposure decreased ($P<.05$) and correlated with DMI ($r=0.76$, $P<.05$), nitrogen intake ($r=0.71$, $P<.05$) and energy intake ($r=0.71$, $P<.05$). The concentration started to decrease at d 3, and reached the significant level at d 7, 10 and 11 ($P<.01$), and at d 12 and 13 ($P<.05$). An exception was found at d 8 and 9 when allantoin concentration became insignificant level again, which can compare with that at 18℃. This will be the effect of slight recovery of DMI at d 8.

Decreasing DMI peaked at d 10 of heat exposure and it remained until the end of the experiment. In contrary, dry matter digestibility (DMD) started to increase at d 1 of heat exposure and peaked at d 11, and then steadied until the end of the experiment. The DMI and DMD negatively correlated ($r = -0.84$), while DMD and organic matter digestibility correlated at 0.99. The DMD during heat exposure significantly increased ($P<.01$) starting at d- 5 and the increase continued until the end of the experiment. Urinary creatinine increased during heat exposure, and it inversely correlated with the changes of BW ($r = -0.83$) and DMI ($r = -0.77$).

The decrease of urinary allantoin excretion during heat exposure indicated decreasing microbial synthesis in the rumen. This figure was considered as a result of decreasing rumen microbial flowed to small intestine due to decreasing DMI[11,14] that would have prolonged the rumen retention time and permitted a greater autolysis of microbial matter in reticulo-rumen, and hence that would have reduced available microbial

