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Different essential oils in diets of broiler chickens: 2. Gut microbes and morphology, immune response, and some blood profile and antioxidant enzymes

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ABSTRACT

A study was conducted to evaluate the effect of three essential oils with different main chemical structures, i.e., cinnamon bark oil (CNO), clove bud oil (CLO) and ajwain seed oil (AJO) as an alternative to antibiotic growth promoter (AGP) on intestinal health, immune response and antioxidant status in broiler chickens. Four hundred one-day-old broiler chicks were randomly divided into five dietary groups comprising of eight replicates (n = 8) in each group. Each replicate contained ten chicks. Five dietary groups consisted of: 1) a basal diet (control; without any antibiotic and essential oils); 2) the basal diet + bacitracin methylene disalicylate added at a dose of 50 mg/kg diet (AGP); 3) the basal diet + CNO at 300 mg/kg diet (CNO); 4) the basal diet + CLO at 600 mg/kg diet (CLO); and 5) the basal diet + AJO at 400 mg/kg diet (AJO). Both AGP and CNO significantly increased the villi height in duodenum ($P = 0.004$), jejunum ($P = 0.008$) or ileuim ($P = 0.003$) compared with the control. The counts of *Escherichia coli* in pre-caecal contents decreased ($P = 0.006$) in the groups receiving AGP and CNO and the counts of *Clostridium* spp. decreased ($P = 0.029$) in the AGP group compared with the control. Population of *Lactobacilli* spp. was not affected ($P = 0.39$) by any diets. Antibody titers against Newcastle disease virus increased with CNO, CLO and AJO supplementation ($P < 0.001$) compared with the control and AGP. The concentrations of cholesterol in serum reduced ($P = 0.030$) in CNO, CLO and AJO groups compared with the control and AGP groups. Supplementation of CNO and CLO increased ($P = 0.001$) the superoxide dismutase activity in serum compared with the AGP. In conclusion, supplementation of CNO could be more advantageous than the AGPs in diets due to its combined beneficial effects on immune response, gut health, antioxidant status and blood cholesterol in broiler chickens.

Abbreviations: AGPs, antibiotic growth promoters; AJO, ajwain oil; BMD, bacitracin methylene disalicylate; CLO, clove bud oil; CNO, cinnamon bark oil; EE, ether extract; CP, crude protein; DM, dry matter; EOs, essential oils; ME, metabolisable energy; NDF, neutral detergent fiber; OM, organic matter; SEM, standard error of mean; VH, villi height; CD, crypt depth; NDV, Newcastle disease virus; SOD, superoxide dismutase

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1. Introduction

Several feed additives including drugs and antibiotic growth promoters (AGPs) are usually included in poultry feeds to improve the efficiency of production, product quality and to prevent diseases (Bedford, 2000; Brenes and Roura, 2010). The poultry industries are currently transforming towards decreased use of AGPs due to increased concerns regarding development of antibiotic-resistant bacteria and transfer of residues of AGPs in meat and eggs, which may cause side-effects in humans (Brenes and Roura, 2010; Landers et al., 2012). In 2006, the European Union (EU) approved a resolution to ban the use of antibiotics as growth promoters for animals (Landers et al., 2012). This restriction has resulted in an increased incidence of enteric disorders such as necrotic enteritis in poultry. Thereafter, numerous biological products, including enzymes, probiotics, prebiotics, synbiotics, organic acids and plant extracts (phytobiotics) have been increasingly evaluated as alternatives to antibiotic feed additives in diets for monogastric animals (Bedford, 2000; Wenk, 2003). The alternative strategies have mainly been focused to prevent proliferation of pathogenic bacteria and modulation of indigenous bacteria so that the health, immune status and performance are improved (Bedford, 2000; Brenes and Roura, 2010).

In recent years, the use of dietary plant derived natural bioactive compounds (phytobiotics) including essential oils (EOs) has attracted increased attention to augment performance and health in poultry production. The knowledge regarding the modes of action and aspects of application of phytobiotics including EOs as feed additives are quite rudimentary (Windisch et al., 2008). Herbs, spices and their EOs can exert antimicrobial, coccidiostatic or anthelmintic activities in monogastric animals (Cowan, 1999; Wenk, 2003). Essential oils also exhibit antioxidant, anti-inflammatory, anti-carcinogenic, and hypolipidemic activities (Viuda-Martos et al., 2010) and could favourably affect gut functions by stimulating endogenous digestive secretions, e.g., enzymes, bile and mucus, and maintaining intestinal epithelial structures (Manzanilla et al., 2004; Jang et al., 2007). Thus, EOs can be used as growth promoters in animal production.

