



# Impact of Rumen-Protected Niacin on Milk Production Performance and Blood Biochemical Indexes of Dairy Cows Under Mild Heat Stress

Yong Chen, Guang Wang, Xingbo Yu, Li Zhen, Yongli Qu

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## ABSTRACT

To study the influence of rumen-protected niacin on mild heat-stressed dairy cow, 30 healthy high-yielding dairy cows with similar parity were selected and randomly divided into three groups were fed the rations with 0g/d, 6g/d and 12g/d rumen-protected niacin respectively. The results showed that the dairy cows were in mild heat stress during the experiment and the time of test day significantly affected the milk yield, FCM and respiration rate of cows. Rumen-protected niacin supplement neither altered the dairy cow's body temperature, skin temperature and respiration rate, nor affected their milk yield and milk quality; however, it reduced the level of triglyceride and urea in serum.

**Key words:** Heat-stressed, Milk yield, Rumen-protected.

## INTRODUCTION

Heat stress is a key factor on milk yield of dairy cows, especially for high yielders. The dairy cow would lose appetite and change feeding behavior (Heinicke *et al.* 2018). When the temperature of stall was over 25°C, the dairy cows drank more water, reduced feed intake and the nutrient digestibility dropped (Sanchez *et al.* 1994). Wheelock *et al.* (2010) indicated that, the decline of 50-60% milk yield was due to the metabolic changes caused by heat stress. To a certain extent, the methods of regulation of nutrition for reducing heat stress were as follows: reducing fiber intake, feeding high-concentration ration and adding niacin to ration (Sinha *et al.* 2019).

Niacin mainly participated in body metabolisms and was applied to prevent or cure metabolic diseases and maintain energy balance (Schwab *et al.* 2005). The studies of Pineda *et al.* (2016) indicated rumen-protected niacin could enhance milk fat percentage and reduce dairy cow body core temperature in high-temperature environment. Niacin added to ration at suitable temperature didn't enhance the milk yield, however, trends towards increased milk yield were observed in cows fed with rumen-protected niacin during higher mean temperature-humidity index (THI) (Pineda *et al.* 2016). The research by Zimbelman *et al.* (2013) found the impact of niacin on milk yield in August was inconsistent with that in September. The different heat stress degree may be one reason for inconsistent result. Heilongjiang province locates in the farthest north and highest latitude of China, and is the most suitable place (between 43° 26' and 53° 33' of North latitude) for dairy cow; its mean temperature is about 20°C and the highest is about 30°C in summer, and July is the hottest month. In recent years, as the climate warms and dairy cows' milk field increases, in Heilongjiang, especially in the eastern region, the dairy cows' heat stress

Heilongjiang Provincial Key Laboratory of Efficient Utilization of Feed Resources and Nutrition Manipulation in Cold Region, College of Animal Science and Veterinary Medicine, Heilongjiang Bayi Agricultural University, Daqing, China

**Corresponding Author:** Yongli Qu, College of Animal Science and Veterinary Medicine, Heilongjiang Bayi Agricultural University, Daqing, China. Email: ylqu007@126.com

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in summer has been paid more and more attention. This experiment mainly studied the influence of niacin on milk performance and blood biochemical parameters of mild heat-stressed dairy cows.

## MATERIALS AND METHODS

### Experimental design and animals

Thirty healthy high-yielding dairy cows (142±7 days in milk) with similar parity were selected and randomly divided into three groups (Niacin 0 Niacin 6 and Niacin 12), which were fed the rations with 0g/d, 6g/d and 12g/d rumen-protected niacin respectively in a commercial dairy farm of Heilongjiang province. The rumen-protected rate of niacin was 85% and the release rate of small intestine was 95%. The experimental period was 60 days (from July 3 to August 31, 2015). The dairy cows were fed with TMR (according to Chen *et al.* 2019) twice a day in free stall, drank water freely and were milked twice a day. The ration compositions and nutritional ingredients were shown in Table 1.

### Thermal environment evaluation of stall

Three points above the ground 1.5m were evenly selected in the stall to record the dry-bulb temperature ( $T_d$ ) and wet-bulb temperature ( $T_w$ ) every 4 days from Day 4. The record time was 5:00, 10:00, 14:00 and 17:00 respectively. The THI was calculated by the formula  $THI = 0.72(T_d + T_w) + 40.6$  (Mcdowell *et al.* 1976). The THIs calculated at 5:00 and 14:00 were the lowest and highest respectively, and the average THI was the mean one of the four time.

