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Effects of the Chinese herbal medicine prescription Suanzaoren decoction on stress response of horses in transportation

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ABSTRACT - The objective of this study was to assess the effect of Suanzaoren decoction on stress response of horses in transportation. A total of six male Ili horses were equally divided into Suanzaoren decoction treatment group (n = 3; basal diet supplemented with Suanzaoren decoction, three times/day) and control group (n = 3; basal diet, three times/day). After feeding for five days, all horses were transported for 8 h to simulate stress. Blood and serum samples were obtained before transport (BT), during transport (T), and after transport (AT). Results showed that there was significant interaction between road transport and dietary Suanzaoren decoction supplementation for the white blood cells (WBC), intermediate cell ratio (MID%), granulocyte ratio (GRAN%), alanine aminotransferase (ALT), glucose (GLU), aspartate transaminase (AST), creatine (CK), cortisol (COR), human growth hormone (HGH), adrenocorticotropic hormone (ACTH) and arginine vasopressin content, insulin (INS), and thyronine 4 (T4) in blood and serum. The highest WBC was found in T group. The highest GRAN%, ALT, GLU, AST, CK, T4, ACTH, and INS contents were observed in serum from AT groups, which was decreased by treatment. In conclusion, dietary Suanzaoren decoction supplementation did relieve horse transportation stress. This study provides a useful clinical therapy for relieving transportation stress of horses.

Keywords: horse, Suanzaoren decoction, transportion stress

1. Introduction

Ili horses are characterized with strong pull performance, beautiful appearance, both speed and strength, high milk yield, and meat production rate. Ili horse breeding has become a pillar industry of animal husbandry in Xinjiang. Nevertheless, due to the fact that there are long distances between farms and hippodromes or slaughter plants, continuous transport for 24 h is common, and some trips last for 48 h or longer (Friend, 2000). Transportation has increasingly become one of the main causes of stress for animals (Pan et al., 2018). There are several studies reporting that transportation has been identified as a stressor for horses, and has been associated with several adverse outcomes including respiratory, gastrointestinal disease, and injury (Riley et al., 2018). For example, transportation can increase heart rate and serum cortisol (COR) concentration, which could further lead to gastric ulceration (Padalino et al., 2020). Moreover, an increased risk of transport-associated respiratory diseases is identified through clinical examination and observation of behavior along the way (Padalino et al., 2018a). Injury resulting from road transport is common in horses and is a potential welfare concern as well as a source of economic loss (Padalino et al., 2018b).

According to the theory of traditional Chinese medicine, Qi and blood are the material basis to maintain the normal function of the body. Qi can drive the movement of blood, blood can carry Qi, and Qi and blood breed mutually. Qi deficiency leads to less blood, and blood deficiency signals less blood. In recent years, using herbal medicines to relieve stress has received the widespread attention of researchers all over the world for the simple reason that herbal medicine exerts no side effects and increases a wide range of biological activity (Jiang, 2017; Liu et al., 2018; Padalino and Raidal, 2020). For example, it is reported that Sini powder, Banxiahoupu decoction, Ganmaidazao decoction, and Guizhigancaolonggumuli decoction could increase the anti-stress ability by affecting the different taches of the hypothalamus-pituitary-adrenal gland axle and changing the stress behaviors, which are based on their respective functions of regulating Qi, dispersing phlegm, tonifying Qi, and warming Yang (Ji et al., 2019). Chinese herbal medicine Yin-Chen-Hao-Tang can reduce the hepatic oxidative stress in obese mice with steatosis (Lee et al., 2010).

