Active components of common traditional Chinese medicine decoctions have antioxidant functions

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ABSTRACT: Many traditional Chinese medicine (TCM) decoctions are proven to have multiple functions in animal production. These decoctions are seldom recognized by the international scientific community because the mechanisms of action are not clearly elucidated. According to TCM theory, Cortex Phellodendri (COP), Rhizoma Atractylodes (RA), Agastache Rugosa (AR), and Gypsum Fibrosum (GF) can be used to formulate a medicinal compound that prevents or cures animal disease caused by heat stress. The aim of this research was to study the regulatory functions of the active components of TCM and to elucidate the effects of different TCM decoctions on antioxidant activity and lipid peroxide content, using in vitro and in vivo models of heat stress. For in vitro experiments, intestinal crypt-like epithelial cell line-6 (IEC-6) cells were employed to evaluate the effects of the active components of COP and to elucidate the effects of different TCM decoctions on antioxidant activity and lipid peroxide content, using in vitro and in vivo models of heat stress. For in vitro experiments, intestinal crypt-like epithelial cell line-6 (IEC-6) cells were employed to evaluate the effects of the active components of COP, RA, AR, and GF. For in vivo experiments, forty-eight 2-mo-old Chinese experimental mini-pigs (7.20 ± 0.02 kg) were randomly assigned to 4 groups: a normal-temperature group (NTG); a high-temperature group (HTG); HTG treated with COP, RA, AR, and GF (1:1:1:1, TCM1); and HTG treated with COP, RA, AR, and GF (1:1:1:0.5, TCM2). Results showed that the active components of the COP, RA, AR, and GF increased (P < 0.05) the proliferation and viability of heat-stressed IEC-6 cells and that the most effective treatment doses of COP alkaloid, RA, Aetherolea, Herba Agastachis Aetherolea, and GF water extract were 200, 100, 100, and 200 µg/mL, respectively. All 4 active components increased (P < 0.05) superoxide dismutase, glutathione peroxidase activities, and glutathione content, and decreased (P < 0.05) malondialdehyde content with respect to the heat-stressed group to concentrations similar to those seen in NTG. In vivo experiments demonstrated that TCM1 and TCM2 improved (P < 0.05) the poor growth performance seen in HTG pigs. The superoxide dismutase, glutathione peroxidase activities, and malondialdehyde content in porcine jejunum treated with TCM1 and TCM2 were not different (P > 0.05) from those seen in the NTG and were better (P < 0.05) than results seen in the HTG. Overall, it appeared that TCM2 was more effective than TCM1 in ameliorating the effects of heat stress in pigs. In conclusion, this study revealed that the active components of common TCM decoctions have antioxidant functions.

Key words: active component, antioxidant, intestinal crypt-like epithelial cell line, lipid peroxide, traditional Chinese medicine

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INTRODUCTION

Because of increasing public interest in biosafety and organic farming, further study into the effects of using plant extracts in animal feed is warranted. Traditional Chinese medicine (TCM) has many advantages including the natural origin of its constituents, few side effects, and multifunctional properties verified through hundreds of years of use (Li et al., 2008). However, investigation into the mechanisms of action of TCM has been performed only recently.

According to TCM theory, Cortex Phellodendron (COP), Rhizome Atractylodes (RA), Agastache Rugosa (AR), and Gypsum Fibrosum (GF) can be combined into a medicinal compound that prevents or cures animal disease caused by heat stress, which is becoming the primary factor influencing animal health and production due to global warming (Leon et al., 2005), especially animal production in tropical areas. The active components of COP, RA, AR, and GF are COP alkaloid (CPA), RA Aetherolea (RAA), Herba Agastachis Aetherolea (HAA), and GF water extract (GFE), respectively. Each of these 4 active components has an antioxidative function against heat stress (Chen et al., 1998; Lu et al., 2006; Yan et al., 2006).