### Measurement of body temperature, skin temperature and respiration rate

The skin temperature (means of waist, shoulder and tail-head) and rumen temperature were measured at 14:00 every week from Day 4 with an infrared temperature measurement replicated 3 times. The body temperature (rectal temperature) was measured with an electronic thermometer. The respiration rate (number of breaths per minute, Bpm) was the mean of three consecutive 1 min flank movement counts.

### Measurement of milk production performance and milk quality

The milk yield data were recorded and milk samples were taken every week to analyze the milk fat, protein, lactose, solid and somatic cells.

**Table 1:** Ingredient and chemical composition of basal diet.

Item	Basal diet
<b>Ingredient, % of DM</b>	
Corn silage	44.32
Alfalfa hay	8.68
Chinese wildrye	1.66
Corn	13.91
Cottonseed	5.40
Palm meal	2.70
Beet pulp	5.95
Soybean meal	14.5
Urea	0.75
Salt	0.21
Calcium bicarbonate premix <sup>1)</sup>	0.12
Total	100.00
<b>Nutrient, % of DM</b>	
Crude protein	17.82
Crude fibre	16.15
Ether extract	4.64
NDF	33.08
ADF	20.71
Ash	5.16
Ca	0.93
P	0.55

<sup>1)</sup>Zinc sulfate; ferrous sulfate; manganese sulfate; copper sulfate; sodium selenite; cobalt chloride; calcium carbonate; vitamin A; vitamin D; vitamin E, etc.

### Measurement of blood indexes

The blood (20 ml) was collected from the jugular vein each cow before feeding in the morning and the serum was prepared by centrifugation every week from Day 4. The content of triglycerides, non-esterified fatty acid (NEFA), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in serum was analyzed by ELASE kits. The content of calcium (Ca), phosphorus (P) and urea in serum were measured by a semi-automatic biochemical analyzer.

### Statistical analyses

Data were analyzed by using SAS 9.0 (SAS Institute, 2002) PROC MIXED procedures. Dependent variables were body temperature, skin temperature, rumen temperature, respiration rate, milk yield, FCM, milk fat rate, milk protein, milk lactose, milk total solids, Somatic cell count, ALT, AST, Triglyceride, NEFA, Ca, P and Urea. The independent variables included treatment, time of sampling day, and the appropriate interactions. Values of the mentioned variables were presented as means and SEM. Statistical significance and trend were set at  $P < 0.05$  and  $0.05 < P < 0.10$  for all main effects and interactions.

## RESULTS AND DISCUSSION

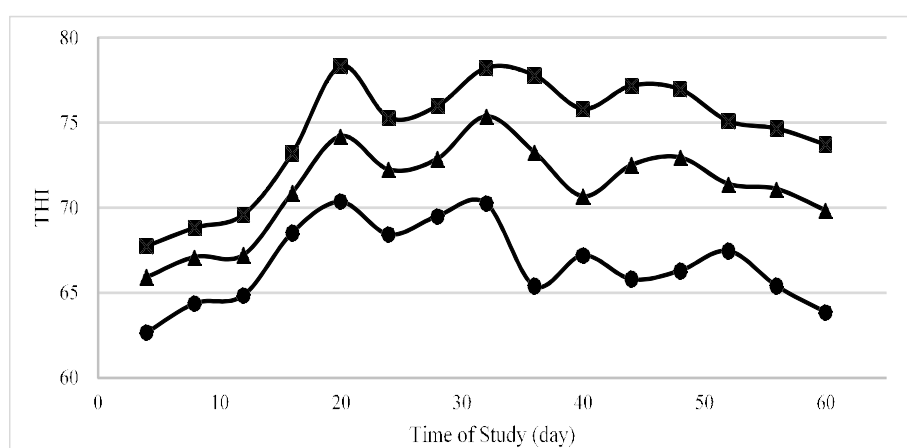
### Evaluation of THI

THI is an effective indicator commonly used to evaluate the heat stress degree. During the experiment, the highest THI in the stall was 78 and the lowest was 63 (Fig 1). Bohmanova *et al.* (2007) had shown that THI of 73–83 were thresholds of declining in milk and milk protein yield in Arizona, USA. However, the threshold of 60–70 was described for Lower Saxony, Germany (Brügemann *et al.* 2012). In present study, it was known that the dairy cows in summer of the northern region were living in the mild heat stress for most time (Sinha *et al.* 2019). Gorniak *et al.* (2014) reported that dry matter intake and milk yield of dairy cow decreased when THI rose above 60, especially above 72, moreover, milk protein and fat decreased.