The Chinese herbal medicine prescription, Suanzaoren decoction, consists of the following components: Ziziphi Spinosae Semen, *Poria cocos* Wolf, Anemarrhena, Szechuan Lovage Rhizome, Licorice, Codonopsis, Radix Aucklandiae, and Polygala. Among them, Ziziphi Spinosae Semen, due to the effects of nourishing the heart and liver, calming the heart and mind, condensing sweat, and promoting body fluid, has been widely used in treating insomnia and anxiety (Cao et al., 2010; Yang and Zhang, 2015). *Poria cocos* Wolf, Radix Aucklandiae, and Anemarrhena have the beneficial efficacy of calmness, anti-inflammation, and antibacterial effect and have been reported to enhance the immune response and inhibit inflammation (Wagner et al., 2015; Lee and Cha, 2018; Ji et al., 2019). Szechuan Lovage Rhizome not only promotes Qi circulation and removes collateral obstruction, but also helps to dissipate coldness and dispel stasis and regulates vital energy and soothing liver (Cui et al., 2013). Licorice has the effects of clearing away heat and detoxification, relieving cough and expectorating, invigorating the spleen and Qi and has been used to treat many ailments including lung diseases, arthritis, kidney diseases, eczema, heart diseases, low blood pressure, gastric ulcer, liver toxicity, allergies, and certain microbial infections (Mamedov and Egamberdieva, 2019). Codonopsis has the effects of nourishing the middle and nourishing Qi and invigorating the spleen and lungs. It can also enhance immunity, dilate blood vessels, lower blood pressure, improve microcirculation, and enhance hematopoietic function (Singh et al., 2004). Polygala has the functions of soothing the nerves, improving the mind, eliminating phlegm, and reducing swelling (Farina et al., 2005). However, whether Suanzaoren decoction can alleviate the stress in horse transportation is unclear.

Herein, we investigated the efficacy of Suanzaoren decoction in relieving stress of horses during transportation. The efficacy and safety of Chinese herbal medicine Suanzaoren decoction was analyzed. Our findings may provide evidence for the use of Suanzaoren decoction as an intervention to alleviate stress of horses caused by transportation.

2. Material and Methods

2.1. Experimental animals

All animal procedures were approved by local Animal Ethics Committee and followed the guidelines in the document 2019020.

A total of six male Ili horses (weighing 450±20 kg) were purchased in Ili (Xinjiang, China). All the horses came from farmers and were of Ili breed for riding (grazing), ranging in age from 7 to 12 years, which had not been transported before under similar conditions. The experiment was carried out in the Urumqi, Xinjiang, China (43°49'07" S, 87°34'23" W).

2.2. Experimental design and diets

After five days of acclimation (horses were kept tied and fed conventional feed, with concentrated feed (about 1 kg) each in the morning and evening, during which a horse is fed 3 kg of alfalfa grass along with wheat straw a time, four times a day), six male Ili horses were randomly assigned to two groups, three horses per group. Horses in the control group were fed a basal diet (concentrated food, alfalfa grass, and wheat straw), while horses in the treatment group were fed a basal diet by adding the Suanzaoren decoction three times a day. The standard formula of Suanzaoren decoction is shown in Table 1. All of the horses were transported using common trucks under the condition of natural ventilation, during which each horse was tied to the rein, with good ventilation at an average speed of 80 km/h. The truck travelled on the common highway, taking 8 h in total. Water and food were not allowed throughout the whole journey.

Table 1 - Standard formula of Suanzaoren decoction

Chinese name	Pharmaceutical name	Percentage by mass
Suan Zao Ren	Ziziphi Spinosae Semen	11~15%
Fu Ling	Poria	24~28%
Zhi Mu	Anemarrhena	6~10%
Chuan Xiong	Szechuan Lovage Rhizome	11~15%
Gan Cao	Licorice	6~10%
Dang Shen	Codonopsis	11~15%
Mu Xiang	Radix Aucklandiae	6~10%
Yuan Zhi	Polygala	11~15%
	Total	100%

2.3. Sample collection

Blood samples were collected from the jugular vein of each horse at 8:00 h (before transport, BT), 12:00 h (during transport, T), and 20:00 h (4 h after transportation, AT). Serum was obtained after centrifugation at 3000 × *g* for 10 min at 4 °C.

2.4. Detection of physiological indexes

The counts of white blood cells (WBC), red blood cells (RBC), hemoglobin (HGB), hematocrit (HCT), neutrophil (GRAN), and lymphocyte (LYM) in blood samples were determined using a PE-6800 automatic hemocyte analyzer (China).

