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Full Length Research Paper

Effects of Chinese herbal formula "Kangshu I" on the blood heat shock protein (HSP70) and levels of some hormones in heat-stressed cows

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In order to investigate the effects of the Chinese herbal formula "Kangshu I" on the mechanism in dairy cows, 10 Holstein dairy cows which had similar weight, age, parity, milk yield were selected, and the experiments were conducted in three stages: the room temperature stage (negative control group), heat stress stage (positive control group) and heat stress stage with drug treatment (drug treatment group). The results indicated that the dairy cows sera heat shock protein (HSP70) concentration in the positive control group increased significantly than those in the negative control group (P<0.01). The plasma aldosterone (ALD) concentration, sera cortisol (COR) and thyroxine (T4) concentrations decreased significantly than those in the negative control group (P<0.01). Adrenocorticotropin (ACTH) decreased significantly than those in the negative control group (P<0.05). Triiodothyronine (T3) decreased non-significantly than those in negative control group (P>0.05). After usage of Chinese herbal formula "Kangshu I", HSP70 levels in drug treatment group increased non-significantly than those in the positive control group (P>0.05). The plasma ALD concentrations, sera COR and T4 concentrations were significantly higher than those in the positive control group (P<0.01). ACTH increased significantly than those in the positive control group (P<0.05), T3 increased non-significantly than those in the positive control group (P>0.05).

Key words: Dairy cow, heat stress, Chinese herbal formula, heat shock protein (HSP70), hormone.

INTRODUCTION

All organisms depend on their environment to survive. Therefore, various environmental factors can cause physiological stresses on animals and adversely affect animal production, of which the most serious impact on animals is heat stress (Wang, 2000). Heat stress is the response of body's thermal integration at a high temperature environment for any request made by non-specific physiology (Wang and Liu, 2006). There is no exception for cows, from an economical point of view, in diverse stress response. The greatest impact on dairy cow is heat stress. Under the heat stress, some blood biochemical indices of cows would change significantly. In heat stress test of dairy cows, the scholars from various countries paid adequate attention to the biochemical components change in blood. Generally speaking, the inorganic ions of blood, cortisol (COR), triiodothyronine (T3), thyroxine (T4), enzyme, hormone etc. had significant impact on dairy cows under the heat stress. In this experiment the changes of heat shock protein (HSP) 70, T3, T4, COR, aldosterone (ALD), adrenocorticotropin (ACTH) in the dairy cows blood were analyzed to provide a theoretical basis for clarifying the heat stress mechanism on dairy cows.

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MATERIALS AND METHODS

*A*nimals

Ten Holstein cows which had similar body weight, age, parity, milk yield were selected at Dacaozhuang dairy farm in Xingtai City from May to August in 2011.

Table 1. Effect of "Kangshu I" on heat shock protein HSP70 in cows (n = 10).

Groups	HSP70 (ng/ml)
Cure group of "Kangshu I"	2.03 ± 0.16^{B}
Positive control group	1.85 ± 0.20^{B}
Negative control group	1.34 ± 0.13^{A}

Note: The same alphabets of column means no significant difference (P>0.05), the different alphabets means significant difference (P<0.01).

Herbs

The herbs: Elsholtzia splendens, Baikal skullcap root, *Ophiopogon japonicus*, Mint, Akabia stem, Rice paper plant pith, Wrinkled gianthyssop herb, Capillary wormwood herb, Tatarinow sweetflag rhizome, Honeysuckle, Spine date seed, Hawthorn, Magnolia officinalis, and Licorice purchased from an herbal shop in Baoding City and authenticated by Hebei Provincial Bureau of herbal medicine, were mixed, minced, soaked in water for an hour, boiled for an hour, and the supernatant collected. Repeat the aforementioned step and collect the supernatant. Put the supernatants together, and concentrate to a desired volume (1 g/ml).

Reagents

HSP70 ELISA kit was obtained from Shanghai Ya-pei Biotechnology Company (Shanghai, China). ^{125}I-T_3 RIA detection kit, ^{125}I-T_4 RIA detection kit, and ^{125}I- COR RIA detection kit were from Shanghai Institute of Biological Products (Shanghai, China). ^{125}I- ACTH RIA detection kit and ^{125}I- ALD RIA detection kit were supplied by Beijing North Biotechnology Institute (Beijing, China).

