

## Effects of Cysteamine Hydrochloride on Production Performance of Mid-lactation Dairy Cows under Heat Stress (Postprint)

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### Abstract

This experiment aimed to investigate the effects of dietary cysteamine hydrochloride supplementation on the production performance of mid-lactation heat-stressed dairy cows under free-stall housing conditions. Forty-eight healthy Holstein dairy cows with body weight ( $562.25 \pm 37.85$ ) kg, days in milk ( $123.64 \pm 10.67$ ) d, parity of  $1.23 \pm 0.42$ , and daily milk yield ( $26.58 \pm 1.58$ ) kg were selected and randomly allocated into 4 groups (12 cows per group) based on similar body weight, days in milk, and milk yield. The control group was fed a basal diet, while the experimental groups were supplemented with cysteamine hydrochloride at 3, 15, and 30 g/kg of the basal diet, respectively. The pre-trial period lasted 12 d, followed by a 30-d formal trial period. The results showed that with increasing supplementation levels of cysteamine hydrochloride, respiratory rate and rectal temperature of dairy cows decreased linearly and significantly ( $P < 0.05$ ). Dry matter intake, fat-corrected milk yield, energy-corrected milk yield, milk fat percentage, and milk protein percentage exhibited a significant quadratic response of initially increasing and then decreasing ( $P < 0.05$ ), with the 3 g/kg group showing the optimal effects. At the end of the formal trial period, serum thyroxine concentration in the 3 and 15 g/kg groups was significantly lower than that in the control group ( $P < 0.05$ ), while serum cortisol concentration in the 15 g/kg group was significantly higher than that in the control group ( $P < 0.05$ ). These results indicate that cysteamine hydrochloride can effectively alleviate heat stress in mid-lactation dairy cows under free-stall housing conditions, improving milk yield and milk quality. Under the conditions of this experiment, dietary supplementation with 3 g/kg cysteamine hydrochloride is recommended.

## Full Text

### Effects of Cysteamine Hydrochloride on Production Performance of Heat-Stressed Mid-Lactating Dairy Cows

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## Abstract

This study investigated the effects of dietary cysteamine hydrochloride (CSH) supplementation on production performance of mid-lactating dairy cows experiencing heat stress under free-stall housing conditions. Forty-eight healthy Holstein cows in mid-lactation were selected with body weight of  $(562.25 \pm 37.85)$  kg, days in milk of  $(123.64 \pm 10.67)$  d, parity of  $1.23 \pm 0.42$ , and daily milk yield of  $(26.58 \pm 1.58)$  kg. Based on similar body weight, lactation stage, and milk production, cows were randomly allocated to four groups ( $n=12$  per group): a control group fed the basal diet, and three treatment groups receiving the basal diet supplemented with CSH at 3, 15, or 30 g/kg. The experiment consisted of a 12-day pre-trial period followed by a 30-day formal trial period.

The results demonstrated that increasing CSH supplementation levels produced a significant linear decrease in both respiratory rate and rectal temperature ( $P < 0.05$ ). Dry matter intake, 4% fat-corrected milk yield, energy-corrected milk yield, milk fat percentage, and milk protein percentage exhibited significant quadratic responses ( $P < 0.05$ ), initially increasing then decreasing with higher doses, with the 3 g/kg group showing the most favorable effects. At the end of the trial, serum thyroxine concentrations in the 3 and 15 g/kg groups were significantly lower than in the control group ( $P < 0.05$ ), while serum cortisol concentration in the 15 g/kg group was significantly higher ( $P < 0.05$ ).

These findings indicate that CSH effectively alleviates heat stress in mid-lactating cows housed in free-stall barns, improving both milk yield and milk quality. Under the conditions of this experiment, dietary supplementation with 3 g/kg CSH was determined to be optimal.

**Keywords:** cysteamine hydrochloride; heat stress; milk yield; milk composition; serum hormone

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## Introduction

Cysteamine functions by depleting somatostatin *in vivo*, thereby attenuating its inhibitory effect on growth hormone secretion, which leads to increased secretion of growth hormone and insulin. It also enhances growth hormone secretion by inhibiting the conversion of dopamine to norepinephrine and regulating  $\beta$ -endorphin levels [1]. Growth hormone plays a crucial physiological role in promoting animal growth and lactation. Recent studies have demonstrated that cysteamine supplementation can enhance growth performance in poultry, swine, fish, meat sheep, and buffalo [2-7]. Numerous studies have investigated the effects of sole cysteamine supplementation in ruminant diets with promising results. Cysteamine promotes mammary gland development and ductal growth [8], thereby increasing milk yield and improving milk quality in dairy cows [9-10]. Additionally, cysteamine elevates heat shock protein (HSP) levels in astrocytes and glioma cells [11] and reduces heat shock-induced sperm damage and oxidative stress in oocytes during *in vitro* fertilization [12].