2.5. Detection of biochemical indicators

Total protein (TP), albumin (ALB), aspartate transaminase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), creatine (CK), lactate dehydrogenase (LDH), and glucose (GLU) were determined using a SUNO AB-1020 automatic biochemical analyzer (China).

2.6. The detection of hormone levels

The levels of triiodothyronine (T3), thyroxine (T4), human growth hormone (HGH), COR, insulin (INS), adrenocorticotropic hormone (ACTH), and adrenaline (AD) were determined by radioimmunoassay.

2.7. Statistics

Data were analyzed by two-way ANOVA with a model including Chinese herbal medicine prescription treatment, road transport, and their interactions as fixed effects with animal as the random effect. Significant differences in treatment means were determined by Duncan's multiple range tests at P<0.05.

Analysis of variance models can be generalized to more than one grouping variable. In case there are two such variables: one representing rows having I categories, and one representing columns having J categories. The two-way ANOVA model has the form:

$$y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ijk},\ i = 1, \dots, I,\ j = 1, \dots, J,\ k = 1, \dots, n_{ij}$$

Parameters α_i and β_j represent the main effects of rows and columns, respectively, and have the same general interpretation as the effect in a one-way ANOVA does. The $(\alpha\beta)_{ij}$ represents an interaction effect.

3. Results

3.1. Physiological indexes

Significant interaction between road transport and Suanzaoren decoction supplementation was found for WBC, MID%, and GRAN% values (P<0.01; Figure 1A-1C). White blood cells and GRAN% were not affected by treatment in BT group. The highest WBC value was found in T group ($9.20\pm1.20\times10^9\cdot L^{-1}$), which was decreased by treatment (P<0.01). The highest GRAN% was found in AT group (59.43±3.22%), which was decreased by treatment (P<0.01). Compared with the control group, horses in the Suanzaoren decoction treatment group had lower WBC, MID%, RBC, HGB, and HCT values, but higher LYM% values (P<0.01) (Table 2). Values of WBC and GRAN% had a significant increase during transportation, and LYM% showed opposite effect (P<0.01; Table 2).

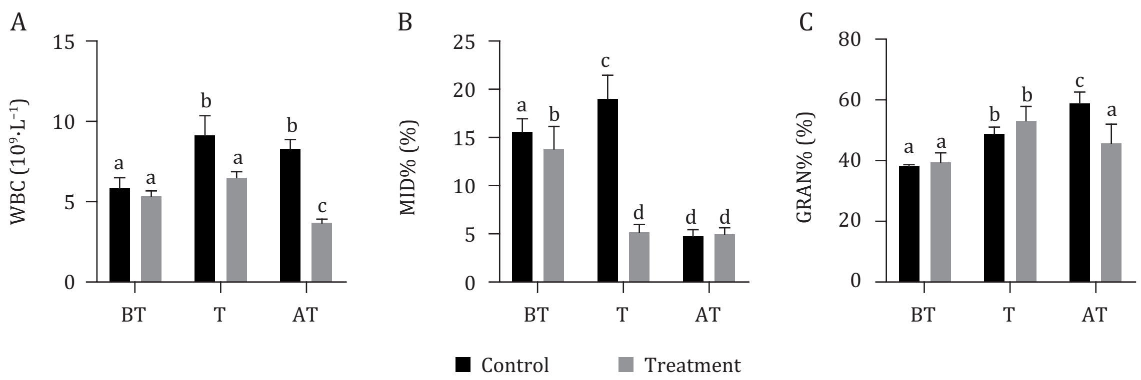

Figure 1 - Interaction effects between road transport and Suanzaoren decoction supplementation on white blood cell (WBC), intermediate cell ratio (MID%), and granulocyte ratio (GRAN%).

