Effects of Lonicera japonica Extract on Serum Hormones and Antioxidant Indices in Heat-Stressed Beef Cattle: Postprint

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Date: 2017-10-10T00:00:00+00:00

Abstract

This study aimed to investigate the effects of dietary honeysuckle extract supplementation on serum hormonal and antioxidant indices in heat-stressed beef cattle under summer high-temperature conditions. Twenty healthy Jinjiang yellow cattle with a body weight of (360±20) kg were selected and allocated into 4 groups, with 0 (control), 0.2%, 0.4%, and 0.6% honeysuckle extract added to the concentrate, respectively. Each group consisted of 5 replicates with 1 animal per replicate. The preliminary feeding period was 10 d, and the formal experimental period was 60 d. The results showed that, compared with the control group: 1) On day 40, the body temperature of cattle in the 0.2% and 0.6% groups was significantly lower than that of the control group ($P<0.05$). Honeysuckle extract had no significant effect on daily weight gain of beef cattle ($P>0.05$). 2) Serum triiodothyronine (T3) and thyroxine (T4) contents in the treatment groups increased, while cortisol (COR) content decreased. 3) Serum total antioxidant capacity (T-AOC), glutathione peroxidase (GSH-Px), and superoxide dismutase (SOD) activities in the treatment groups all increased, while malondialdehyde (MDA) content decreased. These results suggest that honeysuckle extract can improve antioxidant capacity and alleviate heat stress responses in beef cattle, but has no significant effect on weight gain. Based on comprehensive evaluation of all indices, supplementation at 0.2% in concentrate is considered appropriate.

Full Text

Abstract

This study was conducted to investigate the effects of honeysuckle extract on serum hormones and antioxidant indices in beef cattle under summer heat stress

conditions. Twenty healthy Jinjiang yellow cattle with an initial body weight of (360±20) kg were divided into four groups (n=5 per group, one animal per replicate). Honeysuckle extract was supplemented in the concentrate at levels of 0 (control), 0.2%, 0.4%, and 0.6%. The experiment consisted of a 10-day pre-trial period followed by a 60-day formal trial period. The results showed that: (1) On day 40, body temperature in the 0.2% and 0.6% groups was significantly lower than in the control group ($P<0.05$); however, honeysuckle extract had no significant effect on daily weight gain ($P>0.05$). (2) Serum triiodothyronine (T3) and thyroxine (T4) concentrations increased, while cortisol (COR) concentration decreased in the treatment groups. (3) Serum total antioxidant capacity (T-AOC), glutathione peroxidase (GSH-Px) activity, and superoxide dismutase (SOD) activity all increased, while malondialdehyde (MDA) content decreased. These findings indicate that honeysuckle extract can improve antioxidant capacity and alleviate heat stress responses in beef cattle, though it has no significant effect on weight gain. Based on comprehensive evaluation of all indices, supplementation at 0.2% in the concentrate is considered optimal.

Keywords: honeysuckle extract; high temperature; beef cattle; body temperature; hormones; antioxidant enzymes

Introduction

In modern intensive beef cattle production systems, stress has become one of the most important factors affecting animal performance. Among various stressors, heat stress is the most widespread and often unavoidable challenge for beef cattle. Previous studies have reported that heat stress can cause reduced feed intake, poor feed utilization, slow growth, and in severe cases, metabolic disorders or even death, resulting in substantial economic losses to beef production [1]. To mitigate these effects, researchers have investigated both environmental control measures and dietary interventions using nutritional or non-nutritional additives to modulate animal metabolism. Certain vitamins such as vitamin C, vitamin A, and -carotene can alleviate heat stress by scavenging excess oxygen free radicals and enhancing antioxidant enzyme activity [2]. However, vitamin additives are prone to oxidative degradation during use, have low absorption and utilization rates, and their optimal dosage is difficult to estimate. Excessive supplementation of vitamin A and -carotene may also reduce intramuscular fat content and cause yellow discoloration of meat [3].

Honeysuckle (*Lonicera japonica*) has traditional medicinal properties for clearing heat, detoxifying, and resolving dampness. Research by Zhi Dexian et al. [4] demonstrated that the active components of honeysuckle—flavonoids and chlorogenic acid—are natural antioxidants that can scavenge superoxide anion free radicals and protect tissues from oxidative damage. Given these pharmacological properties, honeysuckle may overcome the negative effects reported with other additives used for heat stress relief. However, no studies have yet reported on the anti-heat stress effects of honeysuckle or its extracts in animals. This experiment used Jinjiang yellow cattle to investigate the effects of dietary hon-

eysuckle extract supplementation on body temperature, serum hormones, and antioxidant indices in heat-stressed beef cattle, aiming to provide a theoretical basis for the application of plant extracts in heat stress prevention.

Materials and Methods

1.1.2 Honeysuckle Extract

Honeysuckle extract was purchased from a biotechnology company in Xi' an. High-performance liquid chromatography (HPLC) analysis determined that the main active component, chlorogenic acid, comprised 19.01% of the extract.

