ISSN : 0974 - 7435 Volume 10 Issue 23

2014

BioTechnology

An Indian Journal

FULL PAPER

BTAIJ, 10(23), 2014 [14073-14079]

Shi-he-powder relieve stress caused by weaning to piglet

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ABSTRACT

In this study, the effect of Shi-He-Powder (SHP) on remitting weaning stress of piglet was observation. Each of four treatment groups' piglets were respectively feed by basal diet (control group), and the basal diet add into1 fold SHP (1SHP group), 0.5 fold SHP (0.5SHP group), and 2 fold SHP (2SHP group). The behavior change, feed intake, weight gain and blood routine were observed, the expression of Inflammatory factors were detected by ELISA and RT-PCR, and HE staining was used to observe histology of the liver and jejunum. The result shows that the weaned stress will cause diarrhea and other adverse effects to the piglets. But comparing control group, SHP treatment can relieved diarrhea, significantly increased daily gain ($P < 0.05$), and decreased feed conversion ratio ($P < 0.05$); the tissue morphology of liver and the top of jejunum villi were more complete than control group. The SHP treatment can decreased the expression of IL-1β, IL-6, iNOS and PGE2, but have no significant different with TNF-α. This study demonstrate that weaning stress can cause diarrhea and other adverse effects, and SHP can effectively relieve these phenomenon, and increased daily gain, decreased feed conversion ratio. The mechanism of SHP may is that SHP can play a role in against body inflammatory reaction and protect liver and intestinal from stress injure, ultimately promote digestive absorption and dynamiting to piglet.

KEYWORDS

Shi-he-powder; Weaning stress; Piglets.

INTRODUCTION

The piglet will experience a series change of physiological, environment and population, which can directly cause the weaned stress to piglet, when they leave sows and be column feed. weaned stress can cause the change of intestinal, immunity, growth performance and life habits[1]. Studies have shown that the incidence of stress disorder after weaning piglets is as high as 73.19%, the mortality was 8.25%, and diarrhea was more than 49.48%, the incidence of edema disease accounted for about 2.58%, eating the tail rates were 17.53%. So, piglets weaned stress can cause diarrhea, immunity decline, and eventually led to the piglet growth slow even death, this will greatly hindered the development of pig industry[2]. Therefore, piglets weaned stress not only restricts the development of animal husbandry, but also cause imponderable damage to our country economic growth.

The reasons of causing weaning stress were very complex. At present, the study shown that bacteria increased, dietary changes and immunity decreased all can cause piglet's weaned stress[1]. In order to get the most production speed and efficiency, in the actual production, we must make the piglets quickly adapt to this stress. For a long time, antibiotics have been widely used to treat various animal diseases. Studies have reported that chlortetracycline and terramycin can effectively resist the piglets weaned stress. But, the extensive use of antibiotics can cause the drug resistance of bacteria increase, which will not only cause the animal disease resistance is abate, but also more likely to endanger the human health. Therefore, looking for a kind of alternative methods of natural, healthy low side effect, has become the big trend of the society. In recent years, most of the Chinese herbal medicine that have nutrition and medicinal function be used to promote the growth of weaning piglets, and improve the feed conversion rate[2].

Pomegranate rind is rich in polyphenols, organic acids, flavonoids, alkaloids and other chemicals. It cans antidiarrheal, hemostasis, sterilization, expelling parasite and other functions[3]. Guo *et al* found pomegranate rind tannin have significant bacteriostat effect on against Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, thus pomegranate rind can antibacterial and anti-inflammatory, treating enteritis and diarrhea[4-6]. Terminaliachebula is a traditional Chinese medicine (TCM) to remedy intestinal diarrhea and detoxify, and it was been wide used to bacteriostat and anti- tumor in modern pharmacological clinical applications[7].

Our study was guided by the basic theory of TCM, and SHP was mainly made from pomegranate rind and terminaliachebula. Our previous study found SHP has a good effect to chicken E. coli disease[8]. But there is not report to SHP treating weaned stress to piglet.

MATERIALS AND METHODS

Experimental animal grouping and processing

The experimental procedures involving animals in this study were approved by the Animal Care Center of the Beijing University of Agriculture, Beijing, China. Eighty four at 28 days of age weaning changbai piglets, weight of 10.27 ± 0.55 kg, bought from Beijing HuichuanZhaoliang animal husbandry Co., Ltd. Piglets that weight was not significant difference were randomly divided into four treatment groups, every treatment group set three repeat, each repeat had 7 pigs.