Table 2 - Effects of Chinese herbal medicine on blood physiological indexes of horses before transport (BT), during road transport (T), and after transport (AT)

Chinese herbal medicine	Transport	WBC ($\times 10^9 \cdot L^{-1}$)	LYM% (%)	MID% (%)	GRAN% (%)	RBC ($\times 10^{12} \cdot L^{-1}$)	HGB ($\times 10^{12} \cdot L^{-1}$)	HCT (%)	MCV (fL)	MCH (pg)	MCHC (g/L)	RDW-SD (fL)
Control	BT	5.85±0.75	44.93±1.83	15.80±1.30	38.55±0.05	7.42±0.60	119.00±5.29	36.87±0.93	49.97±3.90	16.07±1.39	322.33±6.66	35.63±3.87
	T	9.20±1.20	34.85±1.75	19.10±2.40	48.90±2.20	6.47±0.38	104.33±5.03	31.83±1.80	49.43±4.45	16.13±1.25	327.67±4.51	34.90±2.60
	AT	8.40±0.50	37.35±2.15	4.93±0.58	59.43±3.22	6.79±0.29	107.00±9.17	33.47±2.58	49.37±3.84	15.70±1.06	319.33±11.06	34.90±4.64
	Total	7.82±1.69	39.04±4.84	13.28±6.57	48.96±9.25	6.90±0.57	110.11±8.95	34.06±2.76a	49.59±3.54	15.97±1.09	323.11±7.75	35.14±3.31
Treatment	BT	5.40±0.30	46.35±5.25	14.00±2.20	39.65±3.05	5.46±0.11	90.33±6.35	27.37±1.05	49.97±2.93	16.17±1.30	323.33±7.64	35.67±2.25
	T	6.65±0.25	41.65±3.95	5.23±0.83	53.25±4.75	6.20±1.11	89.33±4.16	27.70±0.95	50.37±3.37	16.27±0.80	324.00±6.93	36.43±1.27
	AT	3.82±0.13	46.20±2.70	5.00±0.70	45.60±6.60	6.20±0.56	98.00±9.54	30.70±2.15	49.73±3.95	15.77±1.25	318.33±9.29	34.90±2.60
	Total	5.29±1.24	44.73±4.24	8.08±4.61	46.17±7.33	5.95±0.72	92.56±7.35	28.59±2.05	50.02±2.99	16.07±1.01	321.89±7.44	35.67±1.95
P-value												
Chinese herbal medicine		<0.01	<0.01	<0.01	0.153	<0.01	<0.01	<0.01	0.811	0.862	0.750	0.725
Transport		<0.01	<0.01	<0.01	<0.01	0.889	0.170	0.054	0.979	0.774	0.344	0.886
Chinese herbal medicine × Transport		<0.01	0.158	<0.01	<0.01	0.068	0.077	0.120	0.977	0.999	0.879	0.886

WBC - white blood cell; LYM% - lymphocyte ratio; MID% - intermediate cell ratio; GRAN% - granulocyte ratio; RBC - red blood cells; HGB - hemoglobin; HCT - hematocrit; MCV - mean corpuscular volume; MCH - mean corpuscular hemoglobin; MCHC - mean corpuscular hemoglobin concentration.

3.2. Serum biochemical parameters

There was significant interaction between road transport and Suanzaoren decoction supplementation for serum ALT, GLU, AST, and CK ($P<0.01$; Figure 2 and Table 3). Levels of ALT, GLU, AST, and CK in serum remained unchanged in BT groups. The highest ALT (59.05 IU/L), GLU (6.48 mmol/L), AST (328.45 IU/L), and CK (342.51 IU/L) levels were observed in AT groups ($P<0.01$), which were decreased with the treatment for similar values to those in BT groups. Compared with serum from horses without treatment, serum of horses fed Suanzaoren Decoction had lower CK ($P<0.01$) and LDH content ($P<0.01$) and higher ALP ($P<0.01$; Table 3). Road transport increased serum TP, ALT, GLU, and CK levels. Moreover, no treatment effects, road transport effects, or interaction effects between treatment and road transport were found in serum ALB values.

