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Effects of Chinese herbal formula *Heat-stress-releasing* on antioxidant function in dairy cows

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Abstract In order to investigate the effects of a Chinese herbal formula *Heat-stress-releasing* on the antioxidant function in dairy cows, ten dairy cows were randomly divided into the control group and the experimental group, with five cows in each group. All the cows were fed with a basal diet. The animals in the experimental group were given with 220 g of herbs per day in addition to the basal diet. The trial was conducted for 14 days. Blood samples were taken from the vena cava at day 0, day 7, and day 15, respectively. The antioxidant statuses were examined. The results are as follows. (1) *Heat-Stress-releasing* formula can significantly increase the milk yield of dairy cows under heat stress. Compared with the control group, the milk yield of the herb-treated group increased by 14.01% ($P < 0.05$), 14.32% ($P < 0.05$) and 15.01% ($P < 0.05$) in prophase, metaphase and anaphase of the test, respectively. (2) *Heat-Stress-releasing* formula can increase significantly the antioxidant status of the heat stressed dairy cows. Compared with the control group, the superoxide dismutase (SOD) activity increased by 45.93% ($P < 0.01$) at day 7 and by 54.40% ($P < 0.01$) at day 15. The Glutathione-peroxidase (GSH-PX) activity of the test group increased by 17.99% ($P < 0.05$) at day 7 and 25.98% ($P < 0.01$) at day 15. The total antioxidant capacity (T-AOC) of the test group increased by 43.64% ($P < 0.01$) at day 7 and 46.35% ($P < 0.01$) at day 15. The malondaldehyd (MDA) content of test group declined by 23.88% ($P < 0.01$) at day 7 and 25.32% ($P < 0.01$) at day 15.

Keywords dairy cows, heat stress, antioxidant, Chinese herbal medicine

1 Introduction

The dairy cows are animals that can bear lower temperature but cannot endure higher temperature. The ideal temperature

range for dairy cattle is between 0°C and 20°C. Once the temperature goes above 26°C, feed intake and milk production of the cows will fall down with lower pregnancy rate. And the disease rate will be increased (Zhang et al., 2003). Chinese herbal medicines were selected to make a prescription *Heat-Stress-releasing* formula that can clear up summer-heat, nourish *Qi* to strengthen the spleen, and nourish *Yin* to generate body fluid. The results show that the *Heat-stress-releasing* formula can increase significantly the milk yield of heat stressed dairy cows, and improve the physiology and blood biochemical parameters of heat stressed dairy cows. The objective of this study was to find out the effects of *heat-stress-releasing* formula on the antioxidant function of heat stressed dairy cows.

2 Materials and methods

2.1 Preparation of Chinese herbal medicine

The *Heat-stress-releasing* formula was made of *Radix Codonopsis* (50 g), *Radix Ophiopogonis* (50 g), *Fructus Schisandrae Chinensis* (30 g), *Folium Nelumbinis* (50 g), *Herba Lophatheri* (40 g). These herbs were purchased from an herbal medicine shop in Anguo City, Hebei Province, China. They were mixed, shivered, sieved and made into 220-gram packs.

2.2 Reagent

Superoxide Dismutase (SOD) kit, Glutathione-peroxidase (GSH-PX) kit, Total Antioxidant Capacity (T-AOC) kit and Malondaldehyd (MDA) kit were purchased from Jiancheng Biological Engineering Institute of Nanjing, China.

2.3 Test animals

Ten dairy cows of similar age and body weight were randomly divided into a control group and an experimental group. There are five cows in each group. The cows in the control group were fed with a basal diet, the experimental

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group with a basal diet plus 220 g of herbs as Heat-Stress-releasing formula per day. The trial was conducted for 15 days.

2.4 Temperature and humidity

The temperature and humidity of the dairy farm was measured at 9:00, 15:00 and 20:00 daily by dry and wet bulb thermometers. Then the average temperature and temperature-humidity index (*THI*) of test prophase (from day 0 to day 5 of the test), test metaphase (from day 6 to day 10), and test anaphase (from day 11 to day 15) were calculated.

2.5 Antioxidant function examination

The blood samples were taken from the vena cava at day 0, day 7 and day 15, respectively. Serum was obtained through centrifugation. Using SOD kit, GSH-PX kit, T-AOC kit, MDA kit, the activities of SOD, GSH-PX and T-AOC, and the concentration of MDA were assayed following the manufacturer's instructions.