1.1.3 Experimental Diets

The experimental diets consisted of concentrate and roughage. The composition and nutrient levels of the concentrate are shown in Table 1 . Roughage consisted of dried distillers grains and king grass. The comprehensive net energy of the concentrate was calculated according to the *Chinese Feed Composition and Nutrient Value Tables* [5], while other nutrient levels were determined using conventional analytical methods from *Experimental Course of Animal Nutrition* [6]. Crude protein content was determined by the Kjeldahl method, phosphorus by molybdenum yellow colorimetry, and calcium by potassium permanganate titration.

1.2 Experimental Design and Management

Twenty Jinjiang yellow cattle were randomly divided into four groups with five replicates per group (one animal per replicate). The groups received honeysuckle extract supplementation in the concentrate at levels of 0 (control), 0.2%, 0.4%, and 0.6%. The experiment was conducted at Yufeng Agricultural and Livestock Co., Ltd. in Gao' an, Jiangxi Province from July 1 to September 8, 2014, comprising a 10-day pre-trial period and a 60-day formal trial period. During the trial, all cattle were housed in the same barn, tethered individually by nose rings, and managed by the same caretaker. Prior to the experiment, all animals were treated for internal and external parasites using conventional methods. Daily management was identical across groups: feeding occurred twice daily at 07:00 and 18:00. Concentrate and dried distillers grains were fed at restricted amounts of 3.5 kg and 5 kg per animal per day, respectively. After complete consumption of these feeds, king grass was provided *ad libitum*, and water was freely available.

1.3.1 Barn Temperature-Humidity Index (THI)

Thermohygrometers were hung on both side walls of the barn at animal height (approximately 1.5 m from ground) to record temperature and relative humidity at 08:00, 14:00, and 20:00 daily during the formal trial period. THI was calculated using the method reported by Berman [7]:

THI = td - (0.55 - 0.55 × RH) × (td - 58)

where td is temperature in Fahrenheit (td = Celsius × 9/5 + 32) and RH is relative humidity. THI < 72 indicates no heat stress; 72 THI 79 indicates mild heat stress; 79 < THI 88 indicates severe heat stress; and THI > 88 indicates extreme heat stress.

1.3.2 Body Temperature

Rectal temperature of all experimental animals was measured using a digital thermometer on days 1, 20, 40, and 60 of the trial.

1.3.3 Daily Weight Gain

Animals were weighed at 06:00 on days 1 and 60 after overnight fasting to calculate daily weight gain.

1.3.4 Serum Hormone and Antioxidant Index Detection

On days 20, 40, and 60 of the formal trial period, 20 mL of blood was collected from the jugular vein of each animal after overnight fasting. Serum was prepared by centrifugation at 1,048 × g for 15 minutes and stored at -20 °C for subsequent analysis. Serum hormone concentrations [triiodothyronine (T3), thyroxine (T4), and cortisol (COR)] were measured by radioimmunoassay at the Second Affiliated Hospital of Nanchang University. Serum antioxidant indices [total antioxidant capacity (T-AOC), glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD) activities, and malondialdehyde (MDA) content] were determined using assay kits from Nanjing Jiancheng Bioengineering Institute.

1.4 Statistical Methods

Data were analyzed using one-way ANOVA procedure in SPSS 17.0 software. Duncan' s multiple range test was used for post-hoc comparisons. Results are expressed as mean ± standard deviation.

Results

2.1 Barn THI Under Summer High Temperature Conditions

Environmental THI reflects the combined effects of temperature and humidity on heat exchange in cattle. Generally, heat stress occurs when ambient temperature exceeds 25 °C and THI exceeds 72. As shown in Table 2 , barn THI remained above 72 throughout the experimental period, exceeding the critical threshold for heat stress in beef cattle. Therefore, the summer high temperature and humidity environment in this study induced heat stress in the experimental animals.

2.2 Effects of Honeysuckle Extract on Daily Weight Gain

As shown in Table 3 , daily weight gain in the treatment groups showed no significant difference compared with the control group (P>0.05), although an increasing trend was observed, with the 0.6% group showing the highest value.

2.3 Effects of Honeysuckle Extract on Body Temperature

Figure 1 [Figure 1: see original paper] shows that on days 1, 20, and 60, body temperature in the treatment groups did not differ significantly from the control group (P>0.05). However, on day 40, body temperature in the 0.2% and 0.6% groups was significantly lower than in the control group (P<0.05).

2.4 Effects of Honeysuckle Extract on Serum Hormone Concentrations

As shown in Table 4 , compared with the control group, serum T3 concentration in the treatment groups showed no significant difference on day 20 (P>0.05). On day 40, T3 concentrations in the 0.2%, 0.4%, and 0.6% groups increased by 30.53%, 22.11%, and 23.16%, respectively (P<0.05). On day 60, only the 0.2% group showed a 39.08% increase in T3 concentration compared with the control group (P<0.05). Serum T4 concentration in the 0.2% group increased by 14.33%, 45.97%, and 32.98% on days 20, 40, and 60, respectively (P<0.05). The 0.4% group showed 28.68% and 24.37% increases on days 40 and 60 (P<0.05), while the 0.6% group showed 33.60% and 23.53% increases on days 40 and 60 (P<0.05). Serum cortisol concentration in the 0.2% group decreased by 20.45% and 29.41% on days 20 and 40 (P<0.05). The 0.4% group showed a 15.22% decrease on day 20 (P<0.05), and the 0.6% group decreased by 23.26% and 19.23% on days 20 and 60 (P<0.05).