Cysteamine hydrochloride (CSH) is a feed additive encapsulated with  $\beta$ -cyclodextrin using supramolecular technology. Previous research has shown that CSH supplementation in late-lactation heat-stressed cows reduces body temperature and increases milk yield [13-14]. Under tie-stall housing conditions, mid-lactation heat-stressed cows fed CSH exhibited decreased serum triiodothyronine (T<sub>3</sub>) concentrations and increased fat-corrected milk yield and milk fat percentage [15]. However, limited information is available regarding the effects of CSH on lactation performance of heat-stressed dairy cows, necessitating further investigation. This study evaluated the effects of different CSH dosages on production performance, respiratory rate, and rectal temperature in mid-lactating heat-stressed cows under free-stall conditions. The findings possess practical application value and contribute to improving nutrient utilization efficiency and production performance in dairy cattle.

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## Materials and Methods

**1.1 Experimental Material** CSH is a milky-white microparticle produced using microencapsulation technology, with  $\beta$ -cyclodextrin as the main material and an active ingredient content of 30%, manufactured by Shanghai Huakuoda Biochemical Technology Co., Ltd.

**1.2 Experimental Animals** Forty-eight healthy Holstein dairy cows were selected with body weight of  $(562.3 \pm 37.9)$  kg, days in milk of  $(123.6 \pm 10.7)$  d, parity of  $1.23 \pm 0.42$ , and daily milk yield of  $(26.6 \pm 1.6)$  kg.

**1.3 Experimental Design** A randomized block design was employed. Based on similar body weight, parity, lactation stage, and milk yield, the 48 cows were randomly divided into four groups (n=12 per group). The control group received the basal diet, while the treatment groups received the basal diet supplemented with CSH at 3 g/kg (CSH3), 15 g/kg (CSH15), or 30 g/kg (CSH30). The experiment comprised a 12-day pre-trial period and a 30-day formal trial period.

**1.4 Basal Diet and Management** The composition and nutrient levels of the basal diet are presented in Table 1, with nutrient levels meeting or exceeding requirements for cows producing 25 kg of milk daily. Cows were fed total mixed rations (TMR) three times daily at 06:30, 13:30, and 18:30. Milkings occurred three times daily at 05:00, 13:00, and 21:00 using an Afimilk pipeline system. Free-stall housing was employed with partition boards separating feed bunks. CSH was thoroughly mixed into the TMR according to group allocation. Feed was pushed once after feeding, with ad libitum access to feed and water.

## 1.5 Measurements

**1.5.1 Temperature-Humidity Index (THI)** Thermohygrometers were installed 1.5 m above ground level in the middle of each group's feed bunk. Temperature and humidity were recorded daily at 05:30, 13:30, and 21:30 to calculate THI using the formula:  
$$\text{THI} = 0.81T + (0.99T - 14.3)R + 46.3$$
 [16], where T is temperature (°C) and R is relative humidity (%).

**1.5.2 Respiratory Rate and Rectal Temperature** Respiratory rate and rectal temperature were measured for individual cows for three consecutive days during the week before the formal trial and weekly during the trial period at 07:30, 14:00, and 21:30 (including rainy days). Respiratory rate was manually recorded as breaths per minute using a timer, and rectal temperature was measured using an electronic thermometer.

**1.5.3 Dry Matter Intake** During the trial period, individual cow feed intake was measured for three consecutive days each week. Equal amounts of TMR were provided to each cow with fixed feeding positions. After 1.0-1.5 h, feed was pushed once, and residual feed was weighed individually after 2 h of feeding. TMR and residual feed samples were collected daily, quartered, and stored at -20°C. Samples were dried at 65°C for 48 h to determine initial moisture content, ground through a 20-mesh sieve, quartered again, and approximately 300 g was further ground through a 40-mesh sieve. Dry matter content was determined by drying at 105°C for 4 h to calculate dry matter intake.