2.6 Statistics and analysis

All data were calculated and analyzed with Microsoft Excel software and were expressed in mean \pm standard error (*Se*). The differences between the control group and the test group were compared using *t*-test ($P < 0.05$).

3 Results

3.1 Temperature and temperature-humidity index of cottage

According to Table 1, the temperatures in shed at 8:00, 15:00 and 20:00 during the test were all above 27°C which exceeded significantly the cow's suitable temperature range of 4–24°C recommended by Johnson (1976); and the temperature-humidity indices (*THI*) in shed at 8:00, 14:00 and 20:00 during the test were all above 75, which is higher than 69, the threshold for dairy cows to undergo heat stress. These temperature and *THI* data indicated that the dairy cows were under an acute heat stress condition during the test.

Table 1 Temperature and temperature-humidity index (*THI*) during the test

Test time	Temperature (T) and <i>THI</i>	Test prophase	Test metaphase	Test anaphase
9:00	T	28.42 \pm 2.45	28.15 \pm 2.67	27.81 \pm 3.24
	<i>THI</i>	75.86 \pm 3.28	76.83 \pm 2.98	75.12 \pm 3.18
15:00	T	31.11 \pm 2.96	31.04 \pm 3.15	30.43 \pm 2.88
	<i>THI</i>	80.27 \pm 3.76	80.42 \pm 3.68	79.12 \pm 3.56
20:00	T	30.05 \pm 3.78	30.13 \pm 3.65	30.15 \pm 3.54
	<i>THI</i>	80.15 \pm 3.54	80.21 \pm 3.38	79.60 \pm 3.46

Note: $THI = 0.72 \times (T_d + T_w) + 40.6$, T_d and T_w mean the temperatures of dry bulb and humid bulb, respectively.

3.2 Effects of Chinese herbal formula on milk yielding of the dairy cows

There was no significant difference in milk yielding ($P > 0.05$) between the test group and the control group before test. After herbal treatment, the milk yielding in experimental group increased by 14.01% ($P < 0.05$), 14.32% ($P < 0.05$) and 15.01% ($P < 0.05$) in prophase, metaphase and anaphase of the test, respectively, compared with the control group (Table 2).

Table 2 Milk yielding of dairy cows fed with Heat-stress-releasing formula herbs (kg)

Group	Before test	Test prophase	Test metaphase	Test anaphase
Test group	18.78 \pm 1.74 ^a	21.48 \pm 1.86 ^a	21.63 \pm 1.82 ^a	21.69 \pm 1.72 ^a
Contrast group	18.56 \pm 1.82 ^a	18.84 \pm 1.64 ^b	18.92 \pm 1.76 ^b	18.86 \pm 1.69 ^b

Note: The data with the same superscript letter in a row indicate not significant ($P > 0.05$), the data with different superscripts letters indicate significant ($P < 0.05$).

3.3 Effects of Heat-stress-releasing formula on antioxidant function of heat-stressed dairy cows

There were no significant differences ($P > 0.05$) in the activities of SOD, GSH-PX and T-AOC, and MDA content between the test group and the control group before test. After the treatment, the SOD activity of the test group increased by 45.93% ($P < 0.01$) on the seventh day and 54.40% ($P < 0.01$) on the 15th day of treatment compared with the control group. The GSH-PX activity of the test group increased by 17.99% ($P < 0.05$) on the seventh day and 25.98% ($P < 0.01$) on the 15th day. The T-AOC of the test group increased by 43.64% ($P < 0.01$) on the seventh day and 46.35% ($P < 0.01$) on the 15th day. The MDA content of the test group declined by 23.88% ($P < 0.01$) on the seventh day and 25.32% ($P < 0.01$) on the 15th day after herbs feeding (Table 3).

Table 3 Effect of Heat-stress-releasing formula on antioxidant activity of dairy cows

