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Short Communication

EFFECTS OF VARYING DIETARY CHINESE HERB EXTRACTS ON BLOOD IMMUNE STATUS AND ANTIOXIDANT FUNCTION IN DAIRY CATTLE

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ABSTRACT

Chinese herbs of *Astragalus* and *eucommia* have shown the function of raising body immunity and antioxidant in animal production. The current experiment was aimed to study the effects of varying dietary supplemental two Chinese herb extracts (CHEs) on blood immune status and antioxidant function in dairy cattle. CHEs is the mixture of 40% *Astragalus* extract and 60% *eucommia* extract. Forty Holstein dairy cattle were randomly assigned to four treatments of each treatment ten animals, included: (1) NC (basal diet), (2) T1 (NC+400mg/kg CHEs), (3) T2 (NC+600mg/kg CHEs), and T3 (NC+1000mg/kg CHEs). Supplemental CHEs enhanced the blood immunity (P<0.05) with higher concentrations of immunoglobulin (Ig) G, IgM, interleukin (IL)-2, IL-4, a higher proportion of CD4 (T1 treatment), CD8 and ratio of CD4/CD8 (T1 and T2 treatments) and lower content of IL-6. Compared with the NC treatment, dairy cattle in T1, T2 and T3 treatments had higher (P<0.05) enzyme activities of glutathione-peroxidase (934, 798, 779 vs 765U/mL) and superoxide dismutase (86.1, 75.3, 81.7 vs 72.5U/mL), lower (P<0.05) content of malondialdehyde (2.24, 3.55, 3.34 vs 5.64nmol/mL). Collectively, supplemental dietary CHEs at the levels of 400 and 600mg/kg dry matter of concentrate in dairy cattle improved blood immune status and enhanced their antioxidant function. These findings suggested that dietary inclusion of CHEs may be favorable for dairy cattle production.

Key words: Antioxidant function, dairy cattle, herb, immunity

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INTRODUCTION

Research has shown the immunity of high yield dairy cattle was reduced because of increasing exposure to oxidative stress (Löhrke *et al.,* 2005). Recent years, some Chinese herb extractions are also supplemented in the ratio of dairy cattle to regulate rumen endocrine and fermentation (Gu *et al.,* 2010; Liu *et al.,* 2015), improve the utilization of feed and milk production (Jin *et al.,* 2007; Jia *et al.,* 2011), enhance immune status (Zhang *et al.,* 2004; Huang *et al.,* 2008), and increase anti-stress ability (Zhao *et al.,* 2008; Chen *et al.,* 2010). In addition, the herb additive of *Astragalus* and *eucommia* have shown the function of anti-inflammatory and antibacterial for immune enhancers and antibiotic alternatives in animal production (Qi *et al.,* 2011; Wang *et al.,* 2013; Zhu and Sun 2018; Abdallah *et al*. 2019; Xing *et al*. 2019).

Many ruminant researchers have an increasing interest to focus on substitutes for antibiotics or hormones. However, few literatures were reported on their effects of *Astragalus* extract or *eucommia* extract supplemented to diet on blood immune status and antioxidant function in lactating dairy cattle. According to the authors' pilot study, this experiment was conducted to investigate the effects of supplemental varying Chinese herb extracts (CHEs) on blood immune status and antioxidant function in dairy cattle.

MATERIALS AND METHODS

The protocol of this experiment involving animals and their care was approved by Committee for Animal Use and Ethics of Yangzhou University, China [SYXK(Su) IACUC 2012-0029]. Experiment was conducted at Shanghai SHENXING dairy farm during winter. Forty Holstein dairy cattle (2nd lactation number, 101±12 days in milk, and 603±48kg body weight) were randomly assigned to the following four treatments, each treatment had ten cows, included: (1) NC (basal diet), (2) T1 (NC+400 mg/kg CHEs), (3) T2 (NC+600mg/kg CHEs), and T3 (NC+1000mg/kg CHEs). Dairy cattle were fed three times daily at 6:00, 12:00 and 20:00 and drunk enough fresh water freely. Each cattle were housed in a tie stall barn. A total of 5 weeks was involved in this study. Dairy cattle were allowed 7 days for adaptation to CHEs supplementation and then experimental period started and lasted for 4 weeks.