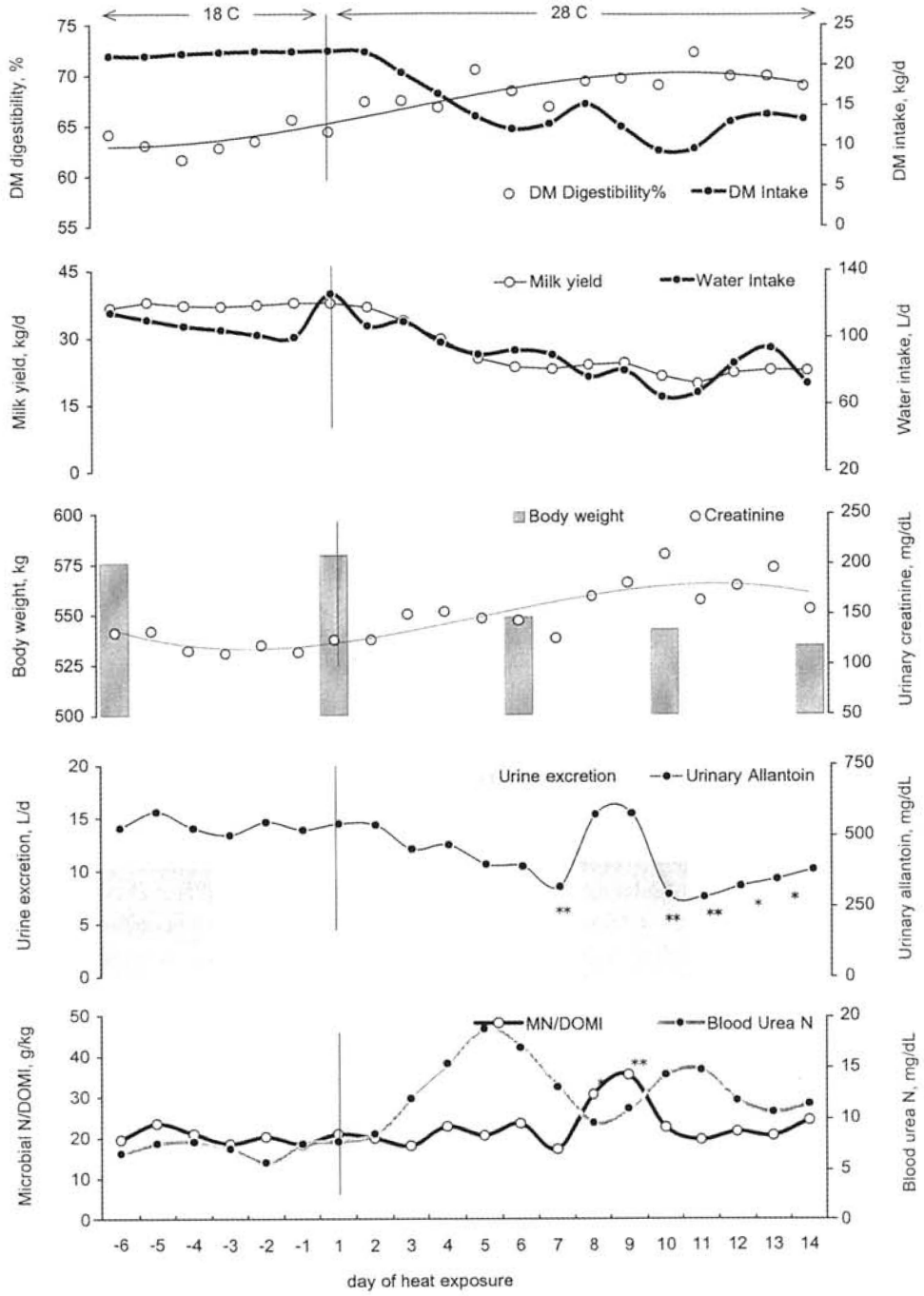

Figure 2. The dry matter intake (DMI; kg/d), dry matter digestibility (DMD; %), water intake (L/d), milk yield (kg/d), body weight (BW; kg), concentration of urinary creatinine (mg/dL), allantoin (mg/dL), total urine excretion (g/d), blood urea N (BUN; mg/dL) and the efficiency of digestible organic matter intake for microbial N synthesis (MN/DOMI; g/kg) during heat exposure. The * and ** marks in 28 ℃ indicated significance at $P<0.05$ and 0.01 after compared to 18℃.

mass[18].

The trend of DMD was in agreement with those previously reported[7, 27] and considered as response for decreasing feed intake, increasing rumen retention time, and decreasing rumen passage rate[7]. The DMD in the whole tract was related to total mean retention time[41] and the volume of rumen fluid[23] that was associated with water intake[17]. The water intake in the present study decreased during heat exposure (fig 2) and therefore the volume of rumen fluid may have decreased, which might have increased the retention time, resulting in the increase of DMD. The daily DMD during heat exposure was observed with non-linear curve, and it was consistent with those

previously reported[13, 27].

3. Estimation of microbial protein synthesis during heat exposure

Total allantoin was determined from total urine excretion that was converted from urinary creatinine. This was based on assumption that creatinine was excreted relatively constant on muscle mass[12, 35]. The present study showed that the concentration of creatinine in spot urine increased during heat exposure although statistical significance was not found, and that it negatively correlated with the decreased body weight as well as DMI. Increasing creatinine, while body weight was decreasing, indicated a rising body protein catabolism[42] because of decreasing DMI. Therefore, the condition in this study showed an exception to converting total urine excretion through creatinine, which was confirmed in the findings of Susmel *et al.* (1995) that the ratio of allantoin to creatinine cannot be used for changing animals' body masses. Therefore, daily excretions of allantoin and creatinine were calculated using total urine collected in the first week of the thermoneutral temperature and in the second week of the hot temperature. Daily urine excretion was not different significantly between the thermoneutral and the hot temperatures (average 10.4 vs 10.8 L/d). Urine excretions at the days between total collections were estimated using the average values of these total excretions. The utilization rate of nitrogen intake for microbial N synthesis during the hot temperature (averaged 75.6%) was higher than that in the termoneutral temperature (63.4%). The weekly averages during the first and the second week of heat exposure were 68.8 and 82.5%, respectively. The efficiency of N intake for microbial N synthesis in the thermoneutral temperature and in the first week of heat exposure was comparable to 43-76% reported from Belgian white-blue bulls fed the diet containing 17-24% CP[16], and to 66.5% observed from Holstein cows fed 14-16% CP[20]. But, those values were much higher than 36-42% reported from Simmental lactating cows fed the diet containing 11% CP[37]. The differences in N utilization rate between the thermoneutral and the hot temperatures in this study were derived from N intake levels, because higher N intake resulted in lower N utilization rates[16].

