



## Original research article

## Differences of hormones involved in adipose metabolism and lactation between high and low producing Holstein cows during heat stress



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## ABSTRACT

The experiment was conducted to evaluate hormonal involvement in the adipose metabolism and lactation between high and low producing dairy cows in a hot environment. Forty Holstein healthy cows with a similar parity were used and assigned into high producing group (average production  $41.44 \pm 2.25$  kg/d) and low producing group (average production  $29.92 \pm 1.02$  kg/d) with 20 cows in each group. Blood samples were collected from caudal vein to determine the difference of hormones related to adipose metabolism and lactation. The highest, lowest, and average temperature humidity index (THI), recorded as 84.02, 79.35 and 81.89, respectively, indicated that cows were at the state of high heat stress. No significant differences between high and low producing groups were observed in the levels of nonesterified fatty acid (NEFA),  $\beta$ -hydroxybutyrate ( $\beta$ -OHB), total cholesterol (TCHO), and insulin (INS) ( $P > 0.05$ ). However, the very low density lipoprotein (VLDL), apolipoprotein B100 (apoB-100), high-density lipoprotein (HDL-C) and estrogen ( $E_2$ ) concentrations in high producing group were significantly higher than those of low producing group ( $P < 0.05$ ). No significant differences between high and low producing groups were observed in the levels of prolactin (PRL) and progesterone (PROG) ( $P > 0.05$ ), whereas high producing group had a rise in the insulin-like growth factor-1 (IGF-1) level compared with low producing group ( $P < 0.05$ ). These results indicated that, during summer, high and low producing dairy cows have similar levels of lipid catabolism, but high producing dairy cows have advantages in outputting hepatic triglyceride (TG).

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## 1. Introduction

The appropriate ambient temperature for lactating cows ranges from 5 to 25°C, above which cows' core body temperature begins to rise, leading to heat stress response (Ray et al., 1992). Over the past several decades, major advances in environmental cooling systems (Ortiz et al., 2015) and nutritional regulation have helped to ameliorate production losses, metabolic disorders and health problems during summer months. However, heat stress continues to be a challenge for global dairy industry (St-Pierre et al., 2003). In

response to heat stress, dairy cows take metabolic adaptation measures to reduce heat load, such as elevating respiration rate (RR) and concomitantly reducing feed intake (Havlin and Robinson, 2015; Baumgard et al., 2011). Due to decreased feed intake, decreased energy intake cannot meet milk energy output with the consequence of negative energy balance, which has traditionally been assumed to be primarily responsible for decreases in milk production. Adipose tissues have been well known as an important regulator in energy balance and body weight, and it is mobilized aiming to compensate for the deficient induced by decreased feed intake in response to heat stress (Lanthier and Leclercq, 2014). Considering the rise in energy requirement of high producing dairy cows in lactation is parallel with increase in milk production, thereby aggravating negative energy balance, we hypothesized that lipolysis in high producing cows is more intense compared with low producing cows to maintain greater performance. In addition, secretions of prolactin (PRL), estrogen ( $E_2$ ) and progesterone (PROG) involved in the formation of mammary gland are inhibited in summer, which would have a negative effect on the formation of acinus and ductal system, and contribute to lower milk production.

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Accordingly, the aim of the present study was to obtain insights into adipose metabolism characteristics and the levels of hormones associated to lactation in high and low producing cows in a hot environment by determining the concentration of factors related to lipid metabolism and lactation in plasma and providing theoretical reference for improving the performance of low producing cows in summer.

## 2. Materials and methods

### 2.1. Experimental design and animals

The experiment was conducted at a farm located at Jiangsu province from 20 July to 20 August. Forty multiparous Holstein cows with similar ages, parities and lactation days were used and randomly assigned into high producing group (average production  $41.44 \pm 2.25$  kg/d) and low producing group (average production  $29.92 \pm 1.02$  kg/d) with 20 cows in each (Table 1).

**Table 1**

Experimental cows were selected by milk production, parity, day in milk (DIM).

Item	Milk production, kg/d	Parity	DIM, d
High producing dairy cows	$41.44 \pm 2.25$	2	$168 \pm 32$
Low producing dairy cows	$29.92 \pm 1.02$	2	$174 \pm 46$

### 2.2. Diet ingredients and herd management

The cows were housed in a tie stall barn. Fans and sprinklers were used to cool the barn. The cows were fed total mixed ration 3 times daily. The ingredient and chemical composition of basal diet are shown in Table 2. The cows were milked 3 times daily (0730, 1130 and 1730 h) and the milk productions were recorded after each milking.

