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RESEARCH ARTICLE

Effects of *Bupleurum* extract on blood metabolism, antioxidant status and immune function in heat-stressed dairy cows



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Abstract

This experiment was conducted to evaluate the effects of *Bupleurum* extract (BE) on blood metabolites, antioxidant status, and immune function in dairy cows under heat stress. Forty lactating Holstein cows were randomly assigned to 1 of 4 treatments. The treatments consisted of 0, 0.25, 0.5, and 1.0 g of BE kg⁻¹ dry matter. Supplementation with BE decreased ($P<0.05$) blood urea nitrogen (BUN) contents and increased blood total protein (TP) and albumin (ALB) levels compared with control cows, but it had no effects ($P>0.05$) on blood glucose (GLU), nonesterified fatty acid (NEFA), total triglyceride (TG), low-density lipoprotein cholesterol (LDL-C), and high density lipoprotein cholesterol (HDL-C). Compared with control cows, cows fed BE had higher ($P<0.05$) superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activity. However, supplementation with BE had no effect ($P>0.05$) on total antioxidant capacity (T-AOC) or malondialdehyde (MDA) levels. The immunoglobulin (Ig) A and G contents increased ($P<0.05$) in cows fed 0.25 or 0.5 g of BE kg⁻¹. Interleukin (IL)-2 and IL-4 levels were higher ($P<0.05$) in cows fed 0.5 and 1.0 g of BE kg⁻¹, and IL-6 was significantly elevated ($P<0.05$) in cows fed 0.5 g of BE kg⁻¹. There were no treatment effects ($P>0.05$) on the CD4⁺ and CD8⁺ T lymphocyte ratios, CD4⁺/CD8⁺ ratio, or tumor necrosis factor- α (TNF- α) level among the groups. These findings suggest that BE supplementation may improve protein metabolism, in addition to enhancing antioxidant activity and immune function in heat-stressed dairy cows.

Keywords: *Bupleurum* extract, blood metabolites, heat stress, dairy cows

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1. Introduction

Heat stress not only reduces production performance (Collier *et al.* 2006; Hammami *et al.* 2012) but also has adverse effects on the health status of dairy cows. These adverse effects include an increased incidence of metabolic disorders (Wheelock *et al.* 2010), in addition to increased

oxidant stress (Lin *et al.* 2006) and impaired immune function (Carroll *et al.* 2012). Although many nutrition strategies, such as increasing the energy density of diets (Wang *et al.* 2010) and rumen-protected niacin supplementation (Zimbelman *et al.* 2013), have been used to alleviate heat stress, heat stress remains a costly issue for the global dairy industry (St-Pierre *et al.* 2003). Consequently, new strategies need to be developed that can help maintain dairy cows' health during hot summers.

The genus *Bupleurum*, a major ingredient of oriental folk medicine, has been widely used in Asia for the treatment of many diseases over the past 2 000 years (Ashour and Wink 2011). It contains many secondary metabolites, such as polysaccharides (Sun *et al.* 1991), saikosaponins (Navarro *et al.* 2001), and essential oil (Martine *et al.* 1993). Saikosaponin constitutes the main class of secondary metabolites in the genus *Bupleurum*, amounting for up to 7% of roots (Ashour and Wink 2011). Plants belonging to the genus *Bupleurum* have various pharmacological functions, with studies reporting antipyretic properties (Huang 1993), anti-inflammatory activity in rats (Martin *et al.* 1993), antioxidant activity in rats (Zhao *et al.* 2012), and immunostimulant activity in mice (Sun 2006). Previous research on *Bupleurum* has focused on humans and mice, whereas there has been little research on the effect of *Bupleurum* supplementation on cows. Our previous study suggested that *Bupleurum* extract (BE) could mitigate negative effects of heat stress on production in lactating Holstein cows (Pan *et al.* 2014), but the effects of direct supplementation with BE on blood metabolites, antioxidant status, and immune responses in heat-stressed lactating Holstein cows are unknown. Therefore, the objective of this study was to investigate the effects of oral administration of BE on blood metabolites, antioxidant status, and immune function of lactating Holstein cows subjected to heat stress.

2. Material and methods

2.1. Animals, diet and experimental design

All animals included in this study were maintained according to the principles of the Animal Care and Use Committee of the Chinese Academy of Agricultural Sciences. Forty lactating Holstein cows, (37.5±1.8) kg of milk d⁻¹, (75±15) d in milk, and (1.7±0.4) parity, were categorized according to average daily milk yield, parity, and day in milk and randomly assigned to 1 of the following 4 treatments (*n*=10): 0 (control), 0.25, 0.5, or 1.0 g of BE kg⁻¹ dry matter. A commercially available BE product was used (Beijing Centre Biology Co., Ltd., China) containing 35.0% BE (a mixture of 6.6% saikosaponins, 2.5% essential oil, and 25.9% polysaccharides) and 65.0% starch mix. In dairy cows, heat

stress occurs when the temperature-humidity index (THI) exceeds 72 (Armstrong 1994). During the experiment, the mean THI in the barn in which the animals were housed was 78.2 (range: 71.9 to 80.8) at 06:00 h, 79.7 (range: 72.7 to 83.3) at 14:00 h, and 78.3 (range: 70.2 to 81.7) at 22:00 h (Pan *et al.* 2014).

The basal diet (Table 1) was formulated to meet or exceed nutrient recommendations (NRC 2001). Feedstuffs were offered (roughage first and then concentrated feed) daily at 05:30, 13:00, and 20:00 h to ensure 5% refusals, and the BE supplements were top dressed in equal portions to concentrated feed in the morning feeding. All the cows were assigned to an individual feeding column cage, with access to fresh water. The experimental period lasted 10 weeks.

2.2. Sampling procedure

Blood samples were collected from the tail vein of each cow before the morning feed on days 0, 21, 42, and 63 of the experimental period. Blood samples were collected in EDTA-treated tubes for the analysis of CD4⁺ and CD8⁺ T lymphocytes. Additional blood samples were collected in

Table 1 Ingredients and chemical composition of basal diets (dry matter (DM) basis)

Composition	Content
Ingredient (% of DM)	
Alfalfa hay	12.7
Chinese wildrye	9.4
Corn silage	12.6
Oat grass	3.7
Cotton seed meal	8.9
Beet pulp	7.1
Corn	23.0
Dry distillers	3.7
Barley	9.4
Soybean	7.4
Sodium bicarbonate	0.64
Dicalcium phosphate	0.54
Salt	0.49
Vitamin-mineral premix ¹⁾	0.43
Chemical composition (% of DM) ²⁾	
NE _L (Mcal kg ⁻¹ of DM)	1.60
CP	17.2
NDF	39.9
ADF	25.1
EE	4.12
Ca	1.00
P	0.48

¹⁾ Contained (kg⁻¹ DM): a minimum of 2 000 000 international unit (IU) of vitamin A; 450 000 IU of vitamin D; 10 000 IU of vitamin E; 3 000 mg of niacin; 4 560 mg of Cu; 4 590 mg of Mn; 12 100 mg of Zn; 200 mg of Se; 270 mg of I; 60 mg of Co.

²⁾ NE_L was calculated according to NRC (2001) based on actual dry matter intake. CP, crude protein; NDF, neutral detergent fiber; ADF, acid detergent fiber; EE, ether extract.

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