Item	Test time/d	Contrast group	Test group
SOD activity /($U \cdot mL^{-1}$)	0	71.23 \pm 12.11 ^a	69.98 \pm 13.45 ^{Ba}
	7	70.76 \pm 14.13 ^a	103.26 \pm 14.32 ^{Bb}
	15	72.12 \pm 13.84 ^a	111.35 \pm 13.64 ^{Bb}
GSH-PX activity /($enzyme \ activity \ unit$)	0	121.13 \pm 15.81 ^a	120.47 \pm 16.18 ^a
	7	120.65 \pm 15.11 ^a	142.36 \pm 14.47 ^b
	15	120.41 \pm 14.46 ^a	151.69 \pm 15.03 ^{Bb}
T-AOC	0	2.77 \pm 0.42 ^a	2.73 \pm 0.37 ^a
	7	2.75 \pm 0.51 ^a	3.95 \pm 0.45 ^{Bb}
	15	2.74 \pm 0.49 ^a	4.01 \pm 0.53 ^{Bb}
MDA content /($nmol \cdot mL^{-1}$)	0	7.70 \pm 0.62 ^a	8.05 \pm 0.78 ^a
	7	7.83 \pm 0.81 ^a	5.96 \pm 0.59 ^{Bb}
	15	7.74 \pm 0.76 ^a	5.78 \pm 0.73 ^{Bb}

Note: The data with the same superscripts letter in a row indicate not significant ($P > 0.05$), the data with different superscripts of small letters indicate significant at 0.05 level, the data with different superscripts of capital letters indicate significant at 0.01 level.

4 Discussion

The traditional Chinese veterinarians believe that summer-heat is the primary pathogen of summer and is one of six exopathogens. Summer-heat evils belong to fire and heat and can consume lots of *Qi* (or body energy) and impair body fluids. However, milk is regulated by *Qi* and blood. The milk yield of heat-stressed dairy cows is decreased because the cows consume *Qi* and impair body fluids in summer. Wang (1963) stated that heat evils might cause an easy exhaustion of *Qi* and *Yin*, and that the nourishing *Qi* could not get final good effects. In this test, the herbal formula composed of *Radix Codonopsis*, *Radix Ophiopogonis*, *Fructus Schisandrae Chinensis* can nourish *Qi* and stop profuse sweating, and nourish *Yin* to generate body fluids, with the main treatment of summer-heat impairment of *Qi*, impairment of *Qi* and *Yin*, and abnormal sweating resulting in body weariness, thirsty and feeble pulse. *Shengmaisan* is the classical prescription to treat deficiency of *Qi* and *Yin*, having a stronger function of resisting heat stress (Li et al., 1997). As *Folium Nelumbinis* can clear away heat and generate body fluids, and *Lophatherum Gracile* Brongn can clear summer-heat, therefore, using these medicines together can clear summer-heat, nourish *Qi* to strengthen the spleen, and nourish *Yin* to generate the body fluids so that the Chinese herbal formula can increase significantly the milk yielding of the heat-stressed dairy cows. The modern studies indicate that *Radix Codonopsis Pilosulae* and *Liriope Spicata* can inhibit significantly the excitation of sympathetic nerves, and greatly decrease blood adrenalin and noradrenalin contents of heat-stressed animals, so they have the stronger function of resisting heat stress (Bai et al., 2002).

In recent years, more and more people have paid much attention to the effects of free radicals under heat stress conditions. At a physiological status, the body keeps a balance between the production and scavenging of free radicals. But under a heat stress condition, the free radical content is produced too much that the vitamins, Cu, Zn, etc. with an antioxidant function are over consumed, exhausting the free radical scavenging system. Therefore, the free radicals become accumulated. And finally tissues are injured by the oxidation of free radicals. In the resisting free radicals and lipid peroxidation system, SOD can clean away the redundant superoxide anion free radicals (O_2^-) in cells, the GSH-PX can catalyze distinctively the glutathione reduction for hydrogen peroxide (H_2O_2) which can block the lipid peroxidation reaction. Catalase (CAT) can remove rapidly the H_2O_2 that can protect the sulfhydryl membrane protein (Li et al., 2000; Liu et al., 2003; Sahin et al., 2004; Ozturk and Gumuslu, 2004; Wu and Hu, 2005). Many tests indicated that *Shengmaisan* or *Shengmai Yin* and their composition medicines can remove the hydroxy-free-radical (OH) and superoxide anion free

radicals (O_2^-) in vitro, and increase the SOD and GSH-PX activities in blood, and decrease the concentration of the lipid peroxide (LPO) and MDA content which is the product of lipid peroxide reaction (Wang et al., 2002; Lü et al., 2005; Ma et al., 2005). In our test, the Chinese herbal formula can increase significantly the SOD, GSH-PX activities and T-AOC of the heat-stressed dairy cows, and decrease the MDA concentration significantly. These results indicate that the Chinese herbal formula has a strong antioxidant function, so it may protect cell membranes from being injured by free radicals, which may be the main mechanism of Chinese herbal formula resisting dairy cows heat stress and improving the milk performance of the heat-stressed dairy cows.

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