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RESEARCH ARTICLE

Development and evaluation of a herbal formulation with anti-pathogenic activities and probiotics stimulatory effects

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Abstract

Searching alternative feed antibiotics is always a study hotspot in the field of animal production. In this study, the anti-pathogenic activities and probiotics stimulatory effects of 30 kinds of herbs were screened through Oxford cup method and Hungate roll method. 15 herbs showed significantly antibacterial activities (P<0.05) against *Escherichia coli*, *Salmonella enteritidis*, *Salmonella typhimurium* and *Staphylococcus aureus*. Seven herbs showed greatly stimulatory promoting effects on *Lactobacillus acidophilus* and *Bifidobacterium longum*. Finally, five herbs were combined for the developed formulation with selective antibacterial properties and they were *Mume Fructus* (35%), *Isatidis Folium* (25%), *Moslae Herba* (20%), *Chrysanthemi Indici Flos* (13%) and *Bupleuri Radix* (7%). The herbal formulation showed significantly antibacterial abilities against four pathogens and stimulatory promoting abilities on two probiotics *in vitro* and the equivalent activities in broiler chickens *in vivo* against *E. coli* and *L. acidophilus*. The toxicity study showed it had no toxicity, which indicated that it would be a kind of preferred candidate for an alternative antibiotic in future animal production.

Keywords: herbal formulation, alternative feed antibiotic, anti-pathogenic activity, probiotic stimulatory effect

1. Introduction

Since the 1950s, the feed antibiotics have been successfully used to kill or inhibit the growth of pathogenic bacteria in livestock and poultry production (Allen *et al.* 2013). Their use has dramatically improved and met the increasing demand for animal foods in a long history period. However, a widespread administration of antibiotics has resulted in the rise of antibiotic-resistant bacteria and reduction of the effective in treating bacterial diseases (Taylor *et al.* 2011). On the other hand, the non-fulfillment of antibiotic withdrawal periods induced the presence of antibiotic residues in subsequent animal foodstuffs which would pose potential health hazards to human, such as allergies, toxic effects, acquisition of drug resistance in pathogens in human body, as well as cancer (Andrew *et al.* 2009; Martínez 2009). Since 1999, European Union (EU) have prohibited the uses of five antibacterial growth promoters, e.g., zinc bacitracin, spiramycin, tylosin, virginiamycin and olaquindox in animal feeding stuffs (Burel 2012). Therefore, there is a growing awareness in different countries and regions to seek safer alternative antibacterial material for better animal production.

In recent years, special attention has been paid to phytogenic and herbal products by many researchers due to their

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remarkable antibacterial properties (Vimal *et al.* 2010; Oh *et al.* 2013). It could cause structural and functional damage to the bacterial cell membrane (Yoo *et al.* 2011; Ye *et al.* 2013), and therefore kill the bacteria or inhibit the bacteria growth. Cho *et al.* (2014) observed that the supplement of a phytogenic feed additive inhibited *Clostridium perfringens* and *Escherichia coli* proliferation in small and large intestines in broiler chicken under oral C. *perfringens* challenge. Beside the antibacterial activities, the herbal-derived feed additives could also improve the growth performance of animal production, such as the body weight, average daily gain, and gain/feed ratio (Landy *et al.* 2011; Hashemi *et al.* 2012).

Normal health intestinal microflora contains a large amount of lactic acid bacteria which serves as the barrier against colonization by potentially pathogenic microorganisms and overgrowth of already present opportunistic microorganisms (Salminen *et al.* 1998; Rashid *et al.* 2012). It is always in a dynamic equilibrium and could be also altered by many factors, such as temperature, diet, drug therapy and environment (Takaishi *et al.* 2008). While the traditional antibiotic would inhibit or kill the pathogenics, and the balance of intestinal microflora would also disturbed during the drug administration. Previous studies showed herbal-derived antibacterial treatment has no adverse influence on the number of intestinal microflora, and even has growth stimulatory effect on it (China *et al.* 2012).