The results reported by Zimbelman *et al.* (2010) showed the threshold of THI was 68 for high-yield milk cows (> 35kg/day). Heilongjiang province located in the northeast of China belongs to a temperate continental monsoon climate with distinct four seasons. In this study, the highest, lowest and average THI roughly increased first and then decreased and the data indicated the dairy cows were in mild heat stress during the summer.

### Impact of rumen-protected Niacin on physiological indexes

As shown in Table 2. Niacin supplementation neither affected body and skin temperature ( $P > 0.05$ ), nor reduced respiration rate ( $P > 0.05$ ) of dairy cow, but the sampling time had the significant influence on respiration rate ( $P < 0.05$ ). Moreover, it also didn't reduce the temperature of the rumen part ( $P > 0.05$ ), however, there was a declining tendency.



**Fig 1:** Temperature-humidity index (THI) in the stall during experiment for minimum THI (●), average THI (▲) and maximum THI (■) of the days.

**Table 2:** Effects of rumen-protected niacin on body, skin, rumen temperature and respiratory rate (breaths per minute, Bpm) in lactating dairy cows.

Item	Niacin 0	Niacin 6	Niacin 12	SEM	P value		
					Niacin	Time	Niacin×Time
Body temperature, °C	38.76	38.52	38.48	0.21	0.592	0.237	0.364
Skin temperature, °C	34.73	33.55	33.80	0.58	0.631	0.293	0.525
Rumen temperature, °C	38.22	37.55	37.49	0.64	0.091	0.350	0.154
Respiration rate, Bpm	68.95	67.43	66.78	3.17	0.127	<0.001	0.093

**Table 3:** Effects of rumen-protected niacin on milk yield and milk composition of dairy cows.

Item	Niacin 0	Niacin 6	Niacin 12	SEM	P value		
					Niacin	Time	Niacin×Time
Milk yield, kg/d	36.95	37.68	37.41	2.61	0.277	0.046	0.163
FCM, kg/d	35.41	36.51	36.46	2.76	0.130	0.037	0.276
Milk fat rate, %	3.70	3.79	3.81	0.20	0.061	0.138	0.087
Milk protein, %	3.05	3.14	3.07	0.08	0.370	0.163	0.595
Milk lactose, %	4.76	4.79	4.80	0.27	0.761	0.328	0.364
Milk total solids, %	12.21	12.48	12.05	1.76	0.371	0.201	0.193
Somatic cell count, $\times 10^3/\text{mL}$	302	324	286	78	0.402	0.284	0.517

High yield dairy cows have a very high metabolic rate and are more likely to be affected by thermal environment. Their respiration rate would be enhanced and rumination and nutrient absorption would be decreased to eliminate the excess heat and maintain body temperature, which might be the result of lack of nutrients and energy for milk production during heat stress. In northeast China, dairy cows are in a mild heat-stressed condition for most time in summer. In this experiment, the niacin added in ration did not change the body temperature and skin temperature of the dairy cows exposed to mild heat stress. The results of this experiment were consistent with those of Lohölter (2013). However, the study of Zimbelman (2010) indicated that supplementing niacin could reduce the dairy cows' rectum and vaginal temperature and alleviate the discomfort

of lactating dairy cows in thermal environment during heat-stressed period and the reason might be the THI was high (THI >72 for 12 of 24 hours/day).

In this study, niacin supplement didn't affect the skin and body temperature, however, the rumen part temperature of cow fed with niacin presented a declining tendency. Zimbelman *et al.* (2010) pointed out the water intake of cows fed with niacin was increased. The increase of water intake probably was the reason of declining tendency for niacin supplement.

#### Impact of rumen-protected Niacin on milk yield and milk composition

Milk yield and milk composition of cows fed with niacin are presented in Table 3. No effects of treatment or treatment ×