The experimental diet was formulated in reference to the Nutrient Requirement of Dairy Cattle (pressed by National Academy of Science, Salt Lake City, 2001). Composition of diets (DM basis) in dairy cattle is shown in Table 1. CHEs composed of *Astragalus*

extract (40%) and *eucommia* extract (60%) were collected for the experiment before firstly ground through a 1 mm screen. The content of main bioactive substances in the CHEs are as follows, Moisture 5.2%, Chlorogenic acid 900ppm, and Astragaloside iv 64ppm, which was detected by Guangzhou Kingmed Testing Center Co., LTD, China. In all cases, all dosage of CHEs supplemented in the ratio were completely consumed by dairy cattle. The doses in this study was based on the authors' pilot study.

Table 1. Composition and content of diets (DM basis) in dairy cattle.

Ingredients		Chemical Composition [2]	
Chinese wild rye hay, %	3.67	NE_L, Mcal /kg	1.66
Corn silage, %	24.7	CP, %	16.9
Alfalfa hay, %	23.4	NDF, %	42.4
Corn, %	26.1	ADF, %	24.6
Cottonseed meal, %	4.00	EE, %	2.71
Soybean meal, %	16.5	Ash, %	8.60
Dicalcium phosphate, %	0.60	Calcium, %	1.13
NaCl, %	0.50	Phosphorus, %	0.45
Premix[1], %	0.53		
Total, %	100		

[1]. One kilogram of premix contains the following: Cu 4080mg, Fe 5500mg, Mn 4980mg, Zn 17500mg，Co 6.2mg, Se 110mg, I 6.25mg, Vitamin A 1500 00 IU, Vitamin D3 1250000IU, Vitamin E 3125mg, niacin 4500mg, choline 125000 mg; [2]. NE_L, net energy of lactation, was obtained from NRC (2001), other nutrition levels were acquired from measured values. NDF, neutral detergent fiber; CP, crude protein; ADF, acid detergent fiber; EE, ether extract.

Blood was sampled from all dairy cattle before feeding via the tail vein on the last day of the experimental period. Samples were stored in vacutainer tubes without anticoagulant, centrifugated at 3190 rpm for 15 min to separate serum. The supernatant stored at -40℃ for analysis the activities of antioxidant enzymes, and the concentration of immunoglobulins and cytokines. Another blood was sampled in EDTA-coated tubes and placed at 4℃ until the T lymphocyte (CD3, CD4 and CD8) were detected.

The determination of serum immunoglobulin (Ig) G, IgA and IgM content was performed using cow IgG, IgA and IgM ELISA kits which was produced by Bethyl Labs (Montgomery, USA). The concentrations of serum IL-2, IL-4, and IL-6 were tested with the cow ELISA kits (Groundwork Biotechnology Diagnosticate Ltd, USA) and the content of tumor necrosis factor (TNF)-α was detected with a cow radioimmunoassay kit (Bejing North Institute of Biotechnology, China). The CD8, CD4 and CD3 T lymphocytes were determined by Flow Cytometer (Beckman Coulter, USA), respectively (Caraher *et al.,* 2000). The serum enzyme activities of superoxide dismutase (SOD), glutathione-peroxidase (GSH-Px), Catalase (CAT), and the content of malondialdehyde (MDA) were determined using a SOD kit, a GSH-Px kit, a CAT kit, and an MDA kit reagent (Nanjing Jiancheng Bioengineering Institute, China).