Animal antioxidant and lipid peroxidation levels may be significantly affected by heat stress (Meerson, 1984). Superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) activities, and malondialdehyde (MDA) content reflect the antioxidant and lipid peroxidation status of cultured cells and animal tissues (Efe et al., 1999).

The aim of this research was to elucidate the effects of TCM decoctions on animal antioxidant activity in vitro by studying the effects of varying doses of CPA, RAA, HAA, and GFE on SOD, glutathione (GSH), GSH-Px activity, and MDA content in heat-stressed intestinal crypt-like epithelial cell line-6 (IEC-6) cells. Furthermore, 2 different decoctions composed of COP, RA, HA, and GF were used in vivo to determine their effects on SOD, GSH-Px activity, and MDA content in heat-stressed porcine jejunum.

MATERIALS AND METHODS

All experimental protocols involving animals were approved by the Committee for the Care and Use of Experimental Animals, Beijing University of Agriculture.

In Vitro Experiments

IEC-6 Cell Culture. The IEC-6 cells (CRL21592, obtained from Peking Union Medical College, Beijing, China) were maintained in culture in Dulbecco’s modified Eagle’s medium (DMEM) containing 5% (vol/vol) fetal bovine serum (HyClone, Logan, Utah), 2 mg/L of insulin, 50 IU/mL of penicillin, and 50 g/mL of streptomycin (complete medium) at 37°C in a 5% (vol/vol) CO₂ atmosphere. After the initial plating of cells, the medium was changed 24 h postplating.

IEC-6 Heat Stress Model. To establish an IEC-6 heat stress model, the proliferation of IEC-6 cells was measured at different temperatures at different time points. When IEC-6 cells reached >95% confluence, cells were digested using 0.25% pancreatic enzyme (Gibco, New York, NY). Subsequently, 100 µL of the IEC-6 cell suspension was loaded into 96-well multi-plates and cultured in DMEM containing 5% fetal bovine serum at a density of 1 × 10⁵ cells/mL. The cells were divided into 10 groups: a normal-temperature control group (NTG) that was cultured at 37°C for 44 h and 9 heat-stressed groups that were first cultured at 37°C and then incubated at 39, 41, or 43°C for the final 3, 2, or 1 h (3 × 3 factorial arrangement of treatments). After the heat-stress period, a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was performed according to the methods of Yu et al. (2010b). Five observations were made on each of the 3 replicates for each group over the treatment period. Cells grown under these optimized conditions were subsequently referred to as the heat-stress condition.

Dosage Screening of 4 Active Components of TCM. To identify the treatment dose that provides the best protection to heat-stressed cells, CPA, RAA, HAA, and GFE were prepared and standardized according to Chinese Pharmacopoeia (2005), which contained 42.0% berberine, 33.6% β-eudesmol, 30.5% patchouli alcohol, and 92% CaSO₄, respectively. Successfully, these 4 solutions were made to concentrations of 200, 100, and 50 µg/mL, respectively. In this experiment, 90 µL of the IEC-6 cell suspension was loaded into 96-well multi-plates and cultured in DMEM containing 5% fetal bovine serum at a density of 1 × 10⁵ cells/mL. The cells were divided into 14 groups: NTG, a high-temperature control group (HTG), and 12 experimental groups of heat-stressed cells treated with 10 µL of CPA, RAA, HAA, or GFE at 3 different concentrations (4 × 3 factorial arrangement of treatments). For control groups, 10 µL of DMEM was added to the cell suspension. Experimental and control groups were maintained and then processed according to the protocol outlined for the previous assay.

Effects of CPA, RAA, HAA, and GFE on IEC-6 SOD, GSH-Px Activities, and GSH and MDA Contents. Optical density (OD) readings obtained from the previous assay were used to identify the concentrations of CPA, RAA, HAA, and GFE that had the greatest influence on the proliferation of IEC-6 cells during heat stress. The effects of predetermined concentrations of CPA, RAA, HAA, and GFE on SOD, GSH-Px activities, and GSH and MDA contents were evaluated using IEC-6 cells.