**1.5.4 Milk Yield and Composition** Daily milk yield was automatically recorded by the Afimilk system, with operational status monitored and abnormalities documented. Milk samples were collected every 7 days, mixing morning,

midday, and evening samples in a 4:3:3 ratio. Fifty milliliters of each composite sample were preserved with potassium dichromate and analyzed for milk fat percentage, milk protein percentage, lactose percentage, and somatic cell count using FOSS milk composition and somatic cell analyzers at the Ministry of Agriculture-Milk and Dairy Product Inspection Center (Beijing). Four percent fat-corrected milk and energy-corrected milk yields were calculated using the following formulas:

$$4\% \text{ FCM yield (kg/d)} = 0.4 \times \text{milk yield (kg/d)} + 15 \times \text{milk fat yield (kg/d)} \quad [17]$$

$$\text{ECM yield (kg/d)} = 0.327 \times \text{milk yield (kg/d)} + 12.95 \times \text{milk fat yield (kg/d)} + 7.20 \times \text{milk protein yield (kg/d)} \quad [18]$$

**1.5.5 Serum Hormone Concentrations** Blood samples were collected from the tail vein one day before and on the final day of the formal trial period. Serum was separated by centrifugation at  $940\times g$  for 15 min at  $4^{\circ}\text{C}$  (CR22G centrifuge, HITACHI). Serum hormone concentrations were measured using assay kits produced by Beijing Northern Biotechnology Institute for cortisol, insulin, triiodothyronine (T<sub>3</sub>), thyroxine (T<sub>4</sub>), and thyroid-stimulating hormone (TSH).

**1.5.6 Dietary Nutrient Levels** Crude protein content was determined using a FOSS Kjeldahl nitrogen analyzer. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) contents were analyzed using a FOSS fiber analyzer. Ether extract content was measured using a FOSS Soxhlet fat extraction apparatus. Phosphorus content was determined by atomic absorption spectrophotometry, and calcium content was measured according to GB/T 13885.

**1.6 Statistical Analysis** Data were analyzed using the PROC MIXED procedure of SAS 9.2 software. Multiple comparisons were performed using Tukey's test, with significance declared at  $P < 0.05$ .

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## Results

**2.1 Temperature-Humidity Index** As shown in Figure 1 [Figure 1: see original paper], THI values remained above 68 for all time points except for a few instances after August 30, 2013, when daily average temperature dropped below  $25^{\circ}\text{C}$ . Daily average THI values exceeded 72 throughout the trial period. Since the optimal THI for dairy cows is below 68, the cows were confirmed to be in a state of heat stress.

**2.2 Rectal Temperature and Respiratory Rate** Table 2 shows that increasing CSH supplementation levels produced a significant linear decrease in both respiratory rate and rectal temperature ( $P < 0.05$ ), with the lowest values

observed in the CSH30 group. The CSH3 group exhibited slightly elevated respiratory rate compared to the control group, though the difference was not significant ( $P>0.05$ ). Rectal temperature showed a decreasing trend in the CSH3 group, but this difference was also not significant ( $P>0.05$ ).

**2.3 Dry Matter Intake and Milk Yield** Dry matter intake in the CSH3, CSH15, and CSH30 groups increased by approximately 1.55, 1.02, and 0.37 kg/d compared to the control group, respectively, with significant differences observed between the CSH3 and CSH15 groups and the control group ( $P<0.05$ ). The CSH30 group was significantly lower than both the CSH3 and CSH15 groups ( $P<0.05$ ) (Table 3). During weeks 0-2 when cows experienced severe heat stress, the CSH3 group maintained higher dry matter intake than other groups despite reductions (Figure 2 [Figure 2: see original paper]).

The 4% fat-corrected milk yield and energy-corrected milk yield were significantly higher in all treatment groups compared to the control group ( $P<0.05$ ), but exhibited significant quadratic responses ( $P<0.05$ ) as CSH supplementation increased (Table 3). Throughout the trial period, the CSH3 group consistently maintained higher milk yield (Figure 3 [Figure 3: see original paper]).

**2.4 Milk Composition** Table 4 reveals that milk fat percentage, milk protein percentage, and milk protein yield showed significant quadratic responses ( $P<0.05$ ), initially increasing then decreasing with higher CSH doses. The CSH3 group increased milk fat percentage by 9.61% compared to the control group ( $P<0.05$ ), while CSH15 and CSH30 groups showed non-significant improvements. Milk protein percentage in the CSH3 and CSH15 groups increased by 5.02% and 3.68%, respectively, compared to the control group ( $P<0.05$ ). All treatment groups significantly increased milk protein yield compared to the control group ( $P<0.05$ ), with a significant linear increase as CSH dosage increased ( $P<0.05$ ). Milk somatic cell count showed a significant linear increase with dosage ( $P<0.05$ ), though differences among groups were not significant ( $P>0.05$ ). No significant differences were observed in lactose percentage or lactose yield among groups ( $P>0.05$ ).