All the data obtained in this study were analyzed using a one-way ANOVA of GLM procedure in SAS version 9.2. The mathematical model used for the analysis was: $Y_{ijk}=\mu+C_j+T_k+E_{ijk}$, Where Y_{ijk} = observed variables; μ = overall mean; C_j = effect of herbs; T_k = effect of blocks; E_{ijk} = random error. Data was presented as adjusted least squares means. The differences among treatments means were set by Duncan's multiple range tests. Indication of significance and tendency level indicated as $P<0.05$ and $0.05<P\leq0.10$, separately.

RESULTS

The concentrations of IgG, IgM, IL-2, IL-4 increased linearly ($P<0.05$) and IL-6 decreased linearly ($P<0.05$) with dietary supplemental CHEs increased. As amount of dietary CHEs increased, it had a trend ($P=0.06$) to increase the serum TNF-α concentrations. Compared with the NC treatment, the proportion of CD4 increased ($P<0.05$) in T1 treatment. The proportion of CD8 and ratio of CD4/CD8 in treatments of T1 and T2 differed ($P<0.05$) from NC treatment (Table 2).

Table 2. Effects of varying dietary CHEs on concentration of blood immunoglobulins, cytokines and proportions of lymphocytes and subsets in dairy cattle.

Items	Treatments[1]				SEM[2]
	NC	T1	T2	T3	
Serum immunoglobulins Concentration					
IgG, g/L	9.33^{b}	11.98^{a}	10.54^{ab}	10.09^{ab}	0.353
IgM, g/L	2.11^{a}	2.50^{b}	3.06^{b}	3.27^{c}	0.101
IgA, g/L	0.78	0.69	0.7	0.79	0.032
Serum cytokines concentrations					
IL-4, pg/mL	55.1^{b}	88.0^{a}	76.3^{a}	58.7^{b}	5.23
IL-6, pg/mL	117^{a}	84.5^{b}	74.5^{b}	92.5^{b}	7.30
IL-2, ng/mL	9.97^{b}	13.1^{a}	10.1^{a}	10.6^{a}	0.29
TNF-α, fmol/mL	47	61	54.2	43.6	2.90

Proportions of whole blood lymphocytes and subsets					
CD3, %	46.3	51.6	54.8	48.5	7.75
CD4, %	39.0[b]	44.8[a]	38.5[b]	38.2[b]	4.35
CD8, %	29.9[a]	24.5[b]	21.6[b]	33.4[a]	1.75
CD4/CD8	1.59[b]	1.98[a]	1.99[a]	1.22[b]	0.181

[1] treatment- NC, basal diet, T1, NC+400mg/kg CHEs, T2, NC+600mg/kg CHEs, T3, NC+1000mg/kg CHEs; [2]SEM = Standard error of mean; [abc] Mean values within a row sharing different superscript differ significantly (P<0.05).

The research data obtained from experimental animals revealed that the enzyme activities of GSH-Px and SOD increased (P<0.05) with CHEs supplementation increased, reversely the MDA content decreased significantly (P<0.05) (Table 3).

Table 3. Effects of varying dietary CHEs on serum antioxidant enzyme activities of GSH-Px, SOD, CAT and MDA in dairy cattle.

Items	Treatments[1]				SEM[2]
	NC	T1	T2	T3	
GSH-Px, U/Ml	765[b]	934[a]	798[b]	779[b]	38.1
SOD, U/Ml	72.5[b]	86.1[a]	75.3[b]	81.7[ab]	2.65
CAT, U/mL	51.1	57.5	53	53	1.87
MDA, nmol/mL	5.64[a]	2.24[b]	3.55[b]	3.34[b]	0.191

[1] treatment- NC, basal diet, T1, NC+400mg/kg CHEs, T2, NC+600mg/kg CHEs, T3, NC+1000mg/kg CHEs; [2]SEM = Standard error of mean; [ab] Mean values within a row sharing different superscript differ significantly (P<0.05).