After centrifugation at 1,000 × g for 20 min at room temperature, SOD activity was determined spectrophotometrically on cell culture supernatants using the hydroxylamine method (Marklund and Marklund, 1974).
with a microplate reader (Bio-Rad Laboratories, Hercules, CA) at a fixed absorption wavelength of 550 nm. The GSH content was determined using the method described by Beutler et al. (1963) at 412 nm.

To measure GSH-Px activity, cultured cells were washed twice with PBS before adding 150 µL of Tris buffer (50 mMol/L of Tris-HCl, pH 7.5, 5 mmol/L ethylenediaminetetraacetic acid, 1 mmol/L of dithiothreitol). After ultra-sonication for 1 min, cellular debris was removed by centrifugation at 14,000 × g at 4°C for 30 min. The supernatant was collected and GSH-Px activity was measured spectrophotometrically using a commercially available GSH-Px kit (Nangjing Jiancheng Bioengineering Institute, Nanjing, China), at a fixed absorption wavelength of 412 nm.

Lipid peroxides in cultured suspensions were measured spectrophotometrically by the thiobarbituric acid method (Uchiama and Mihara, 1978) at a fixed absorption wavelength of 532 nm.

**In Vivo Experiments**

**Animals.** Forty-eight 2-mo-old male Chinese experimental mini-pigs (7.20 ± 0.02 kg) were purchased from the Changping Experimental Pig Farm at China Agricultural University and randomly assigned to 4 groups (12 pigs each): NTG; HTG; HTG treated with COP, RA, AR, and GF (1:1:1:1 dry weight, TCM1); and HTG treated with COP, RA, AR, and GF (1:1:1:0.5 dry weight, TCM2). Each group was balanced for BW and litter origin. Pigs were housed and managed as described by Liu et al. (2009).

**Preparation of TCM Decoctions.** In the present study TCM decoctions were composed of COP, RA, AR, and GF in a dry weight ratio of 1:1:1:1 (TCM1) or 1:1:1:0.5 (TCM2). All raw materials used in the decoctions were purchased from the Chinese Traditional Medicine Pharmacy, Tong Ren Tang. The combined ingredients were immersed in water for 40 min, extracted in boiling water for 2 h, and the aqueous extract separated by filtration (100-mesh). The extract was concentrated by heating to 50°C under reduced pressure until a relative density of 1 g/mL was obtained. The concentrated extract was then dried, combined with excipient (starch), and ground into fine granules to be mixed into the basal feed of the pigs at a dose of 0.15 g/(kg of BW-d). At the same time, the same quantity of excipient was added to the basal feed of the 2 control groups.

**Treatment and Sampling.** Animals were acclimatized to a temperature of 23°C over a period of 7 d. Over the 10-d experimental period, the NTG was maintained at this temperature. For the HTG, TCM1, and TCM2 groups, the temperature was increased to 26°C and maintained at this temperature for the whole experiment, except when the temperature was raised from 26 to 40°C for 5 h from 0400 until 0900 h. Heat-stressed groups were subjected to this procedure for 10 consecutive days.

Body weight and feed intake were measured at the beginning and end of the experiment. For the experimental period, ADFI, ADG, and G:F were calculated.

Four pigs were randomly selected from each group at d 1, 3, 6, and 10 of the experimental period. The pigs were electrically stunned using a head-only electric stun tong apparatus (Xingye Butchery Machinery Co. Ltd., Chengde, Hunan Province, P.R. China) and subsequently exsanguinated. Samples from the middle part of the jejunum were immediately collected and washed with physiological saline. Jejunal samples were then minced, transferred into sample tubes, snap-frozen in liquid nitrogen, and stored at −80°C.