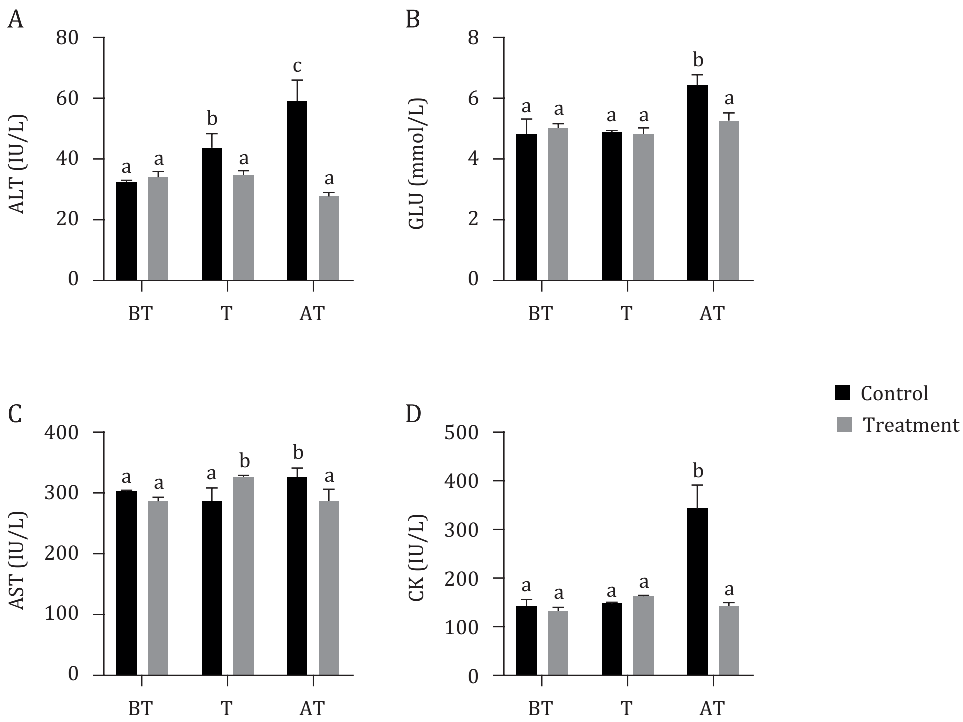

Figure 2 - Interaction effects between road transport and Suanzaoren decoction supplementation on serum alanine aminotransferase (ALT), glucose (GLU), aspartate aminotransferase (AST), and creatine kinase (CK) contents.

3.3. Hormone levels

No interaction effects between road transport and dietary Chinese medicine complex supplementation were found for T3 and AD (Table 4). Significant interaction between road transport and Suanzaoren decoction supplementation was observed in HGH, COR, ACTH, AVP ($P<0.01$; Table 4), and T4 and INS ($P<0.05$; Table 4). The highest T4 levels (21.85 ng/mL), INS (18.39 uIU/mL), and ACTH (25.49 pg/mL) were observed in serum from AT groups ($P<0.01$), which were decreased by treatment ($P<0.01$; Table 4).

Table 3 - Effects of Chinese herbal medicine on serum biochemical parameters of horses before transport (BT), during road transport (T), and after transport (AT)

Chinese herbal medicine	Transport	TP (g/L)	ALB (g/L)	ALT (IU/L)	AST (IU/L)	ALP (IU/L)	GLU (mmol/L)	CK (IU/L)	LDH (IU/L)
BT	Control	77.85±4.25	39.83±0.95	32.9±0.1	302.8±2.4	155.45±7.25	4.84±0.55	146.06±11.64	343.88±18.19
	Treatment	66.9±15.8	39.8±0.95	34.43±0.54	288.8±5.8	215.65±11.35	5.10±0.5	132.87±8.54	285.92±10.64
	Total	72.38±11.96	39.82±0.85	41.8±1.89	295.8±8.63	185.55±34.06	4.97±0.53	139.46±11.64	314.9±34.43
T	Control	107.3±2.69	38.9±1	44.1±4	286.95±21.05	171.05±11.95	4.92±0.1	147.2±2.61	346.1±18.03
	Treatment	112.2±2.75	42.6±22.82	35.17±4.2	325.2±4.8	208.7±12	5.89±0.57	163.64±3.04	324.02±4.3
	Total	109.75±3.62	40.75±16.28	57.8±4.67	306.08±25.01	189.88±23.24	6.91±0.36	155.42±9.35	335.06±16.84
AT	Control	85.1±6	39.5±1.44	59.05±3.7	328.45±13.35	160.5±8.2	6.48±0.33	342.51±47.63	355.13±5.25
	Treatment	83.4±0.96	36.15±0.35	27.9±1.3	286.35±20.75	220.7±6.9	5.32±0.16	142.36±3.73	319.98±9.12
	Total	84.25±3.95	37.83±2.06	43.49±5.9	307.4±27.84	190.6±33.66	5.9±0.68	242.44±113.71	337.55±20.37
P-value									
Chinese herbal medicine		0.467	0.451	0.103	0.373	<0.01	<0.01	<0.01	<0.01
Transport		<0.01	0.345	<0.05	0.308	0.642	<0.01	<0.01	0.14
Chinese herbal medicine × transport		0.209	0.284	<0.01	<0.01	0.114	<0.01	<0.01	0.072