The response of EOs as AGP in chickens to improve growth performance and feed efficiency is not consistent, which has been attributed to dose, source and type of EOs, dietary and management conditions that have always not been investigated systematically (Cross et al., 2007). Plant bioactives with different molecular structures and functional groups greatly vary in their antimicrobial activities due to diverse lipophilic/hydrophilic property, hydrogen-bonding capacity and polarity (Patra, 2012). Cinnamon bark oil (CNO) contains 505–977 g/kg of cinnamaldehyde (Paranagama et al., 2001; Singh et al., 2007), an aromatic aldehyde that has greater antibacterial activity than the other oils of phenolic nature such as carvacrol and thymol. The main compositions of clove bud oil (CLO) are eugenol (831 g/kg; Nunez and Aquino, 2012) that is primarily responsible for bacteriocidal/bacteriostatic properties (Walsh et al., 2003; Tajkarimi et al., 2010; Devi et al., 2010). The major constituents of ajwain seed oil (AJO) are thymol (367 g/kg) and its precursors, γ-terpinene (365 g/kg), and ρ-cymene (211 g/kg) (Goudarzi et al., 2011). Thymol and its precursors, cymene and terpinene exhibit strong antimicrobial activities (Burt, 2004). In a companion study, growth performance, nutrient utilisation, nitrogen excretion, carcass traits, chemical and fatty acid profiles of meat have been investigated in broiler chickens fed diets supplemented with CNO, AJO and CLO (Chowdhury et al., 2017). This paper reports the effects of these three EOs with different chemical structures on intestinal microbiota, gut morphology, immune response, anti-oxidant status and blood biochemical variables in broiler chickens.

2. Materials and methods

2.1. Broiler chickens, experimental design and diets

All the animal experimentation procedures were approved by the Institutional Animal Ethics Committee, West Bengal University of Animal and Fishery Sciences, Kolkata, India, and the study was conducted following the guidelines of the Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA), Government of India. Four hundred one-day-old mixed sex commercial broiler chickens (Vencobb 400, Venkys, Pune, India) were randomly allocated into five dietary groups. Each group comprised of eight replicated pens (n = 8) and each replicate contained ten broiler chickens. The dietary groups consisted of: 1) a basal diet without antibiotic and essential oils (control); 2) the basal diet added with bacitracin methylene disalicylate (BMD; containing 100 g BMD/kg product) at the dose rate of 0.05 g/kg diet (AGP); 3) the basal diet added with CNO (*Cinnamomum zeylanicum*) at the rate of 0.3 g/kg (CNO); 4) the basal diet added with CLO (*Syzgium aromaticum*) at the rate of 0.6 g/kg diet; and the basal diet supplemented with AJO (*Trachyspermum copticum*) at the rate of 0.4 g/kg diet. The doses of the EOs were decided based on an in vitro study on pathogenic culture of *Escherichia coli* and *Salmonella gallinarum* isolated from chickens reared locally. Initially, different EOs and extracts were tested for their inhibitory effects on these pathogenic bacteria, and CNO, CLO and AJO showed most inhibitory properties on the tested bacteria with CNO requiring the lowest minimum inhibitory concentration, followed by AJO and CLO.

The basal diet based on corn and soybean in mash form was formulated to meet or exceed nutritional requirements of broiler starter (d 1–14), grower (d 15–28) and finisher (d 29–39) chickens using a ration formulation software as per the commercial broiler Vencobb chicken recommendations (Vencobb management guide, Venkys, Pune, India). The basal diet contained 228 g/kg of crude protein (CP) with 12.6 MJ/kg of metabolozable energy (ME) for broiler starters (d 1–14), 214 g/kg of CP with 13 MJ/kg of ME for broiler growers (d 15–28), and 195 g/kg of CP with 13.4 MJ/kg of ME for broiler finishers (d 29–39) (Tables 1 and 2). The natural pure EOs were purchased from Allin Exporters, Noida, India. Premixes of the three EOs separately and BMD were prepared, which were added to the basal diet and mixed homogenously in a feed mixer to obtain the EO and AGP diets. The experimental diets were prepared every week to minimise the loss of bioactive compounds in feeds. The blended mash feeds were packed in separate labelled