Each of four treatment groups' piglets were respectively feed by basal diet (control group), and the basal diet add into1 fold SHP (1SHP group, recommend dosage of 1.0g/kg•bw), 0.5 fold SHP (0.5SHP group), and 2 fold SHP (2SHP group). The Behavioral changes, recording intake and weight gain of each piglet were record.

The sample collection and process

8th day trial, each treatment group were randomly selected six test piglets per replicate extracted two piglets, collected before the vena cava blood, test blood indicators and detect associated inflammatory cytokines. Each treatment group were randomly selected three test piglets per replicate extract a piglet, dissection. In the middle of the jejunum collected two 2cm intestine, rinse with saline and placed in 10% neutral formalin fixed. The liver collected about 1.5cm*1cm, placed in 10% neutral formalin fixed. After fixation, observed jejunum, liver histological changes under paraffin-embedded sections with hematoxylin and eosin (HE) staining. Another piece of liver tissue, frozen in liquid nitrogen load, -80 ° C to save.

ELISA analysis

ELISA method using test piglet serum inflammatory cytokines (IL-1β, IL-6, TNF-α, iNOS and PGE2) expression levels were detected. Interleukin (IL)-1β, Interleukin (IL)-6, tumor necrosis factor (TNF)-α, inducible NO synthase (iNOS), prostaglandin E (PGE2) enzyme-linked immunosorbent assay (ELISA) kits were obtained from Wuhan Cusabio Biotech. Co., Ltd. (Wuhan,China).

Total RNA extraction, quantitative real-time PCR

Take piglet liver tissue were extracted RNA, reverse transcription after detect changes in the expression of inflammatory cytokines using Real-Time PCR method. Primers were designed using Primer5.0 software, were synthesized by the Shanghai Shenggong Bioengineering Co., Ltd. (Shanghai, China).

Data analysis

All data were analyzed using SPSS 17.0 software. One-way ANOVAs and SNK multiple comparison methods were used to determine statistical significance with a *P*-value less than 0.05 considered significant.

RESULTS

The effect of weaning stress on piglets

Typical symptoms of piglet's weaning stress include irritability, gathered to get together, diarrhea and other stress response. Among them, the most typical symptom is diarrhea. Before weaning, piglet's perianal were free of dirt, and feces forming the brown, shiny, normal row, (Figure 1A), but after weaning, weaned stress occurs, piglets happen the concrete manifestation of diarrhea, stool into a paste, light gray, and perianal residual contamination (Figure1B). The experiment shows that weanling stress would indeed cause adverse effects such as diarrhea to piglet. Therefore, we apply the SHP to feed weaning piglets and detecting whether SHP can ease this stress caused by weaning.

Figure 1 : Comparation of piglets' defecation. A), B), C) respectively show, at before and after weaning, and 1SHP treatment piglets' defecation

SHP can relieve weaning stress to piglet

Clinical observations show: The SHP treatment can stabilize piglets' mood, relieve diarrhea, and returned to normal fecal situation (Figure 1C).

Comparing with control group, the diarrhea rates of each dose SHP treatment group all reduce (Figure 2A). From Figure 2B to 2D, there are not significant different of Average daily feed intake (ADFI) among 0.5SHP, 1SHP, 2SHP and control groups ($P > 0.05$). While, the daily gain(ADG) of each SHP groups all significant exceed control group ($P < 0.05$), and feed to gain (F/G) were significant lower than control, and 1SHP group has best effect.

As seen from TABLE 1, after weaning stress piglets were treated by SHP, 0.5SHP and 1SHP groups' white blood cell count (WBC) were significantly higher than the control group ($P < 0.05$), and have not significant between 2SHP and control group ($P > 0.05$). The lymphocyte percentages (LYM%) of each group were significant high than control group ($P < 0.05$).

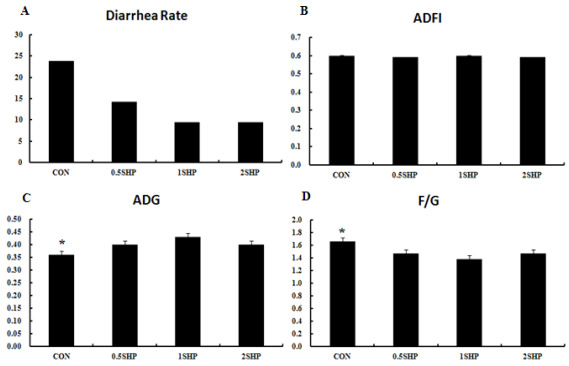

Figure 2 : The effect of SHP treatment to weaning stress piglet. A), B), C), D) respectively represent each group piglets' diarrhea rate, ADFI, ADG and F/G. * indicates $P < 0.05$.