TP - total protein; ALB - albumin; ALT - alanine aminotransferase; AST - aspartate transaminase; ALP - alkaline phosphatase; GLU - glucose; CK - creatine; LDH - lactate dehydrogenase.

Table 4 - Effects of Chinese herbal medicine on hormone levels of horses before transport (BT), during road transport (T), and after transport (AT)

Chinese herbal medicine	Transport	T3 (ng/mL)	T4 (ng/mL)	HGH (ng/mL)	COR (ng/mL)	INS (uIU/mL)	ACTH (pg/mL)	AVP (pg/mL)	AD (pg/mL)
BT	Control	0.56±0.05	11.69±1.26	2.35±0.25	62.53±4.6	14.76±0.09	20.87±0.61	17.56±0.33	96.18±1.74
	Treatment	0.68±0.05	13.57±0.69	3.16±0.18	67.49±6.4	15.91±1.28	21.35±0.17	13.36±1.05	99.13±3.9
	Total	0.62±0.08	12.63±1.37	2.76±0.49	60.01±5.50	15.33±1.03	21.11±0.48	15.46±2.41	97.66±3.15
T	Control	0.52±0.04	14.3±1.79	2.56±0.11	138.84±6.59	17.85±1.67	18.88±1.75	13.8±0.56	95.71±0.13
	Treatment	0.54±0.11	15.42±0.71	1.83±0.01	86.74±1.76	17.95±1.48	18.88±0.59	13.37±0.13	94.9±1.62
	Total	0.53±0.08	14.86±1.37	2.2±0.41	112.79±4.18	17.9±1.41	18.88±1.17	13.58±0.44	95.31±1.12
AT	Control	0.76±0.04	21.85±0.64	2.06±0.16	93.23±0.93	18.39±0.06	25.49±0.44	12.76±0.82	98.82±0.57
	Treatment	0.69±0.09	16±4.59	2.43±0.17	61.73±1.16	15.43±0.2	10.53±0.55	12.22±0.8	101.53±7.39
	Total	0.72±0.07	18.93±4.35	2.25±0.25	77.48±1.05	16.91±1.63	18.01±8.2	12.49±0.78	100.18±4.92
P-value									
Chinese herbal medicine		0.550	0.363	0.073	<0.01	0.274	<0.01	<0.01	0.353
Transport		<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.099
Chinese herbal medicine × Transport		0.094	<0.05	<0.01	<0.01	<0.05	<0.01	<0.01	0.605

T3 - triiodothyronine; T4 - thyroxine; HGH - human growth hormone; COR - cortisol; INS - insulin; ACTH - adrenocorticotropic hormone; AVP - arginine vasopressin; AD - adrenaline.

4. Discussion

The transportation stress of livestock and poultry is referred to as an adaptive and defensive response of livestock under the stimulation of stressors such as fasting and water prohibition, environmental changes (temperature and humidity), and turbulence during transportation, which will affect the production performance, endocrine metabolism, and immunity of the animals (Padalino et al., 2018a). Transportation is considered stressful because horses are confined in a small space, and have higher arousal and the difficulty of maintaining balance during transport (Padalino and Raidal, 2020).