The efficiency of digestible organic matter intake (DOMI) for microbial N synthesis during heat exposure (averaged 23.0 gN/kgDOMI) was higher than that in the thermoneutral temperature (20.4 gN/kgDOMI). During heat exposure, the efficiency of DOMI was 20.8 gN/kg in the first week and 25.3 gN/kg in the second week. These values were comparable to 21.5 gN/kgDOMI[20] or 15.1-23.4 gN/kgDOMI[16], but still higher than 10.9 gN/kgDOMI[37]. The high value in the second week at the hot temperature indicated the increase of allantoin excretion at d 8 and 9 when sudden recovery of DMI would have overflowed allantoin. By eliminating the values at d 8 and 9, the averages of utilization rates of N intake and DOMI during 28 ℃ will be 69.7 % and 21.3 gN/kgDOMI, respectively.

The reason to explain the increase of efficiency for microbial N synthesis in heat exposure may be the increasing rumen retention time and urea recycle in the rumen related to the low protein in feed (CP13%). The retention time leads fermentation rates and raises ammonia in the rumen, and the microbial synthesis would reduce the energy source in the rumen. This condition would lessen the N utilization for microbial N synthesis and increase the excretion of N in urine. This correlates with the increase of digestibility, which was about 3.4-8.8%, during heat exposure. The excessive ammonia would be absorbed from the rumen, transformed into urea in the liver, and then passed into the blood. The greater part would be excreted in the urine and milk[29] and some other part would be recycled to rumen[8]. This recycled urea may supply the N source for generating amino acids or ammonia, which is available for microbes. The recycled urea is also derived from the catabolism of body protein that is induced by low CP intake and decreasing body weight[19, 31]. The occurrence of recycled urea can be detected from increasing concentration of blood urea N during heat exposure. Therefore, the markedly high efficiency of N for microbial N synthesis in the hot temperature would probably be due to the enlarged N source from recycled urea to the rumen that is not included in calculation.

This study concluded that N utilization for microbial N synthesis was increased by heat exposure, correlating with increasing digestibility and increasing rumen retention time, while the environmental temperature was considered as an indirect factor in the microbial N synthesis. Despite the N efficiency for microbial N synthesis is increasing, but the absolute amount of microbial N that can be utilized by host animal is considered decrease due to decreasing DMI during the heat exposure. Moreover, even the excreted allantoin corresponds to the amount of microbial biomass reaching the duodenum rather than that in the rumen[10], urinary allantoin could be used as an indicator for measuring an N utilization of feed for microbial synthesis as shown by the reasonable value compared to the previous studies. This study also showed that the use of creatinine as a conversion factor for calculating total urine excretion cannot be used for animals with highly decreasing in the body weight due to increasing of creatinine from body decomposition. Therefore, as a suggestion, for further study on the use of spot urine sample it needs the study on creatinine excretion per kg body mass in maintenance and in decomposed condition.

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暑熱環境下における泌乳牛の第一胃内微生物態窒素合成量の推定

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摘　要

暑熱環境下における尿中アラントイン排泄量の変動を4頭のホルスタイン種泌乳牛を用いて検討した。供試牛は温度18℃相対湿度60%の人工環境下で14日間飼養した後，28℃60%の環境下でさらに14日間飼養した。その結果，尿中アラントイン排泄量は乾物摂取量及び窒素摂取量との間にそれぞれ，r = 0.76 及び 0.71 の高い相関関係が認められており（$p< 0.05$），一方，クレアチニン濃度は体重減少量との間に正の相関関係（$r = 0.83$，$p< 0.05$）が認められた。暑熱環境下において尿中アラントイン排泄量は減少したが，暑熱期における摂取窒素量に対する微生物態窒素合成量の割合は，18℃ における平均63.4%から75.6%へと増加した。また，可消化有機物摂取量あたりの微生物態窒素合成量は，平均20.4から23.0gNに増加した。窒素利用効率あるいは微生物態窒素合成効率は暑熱負荷が長引くにつれて増大する傾向にあった。

キーワード：アラントイン，微生物態窒素，暑熱，消化率，乳牛