TABLE 1 : The effect of SHP on routine blood to Piglets(n=6)

Item	CON	0.5SHP	1SHP	2SHP
WBC (10^9/L)	14.58±3.95^b	17.60±3.75^a	19.98±2.46^a	15.85±2.90^b
RBC (10^{12}/L)	4.88±0.50	5.03±0.28	4.91±0.76	5.32±0.47
HGB (g/L)	81.50±7.01	85.83±6.01	84.17±6.52	82.67±7.66
PLT (10^9/L)	243.67±45.65	252.50±19.71	260.00±36.38	270.71±56.44
LYM% (%)	42.13±2.57^b	46.30±1.10^a	48.70±2.97^a	48.60±1.55^a
MON% (%)	2.40±0.31	2.47±0.51	2.67±0.49	2.95±0.21
NEUT% (%)	37.03±2.40	38.53±3.55	40.38±3.87	38.00±3.96
EOS% (%)	1.23±0.15	1.30±0.35	1.52±0.33	1.55±0.10

Different lowercase letters represent significant differences in which $P < 0.05$.

After the autopsy, the liver and jejunum of piglets were HE stained to observe tissue morphology under the microscope, the control group piglets' liver tissue happen bleeding and other obvious damage (Figure A1), while the 0.5SHP, 1SHP, and 2SHP group piglets' liver tissue reveal more complete (Figure A2-4). Control group's jejunum villus top part fall off, and the structure is not complete (Figure B1), while the 0.5SHP, 1SHP, and 2SHP group piglets' jejunum villus top structure is intact (Figure B2-4).

Figure 3 : The tissue slices of each group's liver and jejunum (HE stain, × 200). A) The liver portal area slices of each group. B) The jejunum villus top slice of each group. The number of 1, 2, 3, 4 represent respectively control, 0.5SHP, 1SHP, and 2SHP group.

The effect of SHP on the expression of inflammatory factors to weaned stress piglets

ELISA was used to test piglets' inflammatory cytokines (IL-1β, IL-6, TNF-α, iNOS and PGE2) expression levels in serum. As shown from Figure 4, after SHP treatment, IL-1β expression of control group significant higher than 2SHP group ($P < 0.05$), but not significant with the 0.5SHP and 1SHP group ($P > 0.05$). The IL-6 and PGE2 expression of control group was significant higher than 1SHP group ($P < 0.05$), but not significant with the 0.5SHP and 2SHP group ($P > 0.05$). The expression of iNOS of each SHP group were all highly significant with control group ($P <0.01$), but not significant in TNF-α levels ($P >0.05$).

Real-time PCR was used to detect the mRNA levels of inflammatory cytokines (IL-1β, IL-6, TNF-α, iNOS) in piglets' liver. As shown from Figure 5, the expressions of IL-1β and iNOS among 0.5SHP, 1SHP, and 2SHP group were significantly lower than the control group ($P < 0.05$). The expression of IL-6 in 1SHP was significantly lower than the control group ($P < 0.05$). The TNF-α level have not significance among all groups ($P <0.05$).

Figure 4 : The expression levels of inflammatory cytokines of each group piglets. A), B), C), D), E) respectively represent the levels of IL-1β, IL-6, TNF-α, iNOS and PGE2. Different uppercase letters represent significant differences in which $P < 0.01$ and different lowercase letters represent significant differences in which $P < 0.05$

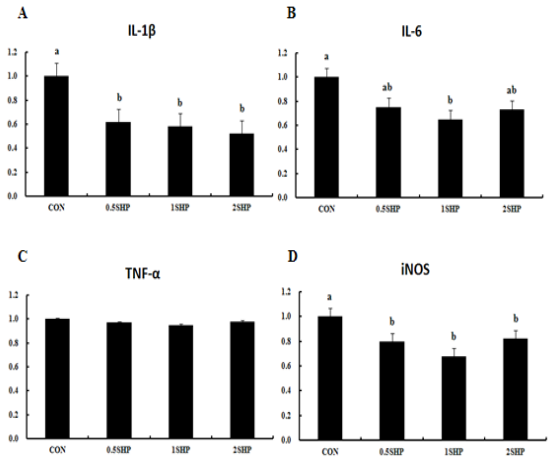

Figure 5 : The mRNA levels of inflammatory cytokines in weanling stress piglets' liver. A), B), C), D), E) respectively represent the expression levels of IL-1*β*, IL-6, TNF-*α*, iNOS and PGE2. Different lowercase letters represent significant differences in which *P* < 0.05

DISSCUSS

Weaned is an integrated stress response for piglets, including the sows and piglets littermates separated, transported to a new environment and sudden changes in dietary[9]. Growth retardation, gastrointestinal disorders and diarrhea are common problems after weaning[10]. In this study, we using natural weaning stress model, studies show that will appear diarrhea after weaning, clusters, irritability and other adverse reactions.