Ziziphi Spinosae Semen, a prototypical hypnotic-sedative herb in oriental medicine, exhibits anxiolytic effects by improving amygdaloid CRF/CRF1 receptor signalling (Li et al., 2019). In addition, the Ziziphi Spinosae Semen can be effectively used as a healthy functional food for preventing or alleviating inflammatory diseases or a therapeutic agent for inflammatory diseases (Kim et al., 2018). Poria extract has the effect of nourishing the liver through regulation of lipid metabolism, inhibiting ER stress, and activating autophagy via AMPK activation (Kim et al., 2019). Anemarrhena and Szechuan lovage rhizome extracts have certain antiplatelet and antithrombotic activities (Lu et al., 2011; Wang and Lu, 2011). Horses in transportation may be inflicted by long-term confining in a limited space, causing prolonged hypoxic exposures and resulting in procoagulant state. Codonopsis extract has the effect of improving the procoagulant state induced by hypoxia (Das et al., 2019). Licorice has the effects of clearing away heat and detoxification (Mohseni et al., 2014).

Biochemical and physiological blood indexes can reflect the physiological changes and are usually used to assess animal welfare and health (Galapero et al., 2015). Among them, WBC, GRAN%, and LYM% are generally used as indicators of immune function. In addition, elevation in serum COR concentration in response to stress decreases LYM count (Morán et al., 2017). Similar to previous studies, horses during transport showed increase in neutrophils (GRAN%), COR, and WBC, but showed a decrease in LYM% in our study. However, Suanzaoren decoction intervention could alleviate these abnormalities in inflammation-related indicators caused by transportation stress.

Serum CK activity is considered an important indicator of muscle damage and muscle exhaustion. When animals are subjected to transport stress, CK from muscles is released into the blood (Alcalde et al., 2017). Similarly, we found that Suanzaoren decoction supplementation decreased serum CK activity, suggesting a lower level of tissue damage or physical fatigue. This is possible due to the antioxidant activity of chlorogenic acids in Poria and Licorice. Previous study has reported that stress can inhibit membrane ATPases activities and damage the integrity and permeability of cell membrane. However, antioxidants can restore the ATPases activities, and dietary antioxidants supplementation can decrease serum CK activity in horses subjected to transport and heat stress (Liu et al., 2016). Elevation in serum COR concentrations can increase serum glucose concentrations by increasing glycogenolysis. In the present study, horses during transport showed increase in serum COR concentrations, and GLU had a significant increase. The rapid increase in GLU in horses after transport may be partially due to the gluconeogenic activity of COR as well as hemoconcentration. Our research showed that Suanzaoren decoction relieved GLU and serum COR reduction caused by transportation stress. This may be achieved by reducing the amount of red blood cells and hematocrit in the blood and via reducing the dehydration of animals. There are some limitations for this study due to factors including geographical location, climate, sample size, and temperament of horses.

5. Conclusions

We intended to investigate the efficacy of Suanzaoren decoction in transport stress of horse, and believe that conscientiously performed studies and positive outcomes can benefit animals during transportation. This study demonstrates that dietary Suanzaoren decoction supplementation relieves horse transportation stress, and can contribute as a useful clinical therapy for relieving transportation stress of horses.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Conceptualization: J. Wang. Data curation: X. Ma, W. Xiang, Y. Lu, Z. Mai, Q. Wang, G. Yao, L. Kuang, H. Zhao, M. Ye and M. Hou. Formal analysis: X. Ma, W. Xiang, Y. Lu, Z. Mai, Q. Wang, G. Yao, L. Kuang, H. Zhao, M. Ye and M. Hou. Funding acquisition: J. Wang. Methodology: X. Ma and W. Xiang. Software: X. Ma, W. Xiang, L. Liu, A. Tang and S. Zhai. Supervision: J. Wang. Writing-original draft: X. Ma and W. Xiang. Writing-review & editing: J. Wang.

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