2.5 Effects of Honeysuckle Extract on Serum Antioxidant Indices

As shown in Table 5 , serum GSH-Px activity in the treatment groups showed an increasing trend on day 20, but the difference was not significant (P>0.05). On day 40, the 0.2% group showed a 12.73% increase compared with the control group (P<0.05), while other treatment groups did not differ significantly (P>0.05). On day 60, the 0.2% and 0.6% groups increased by 17.94% and 15.37%, respectively (P<0.05), while the 0.4% group showed no significant change (P>0.05). Serum T-AOC in the treatment groups showed an increasing trend on day 20 (P>0.05). On day 40, the 0.2% group increased by 17.64% (P<0.05). On day 60, the 0.2%, 0.4%, and 0.6% groups increased by 34.62%, 21.40%, and 20.57%, respectively (P<0.05). Serum MDA content in the 0.2% and 0.6% groups decreased by 34.96% and 34.36% on day 20 (P<0.05), by 51.28% and 35.94% on day 40 (P<0.05), and by 38.20% and 29.07% on day 60 (P<0.05). Serum SOD activity in the 0.2%, 0.4%, and 0.6% groups increased by 17.72%, 13.97%, and 19.11% on day 40, respectively. However, on days 20

and 60, although treatment groups showed an increasing trend, no significant differences were observed compared with the control group (P>0.05).

Discussion

3.1 Anti-Heat Stress Effects of Honeysuckle Extract in Beef Cattle

In this study, barn THI exceeded 79 during the summer high temperature and humidity conditions, indicating that the cattle were under heat stress. The adrenal gland is a stress-sensitive endocrine organ in animals, and stressors can activate the hypothalamic-pituitary-adrenal (HPA) axis, leading to increased secretion of cortisol and corticosterone. Cortisol is a steroid hormone synthesized and secreted by the adrenal cortex that participates in various metabolic processes and facilitates tissue repair [8]. During heat stress, thyroid function decreases, reducing metabolic rate and heat production to enhance adaptation to hot environments, which consequently reduces secretion of T4 and T3 [9]. The results of this study demonstrate that during heat stress, honeysuckle extract supplementation increased serum T3 and T4 concentrations while decreasing cortisol concentration, indicating that honeysuckle extract has beneficial effects in mitigating heat stress in beef cattle.

3.2 Effects of Honeysuckle Extract on Antioxidant Indices in Beef Cattle

Research by Fu Daibo et al. [10] has shown that high temperature stress can induce excessive production of oxygen free radicals, disrupting the antioxidant enzyme system. Lipid peroxidation is a series of oxidative chain reactions initiated by free radical attack on cell membranes, ultimately leading to membrane damage and production of toxic metabolites. GSH-Px and SOD are two important antioxidant enzymes whose activities reflect the body' s ability to scavenge oxygen free radicals. Malondialdehyde is a product of lipid peroxidation, and its content correlates with the degree of lipid peroxidation in tissues [11]. In this study, honeysuckle extract supplementation reduced serum MDA content and significantly increased GSH-Px activity, SOD activity, and T-AOC. These results demonstrate that honeysuckle extract enhanced antioxidant capacity and effectively prevented lipid peroxidation damage induced by high temperature stress. Fang Yuhui [12] reported that honeysuckle compound preparations provide significant protective effects against acute myocardial ischemia, possibly through their flavonoid and chlorogenic acid components scavenging excess free radicals, thereby improving myocardial energy metabolism and enzyme activities, indirectly enhancing hypoxia tolerance, and ultimately protecting and stabilizing myocardial cell membrane permeability and integrity. Honeysuckle extract can increase serum SOD activity while decreasing MDA and nitric oxide content, enhancing antioxidant capacity of pancreatic islet cells and providing protective effects against exogenous nitric oxide-induced damage to NIT-1 pancreatic -cells [4]. Gong Cuicui et al. [13] reported that honeysuckle provides protective effects against oxidative stress injury in RBL cells, with pretreatment

showing better efficacy than post-treatment. The protective mechanism may involve inhibiting heat shock protein 70 and nuclear factor- B expression, suppressing the NF- B signaling pathway, and increasing intracellular antioxidant defense enzyme levels.

Conclusion

Based on the results of this study, two main conclusions can be drawn: First, honeysuckle extract can improve antioxidant capacity and alleviate heat stress responses in beef cattle, but has no significant effect on weight gain. Second, comprehensive evaluation of all indices indicates that supplementation at 0.2% in the concentrate is optimal.

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Note: Figure translations are in progress. See original paper for figures.