DISCUSSION

Specific immunoglobulins and lymphokines could collectively indicate the humoral immunity status and acts as an important part to protect against microbial infections (McKee *et al.,* 2007). Blood IgG plays a major role in regulation of humoral immunity. Dairy cattle rely mainly on the body's immune cells and immune molecules to prevent invasion of pathogenic microorganisms. Many Chinese herbs, such as *Astragalus membranaceus*, *Radix Angelicae sinensis*, *Codonopsis pilosula*, *dandelion*, *Atractylodes macrocephala*, *Flos Lonicerae*, etc., have been reported to improve their immunity by activating immune cells and increase the content of immune molecules (Liu *et al.,* 2011). In the current study, the CHEs, composed of *Astragalus* extract and *Eucommia* extract, increased IgM and IgG content indicative of the cow's nonspecific and specific immune response. This is consistent with previous results that Chinese herbal additives composed of *Astragalus*, and other 4 herbs, can improve the immunity of dairy cattle by increasing the content of serum IgM and IgG content (Jia *et al.,* 2011). Cytokines are released in response to immunological challenge. Cytokines of ILs and TNF-α were important indicators of a host's cellular immunity exposing against microbial infection (Renckens *et al.,* 2006). Many researchers reported that dietary supplemented with flavonoids extracted from plant herbs can enhance the immunity through altering immune factors expressions and IL-4 secretion in cow blood (Hao *et al.,* 2010; Zhan, 2016). These findings are consistent with the present experiments. IL-6 is considered to be a proinflammatory cytokine (Minton, 1994). IL-2 is a cytokine that activates and promotes T cells differentiation and proliferation. The TNF-α is thought to be the first inflammatory mediators appearing in response to infection, initiating and regulating the immune response (Bemelmans *et al.,* 1996). Previous studies were not revealed the effects of herbs on TNF-α, IL-6 and IL-2 secretion in dairy cattle. We observed in current study that dietary supplemented with CHEs increased (P *<0.05*) serum IL-2 concentration and had a trend (P *=0.06*) to increase serum TNF-α concentration. These results indicated an increase in cellular and humoral immunities in the CHEs supplemented in dairy cattle. Both CD8 and CD4 T lymphocytes in peripheral blood are served as surface indicators of T lymphocytes, which could indicate T cells activation and cellular immune status (Park *et al.,* 1993; Uehara *et al.,* 2003). While T cells express surface activation indicators in condition of disease or inflammation, the CD4 and CD8 are released into peripheral blood. Concluded from the current study, the proportions of CD4, CD8 T lymphocyte and ratio of CD4/CD8 increased with dietary supplementation of CHEs in dairy cattle, suggesting that supplemental CHEs enhanced the dairy cow's cellular immunity.

The antioxidant prosperity of herbs may be contribute to protect lipids from oxidative impairment by regulating the activity of specific enzymes (Xiong *et al.,* 2001; Liu, 2010). So anti-oxidative enzymes played an important role in preventing the body against peroxides damage. In anti-oxidation systems, GSH plays a critical role in improving detoxification and immune systems (Brody, 1994). Enzymatic antioxidant defense system was mainly constituted by enzymes of CAT and SOD. MDA is the metabolic end product of lipid peroxidation which is one of the cellular injury indices. Over the years, polysaccharides extracted from Chinese herbals have

been widespread used to be antioxidants in animal production for oxidative damage prevention (Tomida *et al.,* 2009; Zhan, 2016). In the present study, supplemental CHEs at levels of 400 mg/kg dry matter of concentrate increased the enzyme activities of GSH-Px and SOD significantly, whereas the MDA content decreased significantly. The contradictory results between the current study and previous research literatures mainly due to the different species of the experimental animals and herbs.

Scientific studies focused on effects of feeding herbs to ruminants are limited. In addition, the detail regulatory mechanisms of herb feeding affect ruminal immune parameters and antioxidant ability in the current study is still far from understood, and further studies are required.

Conclusion: The findings of the current study concluded that supplemental CHEs at levels of 400 and 600 mg/kg dry matter of concentrate in dairy cattle improved blood immunity and enhanced their antioxidant function. Inclusion of CHEs in the diet may be favorable for dairy cattle production.

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