According to the European Union legislation (EC 2003) on feed additives in animal nutrition, phytogenic compounds have been categorised as “sensory additives” and in particular as flavouring compounds. To our knowledge, individual herb was rarely reported with both significant inhibitory effects against pathogens and effective stimulatory abilities for probiotics growth. The mixture of several herbal extracts, when used in formulation enhances the beneficial effects through synergistic amplification and diminishes any possible adverse effects and offers advantage over a single isolated ingredient (Williamson 2001). So the aim of this study was to develop a kind of herbal formulation showing both anti-pathogenic activities and probiotics stimulatory effect. In this study, the anti-pathogenic activities and probiotics stimulatory effects of 30 kinds of herbs were screened through Oxford cup method and Hungate roll method, and results would be helpful for in-depth study of the novel alternative antibacterial material in animal production.

2. Results

2.1. Antibacterial activities of herbal extracts

The antibacterial activities of aqueous herbal extracts against *E. coli*, *Salmonella enteritidis*, *Salmonella typhimurium*, and *Staphyococcus aureus* were assayed by the inhibition zone observed in mm and the results were summarized in Table 1. There was a significant variation for the antibacterial activities of 30 herbal extracts compared to negative control. For *E. coli*, 11 extracts (37%) showed extremely significant inhibitory effects (P<0.01) and two extracts (7%) showed significant inhibitory effects (P<0.05). For *S. enteritidis*, 11 extracts (37%) showed extremely significant inhibitory effects (P<0.01) and one extract (3%) showed significant inhibitory effect (P<0.05). For *S. typhimurium*, five extracts (17%) exhibited extremely significant antibacterial activities (P<0.01) and five extracts (17%) exhibited significant antibacterial activities (P<0.05). Among these herbs, *Mume Fructus* showed the strongest antibacterial activities for all the Gram-negative bacteria tested. For the Gram-positive pathogen tested, *S. aureu*, 13 extracts (43%) showed extremely significant antibacterial activities (P<0.01) and three extracts (10%) showed significant inhibitory effects (P<0.05). Of them, *Phellodendri Chinensis Cortex* showed the most significant antibacterial activity against *S. aureu* with the diameter of the inhibitory zone (DIZ) value of 21.97 mm.

In general, of the 30 herbs tested, eight herbal extracts exhibited relatively high antibacterial activities against all tested pathogenic bacteria, namely *Mume Fructus*, *Galla Chinensis*, *Caryophylli Flos*, *Chebulae Fructus*, *Schisandrae Chinensis Fructus*, *Houttuyniae Herba*, *Scutellariae Radix* and *Rehmanniae Radix*. Meanwhile, seven herbs displayed specific antibacterial activities. For instance, *Chrysanthemi Indici Flos*, *Forsythiae Fructus* and *Moslae Herba* all showed extremely significant inhibitory effects against *E. coli*, *S. enteritidis* and *S. aureu*, respectively. *Bupleuri Radix* and *Dryopteridis Crassirhizomatis Rhizoma* showed extremely significant (P<0.01) inhibitory effects against *S. aureu*. *Phellodendri Chinensis Cortex* exhibited extremely significant (P<0.01) inhibitory effects against *S. enteritidis* and *S. aureu*. *Isatidis Folium* extract was found to be extremely significant (P<0.01) inhibitory effect against *E. coli*.

2.2. Growth stimulatory activities of herbal extracts

The effects of herbal extracts on probiotics were assayed by the well accepted Hungate roll method and results of colony numbers in triplicate were shown in Table 2. For *Lactobacillus acidophilus*, there were seven herbs whose additions in culture media increased the colony numbers. *Moslae Herba*, *Chrysanthemi Indici Flos*, *Isatidis Folium* and *Rehmanniae Radix* showed extremely significant (P<0.01) effect, and *Forsythiae Fructus*, *Houttuyniae Herba* and *Bupleuri Radix* showed significant (P<0.05) effect. Among them, *Isatidis Folium* extracts showed the strongest probiotic promoting effect for *L. acidophilus*, which enhanced 9.5 folds of colony numbers than negative control. For *Bifidobacterium longum*,