Antioxidants and lipid peroxides present within jejunal homogenates were measured to evaluate the TCM efficacy. Briefly, SOD enzyme activity was measured by the inhibition of pyrogallol autoxidation at 420 nm for 3 min, according to methods described by Marklund and Marklund (1974). Enzyme activity was expressed as units per milligram of protein, where 1 U is the amount of enzyme required to bring about 50% inhibition of pyrogallol autoxidation. The GSH-Px enzyme activity was assayed according to the method of Rotruck et al. (1973) using H2O2 as a substrate. Enzyme activity was expressed as the quantity of GSH oxidized in µg/(min-mg) of protein. For lipid peroxide analysis, the intestinal homogenate (1 mg of protein) was precipitated with trichloroacetic acid and reacted with thiobarbituric acid in a boiling water bath for 15 min. After cooling the sample, the absorbance was measured at 532 nm and the concentration of MDA was calculated using a molar extinction coefficient value of 153,000 M⁻¹·cm⁻¹. The results were expressed as nanomoles of MDA per milligram of protein (Esterbauer and Cheeseman, 1990).

**Statistical Analysis.** All results are presented as the mean ± SE. Statistical analysis was performed by 1-way ANOVA (SPSS Inc., Chicago, IL). Duncan’s multiple range test was used to compare differences among the treatment means. A P-value <0.05 was considered significantly different.

**RESULTS**

**In Vitro Experiments**

**Establishment of an IEC-6 Heat-Stress Model.** The proliferation of IEC-6 cells at different temperatures and at different times is shown in Figure 1. In comparison with the 37°C control group, IEC-6 cell proliferation increased (P = 0.039) when cultured at 39°C for 1 h, whereas IEC-6 cell proliferation decreased when cultured at 41°C for 3 h (P = 0.015), or at 43°C for 1, 2, or 3 h (P = 0.035, 0.030, and 0.011, respectively). All heat-stressed groups exhibited a linear decreasing response when subjected to longer periods of heat stress at the same temperature condition. Similar effects were observed in cells exposed to tem-
Temperatures of 41°C for 3 h, or 43°C for 3 h. A heat-stress model established by culturing cells at 41°C for 3 h was used in subsequent experiments.

Effects of the Active Components of TCM on the Proliferation of IEC-6 Cells (OD$_{570}$). The effects of HAA, RAA, CPA, and GFE on the proliferation of IEC-6 cells were examined using the heat-stress model established in the previous study (Figure 2). In comparison with the NTG, the HTG exhibited less ($P = 0.025$) cell proliferation. Treatment with 200 and 100 µg/mL of HAA gave rise to OD values that were greater ($P = 0.031$ and 0.042) than those measured in the HTG and similar to those measured in the NTG. Treatment with RAA at all 3 concentrations gave rise to OD values that were not different ($P > 0.05$) from those measured in the NTG and HTG. Treatment with 200 and 100 µg/mL of CPA gave rise to OD values that were greater ($P = 0.035$ and 0.012) than those measured in the HTG. Treatment with GFE gave similar results with CPA. In summary, 200 µg/mL of HAA, 100 µg/mL of RAA, 100 µg/mL of CPA, and 200 µg/mL of GFE were the treatment doses most effective at enhancing cell proliferation.

Effects of 4 Active Components in TCM on Antioxidant Status and Lipid Peroxides in Heat-Stressed IEC-6 Cells. The antioxidant status and lipid peroxidation content were measured in cultured cells treated with 200 µg/mL of HAA, 100 µg/mL of RAA, 100 µg/mL of CPA, and 200 µg/mL of GFE. Heat stress reduced ($P = 0.018$) SOD activity when compared with activity observed in cells cultured at normal temperature (Figure 3a). The HAA, RAA, and CPA supplements may have offset the decrease in SOD activity; the values of these 3 groups were not different ($P > 0.05$) from those measured in the NTG. Treatment with GFE gave similar results with CPA. In summary, 200 µg/mL of HAA, 100 µg/mL of RAA, 100 µg/mL of CPA, and 200 µg/mL of GFE were the treatment doses most effective at enhancing cell proliferation.