Experimental design

Ten Holstein cows which had similar body weight, age, parity, milk yield were selected and tested in three stages: The room temperature stage (negative control group), heat stress stage (positive control group) and heat stress stage with drug treatment (drug treatment group). At drug treatment stage, the dairy cows were fed with 1:1 "Kangshu I" decoction 400 g/day for 1 week. All cows were fed by normal standard in all stages. The temperature ranged at negative control stage: 16 to 23℃, relative humidity: 50 to 60% (chosen in mid-May); the temperature range in second and third stages: 32 to 40℃, relative humidity: 72 to 79% (chosen from July to August).

Blood sample collection and processing

Blood collection requirements of cows

The time for blood sample collection was chosen at noon and the cows were not fed, blood sample collected by jugular vein. Five millilitre of blood was taken in centrifuge tube which containing heparin anticoagulant, mixed, the plasma for ALD determination was separated by centrifuge 3000 r/min for 10 min. Fifteen millilitre of anti-coagulation blood was collected, and packaged in 10 ml centrifuge tube, the blood plasma was separated using low-temperature centrifuge 3000 r/min for 10 min, stored in -80℃ freezer for the determination of plasma ALD, HSP70, T3, T4, COR, ACTH.

HSP70, ALD, COR, T3, T4, and ACTH were measured according to the manufacturer's instructions.

Data processing and statistical analysis

The data were taken as mean and standard deviation, and analyzed with SPSS11.0 for Windows. The differences were assessed by student's t-test.

RESULTS

Effect of "Kangshu I" on heat shock protein (HSP70) in cows under heat stress

HSP70 concentrations in the cows sera under heat stress were significantly higher than those in the negative control group (P<0.01). After usage of traditional Chinese medicine prescription "Kangshu I", HSP70 levels in drug treated group increased non-significantly than those in the positive control group (P>0.05) (Table 1).

Effect of "Kangshu I" on the blood hormone in cows under heat stress

Plasma ALD concentration of cows under heat stress were significantly lower than those in the negative control group (P<0.01). Sera COR, T4 concentration decreased significantly (P<0.01). ACTH decreased significantly (P<0.05). T3 had somewhat decreased (P>0.05). After usage of traditional Chinese medicine "Kangshu I", ALD plasma concentration, sera COR, T4 concentration increased significantly than those in the positive control group (P<0.01). ACTH concentration increased significantly (P<0.05). T3 had somewhat increase (P>0.05) (Table 2).

DISCUSSION

Effect of "Kangshu I" on heat shock protein (HSP70) in cows under heat stress

Organisms or cells can become tolerable to lethal temperature (Moseley, 1994) after they were treated in an atmosphere just below the lethal temperature. As a result their heat resistance becomes improved. This heat

Table 2. Effect of "Kangshu I" on blood hormone in cows (n = 10).

Groups	ALD (pg/ml)	COR (μg/ml)	T3 (ng/ml)	T4 (ng/ml)	ACTH (pg/ml)
Cure group of "Kangshu I"	56.67±1.93^{AB}	37.92±1.12^{AB}	1.418±0.18	39.59±1.63^{AB}	1.66±0.3^{ab}
Positive control group	52.98±4.91^{A}	40.97±1.16^{B}	1.410±0.04	37.24±2.94^{A}	1.80±0.47^{b}
Negative control group	63.13±4.78^{B}	35.18±3.12^{A}	1.438±0.07	43.80±430.0^{B}	1.22±0.28^{a}

Note: The same alphabets of perpendicular line means no significant difference (P>0.05), the different majuscule alphabets means highly significant difference (P<0.01), the different lowercase alphabets means significant difference (P<0.05).

resistance is primarily related to HSP70 (He et al., 1998). The structure of HSP70 has a high conservation, which can participate in protein folding, assembling and degradation. The amount of HSP70 synthesized in cells was very low under normal circumstances. However, under stress state, they were synthesized rapidly and transferred to the nucleus, nucleolus and other regions, which could improve the body's heat tolerance and play a role in protecting the body (Santoro, 2000). This heat endurance and HSP70 expression level were positively correlated (Wang et al., 2000). Gourgou et al. (2010) and Gong et al. (2010) also proved that expression of HSP could be induced increasingly under heat stress.