Pomegranate rind has astringent intestinal to relieve diarrhea, stanch bleeding, deworming and other effects. It used for diarrhea, dysentery, Blood in the stool, rectal prolapse, uterine bleeding, vaginal discharge, parasitic abdominal pain. In recent years pomegranate rind become a hot research scientist. Because of its unique physiological activity such as lowering blood pressure, anti-oxidation, anti-viral, anti-tumor, hypoglycemic, anti-diarrhea and anti-bacterial and so on[11]. Pomegranate peel powder was also confirmed with anti-inflammatory[8], immune regulation[12] features. Terminalia is commonly astringent TCM, has astringent intestinal to relieve diarrhea, reconcile herbs, detoxification and other effects. Terminalia whole plants have high medicinal value, is used to treat a variety of human diseases. Folk often used in the treatment of diarrhea, asthma, sore throat, vomiting, hiccups, and other diseases[13]. This test is mainly through diarrhea rate, growth performance and other indicators determine the ability to relieve weaning stress by SHP. The results show that: the SHP each dose group could decrease due to weaning piglet diarrhea caused by stress and improve piglet ADG, lower F/G.

Blood test, including the quality and quantity of blood visible component of red blood cells, white blood cells and platelets these three systems for testing and analysis, It is one of the most common clinical laboratory testing project basis. Blood on the guarantee function of regulating, the body's normal metabolism and homeostasis in vivo plays an important role. Therefore, analysis of blood test and understand the various early lesions, the nature of the disease progression and treatment of the disease has a certain significance[14]. Serum albumin, globulin and total protein content decreased is usually a sign of an animal occurs stress response[15]. white blood cells is a huge family of blood cells, swallowed foreign body can produce antibodies, the body injury heal, resist pathogen invasion and immune diseases plays an important role. When the body occur inflammation or other diseases can cause white blood cells and a variety of white blood cells percentage changes[16]. In this experiment, feeding SHP each dose group weaned WBC, LYM% were higher, indicating SHP can alleviate the stress caused by weaning of piglets for resist inflammatory response plays a role.

The tissue morphology was more direct to see the impact of weaning stress on piglets, in experimental proof weaning stress caused piglets liver and intestinal tissue damage. SHP can effectively alleviate the stress caused due to weaning piglets liver, intestinal tissue damage. It showed SHP may stimulate digestion and absorption of piglets, improve ADG, reduce F/G.

Cytokines are a class of small proteins (less than 50kDa), as an inter-cell communication signal play a role in hematopoiesis, stress, inflammation, immunity and tissue repair process[17]. Has been isolated from 30 kinds of cytokines, which play an important role in immune regulation factors are IL, CSF, IFN, and TNF. After stimulation by pathogens macrophages released IL-1*β*, TNF-*α* can enhance local inflammatory reaction[18-19]. NO is an important signaling molecules and pro-inflammatory mediators, produced by iNOS, in the pathogenesis of inflammation is an important role, its overproduction in exceptional circumstances[20]. PGE2 is another important pro-inflammatory factors expression by COX-2[21]. These inflammatory factors can cause a huge change in metabolism[22]. These factors mainly from a wealth of tissue macrophages, such as: the liver and spleen[23.24]. Studies have shown above, SHP can alleviate the stress caused by weaning of piglets related to inflammatory responses against, and therefore, after the experiments were on plasma and liver tissue of inflammatory cytokines (IL-1*β*, IL-6, TNF-*α*, iNOS and PGE2) expression levels were detected. Experimental study found: in the plasma and liver tissue SHP each dose group of IL-1*β*, IL-6, iNOS, PGE2 expression are subject to varying degrees of

inhibition, while the ADG and expression of inflammatory factors over these four is negative correlation. Therefore, inhibit the expression of cytokine IL-1β, IL-6, iNOS, PGE2 may reduce liver inflammation and reduce the negative effects of cytokines on liver tissue, promote digestion and absorption of piglets, promote weight gain of piglets.

ACKNOWLEDGEMENT

We are thankful for the help from the members of CAU-BUATCVM teaching and research team. This work was supported by grants from the National Twelve-Five Technological Supported Plan of China (No. 2011BAD34B01) and National Natural Science Foundation of China (No.31272478).

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