### 2.3. Blood sampling

Blood samples were collected via caudal vein before feeding on the last day of the experiment. Samples were cooled on wet ice, centrifuged at  $3,000 \times g$  for 10 min at room temperature. The plasmas were aliquoted into 1.5-mL eppendorf tubes and stored at  $-20^\circ\text{C}$  till further analysis.

### 2.4. Parameters and methods

#### 2.4.1. Temperature and humidity index measurements

Five relative humidity and temperature sensors (Tianjin meteorological instrument corporation, DHM-2A type, temperature ranges from  $-36$  to  $+46^\circ\text{C}$ , relative humidity ranges from 10 to 100%) were placed in different locations, 1.5 m above the floor and out of direct sunlight. Data were recorded at 0800, 1400 and 2000 h. Temperature humidity index (THI) was calculated as:  $\text{THI} = 0.72(T_d + T_w) + 40.6$ , where  $T_d$  is dry bulb air temperature, and  $T_w$  is wet bulb air temperature (Thom, 1959).

#### 2.4.2. Rectal temperature and respiratory rate measurements

Rectal temperature (RT) and respiratory rate (RR) were determined at 0800, 1400 and 2000 h daily. Respiratory temperatures were detected using animal thermometer. Respiratory rates were determined by observing flank movements of the cows. Numbers of breaths in a 3-consecutive-minute period were recorded and then converted to breaths/min.

**Table 2**

Ingredient and nutrient composition of basal diets (DM basis).

Item	Amount
<b>Ingredient, %</b>	
Energy feed	
Corn	17.20
Brewer's dried grain	8.99
Corn silage	23.38
Dry alfalfa	13.02
Oat hay	1.80
<i>Leymus chinensis</i>	1.69
Protein feed	
Beet pulp	5.40
Cottonseed	3.75
Soybean meal	8.53
DDGS	12.30
Mineral feed	
NaHCO <sub>3</sub>	0.67
NaCl	10.45
Limestone	0.78
CaHPO <sub>4</sub>	1.25
Premix <sup>1</sup>	0.74
Total	100.00
<b>Nutrient composition, %<sup>2</sup></b>	
CP	15.30
NE <sub>L</sub> , MJ/kg	6.65
EE	3.36
Neutral detergent fiber	46.23
Acid detergent fiber	26.74
Ash	3.74
Ca	0.98
Phosphorus	0.47

DDGS = distillers dried grains with soluble; CP = crude protein; NE<sub>L</sub> = net energy for lactating; EE = ether extract.

<sup>1</sup> Premix provided per kilogram of diet: VA 1,350,000 IU; VD<sub>3</sub> 275,000 IU; VE 330,000 IU; nicotinic acid 2,700 mg; Cu 1,600 mg; Fe 4,700 mg; Mn 4,200 mg; Zn 8,500 mg; Se 80 mg; Co 60 mg.

<sup>2</sup> The value of NE<sub>L</sub> was calculated, other values were measured.

### 2.4.3. Blood biochemical parameters

Nonesterified fatty acid (NEFA) level in serum was detected by chemical colorimetric. High-density lipoprotein (HDL-C) concentration was measured with selective precipitation method. Enzyme treatment was used to determine total cholesterol (TCHO) level. Antibody-sandwich enzyme-linked immunosorbent assay (ELISA) was used to detect the very low density lipoprotein (VLDL), apolipoprotein B100 (apoB-100),  $\beta$ -hydroxybutyrate ( $\beta$ -OHB), insulin-like growth factor-1 (IGF-1), E<sub>2</sub>, PROG, PRL and glucocorticoid concentrations. Insulin (INS) was detected by radioimmunoassay. Above kits were purchased from Nanjing Jiancheng Institute of Biology except INS kit from Beijing North Institute of Biotechnology.

### 2.5. Statistical analysis

All the statistical analysis was conducted using a SPSS statistical software program (version. 17.0 for windows, SPSS; IBM SPSS Company, Chicago, IL, USA). Data were subjected to one-way analysis of variance (ANOVA) and the differences between mean values between the different groups were identified by the least significant difference (LSD). Differences with a *P*-value lower than 0.05 were considered as significant. Data presented are means  $\pm$  SEM.

## 3. Results

### 3.1. Temperature and humidity index

During the experiment period, THI observed were between 76.15 and 88.34. Average daily THI were 81.89 with the lowest of

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