In Vivo Experiments

Growth Performance of Piglets. The effects of TCM1 and TCM2 treatment on the growth performance of heat-stressed piglets are shown in Table 1. The ADFI, ADG, and G:F differed ($P < 0.05$) among groups, even though the final BW was not different ($P > 0.05$) from the initial BW. Growth performance in the HTG was inferior ($P < 0.05$) to that in the NTG. The TCM1 and TCM2 treatments increased ADFI ($P = 0.035$ and 0.018) and ADG ($P = 0.029$ and 0.008) when compared with the HTG. The G:F of pigs treated with TCM1 or TCM2 was not different from that of pigs in the NTG, but was less ($P = 0.038$ and 0.021) than that of pigs in the HTG. Even though there was no difference in growth performance among pigs in the

Figure 1. Effects of different temperatures on intestinal crypt-like epithelial cell line-6 (IEC-6) cell proliferation over time (optical density at 570 nm, OD$_{570}$). For each heat-stressed time, columns without a common letter (a–c) differ ($P < 0.05$).

Figure 2. Effects of the active components of 4 traditional Chinese medicines (TCM) on intestinal crypt-like epithelial cell line-6 (IEC-6) cell proliferation (mean ± SE; optical density at 570 nm, OD$_{570}$). For each active component, columns without a common letter (a,b) differ ($P < 0.05$). NTG = normal-temperature group; HTG = high-temperature group; HAA = Herba Agastachis Aetherolea; RAA = Rhizoma Atractylodi Aetherolea; CPA = Cortex Phellodendri alkaloid; GFE = Gypsum Fibrosum water extract.
NTG, TCM1, and TCM2 groups, those in the TCM2 group showed the best growth performance overall.

**Effects of TCM Decoctions on the Antioxidant Status and Lipid Peroxide Content in Heat-Stressed Porcine Jejunum.** To study the in vivo effects of TCM administration, the antioxidant status and lipid peroxide content were evaluated in heat-stressed porcine jejunum. Heat stress reduced (P = 0.040) SOD activity, whereas the addition of TCM1 and TCM2 increased (P < 0.05) SOD (Figure 4). This increase became more and more evident over the course of the experimental period. From d 6 of the experiment, SOD activity in the TCM2 group became greater (P = 0.045) than in the HTG.

A similar trend was observed when GSH-Px activity was measured at different time points across the experimental period (Figure 5). At d 10, GSH-Px activity in the HTG became less (P = 0.013) than in the NTG. The addition of TCM1 and TCM2 similarly increased (P < 0.05) GSH-Px activity in a time-dependent manner. From d 3, GSH-Px activities in TCM1 and TCM2 groups were no longer significantly different from that measured in the NTG, but GSH-Px activity in the TCM2 group was greater (P = 0.043) than that in the HTG on d 10.

Measurement of MDA content was used to evaluate lipid peroxidation in jejunal mucosa of piglets. Heat stress gave rise to an increase (P < 0.05) in MDA content in the HTG greater than that seen in the NTG over d 1 to 6 of the experiment (Figure 6). The addition of TCM1 and TCM2 reduced MDA content to the extent that MDA content in these groups was not

**Figure 3.** Effects of the active components of 4 traditional Chinese medicine (TCM) decoctions on superoxide dismutase (SOD), glutathione (GSH), and glutathione peroxidase (GSH-Px) activity, and malondialdehyde (MDA) content in heat-stressed intestinal crypt-like epithelial cell line-6 (IEC-6) cells. Within each graph, different uppercase letters (A, B) mean P < 0.01; different lowercase letters (a, b) mean P < 0.05. NTG = normal-temperature control group; HTG = high-temperature control group; HAA = Herba Agastachis Aetherolea; RAA = Rhizoma Atractylodi Aetherolea; CPA = Cortex Phellodendri alkaloid; GFE = Gypsum Fibrosum (GF) in a dry-weight ratio of 1:1:1:1; TCM2 = COP:RA:AR:GF in a dry-weight ratio of 1:1:0.5.