**Table 4:** Effects of niacin on biochemical indexes of lactating dairy cows.

Item	Niacin 0	Niacin 6	Niacin 12	SEM	P value		
					Niacin	Time	Niacin×Time
ALT, U/L	36.59	35.86	36.32	2.58	0.173	0.626	0.250
AST, U/L	80.62	78.17	76.83	4.49	0.146	0.338	0.171
Triglyceride, mmol/L	0.34	0.29	0.26	0.03	0.011	0.156	0.384
NEFA, mmol/L	0.18	0.19	0.18	0.02	0.635	0.320	0.298
Ca, mmol/L	2.01	2.05	2.11	0.07	0.572	0.137	0.620
P, mmol/L	1.28	1.32	1.35	0.09	0.286	0.092	0.403
Urea, mmol/L	5.38	4.76	4.23	0.22	0.044	0.088	0.118

time were observed. The present results were consistent with those of Yuan *et al.* (2011) in Wisconsin and the thermal environment in summer was similar to present study. They reported feeding 12g/d rumen-protected niacin didn't affect the body temperature and milk performance. The research made by Muller *et al.* (1986) indicated that niacin supplement to dairy cows under heat stress could significantly increase their milk yield, but didn't significantly change their milk composition. According to the research of Wrinkle *et al.* (2012), feeding 19g/d rumen-protected niacin did not increase the milk yield but reduced milk fat rate, and they pointed the reason might be niacin reduced the yield of triglycerides in the plasma. The result of Gorniak *et al.* (2014) pointed out dry matter intake decreased when THI rose above 60 and it would be the main reason for milk yield decrease. In present study, the milk and FCM yield were significantly affected by the test times, which were corresponding to the THI changes.

#### Impact of rumen-protected niacin on blood indexes

The effects of rumen-protected niacin supplement on the blood biochemical indexes of dairy cows are showed in Table 4. Feeding niacin did not change the serum content of ALT, AST, NEFA, Ca and P ( $P>0.05$ ), but significantly reduced the level of triglyceride in serum ( $P<0.05$ ) though the time of test didn't affect it. The supplement of niacin ( $P<0.05$ ) reduced the urea content. The time of test didn't influence the blood indexes, though it had a change tendency on Phosphorus (P) and urea content.

Heat stress may lead to changes of some biochemical indexes of the body, especially the content of enzymes (Huang *et al.* 2018). In normal physiological state of dairy cows, intracellular enzymes are not easy to enter blood through the barrier of cell membrane. When dairy cows are in the heat-stressed state, the cells would suffer some damages and the permeability of the cell membrane would increase and it would increase the rate that intracellular enzymes are released into the blood. ALT and AST are intracellular enzymes existing in liver cells. ALT and AST in serum would rise, when liver cells are damaged (McGill, 2016). The studies on milk goats indicated that heat stress reduced the activity of glutamate aminotransferase, glutamate aminotransferase and alkaline phosphatase in the milk goat serum (Hrkovic-Porobija *et al.* 2017). In this

experiment, it didn't observe the feeding niacin affect the level of serum ALT and AST and indicated that the liver wasn't damaged under mild heat stress.

Niacin plays an important role in lowering lipid. It can inhibit the gene expression of diacylglycerylacyltran sferase-2 to reduce the triglycerides content in serum, liver lipid and visceral fat (Hu *et al.* 2012). This characteristic can promote the metabolism of lipids under heat stress to improve body energy balance because NEFA is released in the bloodstream and is acted as an alternative energy source for other tissues (Leroy *et al.* 2008). In this experiment, niacin supplement reduced the triglyceride level in serum but did not affect the NEFA content. Hu *et al.* (2012) reviewed that the inconsistent effect of niacin on TG and NEFA might involve other mechanisms.

Calcium and phosphorus are essential mineral elements for animals. Their content would be insufficient as the decrease of feed intake during heat stress which probably cause physiological functional problems of dairy cows, and eventually lead to the decline of milk performance and reproductive performance. Studies showed that heat stress caused the acid-base imbalance of dairy cow body and the combination of secretive organic acid with  $\text{Ca}^{2+}$ , which resulted the decrease of  $\text{Ca}^{2+}$  in serum (Goff, 2006). Kamiya *et al.* (2010) pointed out the phosphorus absorption in digestive tract of dairy cows was significantly lowered due to heat stress. In the present study, the P-concentration had the change trend by time of sampling ( $P < 0.10$ ), but the contents of Ca and P weren't change as the feeding of niacin.

The urea level in blood can reflect the protein metabolism and nitrogen balance of body. In this research, the decreasing of urea content indicated that the nitrogen availability of dairy cow was improved by feeding of niacin, and it meant the niacin enhanced the protein syntheses of dairy cow to some extent under mild heat stress.

#### CONCLUSION

The dairy cows were under mild heat stress in most of daytime during the summer. Rumen-protected niacin supplement didn't improve the milk yield or milk quality of dairy cows; however, it had the trend to reduce the temperature of rumen skin and increase the milk fat rate. Moreover, the contents of serum triglycerides and urea were decreased as the feeding of niacin was added.

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