In this study ELISA method was used to detect the quantity of HSP70 protein expression in sera under high-temperature and medication to verify the anti-stress effects of traditional Chinese medicine. The results showed that under heat stress, the HSP70 concentration in the cows sera were significantly higher than those in the negative control group (P<0.01). After treatment with Chinese herbal medicine prescription "Kangshu I", HSP70 concentrations were increased than those in the positive control group, but not significantly (P>0.05). The results were in consistent with reports by Chen et al. (2002) and Mao et al. (2008) which indicated that heat stress can induce HSP70 expression increase in the blood. After treatment with Chinese herbal medicine, HSP70 expression in the blood increased significantly. So the body can reach the earlier state of heat stress adaptation to protect the various tissues and organs fast and better from heat stress injuries and to improve the body's anti-stress ability. This adaptation will provide a basic reference for follow-up study of the anti-heat stress injury mechanism of heat shock protein, the heat tolerance capacity and the regulation of Chinese herbal medicine on heat stress proteins.

Effect of "Kangshu I" on the blood hormone in cows under heat stress

Under the heat stress, a series of changes occurred in the animal body metabolism and functions of some organs and system. Therefore changes of physical and chemical indices in the blood of animal body would follow (Pu et al., 2005). At present high temperature on blood indices in dairy cows were reported increasingly over the world. Most studies showed that high temperature affects the levels of blood ALD, COR, T3, T4, ACTH, etc.

ALD is a sort of mineralosteroid which was secreted by adrenal cortex playing significant role in preserving sodium and removal of potassium. The results here showed that under heat stress the plasma ALD concentration in dairy cows were significantly lower than those in negative control group (P<0.01). After treatment with Chinese herbal medicine "Kangshu I", the plasma ALD concentrations were significantly higher than those in the positive control group (P<0.01). This would be beneficial to sodium preservation and potassium removal.

COR is a sort of glucocorticoid which was secreted by adrenal cortex. Increase of COR can not only make the lymphoid tissues shrink and speed up the destruction of lymphocytes, but also reduce the number of eosinophils by decomposition. COR is an important index which can measure the level of heat stress. Under heat stress conditions, the sera COR concentrations were significantly lower than those in the negative control group (P<0.01). Yang et al. (2007) also proved that after treatment with Chinese herbal medicine "Kangshu I", the sera COR concentrations were significantly higher than those in the positive control group (P<0.01). The high level of COR thus alleviates the injury of the immune organs and the disorder of protein metabolism in cows under heat stress.

T3 and T4 are sorts of hormones secreted by the thyroid gland and its peripheral tissues. Heat stress can weaken the cow's function thyroid, reduce the secretion of T3 and T4, decrease metabolism speed, and therefore enhance the body's adaptation to a hot environment. Under heat stress, T3 concentration decreased slightly than those in the negative control group (P>0.05). But T4 decreased significantly (P<0.01). These results are consistent with the studies by other scholars (Wang et al., 2007; Guo et al., 1998; Li et al., 2001). Use of Chinese herbal medicine "Kangshu I" elevated the sera T4 concentration significantly than those in the positive control group (P<0.01).

ACTH is a sort of hormone secreted by animal pituitary gland, which can promote the adrenal cortical cell proliferation. Therefore, it can convert the cholesterol stored in cortisol and corticosterone. In animals, ALD secretion requires the involvement of ACTH. Under heat stress conditions, ACTH were significantly lower than those in negative control group (P<0.05). After usage of

Chinese herbal medicine “Kangshu I”, sera ACTH concentrations were significantly increased than those in negative control group ($P<0.05$). Thus, to promote the secretion of ALD, improve significantly the body′s role to protect sodium and remove potassium, alleviate the electrolyte imbalance caused by heat stress.

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