**2.5 Serum Hormone Concentrations** Prior to the formal trial period, no significant differences existed among groups in serum cortisol, insulin, triiodothyronine, thyroxine, or thyroid-stimulating hormone concentrations ( $P>0.05$ ). At the end of the trial period, serum cortisol concentration exhibited a significant quadratic response ( $P<0.05$ ), initially increasing then decreasing with higher CSH doses. Serum triiodothyronine and thyroxine concentrations also showed significant quadratic responses ( $P<0.05$ ), but with an opposite pattern.

The CSH15 group increased serum cortisol concentration by 27.61% compared to the control group ( $P<0.05$ ). The CSH3 and CSH15 groups reduced serum triiodothyronine concentration compared to the control group, though not significantly ( $P>0.05$ ), while significantly reducing serum thyroxine concentration

by 22.00% and 25.66%, respectively ( $P < 0.05$ ), with no significant difference between these two groups ( $P > 0.05$ ). Serum insulin concentration in the CSH15 and CSH30 groups was lower than the control group, but the difference was not significant ( $P > 0.05$ ).

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## Discussion

Hot summer environments consistently impact dairy cow production performance, with THI serving as a robust indicator of environmental heat load. Heat stress begins when THI exceeds 68, reaching severe levels at 76 [19], with milk yield decreasing by 0.2 kg for every unit increase in THI above 72 [20]. In this study, daily average THI values in the barn remained above 72 throughout the trial period, confirming that cows were under continuous heat stress. Heat stress typically increases respiratory rate and rectal temperature in dairy cows; CSH supplementation significantly reduced both parameters, consistent with findings by Shen et al. [13] that CSH decreased rectal temperature in dairy cows.

Insulin enhances mammary blood flow and improves amino acid utilization and glucose uptake, thereby increasing milk yield and milk protein production [21-22]. In this study, 4% fat-corrected milk yield, energy-corrected milk yield, milk fat percentage, and milk protein percentage were highest in the CSH3 group, with reductions observed in the CSH15 and CSH30 groups. This may be related to lower serum insulin concentrations in the latter two groups compared to both the control and CSH3 groups. Previous research has demonstrated that cysteamine depletes somatostatin and promotes growth hormone synthesis and secretion [23], which enhances mammary gland development and increases milk yield while improving milk fat and protein synthesis. However, some studies indicate that high doses of cysteamine may inhibit growth hormone secretion [24]. The reduced milk yield observed at CSH supplementation levels of 15 and 30 g/kg compared to 3 g/kg may result from growth hormone suppression at these higher doses.

Under heat stress conditions, dairy cows reduce heat production by decreasing thyroid activity, resulting in lower blood triiodothyronine and thyroxine concentrations [19]. In this study, supplementation with 3 and 15 g/kg CSH significantly reduced serum thyroxine concentration and tended to reduce serum triiodothyronine concentration, indicating further reduction in heat production and demonstrating CSH's heat stress-alleviating effects. Research has shown that blood thyroid hormone concentrations are negatively correlated with milk yield in lactating cows [25]. The current study observed that serum triiodothyronine and thyroxine concentrations initially decreased then increased with higher CSH doses, while milk yield showed the opposite pattern, consistent with previous reports.

Cortisol is an important indicator of heat stress, though reports on its changes in heat-stressed dairy cows have been inconsistent. Some studies report signifi-

cantly decreased blood cortisol concentrations [26-27], while others suggest that cortisol increases during acute heat stress but decreases during chronic heat stress [28]. Previous research found no significant effect of CSH on blood cortisol in tie-stall housed heat-stressed cows [15]. In contrast, this study showed that 3 and 15 g/kg CSH increased serum cortisol compared to the control group, with a significant effect at 15 g/kg. Blood cortisol concentration is regulated by both temperature and energy balance, and its elevation may facilitate tissue repair and metabolic adjustment, playing an important role in protecting the body against harmful stimuli.

Under free-stall housing conditions, CSH supplementation significantly increased dry matter intake, 4% fat-corrected milk yield, and energy-corrected milk yield in mid-lactating heat-stressed cows while improving milk fat and protein percentages. The effects of CSH in heat-stressed cows appear to be dose-dependent, with dietary supplementation of 3 g/kg CSH being optimal under the conditions of this study.

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