<table>
<thead>
<tr>
<th>Item</th>
<th>NTG</th>
<th>HTG</th>
<th>TCM1</th>
<th>TCM2</th>
</tr>
</thead>
<tbody>
<tr>
<td>IBW, kg (n = 16)</td>
<td>7.19 ± 0.67</td>
<td>7.18 ± 0.45</td>
<td>7.20 ± 0.58</td>
<td>7.21 ± 0.56</td>
</tr>
<tr>
<td>FBW, kg</td>
<td>9.63 ± 0.65</td>
<td>8.99 ± 0.53</td>
<td>9.62 ± 0.78</td>
<td>9.89 ± 0.54</td>
</tr>
<tr>
<td>ADFI, g</td>
<td>698.8 ± 15.9a</td>
<td>640.0 ± 20.7a</td>
<td>690.2 ± 18.5a</td>
<td>710.2 ± 28.4a</td>
</tr>
<tr>
<td>ADG, g</td>
<td>245.2 ± 8.1a</td>
<td>181.7 ± 19.5a</td>
<td>241.5 ± 21.3a</td>
<td>268.0 ± 10.4a</td>
</tr>
<tr>
<td>Feed:gain</td>
<td>2.85 ± 0.18b</td>
<td>3.52 ± 0.28b</td>
<td>2.86 ± 0.23b</td>
<td>2.65 ± 0.18b</td>
</tr>
</tbody>
</table>

**Table 1.** Effects of TCM1 and TCM2 on growth performance in heat-stressed piglets (mean ± SE)1

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1IBW = initial BW; FBW = final BW; NTG = normal-temperature group; HTG = high-temperature group; TCM1 = Cortex Phellodendron (COP):Rhizome Atractylodes (RA):Agastache Rugosa (AR):Gypsum Fibrosum (GF) in a dry-weight ratio of 1:1:1:1; TCM2 = COP:RA:AR:GF in a dry-weight ratio of 1:1:0.5.
different ($P > 0.05$) from that measured in the NTG. By d 10, no significant differences in MDA content were observed between any of the treatment groups.

**DISCUSSION**

**In Vitro and In Vivo Heat Stress Models**

Global warming is becoming a major public concern as high temperatures in our environment start to impair animal production and health, especially in tropical areas. Heat stress can bring about a series of physiological and metabolic changes in pigs, such as increased body temperature, panting, and respiratory alkalosis (Patience et al., 2005) that lead to poor growth performance, weakened immune responses, and decreased resistance to disease (Spencer et al., 2005). Hinnebusch et al. (2002) reported that at high temperatures, systemic blood flow is redistributed to support the heart, brain, and other vital organs, as well as to dissipate heat via the peripheral circulation, leading to gastrointestinal tract ischemia and damage to the sensitive epithelium lining of the intestinal villi.

The effects of heat stress and its regulation can be studied in vitro and in vivo (Mahmoud et al., 2004; Marai et al., 2007; Yu et al., 2010b). The IEC-6 cell line is commonly used as a model to study intestinal epithelial cell status (Wood et al., 2003), wherein the MTT assay may be used to screen inhibitory effects of different culturing temperatures on IEC-6 growth over time. Our findings corroborate previous studies showing that IEC-6 cells subjected to temperatures between $41$ and $43^\circ C$ for $2$ to $5$ h demonstrated heat stress characteristics. The fact that the proliferation and viability of IEC-6 cells cultured at $37^\circ C$ remained constant was testament to the reproducibility of the cell culture.
Effects of the Active Components of TCM on IEC-6 Proliferation

Since the establishment and characterization of the IEC-6 cell line, these cells have been used extensively to elucidate mechanisms of cell growth and wound healing; to investigate the influences and actions of cytokines and growth factors; and to explore autocrine and paracrine regulation of growth, extracellular matrix regulation of differentiation, and the effects of endotoxins and infection (McCormack et al., 1992; Park et al., 1992; He et al., 1993; Dignass et al., 1994; Meyer et al., 1994). In the present study, the proliferation and viability of IEC-6 cells were monitored to verify the functions of the active components of TCM and to establish the most effective treatment doses. The results showed that all 4 active components could improve IEC-6 proliferation impaired by heat stress. Treatment doses of 200 µg/mL of HAA, 100 µg/mL of RAA, 100 µg/mL of CPA, and 200 µg/mL of GFE effectively promote IEC-6 proliferation. Previously, Yin et al. (2008) reported that 1 µg/mL of Atractylodes lancea essential oil had proliferative effects on osteoblast-UMR-106 cells, whereas Chen et al. (2007) demonstrated that CPA treatment could upregulate splenic lymphocyte proliferation and dramatically rebalance aberrant interferon-γ and IL-4 expression induced by heat stress.

Morrison et al. (2005) and Zhang et al. (2006) previously reported that high temperatures led to changes in cell oxidative status and the accumulation of reactive oxygen species (ROS). The SOD and GSH-Px are 2 important antioxidant scavenger enzymes in animals, which help to eliminate ROS and maintain ROS at manageable concentrations (Kimnula et al., 1995). During periods of heat stress, the ROS generated through nonenzymatic and enzymatic reactions as part of normal cellular metabolism cannot be removed efficiently by antioxidants (SOD, GSH-Px), leading to an imbalance between ROS production and elimination (Circu and Aw, 2010). Lipid peroxidation is a well-characterized mechanism of oxidative damage caused by ROS (Barazzone and White, 2000). Measurement of MDA content provides a convenient index of lipid peroxidation occurring in a system (Efe et al., 1999).

Effects of TCM on Antioxidant Status and Lipid Peroxidation in Jejunal Epithelial Tissues

In TCM, medicines are combined according to the principia of principal, associate, adjuvant, and messenger. With respect to the decoctions used in this study, COP and RA are considered principal medicines and AR and GF are considered associate medicines. The COP, RA, AR, and GF were mixed at a ratio of 1:1:1:0.5. Previous trials have proven that the compound formed by combining these 4 TCM improves growth performance (Song et al., 2008), jejunal repair (Liu et al., 2009), intestinal mucosa immune function (Yu et al., 2010a), and intestinal absorption and transportation (Song et al., 2010) in pigs exposed to heat stress. Although in the present study, CPA, RAA, HAA, and GFE all independently enhanced the antioxidant status and reduced lipid peroxidation in IEC-6 cells, the relative ratio of these components in a combined prescription is pertinent to the efficacy of TCM. Our in vivo experiments, showing that both TCM1 and TCM2 improve growth performance in pigs exposed to heat stress, is in accordance with the findings of Song et al. (2010), who reported that decoctions containing COP, RA, AR, and GFE at a ratio of 1:1:1:0.5 improved porcine growth performance and glucose absorption in the small intestine.

In conclusion, IEC-6 cells cultured at 41°C for 3 h exhibited heat stress characteristics. The HAA, RAA, CPA, and GFE were highly effective at enhancing IEC-6 cell proliferation and viability, particularly at treatment doses of 200, 100, 100, and 200 µg/mL, respectively. The 4 active components could increase SOD, GSH-Px activities, and GSH content and significantly...
decrease MDA content. The TCM1 and TCM2 increased growth performance in pigs exposed to heat stress. Both these decoctions significantly increased SOD and GSH-Px activity, and decreased MDA content in porcine jejunum, with TCM2 appearing to be more effective than TCM1. This work provides insight into the antioxidant function of TCM using in vitro and in vivo models of heat stress. Mechanistic studies on the active components of TCM decoctions and further proof of its efficacy may help to increase international recognition of TCM.

**LITERATURE CITED**


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